

**EFFECTS OF DIEL-CYCLING HYPOXIA AND pH ON GROWTH AND
BEHAVIOR OF JUVENILE WEAKFISH (*Cynoscion regalis*)**

by

Danielle M. Lifavi

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Marine Studies

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**INTERACTIVE EFFECTS OF DIEL-CYCLING HYPOXIA AND pH ON
GROWTH AND BEHAVIOR OF JUVENILE WEAKFISH (*Cynoscion regalis*)**

by

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ABSTRACT

During summertime, dissolved oxygen (DO) and pH cycle daily in shallow estuarine waters as a result of organisms' photosynthesis and respiration. Eutrophication exacerbates these physiochemical cycles, which can impact growth and behavior of young fishes which use estuaries as nursery habitat. Laboratory experiments were conducted on juvenile weakfish (*Cynoscion regalis*) to determine the individual and interactive impacts of natural levels of diel-cycling hypoxia and co-varying pH on growth rate and behavior. Computer-controlled, recirculating, aquarium systems were used to expose fish to three DO treatments [extreme (1-11 mg O₂ l⁻¹) and moderate (3-9 mg O₂ l⁻¹) cycling DO, and constant normoxia (7.5 mg O₂ l⁻¹)] and three pH treatments [extreme (6.8-8.1) and moderate (7.2-7.8) cycling pH, and constant pH (7.5)] in a 3X3 factorial design. Fish growth was not significantly impacted by either diel-cycling DO or pH conditions, nor was there any interactive effect, thus demonstrating that this species is physiologically tolerant of the harsh environments encountered by juvenile stages. However, all fish experienced mortality after exposure to a period of extreme diel-cycling DO lasting at least 10 days. Juvenile weakfish exhibited a variety of behavioral responses to diel-cycling DO, such as reduced swimming, that may have allowed fish to maintain high growth rates. By reducing swimming, fish were able to allocate more energy towards growth as opposed to movement when subjected to low DO levels. When fish were exposed to varying

levels of diel-cycling pH, their behavior did not change significantly, indicating that juvenile weakfish are not stressed by fluctuating pH conditions.

Chapter 1

EFFECTS OF DIEL-CYCLING HYPOXIA AND pH ON GROWTH AND BEHAVIOR OF JUVENILE WEAKFISH (*Cynoscion regalis*)

Introduction

Estuary-dependent fishes reside in estuaries containing necessary conditions for rapid growth and survival before they recruit to adult populations (Weinstein, 1979; Lubbers et al., 1990; Peterson & Ross, 1991; Able, 1999; Minello, 1999). However, during summer months, shallow estuarine waters can experience diel-cycling hypoxia, whereby DO concentrations vary substantially over a diel cycle (Kemp & Boynton, 1980; D'Avanzo & Kremer, 1994; Tyler & Targett, 2007; Diaz and Rosenberg, 2008; Tyler et al., 2009). During the day, photosynthesis produces oxygen, resulting in maximum DO concentrations ($>10 \text{ mg O}_2 \text{ L}^{-1}$) in late afternoon. At night, aerobic respiration consumes oxygen, causing DO to fall to its minimum ($<2 \text{ mg O}_2 \text{ L}^{-1}$) just after dawn (Kemp & Boynton, 1980; D'Avanzo & Kremer, 1994; Tyler et al., 2009). Diel cycles of photosynthesis and respiration also cause diel fluctuations in CO_2 concentrations and therefore pH (7.5-8.1) (Orr et al., 2005; Pörtner, 2008; Denman et al., 2011; Howarth et al., 2011; Baumann et al., 2105). During the daylight hours primary producers take up CO_2 and produce O_2 during photosynthesis, increasing pH, whereas night-time respiration produces CO_2 and consumes O_2 , reducing pH (Pörtner, 2008; Howarth et al., 2011).

Anthropogenic influences are exacerbating these biogeochemical cycles in estuaries and ocean acidification is an increasing issue worldwide (Heuer and Grosell, 2014; Greene et al., 2015; Duarte et al., 2013). Agricultural and industrial runoff increases concentrations of nitrogen and phosphorous in nearby estuaries, sometimes leading to eutrophication (Cloern, 2001; Diaz, 2001). Large amounts of nutrients entering estuarine waters gives rise to higher abundances of primary producers and therefore intensifies naturally occurring diel-cycles of DO and pH (Boynton et al., 1996; Paerl et al., 1998; Cloern, 2001; Duarte et al., 2013; Howarth et al., 2011). These extreme diel-cycles have the potential to force estuarine organisms to cope with several potential stressors simultaneously (Orr et al., 2005; Denman et al., 2011).

Little is known about the impacts of diel-cycling hypoxia on growth of young fishes and nothing is known about the independent and combined effects of diel-cycling pH (Duarte et al., 2013). Recent studies have examined how growth rates of estuary-dependent juvenile fishes respond solely to diel cycling-hypoxia. Stierhoff et al. (2009a) found that growth rates of wild-caught juvenile summer flounder (*Paralichthys dentatus*) and weakfish (*Cynoscion regalis*) are negatively impacted by diel cycling hypoxia (<2-15 mg O₂ L⁻¹) throughout the summer. However, Stierhoff et al. (2009b) did not find a significant effect of constant hypoxia (2, 3.5, 5, 7 mg O₂ L⁻¹) or diel-cycling hypoxia (2-11 mg O₂ L⁻¹), on growth rates of juvenile weakfish in the laboratory. Additionally, juvenile spot (*Leiostomus xanthurus*), and juvenile Atlantic menhaden (*Brevoortia tyrannus*) did not show a decrease in growth rate when exposed to diel-cycling hypoxia (2-10 mg O₂ L⁻¹) until DO concentrations reached

near-lethal limits ($1.5 \text{ mg O}_2 \text{ L}^{-1}$) (McNatt & Rice, 2004). In contrast, growth rates of other juvenile estuary-dependent fishes exposed to diel-cycling hypoxia ($2\text{-}11 \text{ mg O}_2 \text{ L}^{-1}$), such as summer flounder and winter flounder (*Pseudopleuronectes americanus*), were lower starting at only moderate levels of hypoxia (between 2 and $5 \text{ mg O}_2 \text{ L}^{-1}$) (Stierhoff et al., 2006). Overall, some studies have been conducted examining the effects of diel-cycling hypoxia on growth rates of juvenile fishes, yet none of them incorporate the potential independent and synergistic effects of co-varying pH on growth and behavior. It is important to understand the effects of these biogeochemical cycles on growth rates of juvenile fishery species to preserve the ecology and quality of shallow estuarine nursery grounds worldwide.

This study exposed juvenile weakfish (*Cynoscion regalis*) to different degrees of diel-cycling hypoxia and pH to examine how these factors affect fish growth rate and behavior. The goals of this study were to determine if a combination of diel-cycling DO and pH will reduce weakfish growth rate more than either stressor alone. Weakfish were also subjected to short term diel-cycling treatments (for 10 days) before being returned to control conditions (for 20 days) to assess if short term exposure resulted in residual impacts on longer term growth and/or survival. In addition, we examined weakfish activity levels as the DO and pH treatments became more extreme.

Materials and Methods

Weakfish collection and acclimation

Juvenile weakfish were collected using an otter trawl from Pepper Creek, a tributary of Indian River Bay, DE. Fish acclimated in ~450L recirculating aquaria at 25°C, 25 ppt salinity, 14h light beginning at 6:00, 10h dark beginning at 20:00, and normoxia (7.0 mg O₂ L⁻¹) for ≥ 14 days. Fish were fed frozen mysid shrimp (*Mysis relicta*) ad libitum once per day (Stierhoff et al., 2006; Stierhoff et al., 2009b). After acclimation, fish were selected by size to be transferred to the computer-controlled, recirculating aquaria for growth experiments.

Experimental laboratory design

Growth experiments were conducted in five air-tight recirculating aquaria systems modified by Bogue (2013) and Davidson (2015) to study impacts of diel-cycling hypoxia and pH on juvenile estuarine fishes. The temperature (25°C) and photoperiod (14 hours light beginning at 6:00: 10 hours dark beginning at 20:00) were controlled in the laboratory to mimic conditions in the field. Each aquarium system (Figure 1) held a total volume of ~415 L between 10 separate, 18 L polyethylene holding tanks, a surrounding overflow tray, and a sump tank. Water flowed into each holding tank through a supply manifold and eventually overflowed into the surrounding tray, where it then entered the sump and was recirculated.

The aquarium systems were capable of manipulating both DO and pH in each of the five separate treatment aquaria simultaneously (Grecay & Stierhoff, 2002;

Stierhoff et al. 2006; Stierhoff et al., 2009b; Bogue, 2013; Davidson, 2015). A PC running LabVIEW instrumentation software, interfaced with a Hach sc200 Universal Controller, a Hach LDO dissolved oxygen probe and a Hach Differential pH/ORP sensor, regulated the DO and pH of each system by adding gases (air, CO₂, N₂, and O₂) as needed into the sump tank. Solenoid-controlled flow of aquaria water past the sensory ends of the DO and pH probes allowed the LabVIEW program to measure current DO and pH levels within each system. After sampling the aquaria water, the program activated the appropriate solenoids to bubble in gases at appropriate amounts in order to achieve desired levels. DO levels were controlled by bubbling in N₂ and O₂, while pH levels were controlled by bubbling in CO₂ and air (Miller, 2009).

While the systems were able to control DO and pH simultaneously, there were some limitations. Compressed air was used to raise the pH of each system. While air was bubbled into the system to increase pH levels, it became impossible to maintain supersaturated DO concentration. Therefore, in diel-cycling treatments, where both DO and pH fluctuated, the system had to wait for the pH to reach desired levels before attempting to supersaturate DO. In order to control the stability of the pH, the water was adequately buffered to prevent the pH from varying too quickly after the desired level had been reached. To establish a higher buffering capacity, the water used in the experiments was treated with Kent Marine SuperbufferdKh™ until the carbonate alkalinity of the water reached 14 dKh.

Growth trials

Experimental treatments mimicked DO and pH diel-cycles observed in shallow estuarine tributaries and coastal waters. A set of appropriate DO and pH treatments was developed reflecting natural DO/pH ranges from water quality data collected from Pepper Creek (tributary of Indian River Bay, DE) and the Chesapeake Bay (Tyler et al., 2009; MD DNR 2012). Fish were exposed to a combination of different DO and pH diel-cycles in order to determine changes in growth rate and behavior. DO treatments included: extreme diel-cycling hypoxia (1.0 – 11.0 mg O₂ L⁻¹), moderate diel-cycling hypoxia (3.0 – 9.0 mg O₂ L⁻¹), and a static normoxic control (7.5 mg O₂ L⁻¹). pH treatments included: extreme diel-cycling pH (6.8 – 8.1), moderate diel-cycling pH (7.2-7.8), and a static pH control (7.5). Each DO and pH cycle was crossed, yielding a 3 x 3 matrix including 8 different DO/pH treatments and a static control (Table 1). Daily DO and pH minima and maxima values were recorded throughout the trials to ensure accurate reporting of the DO and pH treatments (Table 2, Appendix A).

After fish were acclimated to laboratory conditions (≥ 14 days), fish of similar sizes were selected and assigned to each of the 50 holding tanks (1 fish per tank, 10 tanks per system). Trial 1 (moderate diel-cycling hypoxia/ moderate diel-cycling pH, extreme diel-cycling hypoxia/ extreme diel-cycling pH, and a control) started with 10 fish per system, Trial 2 (moderate diel-cycling hypoxia/ static pH control, moderate diel-cycling hypoxia/ extreme diel-cycling pH, extreme diel-cycling hypoxia/ static pH control, extreme diel-cycling hypoxia/ moderate diel-cycling pH, and a control)

started with 6 fish per treatment and 9 fish in the control, and Trial 3 (static normoxic control/ moderate diel-cycling pH, static normoxic control/ extreme diel-cycling pH, and a control) started with 5 fish per system (Table 1). All weakfish in a given trial had similar body masses prior to the start of each trial (confirmed by one-way ANOVA). Fish were given 3 days to acclimate to the new aquaria holding tanks under control DO ($7.5 \text{ mg O}_2 \text{ L}^{-1}$) and pH (7.5) conditions. Fish were fed mysid shrimp *ad libitum* twice a day; once at 09:00 and again at 17:00. 24 hours prior to the end of acclimation, food was withheld in order to minimize the effect of stomach content on body mass. On day 0, 10, and 20 the mass and standard length (SL) of the fish were measured prior to the 09:00 feeding. To measure mass, each individual fish was placed on a damp sponge and the total mass (fish + sponge) was determined. The mass of the sponge was subtracted from the total mass of the sponge and the fish. Standard length was measured (0.1mm) using digital calipers. Mass and standard length of each fish was recorded in order to examine growth throughout the trial periods. Feces and uneaten food were removed each morning before the 09:00 feeding from the holding tanks to reduce risk of water quality degradation and ammonia and nitrite levels were monitored and treated if necessary.

A final 30 day trial was run to determine if short term exposure to diel-cycles impacts longer term growth rate and/or survival. Fish were exposed to extreme diel-cycling DO and pH ($1-11 \text{ mg O}_2 \text{ L}^{-1}$, pH 6.8 – 8.1) conditions and moderate diel-cycling DO and pH ($3-9 \text{ mg O}_2 \text{ L}^{-1}$, pH 7.2-7.8) conditions for 10 days, after which normoxia and static pH were reinstated for 20 days. Residual effects of short term

exposure were shown if fish displayed statistically lower growth rate and/or an increase in mortality during the final 20 days when compared to the control.

Acclimation, feeding, and measuring routines were identical to the methods described previously.

Activity observation and mortality assessment

Throughout the trials, weakfish activity levels were monitored every other day before morning and evening feedings. Five fish were randomly selected and observed from each treatment and their activity level was recorded. Activity levels were qualitatively determined using a scale from 1-10; 1 being stagnant, 10 being hyperactive. In addition, mortality was documented in order to detect any trends in fish mortality over time throughout the treatments. In the event of multiple mortalities, the laboratory system was thoroughly inspected and water parameters (DO, pH, and salinity, ammonia, nitrite, and nitrate) were monitored to ensure “natural mortality”.

Data analysis

Changes in fish mass were calculated over two time intervals, 0-10d and 10-20d, during each trial. Daily specific growth rate (SGR; % mass growth d⁻¹) was calculated using the formula $SGR = (e^G - 1) * 100\%$, where G (instantaneous growth rate) = $[(\ln W_{t_f} - \ln W_{t_i})/d]$; W_{t_f} = final weight, W_{t_i} = initial weight and d = number of days between measurements. Linear growth rate (LGR; mm d⁻¹) was calculated using the formula $LGR = [(L_f - L_i)/d]$, where L_f = final SL, L_i = initial SL and d = number of

days between measurements. The normal distribution of the data was evaluated utilizing the Shapiro-Wilk test.

Statistical routines implemented in R were used to analyze treatment effects on fish growth and behavior throughout the trials. For each time interval, the effect of treatment on growth (change in mass or SGR) was determined using linear models with initial mass as a covariate. For SGR, the interaction between initial mass and treatment complicated a clear interpretation of treatment effect on SGR. However, for absolute growth (change in mass), no interaction between treatment and initial mass was observed, favoring absolute growth as the primary growth metric for statistical analysis.

To analyze growth throughout an individual trial, the R package “lme4” was used to conduct growth curve analyses (GCA) using linear mixed-effect models (Mirman 2014). When significant heterogeneity in growth curves among treatments was observed, pairwise GCA was used to compare Control and Treatment, adjusting for multiple testing using the Hochberg sequential Bonferroni method. During the final trial changes in mass during a 20 day control period following short term (10 day) exposure to diel-cycling hypoxia/ pH treatments (Figure 3) were compared to the control group by a one-way ANCOVA.

Weakfish activity was analyzed for each trial to establish trends and significant findings. For each trial, differences in activity level among treatments were analyzed using RxC chi-squared tests of independence. Additionally, the correlation between mean activity and SGR across treatments was calculated for each time interval (d0-10

and d10-20). Furthermore, the effect of DO on mean activity for each interval was evaluated by a two way mixed model with DO level and individual fish as predictor variables. Differences in mean activity level between intervals were also examined using a two way mixed model with fish and activity as predictor variables. As a rough approximation, the activity level data for the weakfish in the moderate diel-cycling DO were combined as well as the activity level data for weakfish held in normoxic conditions across treatments. An ANOVA was used to compare the mean activity level between these two groups.

Measuring pCO₂ in Pepper Creek and in the laboratory

Water samples were collected from Pepper Creek on September 5, 2014 to compare carbonate chemistry in the field and laboratory. In order to calculate how the injection of CO₂ gas affected the pCO₂ of the treatment water in the laboratory, dissolved inorganic carbon (DIC) and pH were measured simultaneously. Two water samples were taken from each of the five running treatment, three times a day on measurement days. Water was collected in 25 mL scintillation vials during pH minima, mid-range, and maxima for each diel-cycling treatment and the pH during those times was recorded. Each vial was sterilized with 0.2 mL of 5% HgCl₂ and refrigerated to prevent further production of CO₂. During analysis, 0.5-1-ml samples were acidified and the obtained CO₂ gas was measured using an infrared CO₂ gas detector (Li-Cor 7000). The DIC values were then checked against certified reference material from A. Dickson. CO₂SYN software (Lewis et al., 1998) then computed

pCO₂ values using known values of salinity, temperature, pH, and DIC for subsequent analysis.

Results

Change in mass and SGR

Before the start of each trial, a one-way ANOVA was conducted to confirm that the fish in each treatment had a similar mean initial body mass. Treatment effects were then measured as change in body mass during a given time period, with initial mass as a covariate. Growth of all diel-cycling DO and/or pH treatment groups appeared to be slower than the control of each trial (Figure 2). However, after conducting growth curve analyses paired with the Hochberg sequential Bonferroni method, the only significant difference in slope ($p = 6.92e-05$) was found between the control and the “Moderate DO cycle & Extreme pH cycle” from d0-10. Treatment effects on specific growth rate (SGR) were similar to the treatment effects on change in body mass for each trial. The average SGR of all treatment groups was generally lower than the control, but only the “Moderate DO cycle & Extreme pH cycle” treatment in Trial 2 from d0-10 differed significantly from the control (Dunnett’s test, $p = 0.0154$).

When changes in mass during a 20 day control period following short term (10 day) exposure to diel-cycling hypoxia/ pH treatments (Figure 3) were compared to the control group no differences were observed during d0-10 (Figure 3). Following return to control conditions (d10-30), no growth detriment was observed. Furthermore, the

fish subjected to 10 days exposure to extreme diel-cycling DO and pH survived throughout the 30 day trial, unlike the fish held at extreme diel-cycling DO and pH for 20 days (Trial 1), all of which died between d10-20.

Behavioral observations and mortality

Weakfish exhibited ‘unusual’ behavior in the moderate diel-cycling hypoxia treatments and 100% mortality in the extreme diel-cycling hypoxia treatments. In the moderate diel-cycling hypoxia treatments, weakfish displayed both sluggish behavior and reduced activity when fed. Overall, fish in the moderate diel-cycling hypoxia treatments behaved the most differently compared to those in normoxic conditions (Figure 4). However, no significant correlation was detected between SGR and mean activity level during any time interval. Mean activity level did not differ between high and low DO periods, or between intervals (d0-10 and d10-20). Throughout the trials, all of the weakfish in the extreme diel-cycling hypoxia conditions died: extreme cycling hypoxia and pH treatment [3 fish died on d2 (DO minimum = 1.3 mg O₂ L⁻¹), 7 fish died on d10 (DO minimum = 1.11 mg O₂ L⁻¹)], extreme cycling hypoxia and moderate cycling pH treatment [6 fish died on d1 (DO minimum = 1.49 mg O₂ L⁻¹)], extreme cycling hypoxia and static pH treatment [6 fish died on d3 (minimum DO = 1.8 mg O₂ L⁻¹)], and moderate cycling hypoxia and extreme cycling pH treatment [1 fish died on d7 (minimum DO = 2.92 mg O₂ L⁻¹), 1 fish died on d9 (minimum DO = 2.94 mg O₂ L⁻¹), 1 fish died on d10 (minimum DO = 2.85 mg O₂ L⁻¹), 2 fish died on

d11 (minimum DO = 2.81 mg O₂ L⁻¹), and 1 fish died on d12 (minimum DO = 2.84 mg O₂ L⁻¹)] (Table 2, Appendix A).

pCO₂ in Pepper Creek and in the laboratory

Throughout the treatments, we observed a consistent relationship between pCO₂ and pH measurements. In the laboratory, pCO₂ and pH measurements ranged from 6.87 and 15,035 μatm to 7.79 and 1,794 μatm, respectively (Figure 5). Water samples taken from Pepper Creek, Delaware (a weakfish nursery habitat) on September 5, 2014 at 08:09, 12:03, and 16:41 had temperature and salinity measurements comparable to laboratory conditions (~25°C and ~22.5 ppt). The water samples also displayed a temporal increase in pH and decrease in pCO₂, yielding values of 7.35 and ~2,080 μatm at 08:09, 7.6 and 1080 μatm at 12:03, and 7.79 and ~683 μatm at 16:41, similar to those of the moderate diel-cycling pH treatments.

Discussion

Effects of diel-cycling hypoxia and pH on weakfish growth

The results of this laboratory experiment demonstrate that juvenile weakfish are both physiologically and behaviorally tolerant of diel-cycling hypoxia and pH. Stierhoff et al. (2009b) also observed a lack of growth detriment in juvenile weakfish when fish were exposed to constant hypoxia and diel-cycling hypoxia at different temperatures in the laboratory. Furthermore, weakfish did not avoid hypoxia at DO levels higher than that which negatively impacts their growth rate (<2 mg O₂ L⁻¹) (Stierhoff et al., 2009b). Additionally, juvenile spot, a closely related sciaenid fish, did

not show a decrease in growth rate until DO concentrations reached near-lethal limits ($1.5 \text{ mg O}_2 \text{ L}^{-1}$) (McNatt & Rice, 2004). These results indicate that some fish within the family Sciaenidae are physiologically capable of enduring diel-cycling hypoxia currently seen during the summer in estuarine nurseries. In contrast, growth of other juvenile estuary-dependent fishes, such as summer flounder and winter flounder, was lower starting at only moderate levels of hypoxia (between 3.5 and $5.0 \text{ mg O}_2 \text{ L}^{-1}$) (Stierhoff et al., 2006).

Growth rates were not significantly different from the control throughout laboratory trials, with the exception of the “moderate DO cycle & extreme pH cycle” treatment. However, the number of live weakfish decreased from 6 on day 0 to 2 on day 10 in this treatment whereas the number of weakfish remained at 9 in the control. This decline in fish number may have caused the growth rate of the “moderate DO cycle & extreme pH cycle” treatment to significantly differ from the control because the average mass on day 10 was derived from fewer fish compared to day 0.

Juvenile weakfish are physiologically tolerant of moderate-cycling DO and pH in terms of growth and survival. Their growth and survival are also tolerant of short term exposure to extreme diel-cycles of DO and pH for a period of at least 10 days. These findings suggest that juvenile weakfish can experience up to 10 days of extreme diel-cycling in the field before their survival is affected. To further analyze the independent and interactive effects of diel-cycling hypoxia and pH, future studies should increase the number of fish used in both the treatment groups and the control to improve the power of statistical analyses.

The growth rate of juvenile weakfish in the field is less than would be predicted based on previous laboratory results on DO effects (Steinhoff et al. 2009a). These findings suggest that juvenile weakfish may conserve energy by decreasing swimming speed in the laboratory when exposed to long term diel-cycling hypoxia (Stierhoff et al., 2009b). In addition, laboratory weakfish were fed twice a day *ad libitum*, and did not face the risk of predation, decreasing their need to expend energy foraging and escaping predators, which may have contributed to the similarity of growth rates throughout treatments (Stierhoff et al., 2009b). However, in the field, weakfish must forage and avoid predators to survive, reducing their chance to decrease swimming speed when necessary to conserve energy. Weakfish generally avoided hypoxic areas by increasing swimming speed and using tidal flow to facilitate escape (Brady & Targett, 2013). Tyler and Targett (2007) found that juvenile weakfish have an avoidance threshold of $\sim 2.0 \text{ mg O}_2 \text{ L}^{-1}$ and demonstrate dynamic temporal movement related to DO concentrations in the field. When weakfish swim away from hypoxic and anoxic conditions, they diminish their foraging time, increase their time exposed to predation, and suffer the energetic cost of long distance movements, causing lower growth rates (Stierhoff et al., 2009a).

The potential contribution of a synergistic effect of diel-cycling pH in the field was investigated in this work. Experiments did not detect significant changes in weakfish growth when subjected to the large pH swings they can encounter in the field, nor was there an interaction with DO cycles. Both Bogue (2013) and Davidson (2015) reported similar findings about the effects of diel-cycling pH on growth rates of

juvenile mummichogs and summer flounder. Studies conducted exploring chronic ocean acidification, on the other hand, report highly variable results in terms of juvenile fish growth (Heuer and Grosell, 2014). Several juvenile fish species have been found to be resistant to decreased growth when exposed to prolonged reduced pH conditions. However, other species have shown decreased growth or increased growth due to modifications in energy allocation when exposed chronically to ocean acidification (Heuer and Grosell, 2014).

Behavioral observations and mortality assessment

Overall, behavior of weakfish was unusual in the diel-cycling hypoxia treatments when compared to the control. During low DO conditions, the weakfish exposed to both moderate and extreme diel-cycling hypoxia treatments displayed either sluggish behavior or reduced activity when fed. Similar findings reported by Brady et al. (2009) and Stierhoff et al. (2009b), suggest that reduced swimming speeds and reduced foraging or feeding rates during low DO periods may permit weakfish to allocate more energy towards growth as opposed to activity, resulting in similar growth rates among treatments in this study.

Similar to weakfish, reduced swimming speed in response to low DO concentrations was observed in Atlantic cod (*Gadus morhua*) (Schurmann & Steffensen, 1997), white sturgeon (*Acipenser transmontanus*) (Crocker & Cech, 1997), and juvenile weakfish (Brady et al., 2009). In addition, Stierhoff et al. (2009b) reported comparable behavioral results when weakfish were exposed to DO levels

between 1 and 2 mg O₂ L⁻¹. However, Stierhoff et al. (2009b) did not find weakfish behavior to differ significantly from fish in the control when DO levels were ≥ 2 mg O₂ L⁻¹. Similar avoidance responses and thresholds have been reported under similar experimental conditions for other estuarine fish, including Atlantic croaker (*Micropogonias undulatus*), juvenile spot (*Leiostomus xanthurus*), Atlantic menhaden (*Brevoortia tyrannus*), and white mullet (*Mugil curema*) (Wannamaker & Rice, 2000). Therefore, weakfish and other species of estuarine fish may start swimming slower at a particular DO threshold to cope with the detrimental effects of hypoxic conditions.

Numerous field studies report a sharp decline in fish abundance when DO concentrations range from 1.5-3 mg O₂ L⁻¹ (Garlo et al., 1979; Baden et al., 1990; Parker & O'Reilly, 1991; Pihl et al., 1991; Rabalais & Turner, 2001; Eby and Crowder, 2002). Furthermore, Tyler and Targett (2007) found a significant decrease in the number of weakfish caught in an estuarine tributary experiencing diel-cycling hypoxia when DO concentrations were < 2 mg O₂ L⁻¹, indicating that weakfish use an active escape strategy as DO decreases. However, weakfish abundance rebounded within minutes to a few hours after DO conditions improved, suggesting that weakfish may have a higher avoidance threshold in the field than observed in the laboratory.

Weakfish behavior did not significantly change when exposed to diel-cycling pH, nor was there an interaction with diel-cycling hypoxia. Although this experiment only focuses on acute exposure to reduced pH levels, we find it critical to mention that most behavioral changes during chronic ocean acidification are due to a variety of

internal disturbances (Duarte et al., 2013; Heuer and Grosell 2014). Sensory systems including olfaction, hearing, and vision and other processes related to cognitive function including lateralization, activity, boldness, and learning have been found to be impaired in numerous species when exposed to prolonged decreased pH conditions. These findings provide evidence that fish are less tolerant to chronic ocean acidification when compared to acute exposure to reduced pH levels (Duarte et al., 2013; Heuer and Grosell 2014).

The lower lethal DO limit is unknown for juvenile weakfish (Brady & Targett, 2013). However, this experiment suggests weakfish cannot withstand the accumulated stress of daily DO concentrations reaching much lower than $1 \text{ mg O}_2 \text{ L}^{-1}$ because all weakfish exposed to extreme diel-cycling hypoxia treatments died before the end of each trial. The systems exhibited normal pH and salinity values, and the ammonia, nitrite, and nitrate levels were ~ 0 ppm. DO readings indicate that some mortality may have been caused by a slight system malfunction, which brought DO levels slightly below $1 \text{ mg O}_2 \text{ L}^{-1}$. However, Brady (2009) reported weakfish survival at levels as low as $0.4 \text{ mg O}_2 \text{ L}^{-1}$, suggesting that DO levels were accidentally brought below that.

Effects of diel-cycling hypoxia and pH on nursery habitat quality

Many fishery species rely on estuaries during their juvenile stages as nursery habitat (Stierhoff et al, 2009a). If exacerbated diel-cycles of hypoxia and pH, mainly caused by eutrophication, degrade these nursery habitats and/or the physiological fitness of the species residing in them, the cycles could have significant negative

effects on nursery habitat quality and quantity and therefore recruitment. Our study suggests that extreme diel cycles of hypoxia and pH caused by eutrophication in estuaries do not degrade nursery habitat for juvenile weakfish. This experiment demonstrates that juvenile weakfish are physiologically capable of growing well under extreme diel-cycling pH conditions and diel-cycling hypoxic conditions above 1 mg O₂ L⁻¹. In order to survive these conditions, weakfish utilize a variety of behavioral strategies that allow them to conserve energy and maintain high growth rates when exposed to near-lethal DO concentrations. Such reasonably hardy fish may be able to take advantage of an otherwise suboptimal habitat for feeding opportunities and predator avoidance. Additional laboratory and field studies further investigating weakfish and other tolerant species in Sciaenidae will help determine if these results are representative of all species within the family.

Table 1 3 x 3 matrix of diel-cycling DO and pH treatments. Moderate gray shading indicates treatments used in Trial 1, light gray shading indicates treatments used in Trial 2, and dark gray shading indicates treatments used in Trial 3. In addition, a control (unshaded) was used in all three trials.

	DO: 7.5 mg O ₂ l ⁻¹	DO: 3-9 mg O ₂ l ⁻¹	DO: 1-11 mg O ₂ l ⁻¹
pH: 7.5	Control Normoxia, Static pH	Moderate DO Cycle Static pH	Extreme DO Cycle Static pH
pH: 7.2-7.8	Normoxia, Moderate pH Cycle	Moderate DO Cycle Moderate pH Cycle	Extreme DO Cycle Moderate pH Cycle
pH: 6.8-8.1	Normoxia, Extreme pH Cycle	Moderate DO Cycle Extreme pH Cycle	Extreme DO Cycle Extreme pH Cycle

Table 2 Mean daily minimum and maximum DO and pH \pm SE values, during each 10 day period, for each treatment.

Trial	Treatment	Interval	Average DO Maximum \pm SE	Average DO Minimum \pm SE	Average pH Maximum \pm SE	Average pH Minimum \pm SE
1	Control	d0-10	7.95 \pm 0.04	6.58 \pm 0.10	7.57 \pm 0.00	7.45 \pm 0.00
1	Moderate DO & pH Cycle	d0-10	9.38 \pm 0.03	3.30 \pm 0.33	7.79 \pm 0.00	7.24 \pm 0.02
1	Extreme DO & pH Cycle	d0-10	9.09 \pm 0.31	1.75 \pm 0.47	8.01 \pm 0.00	6.99 \pm 0.04
1	Control	d11-20	7.64 \pm 0.01	7.26 \pm 0.02	7.51 \pm 0.00	7.46 \pm 0.00
1	Moderate DO & pH Cycle	d11-20	9.25 \pm 0.05	3.06 \pm 0.01	7.78 \pm 0.00	7.22 \pm 0.00
1	Extreme DO & pH Cycle	d11-20	7.78 \pm 0.07	6.25 \pm 0.70	7.62 \pm 0.06	7.40 \pm 0.06
2	Control	d0-10	7.88 \pm 0.02	5.94 \pm 0.06	7.62 \pm 0.01	7.24 \pm 0.02
2	Moderate DO Cycle & Static pH	d0-10	9.49 \pm 0.04	3.08 \pm 0.04	7.54 \pm 0.00	7.47 \pm 0.00
2	Moderate DO Cycle & Extreme pH Cycle	d0-10	7.76 \pm 0.03	3.00 \pm 0.07	7.73 \pm 0.01	7.03 \pm 0.04
2	Extreme DO Cycle & Static pH	d0-10	8.95 \pm 0.53	5.45 \pm 0.79	7.57 \pm 0.01	7.45 \pm 0.00
2	Extreme DO Cycle & Moderate pH Cycle	d0-10	8.60 \pm 0.47	5.75 \pm 0.72	7.64 \pm 0.03	7.38 \pm 0.02
2	Control	d11-20	7.68 \pm 0.23	7.02 \pm 0.17	7.50 \pm 0.03	7.36 \pm 0.03
2	Moderate DO Cycle & Static pH	d11-20	9.51 \pm 0.07	3.16 \pm 0.03	7.53 \pm 0.00	7.48 \pm 0.00
2	Moderate DO Cycle & Extreme pH Cycle	d11-20	8.06 \pm 0.08	6.35 \pm 0.59	7.55 \pm 0.02	7.41 \pm 0.06
2	Extreme DO Cycle & Static pH	d11-20	7.61 \pm 0.02	7.40 \pm 0.02	7.51 \pm 0.01	7.46 \pm 0.00
2	Extreme DO Cycle & Moderate pH Cycle	d11-20	7.66 \pm 0.02	7.32 \pm 0.05	7.51 \pm 0.02	7.45 \pm 0.00
3	Control	d0-10	7.31 \pm 0.03	7.02 \pm 0.05	7.60 \pm 0.01	7.23 \pm 0.04
3	Normoxia & Moderate pH Cycle	d0-10	7.39 \pm 0.01	7.13 \pm 0.07	7.76 \pm 0.00	7.17 \pm 0.00
3	Normoxia & Extreme pH Cycle	d0-10	7.37 \pm 0.01	7.08 \pm 0.05	7.83 \pm 0.00	6.87 \pm 0.01
3	Control	d11-20	7.26 \pm 0.03	6.83 \pm 0.19	7.58 \pm 0.00	7.21 \pm 0.01
3	Normoxia & Moderate pH Cycle	d11-20	7.38 \pm 0.03	7.19 \pm 0.03	7.71 \pm 0.01	7.17 \pm 0.00
3	Normoxia & Extreme pH Cycle	d11-20	7.32 \pm 0.03	7.08 \pm 0.03	7.80 \pm 0.00	6.86 \pm 0.01

Table 2 Continued

Trial	Treatment	Interval	Average DO Maximum ±SE	Average DO Minimum ±SE	Average pH Maximum ±SE	Average pH Minimum ±SE
Residual Effects	Control	d0-10	7.95 ± 0.04	6.58 ± 0.10	7.57 ± 0.00	7.45 ± 0.00
Residual Effects	Moderate DO & pH Cycle to Control	d0-10	9.58 ± 0.06	3.31 ± 0.33	7.79 ± 0.00	7.22 ± 0.02
Residual Effects	Extreme DO & pH Cycle to Control	d0-10	10.60 ± 0.37	1.68 ± 0.48	8.02 ± 0.00	6.95 ± 0.04
Residual Effects	Control	d11-20	7.64 ± 0.01	7.26 ± 0.02	7.51 ± 0.00	7.46 ± 0.00
Residual Effects	Moderate DO & pH Cycle to Control	d11-20	7.86 ± 0.02	6.72 ± 0.06	7.57 ± 0.00	7.44 ± 0.00
Residual Effects	Extreme DO & pH Cycle to Control	d11-20	7.69 ± 0.03	7.15 ± 0.11	7.52 ± 0.01	7.46 ± 0.00
Residual Effects	Control	d21-30	7.73 ± 0.05	7.16 ± 0.07	7.50 ± 0.00	7.46 ± 0.00
Residual Effects	Moderate DO & pH Cycle to Control	d21-30	7.84 ± 0.09	6.73 ± 0.26	7.52 ± 0.01	7.45 ± 0.00
Residual Effects	Extreme DO & pH Cycle to Control	d21-30	7.70 ± 0.04	7.24 ± 0.04	7.51 ± 0.01	7.46 ± 0.00

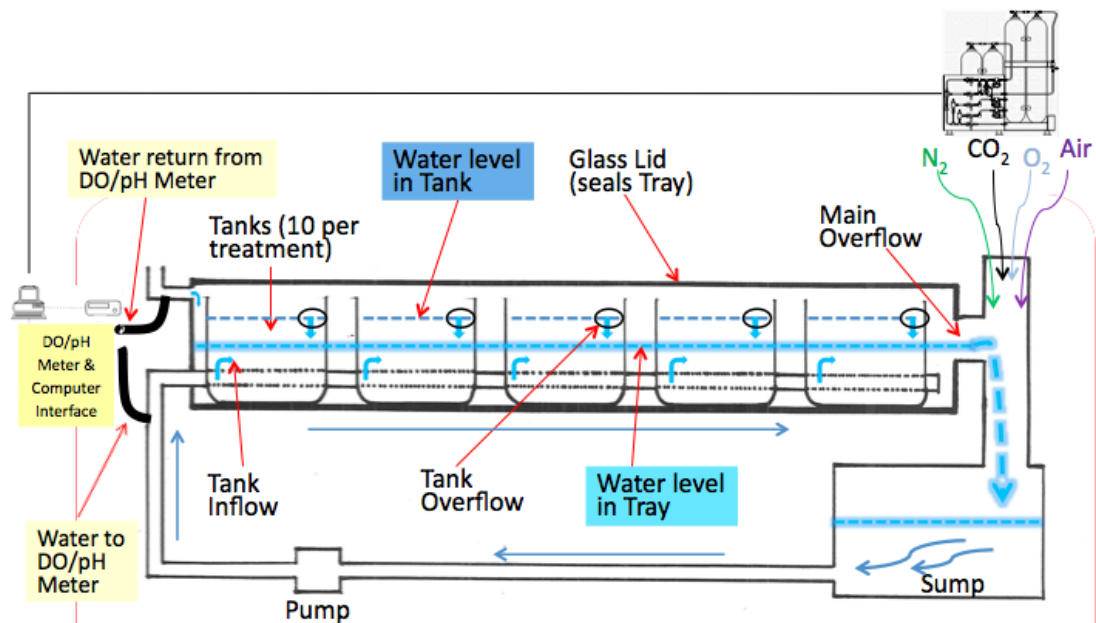


Figure 1 Computer interfaced treatment aquarium. Each system contained ten 18L holding tanks. Water flowed into each holding tank through an inflow, and out through an overflow into the surrounding tray. The water was then treated in the sump and pumped back through the tank supply line. Water samples were directed to the probes to be monitored to ensure DO and pH levels were in agreement with treatment levels, and then returned to the tank supply line. A PC running LabVIEW received DO and pH readings from the Hach sc200 sensor, and in turn activated gas solenoids accordingly in order to maintain desired treatment levels. DO levels were controlled by bubbling in N_2 and O_2 , while pH levels were controlled by bubbling in CO_2 and air (Figure courtesy of Dr. Paul Gre cay and Timothy Targett).

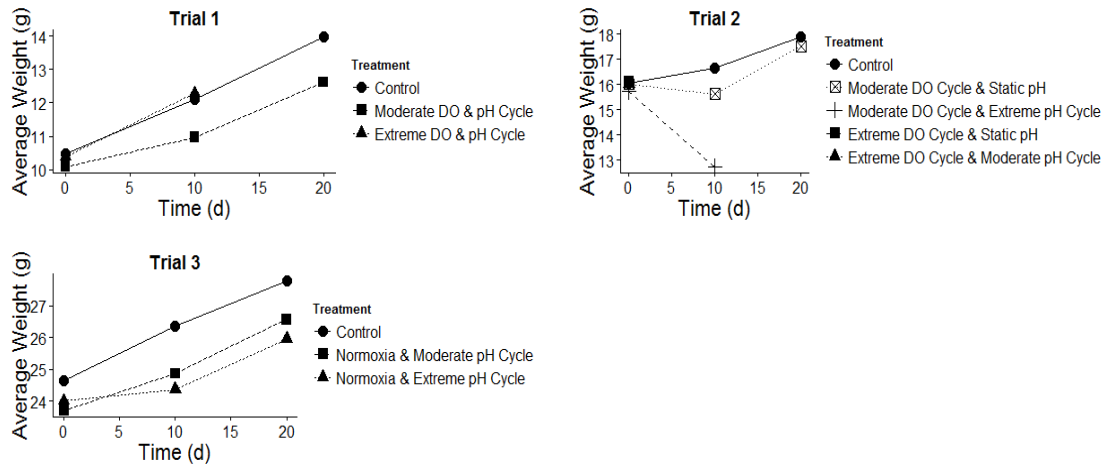


Figure 2 Mean changes in body mass of individual weakfish over time per trial. In Trial 2, all fish in the “Extreme DO Cycle & Static pH” and the “Extreme DO Cycle & Moderate pH Cycle” treatments died before day 10. In addition, the “Moderate DO & Static pH” treatment in Trial 2 was significantly different from the Trial 2 control.

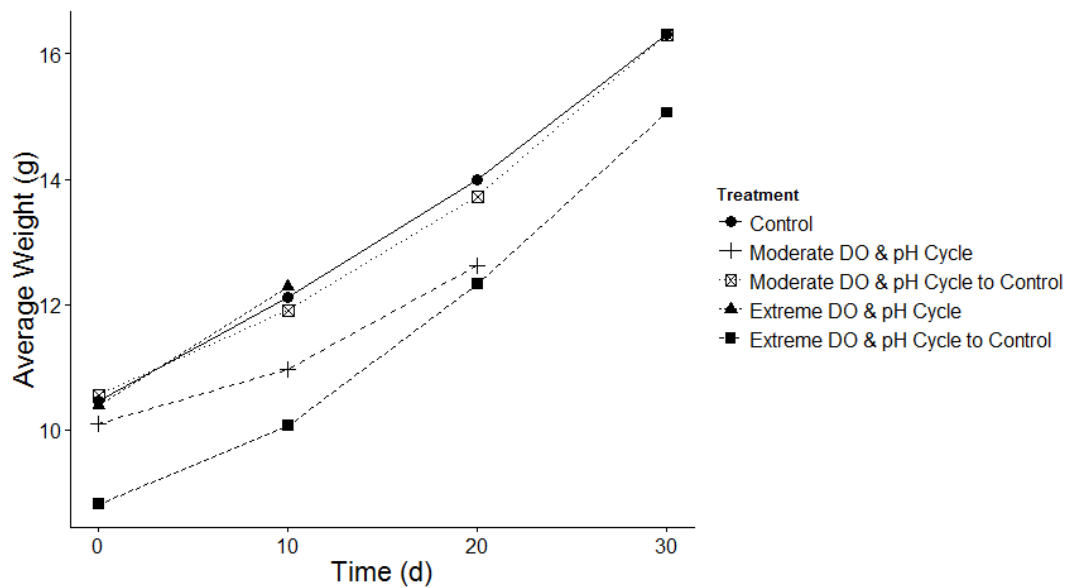


Figure 3 Residual effects of short term exposure. Mean weight (g) of fish per treatment are shown at four intervals (days 0, 10, 20, and 30) over the course of the trial. Fish were exposed to the “Moderate DO & pH Cycle” and the “Extreme DO & pH Cycle” treatments from day 0-10 and then returned to control conditions from day 10-30. Growth curves of prolonged (20d) moderate and extreme diel-cycling pH treatments from Trial 1 are included for comparison. Fish did not show significant growth detriment and/or mortality when compared to the control. However, fish held in the short term extreme diel-cycling DO treatment differed significantly from the fish held in the prolonged extreme diel-cycling DO treatment in terms of mortality.

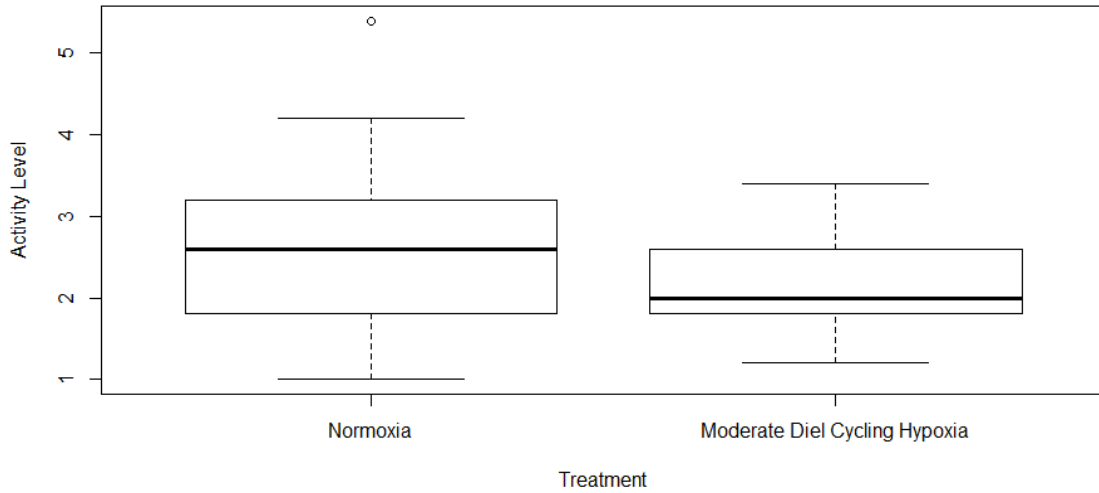


Figure 4 Average activity level of combined normoxia and moderate diel-cycling hypoxia data across trials. The average activity level of the weakfish under normoxic conditions is significantly greater than the average activity level of the weakfish subjected to moderate diel-cycling hypoxia.

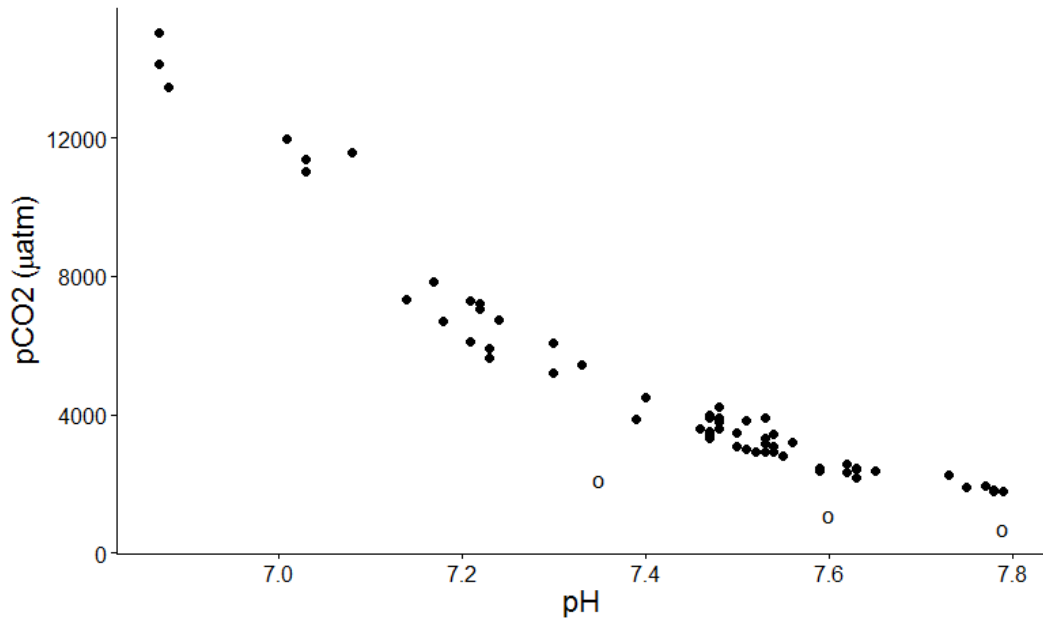


Figure 5 pCO₂ and pH values. Black diamonds represent pCO₂ and pH values from water samples taken from the aquarium systems during the trials and open circles represent pCO₂ and pH values from water samples taken from Pepper Creek.

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Appendix A

DAILY MAXIMUM AND MINIMUM DO AND pH VALUES

Trial	Treatment	Day	DO Maximum	DO Minimum	pH Maximum	pH Minimum
1	Control	0	8.12	7.3	7.54	7.51
1	Extreme DO & pH Cycle	0	10.97	6.49	8.01	7.39
1	Moderate DO & pH Cycle	0	9.3	6.57	7.79	7.47
1	Control	1	7.99	7.01	7.56	7.45
1	Extreme DO & pH Cycle	1	9.49	1.51	8.02	6.97
1	Moderate DO & pH Cycle	1	9.36	3.08	7.79	7.23
1	Control	2	7.92	6.65	7.56	7.45
1	Extreme DO & pH Cycle	2	10.29	1.3	8.02	6.95
1	Moderate DO & pH Cycle	2	9.29	3.01	7.79	7.22
1	Control	3	7.83	6.67	7.56	7.45
1	Extreme DO & pH Cycle	3	9.91	1.25	8.02	6.95
1	Moderate DO & pH Cycle	3	9.26	2.96	7.79	7.22
1	Control	4	8.01	6.6	7.57	7.45
1	Extreme DO & pH Cycle	4	9.43	1.34	8.02	6.98
1	Moderate DO & pH Cycle	4	9.54	2.91	7.79	7.22
1	Control	5	7.76	6.55	7.56	7.45
1	Extreme DO & pH Cycle	5	8.01	1.17	8.01	6.96
1	Moderate DO & pH Cycle	5	9.5	2.87	7.79	7.22
1	Control	6	8.18	6.47	7.58	7.45
1	Extreme DO & pH Cycle	6	8.03	1.45	8	6.97
1	Moderate DO & pH Cycle	6	9.33	3.04	7.79	7.22

1	Control	7	7.88	6.56	7.57	7.45
1	Extreme DO & pH Cycle	7	8.26	1.27	8	6.94
1	Moderate DO & pH Cycle	7	9.35	2.98	7.79	7.22
1	Control	8	7.76	6.3	7.58	7.44
1	Extreme DO & pH Cycle	8	8.17	1.25	8	6.96
1	Moderate DO & pH Cycle	8	9.33	2.98	7.79	7.22
1	Control	9	7.88	6.23	7.59	7.44
1	Extreme DO & pH Cycle	9	8.2	1.17	8.01	6.96
1	Moderate DO & pH Cycle	9	9.55	2.95	7.78	7.22
1	Control	10	8.08	6.09	7.59	7.44
1	Extreme DO & pH Cycle	10	9.23	1.09	8.01	6.92
1	Moderate DO & pH Cycle	10	9.43	2.92	7.8	7.22
1	Control	11	7.66	7.37	7.52	7.47
1	Extreme DO & pH Cycle	11	7.64	1.84	7.96	6.99
1	Moderate DO & pH Cycle	11	9.41	3.08	7.79	7.22
1	Control	12	7.64	7.28	7.52	7.46
1	Extreme DO & pH Cycle	12	8.45	2.27	7.98	7.15
1	Moderate DO & pH Cycle	12	9.32	3.11	7.78	7.22
1	Control	13	7.59	7.25	7.5	7.46
1	Extreme DO & pH Cycle	13	7.71	7.36	7.53	7.49
1	Moderate DO & pH Cycle	13	9.3	3.08	7.78	7.22
1	Control	14	7.61	7.28	7.51	7.46
1	Extreme DO & pH Cycle	14	7.7	7.32	7.53	7.48
1	Moderate DO & pH Cycle	14	9.29	3.06	7.79	7.22
1	Control	15	7.59	7.32	7.5	7.46
1	Extreme DO & pH Cycle	15	7.67	7.31	7.53	7.48

1	Moderate DO & pH Cycle	15	9.33	3.07	7.78	7.22
1	Control	16	7.66	7.28	7.51	7.46
1	Extreme DO & pH Cycle	16	7.75	7.29	7.54	7.48
1	Moderate DO & pH Cycle	16	9.3	3.04	7.78	7.22
1	Control	17	7.68	7.18	7.49	7.46
1	Extreme DO & pH Cycle	17	7.73	7.29	7.54	7.49
1	Moderate DO & pH Cycle	17	9	3.03	7.78	7.22
1	Control	18	7.69	7.2	7.5	7.46
1	Extreme DO & pH Cycle	18	7.72	7.31	7.54	7.48
1	Moderate DO & pH Cycle	18	9.04	3.03	7.78	7.21
1	Control	19	7.65	7.22	7.5	7.46
1	Extreme DO & pH Cycle	19	7.72	7.26	7.54	7.47
1	Moderate DO & pH Cycle	19	9.38	3.03	7.79	7.22
1	Control	20	7.63	7.27	7.52	7.46
1	Extreme DO & pH Cycle	20	7.74	7.27	7.55	7.48
1	Moderate DO & pH Cycle	20	9.08	3.07	7.78	7.23
2	Control	0	7.89	6.58	7.55	7.43
2	Extreme DO Cycle & Moderate pH Cycle	0	10.1	3.15	7.78	7.39
2	Extreme DO Cycle & Static pH	0	11.21	2.55	7.56	7.46
2	Moderate DO Cycle & Extreme pH Cycle	0	7.89	3.71	7.76	7.38
2	Moderate DO Cycle & Static pH	0	9.28	2.93	7.55	7.47
2	Control	1	7.97	5.85	7.58	7.29
2	Extreme DO Cycle & Moderate pH Cycle	1	11.49	1.49	7.78	7.22
2	Extreme DO Cycle & Static pH	1	11.34	2.52	7.6	7.44
2	Moderate DO Cycle & Extreme pH Cycle	1	7.87	2.89	7.78	7
2	Moderate DO Cycle & Static pH	1	9.28	2.97	7.55	7.46

2	Control	2	7.92	5.83	7.61	7.29
2	Extreme DO Cycle & Moderate pH Cycle	2	11.3	1.57	7.77	7.22
2	Extreme DO Cycle & Static pH	2	11.09	1.71	7.61	7.43
2	Moderate DO Cycle & Extreme pH Cycle	2	7.85	2.97	7.77	7.02
2	Moderate DO Cycle & Static pH	2	9.42	3.08	7.56	7.46
2	Control	3	7.8	5.94	7.63	7.25
2	Extreme DO Cycle & Moderate pH Cycle	3	7.66	7.17	7.57	7.43
2	Extreme DO Cycle & Static pH	3	10.99	1.8	7.56	7.45
2	Moderate DO Cycle & Extreme pH Cycle	3	7.78	2.98	7.76	6.97
2	Moderate DO Cycle & Static pH	3	9.61	2.99	7.54	7.48
2	Control	4	7.83	5.96	7.63	7.21
2	Extreme DO Cycle & Moderate pH Cycle	4	7.67	7.29	7.58	7.44
2	Extreme DO Cycle & Static pH	4	7.66	7.34	7.53	7.45
2	Moderate DO Cycle & Extreme pH Cycle	4	7.83	2.95	7.75	6.97
2	Moderate DO Cycle & Static pH	4	9.73	3.06	7.52	7.47
2	Control	5	7.84	5.87	7.62	7.21
2	Extreme DO Cycle & Moderate pH Cycle	5	7.69	7.18	7.59	7.43
2	Extreme DO Cycle & Static pH	5	7.68	7.32	7.56	7.45
2	Moderate DO Cycle & Extreme pH Cycle	5	7.64	2.93	7.74	6.99
2	Moderate DO Cycle & Static pH	5	9.4	3.05	7.54	7.48
2	Control	6	7.82	5.89	7.63	7.21
2	Extreme DO Cycle & Moderate pH Cycle	6	7.74	6.86	7.59	7.37
2	Extreme DO Cycle & Static pH	6	7.73	7.25	7.56	7.45
2	Moderate DO Cycle & Extreme pH Cycle	6	7.61	2.92	7.73	7.02
2	Moderate DO Cycle & Static pH	6	9.41	3.02	7.54	7.47
2	Control	7	7.89	5.84	7.63	7.22

2	Extreme DO Cycle & Moderate pH Cycle	7	7.67	7.18	7.57	7.43
2	Extreme DO Cycle & Static pH	7	7.64	7.39	7.55	7.45
2	Moderate DO Cycle & Extreme pH Cycle	7	7.66	2.92	7.71	7.02
2	Moderate DO Cycle & Static pH	7	9.51	3.36	7.55	7.46
2	Control	8	7.88	5.85	7.62	7.15
2	Extreme DO Cycle & Moderate pH Cycle	8	7.75	7.17	7.61	7.43
2	Extreme DO Cycle & Static pH	8	7.73	7.37	7.56	7.46
2	Moderate DO Cycle & Extreme pH Cycle	8	7.69	2.97	7.71	6.94
2	Moderate DO Cycle & Static pH	8	9.46	3.08	7.51	7.46
2	Control	9	7.96	5.83	7.69	7.14
2	Extreme DO Cycle & Moderate pH Cycle	9	7.78	7.16	7.6	7.44
2	Extreme DO Cycle & Static pH	9	7.68	7.36	7.57	7.45
2	Moderate DO Cycle & Extreme pH Cycle	9	7.72	2.94	7.7	6.96
2	Moderate DO Cycle & Static pH	9	9.6	3.08	7.55	7.47
2	Control	10	7.85	5.93	7.69	7.21
2	Extreme DO Cycle & Moderate pH Cycle	10	7.76	7.06	7.58	7.43
2	Extreme DO Cycle & Static pH	10	7.7	7.33	7.57	7.44
2	Moderate DO Cycle & Extreme pH Cycle	10	7.78	2.85	7.67	7.04
2	Moderate DO Cycle & Static pH	10	9.65	3.22	7.54	7.46
2	Control	11	7.79	5.98	7.69	7.17
2	Extreme DO Cycle & Moderate pH Cycle	11	7.71	7.06	7.6	7.43
2	Extreme DO Cycle & Static pH	11	7.66	7.37	7.58	7.44
2	Moderate DO Cycle & Extreme pH Cycle	11	7.88	2.81	7.67	7.03
2	Moderate DO Cycle & Static pH	11	9.71	3.08	7.55	7.46
2	Control	12	7.79	6.05	7.69	7.15
2	Extreme DO Cycle & Moderate pH Cycle	12	7.78	7.03	7.61	7.44

2	Extreme DO Cycle & Static pH	12	7.73	7.25	7.58	7.44
2	Moderate DO Cycle & Extreme pH Cycle	12	8.36	2.84	7.67	7.02
2	Moderate DO Cycle & Static pH	12	9.62	3.02	7.56	7.46
2	Control	13	7.71	7.17	7.57	7.49
2	Extreme DO Cycle & Moderate pH Cycle	13	7.65	7.35	7.52	7.47
2	Extreme DO Cycle & Static pH	13	7.61	7.44	7.48	7.45
2	Moderate DO Cycle & Extreme pH Cycle	13	7.93	7.16	7.52	7.5
2	Moderate DO Cycle & Static pH	13	9.39	3.05	7.5	7.48
2	Control	14	7.65	7.29	7.49	7.44
2	Extreme DO Cycle & Moderate pH Cycle	14	7.67	7.36	7.48	7.45
2	Extreme DO Cycle & Static pH	14	7.59	7.41	7.48	7.46
2	Moderate DO Cycle & Extreme pH Cycle	14	8.04	7.24	7.53	7.51
2	Moderate DO Cycle & Static pH	14	9.35	3.07	7.53	7.49
2	Control	15	7.67	7.33	7.44	7.41
2	Extreme DO Cycle & Moderate pH Cycle	15	7.59	7.43	7.48	7.45
2	Extreme DO Cycle & Static pH	15	7.56	7.43	7.49	7.46
2	Moderate DO Cycle & Extreme pH Cycle	15	8.19	7.24	7.52	7.5
2	Moderate DO Cycle & Static pH	15	9.62	3.29	7.51	7.49
2	Control	16	7.64	7.25	7.41	7.39
2	Extreme DO Cycle & Moderate pH Cycle	16	7.63	7.41	7.48	7.45
2	Extreme DO Cycle & Static pH	16	7.61	7.39	7.49	7.47
2	Moderate DO Cycle & Extreme pH Cycle	16	8.23	7.21	7.52	7.5
2	Moderate DO Cycle & Static pH	16	9.63	3.26	7.52	7.49
2	Control	17	7.63	7.19	7.43	7.38
2	Extreme DO Cycle & Moderate pH Cycle	17	7.66	7.38	7.49	7.46
2	Extreme DO Cycle & Static pH	17	7.59	7.42	7.5	7.48

2	Moderate DO Cycle & Extreme pH Cycle	17	8.15	7.26	7.52	7.5
2	Moderate DO Cycle & Static pH	17	9.58	3.2	7.52	7.49
2	Control	18	7.67	7.2	7.44	7.38
2	Extreme DO Cycle & Moderate pH Cycle	18	7.65	7.41	7.49	7.45
2	Extreme DO Cycle & Static pH	18	7.57	7.44	7.5	7.47
2	Moderate DO Cycle & Extreme pH Cycle	18	8.19	7.23	7.52	7.5
2	Moderate DO Cycle & Static pH	18	9.59	3.23	7.52	7.48
2	Control	19	7.67	7.28	7.42	7.38
2	Extreme DO Cycle & Moderate pH Cycle	19	7.61	7.42	7.48	7.45
2	Extreme DO Cycle & Static pH	19	7.56	7.45	7.49	7.47
2	Moderate DO Cycle & Extreme pH Cycle	19	8.11	7.18	7.53	7.5
2	Moderate DO Cycle & Static pH	19	9.62	3.19	7.54	7.49
2	Control	20	7.54	7.44	7.41	7.38
2	Extreme DO Cycle & Moderate pH Cycle	20	7.62	7.4	7.48	7.45
2	Extreme DO Cycle & Static pH	20	7.62	7.41	7.51	7.47
2	Moderate DO Cycle & Extreme pH Cycle	20	7.48	7.3	7.54	7.51
2	Moderate DO Cycle & Static pH	20	8.97	3.26	7.53	7.49
3	Control	0	7.19	6.99	7.64	7.16
3	Normoxia & Moderate pH Cycle	0	7.4	7.25	7.77	7.17
3	Normoxia & Extreme pH Cycle	0	7.41	7.27	7.83	7.01
3	Control	1	7.15	6.99	7.64	6.99
3	Normoxia & Moderate pH Cycle	1	7.33	7.17	7.77	7.15
3	Normoxia & Extreme pH Cycle	1	7.35	7.13	7.84	6.87
3	Control	2	7.47	6.57	7.61	7.01
3	Normoxia & Moderate pH Cycle	2	7.38	6.43	7.78	7.16
3	Normoxia & Extreme pH Cycle	2	7.34	6.58	7.83	6.88

3	Control	3	7.39	7.12	7.59	7.36
3	Normoxia & Moderate pH Cycle	3	7.47	7.26	7.78	7.18
3	Normoxia & Extreme pH Cycle	3	7.4	7.2	7.84	6.84
3	Control	4	7.28	7.04	7.58	7.33
3	Normoxia & Moderate pH Cycle	4	7.38	7.19	7.77	7.18
3	Normoxia & Extreme pH Cycle	4	7.34	7.08	7.83	6.84
3	Control	5	7.33	7.02	7.58	7.31
3	Normoxia & Moderate pH Cycle	5	7.36	7.13	7.77	7.18
3	Normoxia & Extreme pH Cycle	5	7.35	7.07	7.83	6.84
3	Control	6	7.29	7.11	7.59	7.31
3	Normoxia & Moderate pH Cycle	6	7.35	7.15	7.76	7.18
3	Normoxia & Extreme pH Cycle	6	7.35	7.13	7.83	6.85
3	Control	7	7.34	7.11	7.59	7.28
3	Normoxia & Moderate pH Cycle	7	7.38	7.21	7.75	7.18
3	Normoxia & Extreme pH Cycle	7	7.36	7.15	7.82	6.84
3	Control	8	7.38	7.12	7.58	7.26
3	Normoxia & Moderate pH Cycle	8	7.43	7.24	7.75	7.18
3	Normoxia & Extreme pH Cycle	8	7.41	7.16	7.82	6.85
3	Control	9	7.39	7.13	7.59	7.28
3	Normoxia & Moderate pH Cycle	9	7.41	7.29	7.75	7.17
3	Normoxia & Extreme pH Cycle	9	7.39	7.15	7.82	6.85
3	Control	10	7.23	7	7.59	7.28
3	Normoxia & Moderate pH Cycle	10	7.36	7.1	7.75	7.17
3	Normoxia & Extreme pH Cycle	10	7.36	7	7.82	6.87
3	Control	11	7.22	7	7.58	7.25
3	Normoxia & Moderate pH Cycle	11	7.28	7.14	7.74	7.17

3	Normoxia & Extreme pH Cycle	11	7.23	7.06	7.81	6.84
3	Control	12	7.16	6.98	7.59	7.25
3	Normoxia & Moderate pH Cycle	12	7.23	7.1	7.73	7.17
3	Normoxia & Extreme pH Cycle	12	7.2	7	7.8	6.86
3	Control	13	7.16	6.99	7.58	7.25
3	Normoxia & Moderate pH Cycle	13	7.26	7.06	7.73	7.17
3	Normoxia & Extreme pH Cycle	13	7.17	7	7.81	6.86
3	Control	14	7.23	7	7.58	7.24
3	Normoxia & Moderate pH Cycle	14	7.37	7.22	7.72	7.17
3	Normoxia & Extreme pH Cycle	14	7.35	7.09	7.8	6.85
3	Control	15	7.34	7.03	7.58	7.22
3	Normoxia & Moderate pH Cycle	15	7.44	7.29	7.72	7.17
3	Normoxia & Extreme pH Cycle	15	7.34	7.18	7.8	6.85
3	Control	16	7.23	7	7.58	7.21
3	Normoxia & Moderate pH Cycle	16	7.38	7.14	7.71	7.17
3	Normoxia & Extreme pH Cycle	16	7.29	7.1	7.79	6.84
3	Control	17	7.37	7.03	7.58	7.2
3	Normoxia & Moderate pH Cycle	17	7.53	7.28	7.71	7.17
3	Normoxia & Extreme pH Cycle	17	7.5	7.16	7.79	6.84
3	Control	18	7.39	7.09	7.58	7.17
3	Normoxia & Moderate pH Cycle	18	7.5	7.36	7.7	7.17
3	Normoxia & Extreme pH Cycle	18	7.5	7.27	7.8	6.84
3	Control	19	7.19	7	7.57	7.17
3	Normoxia & Moderate pH Cycle	19	7.37	7.11	7.7	7.17
3	Normoxia & Extreme pH Cycle	19	7.36	6.94	7.79	6.87
3	Control	20	7.34	5.15	7.56	7.17

3	Normoxia & Moderate pH Cycle	20	7.43	7.22	7.68	7.16
3	Normoxia & Extreme pH Cycle	20	7.31	7.05	7.77	6.92
Residual Effects	Control	0	8.12	7.3	7.54	7.51
Residual Effects	Extreme DO & pH Cycle to Control	0	11.81	6.45	8.02	7.36
Residual Effects	Moderate DO & pH Cycle to Control	0	9.71	6.62	7.79	7.45
Residual Effects	Control	1	7.99	7.01	7.56	7.45
Residual Effects	Extreme DO & pH Cycle to Control	1	11.44	1.45	8.02	6.93
Residual Effects	Moderate DO & pH Cycle to Control	1	9.6	3.09	7.8	7.22
Residual Effects	Control	2	7.92	6.65	7.56	7.45
Residual Effects	Extreme DO & pH Cycle to Control	2	11.58	1.25	8.02	6.9
Residual Effects	Moderate DO & pH Cycle to Control	2	9.43	3.04	7.79	7.21
Residual Effects	Control	3	7.83	6.67	7.56	7.45
Residual Effects	Extreme DO & pH Cycle to Control	3	11.6	1.17	8.02	6.9
Residual Effects	Moderate DO & pH Cycle to Control	3	9.27	2.99	7.79	7.2
Residual Effects	Control	4	8.01	6.6	7.57	7.45
Residual Effects	Extreme DO & pH Cycle to Control	4	10.65	1.2	8.02	6.94
Residual Effects	Moderate DO & pH Cycle to Control	4	9.63	2.96	7.79	7.21
Residual Effects	Control	5	7.76	6.55	7.56	7.45
Residual Effects	Extreme DO & pH Cycle to Control	5	9.33	1.08	8.02	6.9
Residual Effects	Moderate DO & pH Cycle to Control	5	9.58	2.9	7.79	7.21
Residual Effects	Control	6	8.18	6.47	7.58	7.45
Residual Effects	Extreme DO & pH Cycle to Control	6	8.6	1.36	8.01	6.92
Residual Effects	Moderate DO & pH Cycle to Control	6	9.42	3.01	7.79	7.21
Residual Effects	Control	7	7.88	6.56	7.57	7.45
Residual Effects	Extreme DO & pH Cycle to Control	7	9.93	1.2	8.02	6.89
Residual Effects	Moderate DO & pH Cycle to Control	7	9.67	2.99	7.79	7.19

Residual Effects	Control	8	7.76	6.3	7.58	7.44
Residual Effects	Extreme DO & pH Cycle to Control	8	8.73	1.13	8.02	6.89
Residual Effects	Moderate DO & pH Cycle to Control	8	9.66	2.99	7.79	7.2
Residual Effects	Control	9	7.88	6.23	7.59	7.44
Residual Effects	Extreme DO & pH Cycle to Control	9	11.19	1.17	8.02	6.91
Residual Effects	Moderate DO & pH Cycle to Control	9	9.96	2.96	7.8	7.19
Residual Effects	Control	10	8.08	6.09	7.59	7.44
Residual Effects	Extreme DO & pH Cycle to Control	10	11.78	1.05	8.02	6.9
Residual Effects	Moderate DO & pH Cycle to Control	10	9.43	2.87	7.8	7.19
Residual Effects	Control	11	7.66	7.37	7.52	7.47
Residual Effects	Extreme DO & pH Cycle to Control	11	7.9	6.58	7.6	7.45
Residual Effects	Moderate DO & pH Cycle to Control	11	7.86	6.91	7.57	7.42
Residual Effects	Control	12	7.64	7.28	7.52	7.46
Residual Effects	Extreme DO & pH Cycle to Control	12	7.73	6.45	7.58	7.44
Residual Effects	Moderate DO & pH Cycle to Control	12	8.04	6.92	7.56	7.45
Residual Effects	Control	13	7.59	7.25	7.5	7.46
Residual Effects	Extreme DO & pH Cycle to Control	13	7.62	7.38	7.51	7.46
Residual Effects	Moderate DO & pH Cycle to Control	13	7.76	6.88	7.56	7.45
Residual Effects	Control	14	7.61	7.28	7.51	7.46
Residual Effects	Extreme DO & pH Cycle to Control	14	7.6	7.34	7.52	7.46
Residual Effects	Moderate DO & pH Cycle to Control	14	7.8	6.86	7.56	7.45
Residual Effects	Control	15	7.59	7.32	7.5	7.46
Residual Effects	Extreme DO & pH Cycle to Control	15	7.61	7.37	7.5	7.47
Residual Effects	Moderate DO & pH Cycle to Control	15	7.89	6.82	7.57	7.45
Residual Effects	Control	16	7.66	7.28	7.51	7.46
Residual Effects	Extreme DO & pH Cycle to Control	16	7.64	7.23	7.52	7.46

Residual Effects	Moderate DO & pH Cycle to Control	16	7.83	6.73	7.57	7.45
Residual Effects	Control	17	7.68	7.18	7.49	7.46
Residual Effects	Extreme DO & pH Cycle to Control	17	7.65	7.33	7.5	7.47
Residual Effects	Moderate DO & pH Cycle to Control	17	7.83	6.59	7.57	7.44
Residual Effects	Control	18	7.69	7.2	7.5	7.46
Residual Effects	Extreme DO & pH Cycle to Control	18	7.7	7.27	7.5	7.46
Residual Effects	Moderate DO & pH Cycle to Control	18	7.84	6.53	7.57	7.45
Residual Effects	Control	19	7.65	7.22	7.5	7.46
Residual Effects	Extreme DO & pH Cycle to Control	19	7.73	7.28	7.51	7.46
Residual Effects	Moderate DO & pH Cycle to Control	19	7.84	6.35	7.57	7.44
Residual Effects	Control	20	7.63	7.27	7.52	7.46
Residual Effects	Extreme DO & pH Cycle to Control	20	7.68	7.29	7.5	7.46
Residual Effects	Moderate DO & pH Cycle to Control	20	7.91	6.6	7.57	7.44
Residual Effects	Control	21	7.77	7.18	7.51	7.46
Residual Effects	Extreme DO & pH Cycle to Control	21	7.79	7.02	7.51	7.46
Residual Effects	Moderate DO & pH Cycle to Control	21	7.84	6.46	7.58	7.44
Residual Effects	Control	22	7.77	7.04	7.51	7.46
Residual Effects	Extreme DO & pH Cycle to Control	22	7.74	7.04	7.54	7.46
Residual Effects	Moderate DO & pH Cycle to Control	22	7.87	6.21	7.57	7.44
Residual Effects	Control	23	8.14	7.05	7.49	7.46
Residual Effects	Extreme DO & pH Cycle to Control	23	8.06	7.08	7.51	7.46
Residual Effects	Moderate DO & pH Cycle to Control	23	8.28	6.09	7.56	7.43
Residual Effects	Control	24	7.86	6.56	7.5	7.46
Residual Effects	Extreme DO & pH Cycle to Control	24	7.74	7.2	7.5	7.46
Residual Effects	Moderate DO & pH Cycle to Control	24	8.38	4.82	7.51	7.46
Residual Effects	Control	25	7.62	7.29	7.5	7.46

Residual Effects	Extreme DO & pH Cycle to Control	25	7.62	7.29	7.53	7.46
Residual Effects	Moderate DO & pH Cycle to Control	25	7.7	7.26	7.49	7.46
Residual Effects	Control	26	7.61	7.32	7.49	7.46
Residual Effects	Extreme DO & pH Cycle to Control	26	7.61	7.38	7.5	7.46
Residual Effects	Moderate DO & pH Cycle to Control	26	7.67	7.29	7.49	7.46
Residual Effects	Control	27	7.65	7.32	7.51	7.46
Residual Effects	Extreme DO & pH Cycle to Control	27	7.61	7.34	7.5	7.46
Residual Effects	Moderate DO & pH Cycle to Control	27	7.68	7.25	7.5	7.46
Residual Effects	Control	28	7.61	7.26	7.5	7.46
Residual Effects	Extreme DO & pH Cycle to Control	28	7.6	7.32	7.49	7.46
Residual Effects	Moderate DO & pH Cycle to Control	28	7.65	7.28	7.49	7.46
Residual Effects	Control	29	7.63	7.28	7.49	7.46
Residual Effects	Extreme DO & pH Cycle to Control	29	7.63	7.32	7.49	7.46
Residual Effects	Moderate DO & pH Cycle to Control	29	7.68	7.28	7.48	7.46
Residual Effects	Control	30	7.64	7.31	7.54	7.46
Residual Effects	Extreme DO & pH Cycle to Control	30	7.61	7.39	7.54	7.46
Residual Effects	Moderate DO & pH Cycle to Control	30	7.63	7.36	7.51	7.46

Appendix B

IACUC

The following contain the annual review from the University of Delaware Institutional Animal Care and Use Committee pertaining to the animals used in these experiments.

**University of Delaware
Institutional Animal Care and Use Committee
Annual Review**

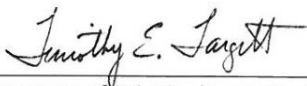


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Title of Protocol: Ecology and Physiological Ecology of Estuarine and Coastal Marine Fishes	
AUP Number: 1131-2014-1	← (4 digits only)
Principal Investigator: Dr. Timothy Targett	
Common Name: Juvenile Weakfish, Juvenile Summer Flounder, Juvenile Striped Bass, Mummichog, Atlantic silverside, Bay anchovy, Silver perch, Atlantic Croaker, Spot, Mullet, Striped killifish, Atlantic menhaden, Halfbeak, Northern Kingfish	
Genus Species: : <i>Cynoscion regalis</i> , <i>Paralichthys dentatus</i> , <i>Morone saxatilis</i> , <i>Fundulus heteroclitus</i> , <i>Menidia menidia</i> , <i>Anchoa mitchilli</i> , <i>Bairdiella chrysoura</i> , <i>Micropogonias undulatus</i> , <i>Leiostomus xanthurus</i> , <i>Mugilidae sp.</i> , <i>Fundulus majalis</i> , <i>Brevoortia tyrannus</i> , <i>Hemiramphus sp.</i> , <i>Menticirrhus saxatilis</i>	
Pain Category: (please mark one)	
USDA PAIN CATEGORY: (Note change of categories from previous form)	
Category	Description
<input type="checkbox"/> B	Breeding or holding where NO research is conducted
<input type="checkbox"/> C	Procedure involving momentary or no pain or distress
<input type="checkbox"/> D	Procedure where pain or distress is alleviated by appropriate means (analgesics, tranquilizers, euthanasia etc.)
<input checked="" type="checkbox"/> E	Procedure where pain or distress cannot be alleviated, as this would adversely affect the procedures, results or interpretation

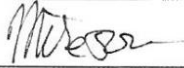


Official Use Only	
IACUC Approval Signature:	
Date of Approval:	4-1-2014

Principal Investigator Assurance

1. I agree to abide by all applicable federal, state, and local laws and regulations, and UD policies and procedures.	
2. I understand that deviations from an approved protocol or violations of applicable policies, guidelines, or laws could result in immediate suspension of the protocol and may be reportable to the Office of Laboratory Animal Welfare (OLAW).	
3. I understand that the Attending Veterinarian or his/her designee must be consulted in the planning of any research or procedural changes that may cause more than momentary or slight pain or distress to the animals.	
4. I declare that all experiments involving live animals will be performed under my supervision or that of another qualified scientist listed on this AUP. All listed personnel will be trained and certified in the proper humane methods of animal care and use prior to conducting experimentation.	
5. I understand that emergency veterinary care will be administered to animals showing evidence of discomfort, ailment, or illness.	
6. I declare that the information provided in this application is accurate to the best of my knowledge. If this project is funded by an extramural source, I certify that this application accurately reflects all currently planned procedures involving animals described in the proposal to the funding agency.	
7. I assure that any modifications to the protocol will be submitted to the UD-IACUC and I understand that they must be approved by the IACUC prior to initiation of such changes.	
8. I understand that the approval of this project is for a maximum of one year from the date of UD-IACUC approval and that I must re-apply to continue the project beyond that period.	
9. I understand that any unanticipated adverse events, morbidity, or mortality must be reported to the UD-IACUC immediately.	
10. I assure that the experimental design has been developed with consideration of the three Rs: reduction, refinement, and replacement, to reduce animal pain and/or distress and the number of animals used in the laboratory.	
11. I assure that the proposed research does not unnecessarily duplicate previous experiments. <i>(Teaching Protocols Exempt)</i>	
12. I understand that by signing, I agree to these assurances.	
 _____ Signature of Principal Investigator	<u>2-23-2014</u> _____ Date

SIGNATURE(S) OF ALL PERSONS LISTED ON THIS PROTOCOL

I certify that I have read this protocol, accept my responsibility and will perform only the procedures that have been approved by the IACUC.

Name	Signature
1. Michael P. Torre	
2. Rachel L. Dixon	
3. Max Davidson	
4.	
5.	
6.	
7.	
8.	
9.	
10.	
11.	
12.	
13.	
14.	
15.	

IACUC approval of animal protocols must be renewed on an annual basis.

1. Previous Approval Date: 4/1/2013

Is Funding Source the same as on original, approved AUP?

Yes No

If no, please state Funding Source and Award Number:

2. Record of Animal Use:

Common Name	Genus Species	Total Number Previously Approved	Number Used To Date
1. Weakfish	Cynoscion regalis	350	20
2. Summer flounder	Paralichthys dentatus	220	50
3. Mummichog	Fundulus heteroclitus	900	400
4. Striped Bass	Morone saxatilis	400	200
5. Atlantic silverside	Menidia menidia	3600	1500
6. Silver Perch	Bairdiella chrysoura	1300	250
7. Striped killifish	Fundulus majalis	250	150
8. Atlantic croaker	Micropogonias undulatus	450	300
9. Atlantic menhaden	Brevoortia tyrannus	240	40
10. Bay anchovy	Anchoa mitchilli	1200	250
11. Spot	Leiostomus xanthurus	400	300

12. Mullet sp.	Mugil sp.	350	50
13 Northern kingfish	Menticirrhus saxitilis	180	40
14. Halfbeak	Hemiramphus sp.	220	40
12. Various species		500	100

3. Protocol Status: *(Please indicate by check mark the status of project.)*

Request for Protocol Continuance:

- A. Active: Project ongoing
- B. Currently inactive: Project was initiated but is presently inactive
- C. Inactive: Project never initiated but anticipated starting date is:

Request for Protocol Termination:

- D. Inactive: Project never initiated
- E. Completed: No further activities with animals will be done.

4. Project Personnel: Have there been any personnel changes since the last IACUC approval? Yes No

If Yes, fill out the Amendment to Add/Delete Personnel form to “Add” Personnel.

Project Personnel Deletions:

Name	Effective Date
1. Katherine A. Bogue	Dec 1 2013
2.	

- 5. Progress Report:** If the status of this project is 3.A or 3.B, please provide a brief update on the progress made in achieving the aims of the protocol.

For project (A): The fish assemblage was sampled biweekly during 2013 at 3 locations (Lewes Ferry Terminal, Mispillion, Port Mahon) from May-October. Sampling at each location occurred at least one hour after sunrise and one hour before sunset. Sampling took place at 2 sites for each stretch of shoreline. Sites are randomly chosen from the 5 possible sites along each stretch of shoreline. Fish were collected a single tow of a 36m bag-seine net deployed by boat. Captured fish are counted, and a subsample of up to 20 individuals per species is measured (fork length for fishes with forked tails, total length for everything else). Predatory fish are stunned with a blow to the head and immediately placed on dry ice and later stored in a -80c freezer for diet analysis. The rest of the catch is released alive adjacent to the shoreline where they were collected. Night sampling was also be conducted weekly, but only at the Lewes site. Sampling methodology was identical to that described above. The other component of our work in 2013 was tagging spot (256) and Atlantic croaker (137) with Visible Implant Elastomer (Northwest Marine Technology), using standard procedures, to study movement patterns. VIE tags are injected as a 1mmX2mm spot of liquid that soon cures into a pliable, biocompatible solid. Fish will be tagged just under the skin using an NMT syringe injector and immediately released alive at the capture location. None were recaptured.

For Project (B): Juvenile striped bass were obtained from aquaculture facilities at GenOn's Patuxent River Chalk Point Generating Station in MD. Planned growth experiments were attempted in the temperature- and photoperiod-controlled room, using the computer-controlled recirculating aquarium systems, described in the latest approved protocol #1131. Survivorship was poor, due largely to a disease issue in the aquacultured fish, and the experiment had to be abandoned. Instead, mummichogs, striped killifish, and Atlantic silversides were collected from the field and, along with the remaining striped bass, were used in a series aquatic surface respiration (ASR) experiments. These behavioral observations of ASR were conducted in the same temperature and photoperiod-controlled lab as used in growth experiments. Fish were exposed to replicate DO/pH treatment combinations over a period of 48 hours, as described in the latest approved protocol #1131. Fish were subsequently euthanized via cranial concussion and pithing for tissue analyses.

- 6. Problems or Adverse Effects:** If the status of this project is 3.A or 3.B, please describe any unanticipated adverse events, morbidity, or mortality, the cause if known, and how these problems were resolved. If there were none, this should be indicated.

As noted above, survivorship of juvenile striped bass obtained from the aquaculture facilities at GenOn's Patuxent River Chalk Point Generating Station in MD was poor, due largely to a disease issue. The growth experiment had to be abandoned.