Swimming Performance of Coastal and Inland Largemouth Bass at Varying Salinities

by

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Abstract

Coastal estuaries represent an interface between marine and fresh waters, and, as such, they are exposed to seasonal and annual fluctuations in salinity. All of the organisms that live in estuaries must emigrate or endure these variations. Largemouth Bass (*Micropterus salmoides*) is a freshwater fish that lives in coastal estuaries and has been shown to not migrate/move to avoid moderate seasonal increases in salinity. Additionally, these coastal Largemouth Bass have growth rates, condition factors, and life history strategies that differ from their inland counterparts. These differences suggest physiological adaptations to tolerate and even thrive in an estuarine environment. I compared swimming performance of Largemouth Bass from an Alabama estuary versus an inland reservoir at 0, 4, 8 and 12 ppt salinities to test for physiological performance-based adaptation to tolerate elevated salinities. I quantified critical swimming speed (CSS) of Largemouth Bass as a measure of swimming performance. I also took scanning electron microscopy (SEM) photos of gill filaments and lamellae of fish after swim tests to determine if there were any morphological differences among salinity treatments and between origin of the fish. CSS did not differ between inland and coastal Largemouth Bass nor were there significant effects of salinity. In addition, 12 inland Largemouth Bass were captured and tested during their spawning period and had significantly higher CSSs compared to non-spawning fish. SEM photos showed no evidence of adaptive gill remodeling or other morphological changes at any salinity or between inland and estuarine populations. It is still possible that inland and estuarine Largemouth Bass have different physiological mechanisms for

tolerating salinity; however, if differences exist they were not measurable using swimming performance as a response metric.

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Introduction

Coastal estuaries are important ecotones at the interface between coastal saltwater and inland freshwater ecosystems (Attrill and Rundle 2002). The relative amount of saltwater and freshwater in these estuaries is strongly dependent on freshwater input from local runoff and river discharge that can vary strongly with season (Kuo and Nielson 1987; Kurup et al.1998; Linderfelt and Turner 2001). During the dry months when river flow is lowest, saline water in the form of a salt wedge can move upstream at the bottom moving counter to the flow of fresher waters at the surface (Ibanez et al. 1996, 1997; Chadwick and Feminella 2001; Kasai et al. 2010). Increases in salinity can significantly influence the biotic components of the estuarine community (Van Den Ayyle and Maynard 1994; Newton 1996; Abookire et al. 1999). Some saltwater fish species and other organisms intolerant of freshwater can migrate upstream during these periods of saltwater intrusion (Mclnerney 1964; Deegan 1993; Juanes et al. 1993; Marshall and Elliott 1998). Food availability for fish during this time may increase due to smaller saltwater tolerant organisms migrating into the estuary (Odum 1980; MacAvoy et al. 2000; Peer et al. 2006). This increase in smaller organisms that recruit to the estuary during periods of elevated salinity may be beneficial as it increases overall food availability in the estuarine system (MacAvoy et al. 2000; Kimmerer 2002); but, the salinity may increase costs to some fishes (freshwater species) as they must deal with increased osmotic stress (Peterson 1988; Morgan and Iwama 1991; Dunson et al. 1993). Estuaries can contain many fish species, including many that mainly thrive in freshwater. Those that do not move to avoid saline water in the estuary must overcome physiological challenges to survive.

One important physiological challenge that occurs for fishes, is osmoregulation. Freshwater fish typically have a blood salinity between 8-9 ppt (Bond 1979; Egna and Boyd

1997). The blood of freshwater fish contains more solutes/ions than the freshwater in which they live. Freshwater fish must counter two problems caused by being placed in a hypotonic solution: water influx and ion loss. Water diffuses in due to a higher concentration of ions in the blood. As a result fish produce large amounts of extremely dilute urine at the expense of the accompanying ion loss (Whitfield 2015). Ions are also lost through diffusion as ion concentration gradients favor their movement out of the body. When freshwater fish are placed in a hypertonic solution the opposite occurs- water leaves and ions diffuse into the blood. Freshwater fish must regulate their blood osmolality by active and passive transport of sodium and other essential ions across the epithelial membrane on the gill lamellae (Gonzalez 2011). Active transport of ions in or out of the blood is primarily achieved through use of ATP/energy (oxygen consuming) (Evans et al. 2005). Therefore when a fish is placed in water that is not isotonic with its blood, it incurs osmoregulatory costs.

Estimates of increased osmoregulatory energy costs due to elevated salinity can vary across species and studies. Specific estimates of osmoregulatory energy costs for freshwater fish swam in salinities greater than 0, generally, are more than 20% of their energetic maintenance costs (Rao 1968; Farmer and Beamish 1969; Furspan et al. 1984), although there are exceptions. One exception is when a fish is placed in a solution that is isotonic with its blood. Some studies have found that metabolic oxygen consumption rates decreased for fish when the water salinity was isotonic with their blood (Farmer and Beamish 1969; Glover et al. 2012). The decrease in metabolic oxygen consumption at isotonicity has been speculated to be the result of decreased osmoregulatory costs. It is believed that there would be a reduction in the active transport of ions resulting in reduced energy expenditures due to decreased electrochemical and diffusion gradients. Studies have shown that freshwater fish that inhabit saline portions of estuaries

express decreased ATPase enzymatic activity, tolerate higher blood plasma levels, and acclimate to saline waters more quickly than freshwater fish that had never experienced salinity (Peterson 1988; Meador and Kelso 1990). Freshwater fish species living in estuaries may have the ability to develop chloride cells, as chloride cells improve ion uptake and facilitate salt excretion at salinities greater than isotonicity (Avella et al. 1993; Whitfield 2015). These previous studies suggest that freshwater fish in estuaries may possess physiological mechanisms to reduce osmoregulatory costs during periods of increased salinities.

Reducing active ion transport may not be the only physiological mechanism responsible for the relative reduction in metabolic oxygen consumption observed at isotonicity. Fishes may also conserve energy that would be required to control ion transport by relying on morphological changes at the site of oxygen uptake, the gill. Freshwater fish living in estuaries may reduce the gill surface area or shunt blood flow away from the secondary lamellae to decrease blood flow to prevent water loss or to limit fluctuations in plasma osmolality if salinities increase past isotonicity (Febry and Kultz 1987; Peterson 1988). This reduction in blood flow would reduce the ability of the fish to exchange oxygen and eliminate waste products such as carbon dioxide and urea. It is still unclear whether these physiological mechanisms for reducing osmoregulatory costs and dealing with elevated salinities represent an energetic advantage, disadvantage, or neither.

Performance testing such as swimming can be used to determine if a stressor such as increased salinity can result in decreased scope for activity (Plaut 2001). Swimming requires the use of ATP (for muscle contraction and ion uptake), oxygen, and other nutrients (Taylor et al. 1997; Evans and Claiborne 2006). Therefore, estimates of fish swimming capabilities can provide an integrated measure of the effects of salinity on fish. The most commonly used method

of measuring swimming performance for fish is to measure critical swimming speed (CSS) (Plaut 2001). Critical swimming speed is an index of swimming performance measured by forcing a fish to swim against current which is increased after a predetermined amount of time if the fish does not fatigue. Critical swimming speed measurements can be affected by many factors such as; temperature, body size, growth rate, salinity, age, and species (Bell and Terhune 1970; Keen and Farrell 1994; Nelson et al. 1996; Gregory and Wood 1998; Kolok 1999; Plaut 2000b).

Largemouth Bass *Micropterus salmoides* is an important sport fish species in coastal estuaries such as the Mobile-Tensaw River Delta, Alabama (Swingle and Bland 1974; Guier et al. 1978; Tucker 1985; Krause 2002; Farmer et al. 2010). Largemouth Bass in the Mobile-Tensaw River Delta (referred to hereafter as "Delta") exhibit high body condition factor, high lipid reserves, are shorter at a given age, grow more slowly, and have a shorter length at maturity compared to freshwater populations of Largemouth Bass (Norris et al. 2010; Glover et al. 2012). Estuarine Largemouth Bass do not appear to move to avoid increasing salinity (Meador and Kelso 1989; Lowe et al. 2009; Farmer et al. 2013) and their high condition body factors, high lipid reserves, and generally shorter length of Delta Largemouth Bass are thought to help meet increased metabolic demands during summer and times of elevated or fluctuating salinities (Glover et al. 2013). Meador and Kelso (1990) found that coastal Largemouth Bass that had been acclimated to water at 8 ppt exhibited reduced active ion transport and tolerated higher blood ion plasma levels compared to freshwater Largemouth Bass. These results suggest that estuarine Largemouth Bass may possess physiological mechanisms that allow them to save energy by conserving ATP and tolerating elevated blood ion plasma levels at elevated salinities.

Largemouth Bass from the Delta may possess different physiological mechanisms for osmoregulating and saving energy at elevated salinities than freshwater Largemouth Bass.

The purpose of this study was to use a performance challenge approach to determine if Largemouth Bass living in an estuarine environment express differences in performance relative to inland populations that may be the result of underlying physiological adaptations for tolerating salinity variation from freshwater to that exceeding their blood osmotic concentration. To test these questions, I compared critical swimming speeds (often referred to as U_{crit} in the literature) of Mobile-Tensaw River Delta (coastal) and inland Largemouth Bass from 0 to 12 ppt salinity. I also compared length at age 1, length, girth, relative weight, and age from coastal and inland Largemouth Bass with swimming performance to determine if there was significant interaction among origin of the fish, salinity, and swimming performance. Further, I compared the gill structure of individual coastal and inland Largemouth Bass held and swam at the same range of salinities using scanning electron microscopy (SEM) to determine if any apparent morphological changes occurred.

Methods

Sampling Locations

Largemouth Bass were collected from two sites, one estuarine (seasonally exposed to saltwater) and one inland (never exposed to saltwater). Estuarine Largemouth Bass were collected from Bay Minette Basin in the Mobile-Tensaw River Delta near Mobile, Alabama (Baldwin County) (Figure 1). Inland Largemouth Bass were collected from Yates Reservoir located near Tallassee, Alabama (Tallapoosa County). Fish were collected year round but only males and females without ripe gonads (determined in the field and laboratory) were used in our study, given that swimming performance results might be affected, possibly due to energy being allocated into gonadal and egg development around the spawning period (Plaut 2002; Brown and Murphy 2004). However, we did find that 12 individuals from Yates Reservoir were in spawning condition after they had been tested. These fish were not included in our main data set, but we do consider their swimming performance separately in the results below.

Sampling methods

Adult Largemouth Bass between 280-404 mm (size range at which fish experience laminar flow in swim chamber) were collected via electrofishing. Fish were transported back to Auburn University's E.W. Shell Fisheries Station in a large aerated tank containing 1% salt (Crystal Sea Bioassay MarineMix) to enhance survival (Wright and Kraft 2012). Once in the lab, they were placed into a large holding tank until they were transferred into individual acclimation tanks. Fish of different origin were kept separate.

Laboratory acclimation

Critical swimming speeds for Largemouth Bass were determined at four different salinities (0, 4, 8, 12 ppt) at 25°C. The acclimation/testing room contained twelve 80 L glass

acclimation tanks. Water quality was maintained by using a recirculating biofiltration subpump system and water quality was tested every other day. City water (dechlorinated using sodium thiosulfate or API® de-chlorination solution) was used (throughout the experiment) to fill the acclimation tanks and swim chamber. Initially, acclimation tanks contained water at the same temperature and salinity as the large flow-through holding tank. Water temperature was controlled by maintaining air temperature in the room. Salinity concentrations were increased or decreased between 1.5-2 ppt per day using Crystal Sea Bioassay MarineMix (Marine Enterprises International Inc.) or by adding freshwater, respectively. Water temperatures below 22°C were initially obtained in the acclimation room system by using ice (added to water then dechlorinated). Typically water reached desired temperature within 48 hours and did not induce any fish mortality. Once the desired salinity and temperature combination was reached, fish were held there for at least 72 hours before testing. Order of testing individual fish at the desired salinity was determined randomly. Largemouth Bass were fed minnows twice a week. Feeding was stopped 48 hours before swim runs to allow fish to clear their stomachs (Beamish 1964b). A 16L:8D photoperiod was maintained throughout the experiment, with testing conducted in the light.

Swimming apparatus

CSS of Largemouth Bass was measured in a 2133 x 609 mm swim tank (Figure 2). The swim tank contained a rectangular area (125 mm long and 50 mm wide) in which a clear acrylic test chamber was inserted that contained the fish being tested (146 mm dia, 546 mm long). A plastic funnel (190.5 mm diameter) on the front of the chamber and square stainless steel mesh (12 mm by 12 mm openings) helped to straighten the flow before it entered the chamber. Semi-laminar flow was created in the rectangular section by passing the water through a grid of 105

mm long 12.7 mm PVC pipes combined with circular straightener vanes at the front end of the tank. Half of the rectangular test section was covered with black plastic, with a light shining on the back half, to ensure that the fish would swim towards the front of the clear acrylic tube during testing. Water was propelled by a modified 40 lb thrust Minn Kota trolling motor. Water velocity measurements were obtained by using a Veriner flow meter connected to a computer.

Swimming tests

Largemouth Bass were placed in the test chamber 1.5 hours before testing to allow recovery from handling (Kolok 1991). After 1.5 hours, fish were habituated to swimming in the test chamber by initiating a current of 15 cm/s for 1.5 hours (Kolok 1992b). At the end of the habituation period, water velocity was increased by 5 cm/s every 30 minutes. Fish were swum until they started to exhibit unsteady swimming patterns (burst and coast, erratic behavior, continually resting on back mesh) indicating anaerobic energy consumption was occurring due to oxygen depletion (Peake and Farrell 2004). Once a fish exhibited these variable swimming patterns, testing would stop when the fish was not able to continually swim in the test chamber for more than ten seconds. A rod was used to prod fish that attempted to rest periodically on mesh at the downstream end of the tube. Critical swimming speed was calculated by the formula used by Brett (1964):

$$\mathbf{CSS} = \mathbf{V_{-1}} + \left(\frac{\mathbf{t}}{\mathbf{\Delta t}}\right)\mathbf{\Delta v}$$

Where CSS is critical swimming speed in cm/s, Δt is time increment in minutes before increasing flow velocity, Δv is the prescribed velocity increment in cm/s, t is time elapsed at final velocity, and V_{-1} is the highest velocity maintained for the full prescribed time period (cm/s). Critical swimming speeds were corrected for the solid blocking effect, an increase in test chamber water velocity caused by a fish having a cross sectional area greater than 10% of the

cross sectional area of the test chamber, using the method described by Bell and Terhune (1970). Largemouth Bass distributed across the size range were tested from each site so that the mean critical swimming speed estimates were not size biased.

Laboratory processing

After each swimming test was completed, the test Largemouth Bass was euthanized. Measurements of length (mm TL), body depth (mm), width (mm), girth/circumference around widest point on fish (mm), liver weight (nearest gram), and total wet weight (nearest gram) were recorded. Saggital otoliths were extracted and stored dry for age-and-growth determination. Otoliths from Largemouth Bass that appeared to be five years or older were sectioned for age-and-growth analysis.

Analysis

Analysis of covariance (ANCOVA) was used to compare the mean critical swimming speeds among salinity treatments and locations. Backcalculation from otolith radius was used to determine length-at-age 1. Backcalculated total length at the *i*th age was estimated using the direct proportion method (Le Cren 1947):

$$L_i = \frac{Si}{Sc} \times L_c,$$

where L_i is the back calculated length of the fish at the formation of the *i*th increment, L_c is the length of the fish at capture, S_c is the radius of the otolith at capture, and S_i is the radius of the otolith at the *i*th increment. Linear regression was used to compare length at age 1, relative weight, girth, and age with critical swimming speeds between populations.

Gill Morphology

Gill morphology was examined with scanning electron microscopy (SEM) (Kirk 1993; Olson 1996). The second gill arch on the fish's right side was extracted after swim testing and preserved in a 10% formalin solution. Segments of the gills were removed, dehydrated, and prepared for SEM analysis using the critical point drying method, followed by gold sputter coating (Boyde and Wood 1969). SEM photos of gill filaments and lamellae were taken at 200 x magnifications from the center of randomly chosen gill filaments on each specimen. Cellular level gill SEM photos were taken on the trailing edge (where the afferent arteries are situated) of gill filaments at 2000 x and 4000 x magnification from randomly chosen locations. Photos were qualitatively analyzed to determine if adaptive gill remodeling was occurring. Adaptive gill remodeling can be identified if an intercellular mass, a proliferation of cells filling the space between the gill lamellae (Sollid and Nilsson 2006), is present. Chloride cells, freshwater mitochondria rich cells (MRCs), and pavement cells were also identified, and chloride cell quantification by counting apical crypts was attempted (King and Hossler 1991).

Results

Site differences

Largemouth Bass from Bay Minette Basin had significantly greater ($p<0.0001$) relative weight and longer length at age 1 relative to Yates Lake (Table 2). Fish from Yates Reservoir were significantly older ($p<0.0001$) than Largemouth Bass from Bay Minette Basin (Table 2).

Origin and Salinity Effects

Critical swimming speed (CSS) values for Largemouth Bass from Yates Reservoir (inland) and Bay Minette Basin (coastal) did not differ significantly ($p=0.11$). In addition, CSS values did not differ across salinities ($p=0.27$) (Table 1, Figure 3) and the interaction effect between origin of fish and salinity on CSS was not statistically significant ($p=0.14$).

Other correlates

There were no significant relationships between CSS values and fish length ($p=0.96$), girth ($p=0.27$), or age ($p=0.53$). However, Largemouth Bass relative weight was positively correlated ($r^2=0.12$; $p=0.0008$) with CSS values (Figure 4). Bay Minette Largemouth Bass length at age 1 was positively correlated with CSS ($p=0.01$), but Yates Reservoir Largemouth Bass length at age 1 was not (Figure 5). CSS values did not differ significantly between males and females ($p=0.26$).

Spawning fish

Twelve Yates Reservoir Largemouth Bass that were collected in spring 2015 were found to have ripe gonads after having been tested and transferred to the laboratory. These individuals were found to be close to spawning given that they did not eat in the laboratory and exhibited spawning behaviors (e.g., trying to build nests, over aggressive behavior) in their tanks. CSS values for these fish were significantly higher than the CSS values from non-spawning fish

($p<0.0001$; Figure 6). Eight out of these 12 fish were tested at 12 ppt and the rest were tested at 0 ppt. There were no significant differences ($p=0.37$) in CSS between these two salinities.

Gill Morphology

Scanning electron microcopy (SEM) photos showed no evidence of adaptive gill remodeling. All of the gill lamellae were separated and none of the spaces between them were filled with an intercellular mass at any salinity (e.g., Figure 7).

Estuarine and inland Largemouth Bass can develop seawater MRCs (chloride cells) at elevated salinities as apical crypts were identified (Figure 8). Apical crypts were few, not consistent from sample to sample, and difficult to distinguish from surface artifacts and were not quantifiable. It did not appear though that inland and estuarine Largemouth Bass had differences at the cellular level at any salinity.

Discussion

Effects due to salinity

Largemouth Bass swimming performance as measured by CSS did not differ across salinities, despite the fact that metabolic oxygen consumption has been shown to increase at both 4 and 12 ppt for estuarine Largemouth Bass (Glover et al. 2012). The increases in oxygen consumption are believed to be due to increased osmoregulatory costs, particularly at 12 ppt when reversed electrochemical gradients change direction of ion movement and alter osmoregulatory functions. I expected any increase in osmoregulatory costs to be sufficiently large so as to impact swimming performance, given that osmoregulatory costs may constitute as much as 20% of a fish's metabolic costs when swimming (Rao 1968; Farmer and Beamish 1969; Furspan et al. 1984; Toepfer and Barton 1992; Boeuf and Payan 2001). Swimming performance not being impacted by elevated salinities may have been because Largemouth Bass in this experiment were exposed to salinities that are common in the Mobile Tensaw Delta. Critical swimming speeds of fish species that are known to thrive in saline or brackish waters have only been affected by salinities that are extreme relative to those found in their natural environment, leading to osmoregulatory failure/stress (Kolok and Sharkey 1997; Swanson 1998; Plaut 2000b). It appears that Largemouth Bass, regardless of origin, were not under osmotic stress at any salinity. Fish that are under a significant level of osmotic stress typically exhibit osmoregulatory failure/dysfunction, or expend large amounts of energy trying to maintain ionic balance, which results in decreased swimming performance (Brauner et al. 1992, 1994; Kolok and Sharkey 1997; Plaut 2000b). Largemouth Bass in this experiment did not appear to expend large amounts of energy due to osmotic stress caused by elevated salinities as their swimming performance was not negatively impacted.

It also appears that Largemouth Bass did not have an energetic advantage at any salinity, even isotonicity, that would improve their swimming performance. It is believed that fish at isotonicity should have reduced osmoregulatory costs (Febry and Lutz 1987; Pérez-Pinzón and Lutz 1991; Glover et al. 2012); as such, I expected Largemouth Bass to have more energy available for swimming at isotonicity. Estuarine Largemouth Bass have reduced ATPase activity and oxygen consumption rates at isotonicity (Meador and Kelso 1990; Glover et al. 2012), which may represent an osmoregulatory energy saving because electrochemical and diffusion gradients are reduced. Explanations for the drop in metabolic oxygen consumption at isotonicity are highly debated and this phenomenon has not been consistently observed among all fish species (Job 1969a; Nordlie 1978; Morgan and Iwama 1991; 1998). Some researchers think that reduced oxygen consumption at isotonicity is not due to decreased osmoregulatory costs (Potts and Parry 1964; Fang 1982; Morgan and Iwama 1991; Altinok and Grizzle 2003). Rather, a physiological change at the gill may occur at 8 ppt to conserve water and reduce ion fluctuations in the event that salinity would increase beyond isotonicity (Peterson 1988; Glover et al. 2012). If this physiological change at isotonicity or higher salinities is present, it did not affect Largemouth Bass swimming performance in my study. In order to be able to complete aerobic activities, some environmentally related physiological changes in the gill are known to reverse once swimming has commenced (Brauner et al. 2011; Perry et al. 2012).

Largemouth Bass may have energetic savings or deficits at elevated salinities, but instead underwent physiological compensatory changes when swimming. For example, Adriatic Sturgeon *Acipenser naccarii* expended more energy in brackish water than in freshwater, but their CSS was not affected because they increased active metabolic rate, ventilation rate, and tail beat frequency (McKenzie et al. 2001). Other studies have also shown that despite elevated

oxygen consumption and metabolic energy expenditures at different salinities, swimming performance is not necessarily affected (Nelson et al. 1996, Wagner et al. 2006). It is also possible that Largemouth Bass devoted energy savings to different parts of the body for other energetically costly processes (e.g., growth, gonad development, lipid accumulation, etc.) instead of swimming. It is unknown how increased or decreased energy expenditures that were not apparent in our swim performance tests would affect Largemouth Bass energetic recovery, long term somatic growth, and survival after prolonged swimming.

Effects due to source

Largemouth Bass from coastal and inland populations did not differ in their swimming performance ability. Numerous studies have found that estuarine Largemouth Bass have higher body condition, greater lipid reserves, and shorter lengths compared to inland Largemouth Bass (Colle et al. 1976; Meador and Kelso 1990; Norris 2010; Glover et al. 2012). Estuarine Largemouth Bass also have reduced ion transport at 8 ppt and can tolerate elevated plasma levels compared to inland Largemouth Bass (Meador and Kelso 1990). Due to these physiological differences, and the fact that salinity can be a strong selective pressure (Susanto and Peterson 1996; Whitfield 2015), I expected that estuarine Largemouth Bass had evolved physiological mechanisms to allow them to conserve energy when osmoregulating at elevated salinities. Although resting osmoregulatory metabolic costs are typically low (Kirschner 1995; Morgan and Iwama 1999), osmoregulatory costs increase when fish undergo sustained swimming (Febry and Lutz 1987). If Bay Minette Basin Largemouth Bass are adapted to greatly reduce their osmoregulatory costs during acclimation and swimming, their CSS may have been higher as more energy would be available for other uses. However, previous studies with brackish and freshwater populations of the same fish species typically have not demonstrated differences in

critical swimming speeds between them at different salinities (Nelson et al. 1996; A. Haukenes, University of Arkansas at Pine Bluff, personal communication). Most likely some fish species have the ability to osmoregulate and maintain ionic balance during swimming at elevated salinities without any loss in performance. Fish that can occur in freshwater and estuarine environments typically already have physiological mechanisms such as the ability to develop chloride cells and change gill permeability which allow them to osmoregulate and maintain water balance in saline environments (Kultz and Onken 1993; McCormick 1994; Uchida et al. 2000; Laverty and Skadhauge 2012; Kizak 2013; Whitefield 2015). This conjecture is further supported by our scanning electron microscopy results showing Bay Minette Basin and Yates Reservoir Largemouth Bass have the ability to develop chloride cells at higher salinities (Figure 8).

While there were no significant differences in swimming performance between estuarine and inland Largemouth Bass populations at any salinity, it is still possible that physiological differences exist relative to osmoregulating and tolerance for elevated salinities. Studies in which different populations of similar fish species were abruptly exposed to saline water have demonstrated reduced critical swimming speeds due to differences in their ability to osmoregulate (Glova and McInerney 1977; Randall and Brauner 1991; Brauner et al. 1992, 1993). Estuarine and inland Largemouth Bass may have different abilities to rapidly acclimate to saline water influxes; for example, Peterson (1988) found that centrarchid survival and distribution was affected by their ability to quickly acclimate to saline waters in Mississippi estuaries. This also may help explain why metabolic oxygen consumption drops near isotonicity for estuarine Largemouth Bass as there may be a physiological mechanism to help them to gradually acclimate to changes in salinities caused by estuarine tidal influxes of saline water.

While notable differences in body condition, lipid storage, and growth have been found between estuarine and inland Largemouth Bass (Colle et al. 1976; Guier et al. 1978; Meador and Kelso 1990; Norris et al. 2010), my results suggest that Bay Minette Basin Largemouth Bass have not evolved a physiological mechanism for tolerating elevated salinities that would be reflected by critical swimming speed. Neither population of Largemouth Bass in this experiment appeared to be under osmotic stress or have substantial energetic differences at any salinity given that swimming performance did not differ across salinities. Despite high variability seen in this experiment, other studies measuring critical swimming speed with similar levels of variability did see declines in CSS when fish were expending energy maintaining ionic balance or were otherwise stressed (Kolok and Sharkey 1997; Plaut 2000b). It is important to note that our fish were run at an optimal temperature and osmoregulatory/metabolic costs will increase at higher more stressful temperatures (Sardella et al. 2004; Sardella and Brauner 2007). At higher temperatures any osmoregulatory differences between these two populations might have yielded differences in swimming performance. While we cannot rule out salinity in having an influence on the observed morphological and physiological differences between inland and estuarine Largemouth Bass, salinity most likely has a complex interaction with other abiotic and biotic factors which can affect Largemouth Bass survival and life history strategies in estuaries. Clearly, elevated salinities between 0-12 ppt alone are sublethal for adult inland and estuarine Largemouth Bass and can be tolerated over prolonged periods of time, most likely as a result of pre-existing physiological adaptations.

Other Correlates

Length and girth were not correlated with CSS, likely as a result of Largemouth Bass all being similar in size. Previous studies have also found CSS to not be correlated with length when fish were similar in size (Kolok 1992b). Critical swimming speed was also not related to Largemouth Bass age. Only one other study has compared age of adult fish with critical swimming speed, and it too did not find a correlation (Reidy et al. 2000). Even though age was not correlated with CSS, length at age 1 was positively correlated with critical swimming speed for Bay Minette Basin Largemouth Bass. We expected that the length that a Largemouth Bass reached at age 1 most likely would not affect its swimming performance given that age-1 Largemouth Bass have different growth rates, diets, and physiology compared to older fish. Therefore it is unclear why length at age 1 would correlate with critical swimming speed for Bay Minette Basin Largemouth Bass.

Largemouth Bass relative weight was positively correlated with critical swimming speed. Previous studies have correlated higher relative weights to good fish health, increased fat storage, and high caloric diets (Blackwell et al. 2000; Coughlan et al. 1996; Legler 1977; Rose 1989; Kohler and Kelly 1991; Wedge and Anderson 1978). As a result, fish with higher relative weights most likely had more available energy to swim. Even though fish with lower relative weights may have had different body shapes which have been correlated to fish swimming performance (Hawkins and Quinn 1996; Walker and Westneat 2002; Fisher and Hogan 2007), girth, a metric of body shape was not found to be significantly related to critical swimming speed. Furthermore, the cross-sectional area of most fish tested filled $\geq$ 10% of the swim chamber cross sectional area and body morphological parameters are accounted for when critical swimming speed is corrected for the solid blocking effect. The solid blocking effect is corrected

by using body depth, width, length, and thickness measurements to calculate a correction factor (Bell and Terhune 1970). This correction factor is then multiplied by the original CSS to obtain the corrected CSS that takes into account an increase in water velocity around the fish (Bell Terhune 1970; Williams and Brett 1987).

Spawning Fish

The higher critical swimming speed for Yates Reservoir Largemouth Bass in spawning condition was most likely caused by physiological and behavioral changes resulting from elevated hormone levels. Previous swim performance studies have found that fish that have energetically demanding spawning roles (nest building and defense, traveling, searching for mates) typically have increased swimming performance immediately before or during the spawning period (Williams and Brett 1987; Adams and Parsons 1998; Cooke et al. 2010). Elevated hormone levels during spawning that can improve cardiovascular performance, increase red muscle, and change fish behavior (Liley and Stacey 1983; Kindler et al. 1989, 1991; Thorarensen et al. 1996; Sandblom et al. 2009; Cooke et al. 2010) are most likely responsible for the increased swimming performance seen in spawning Yates Reservoir Largemouth Bass.

Gill Morphology

Scanning electron microscopy photos revealed no evidence of adaptive gill remodeling as there was no interlamellar cell mass (ILCM) on any gill lamellae at any salinity. An ILCM is a proliferation of cells between the gill lamellae (Sollid and Nilsson 2006; Nilsson 2007), and its formation reduces osmoregulatory costs and the impact of environmental stressors by decreasing the amount of gill surface area and increasing the distance between the blood and external environment (Sollid and Nilsson 2006; Tzaneva et al. 2011; Sinha et al. 2014). Largemouth Bass have the ability to form an ILCM in response to low temperatures and acidic water (Leino and

McCormick 1993). Therefore, I expected that Largemouth Bass may be able to develop an ILCM, especially between 8-12 ppt to prevent water loss and ions from coming into the blood stream. I also expected that an ILCM might explain why oxygen consumption declined at isotonicity as an increase in the distance between the blood and external environment decreases oxygen uptake (Sollid and Nilsson 2006; Glover et al. 2012).

While Largemouth Bass may still form an ILCM in response to elevated salinities, its absence in our SEM photos may have been caused by testing Largemouth Bass at their optimal temperature. An ILCM will form in cold water to conserve ions because oxygen saturation is high and metabolic rates are low (Sollid and Nilsson 2006; Nilsson et al. 2012; Barnes et al. 2014). At 25° C oxygen saturation would be lower and Largemouth Bass metabolic demands would be higher. Therefore, meeting increased oxygen demands may be more important to Largemouth Bass than reducing osmoregulatory costs.

Based the SEM photos, I believe that I have located freshwater and seawater mitochondria rich cells (MRCs) in both Yates Reservoir and Bay Minette Basin Largemouth Bass (Figure 8). Seawater MRCs (also known as chloride cells) typically regress and form an apical crypt when exposed to saline water (Evans 2005). Apical crypts expose the surface of the chloride cell to the external environment, and are believed to improve salt secretion and active transport of ions (Perry 1997; Hossler et al. 1985). Fish that are able to tolerate saline waters typically have the ability to develop apical crypts (Uchida et al. 2000; Whitfield 2015). It appears that Yates Reservoir and Bay Minette Basin Largemouth Bass have the ability to develop these cells, which may explain why they are able to swim as well at salinities above versus at isotonicity.

It was unclear in this experiment how the amount, type, or shape of MRCs differed between Largemouth Bass populations and among salinity treatments. Part of the reason for the confusion was due to the inability to identify freshwater or seawater MRCs with 100 percent certainty. Many experiments analyzing freshwater and seawater MRCs typically use histology, transmission electron microscopy, or confocal scanning electron microscopy to first identify the MRCs then use SEM to quantify them (Perry 1997). There is also the possibility that at salinities higher than 12 ppt they would become more apparent as 12 ppt for Largemouth Bass may not cause measurable proliferation of apical crypts. Another potential reason for why I experienced difficultly quantifying MRCs was due to unknown organic matter covering the epithelial cells at all of the salinities (Figure 9). It is unknown if this organic covering was in fact enlarged MRCs, bacteria, or some sort of response to elevated salinities or stress.

Conclusion:

In conclusion salinities between 0- 12 ppt alone did not significantly stress or impact estuarine or inland largemouth bass swimming performance. However, these results do not rule out the possibility that estuarine and inland Largemouth Bass have different physiological mechanisms for tolerating elevated salinities, but it does demonstrate that they were not expressed in CSS measures. CSS measurements in this experiment were highly variable and identifying sources of variability and performing repeated tests on the same individuals might be useful in future experiments. The absence of gill morphological changes between site and salinity demonstrate that between 0-12 ppt if any physiological differences are present they are unable to be detected with SEM (at 25° C) after swimming Largemouth Bass. Studies should also use SEM in combination with other methods for identifying cells, as MRCS were difficult to distinguish from surface artifacts. Lastly, CSS is improved when Largemouth Bass are taken

close to or during spawning. It is possible that Largemouth Bass during spawning undergo physiological changes that improve aerobic activity during this important time period.

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Table 1- Mean critical swimming speed values (CSS) from estuarine and inland Largemouth Bass at 0, 4, 8, 12 ppt salinity treatments. Estuarine Largemouth Bass were collected from Bay Minette Basin in the Mobile-Tensaw River Delta near Mobile, Alabama. Inland Largemouth Bass were collected from Yates Reservoir located near Tallassee, Alabama. No significant differences in CSS (cm/s) values were found between site or among salinities. An alpha level of 0.05 was used to determine significance. Data are presented as means ± 1 standard error.

Salinity (ppt)	Yates (N=)	Bay Minette Basin (N=)
0	53.62 ± 1.92 (10)	55.95 ± 1.89 (10)
4	52.54 ± 1.74 (10)	49.39 ± 1.35 (10)
8	48.64 ± 2.10 (11)	54.47 ± 2.54 (10)
12	49.73 ± 1.90 (10)	50.62 ± 1.77 (9)

Table 2- Mean relative weight, length at age 1 (Age-1) and age from estuarine and inland Largemouth Bass. Estuarine Largemouth Bass were collected from Bay Minette Basin in the Mobile-Tensaw River Delta near Mobile, Alabama. Inland Largemouth Bass were collected from Yates Reservoir located near Tallassee, Alabama. Averages that were significantly higher between source populations are bolded. An alpha level of 0.05 was used to determine significance. Data are presented as means ± 1 standard error.

Variable	**Yates (N=60)**	**Bay Minette Basin (N=43)**
Relative weight	74.51 ± 1.09	**83.16 ± 1.01**
Age-1	152.66 ± 4.83	**190.46 ± 4.24**
Age	**4.35 ± 0.20**	3.09 ± 0.13

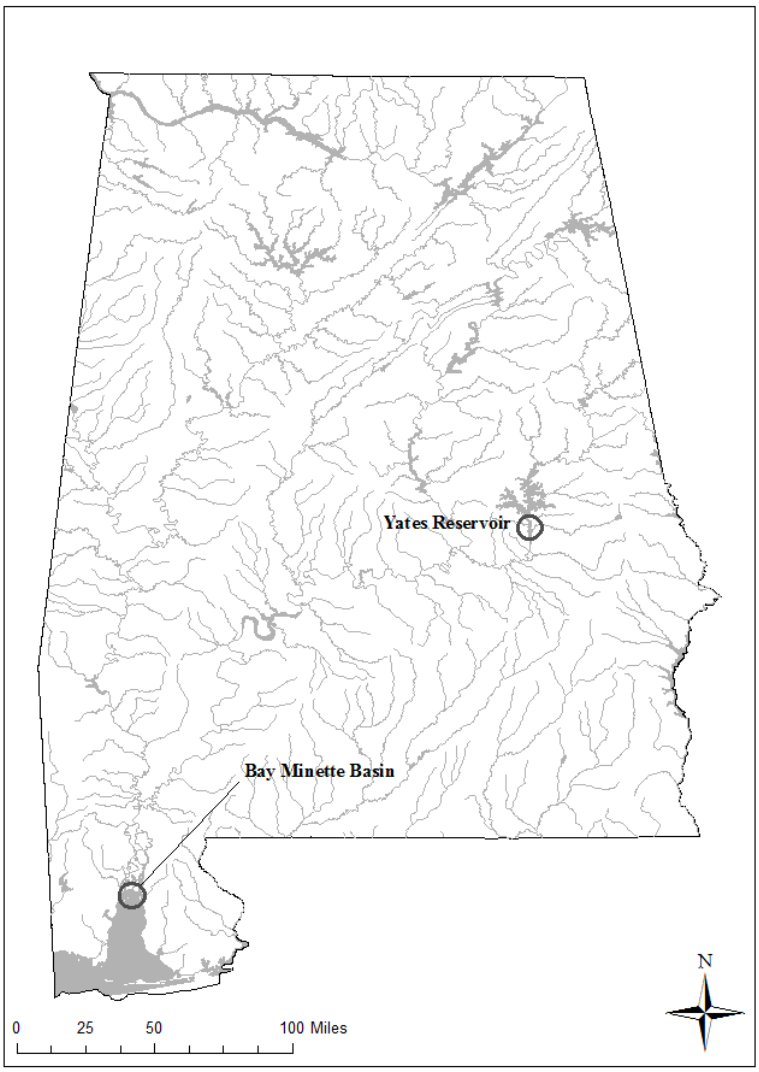


Figure 1.

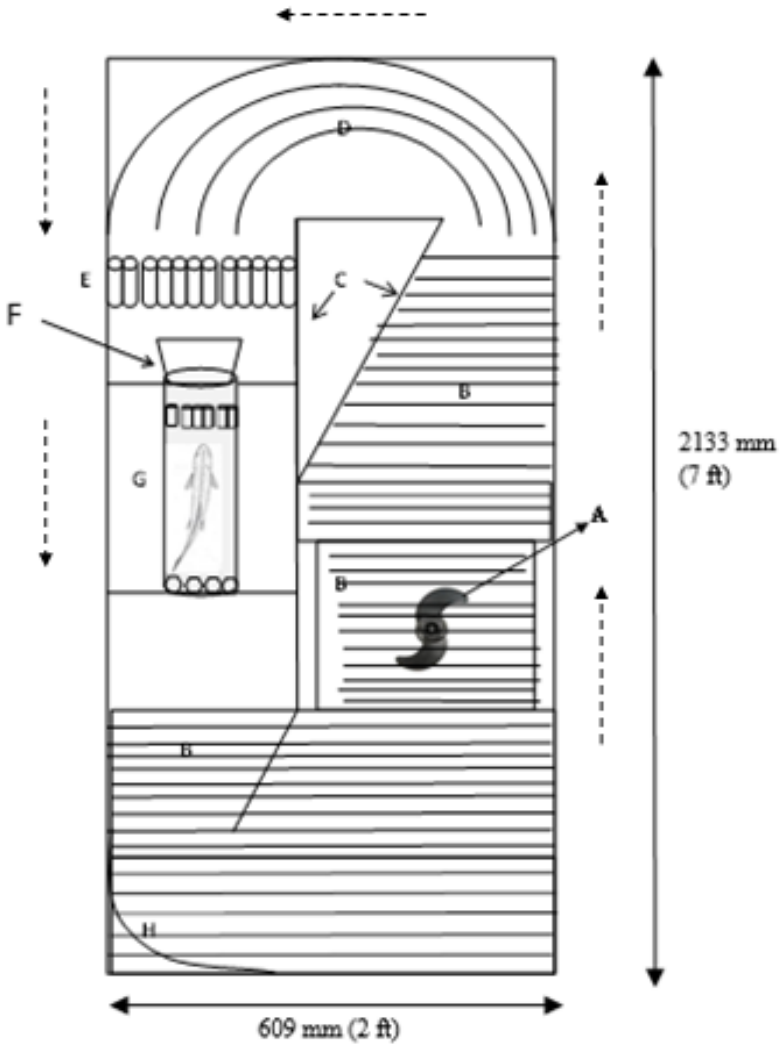
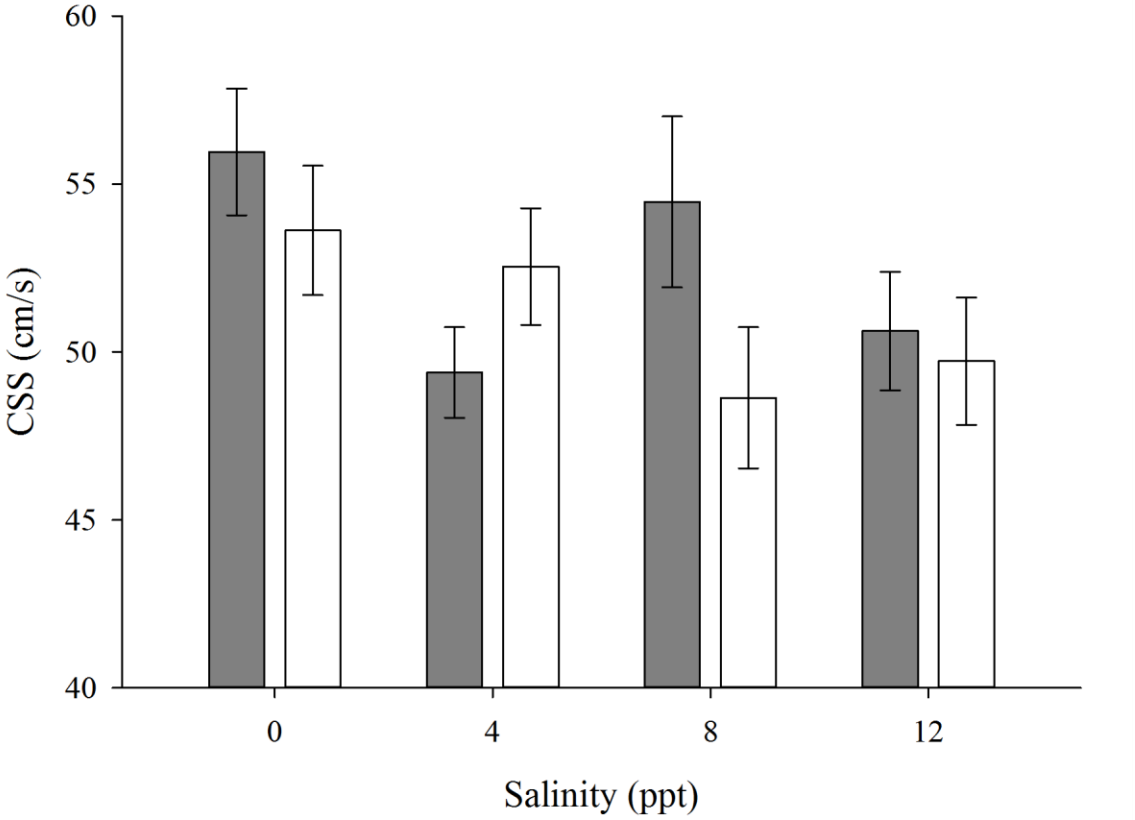


Figure 2.



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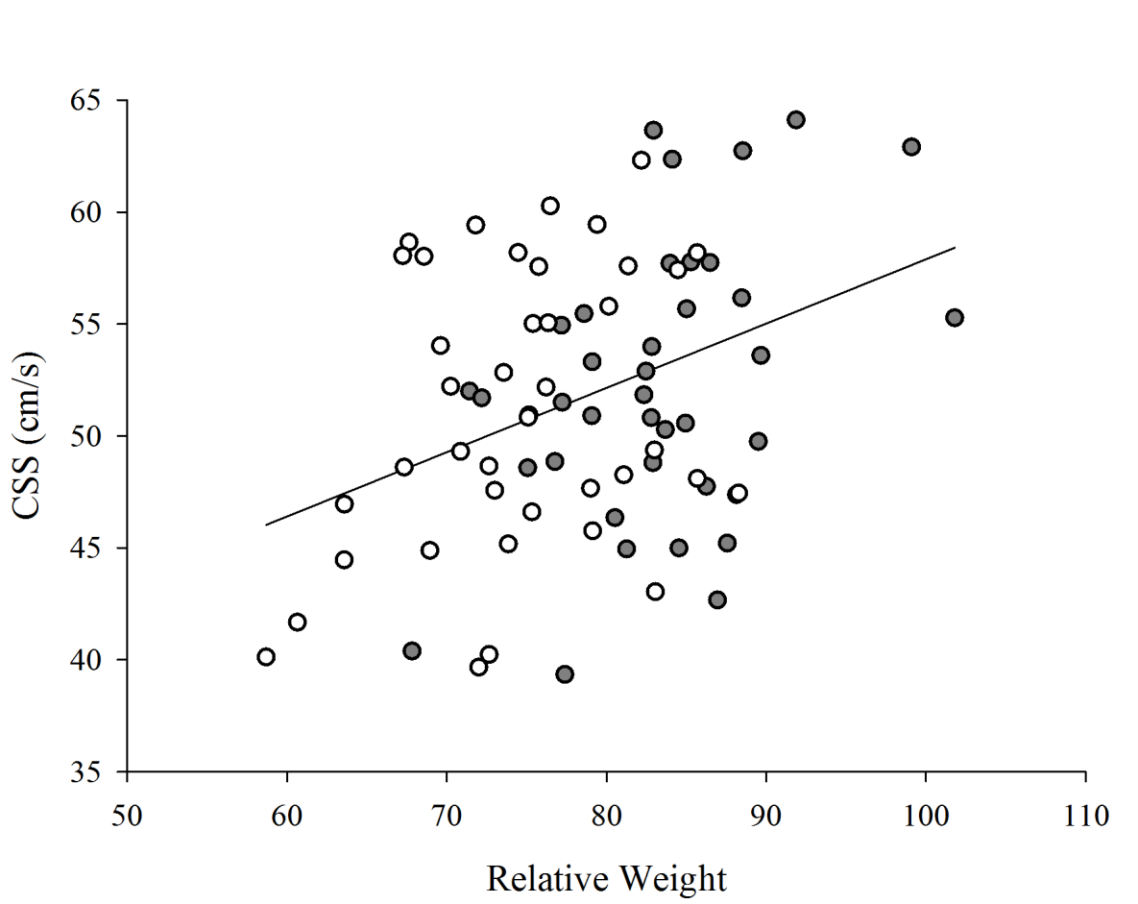

65
60
55
50
45
40
35
CSS (cm/s)
50
60
70
80
90
100
110
Relative Weight

Figure 4.

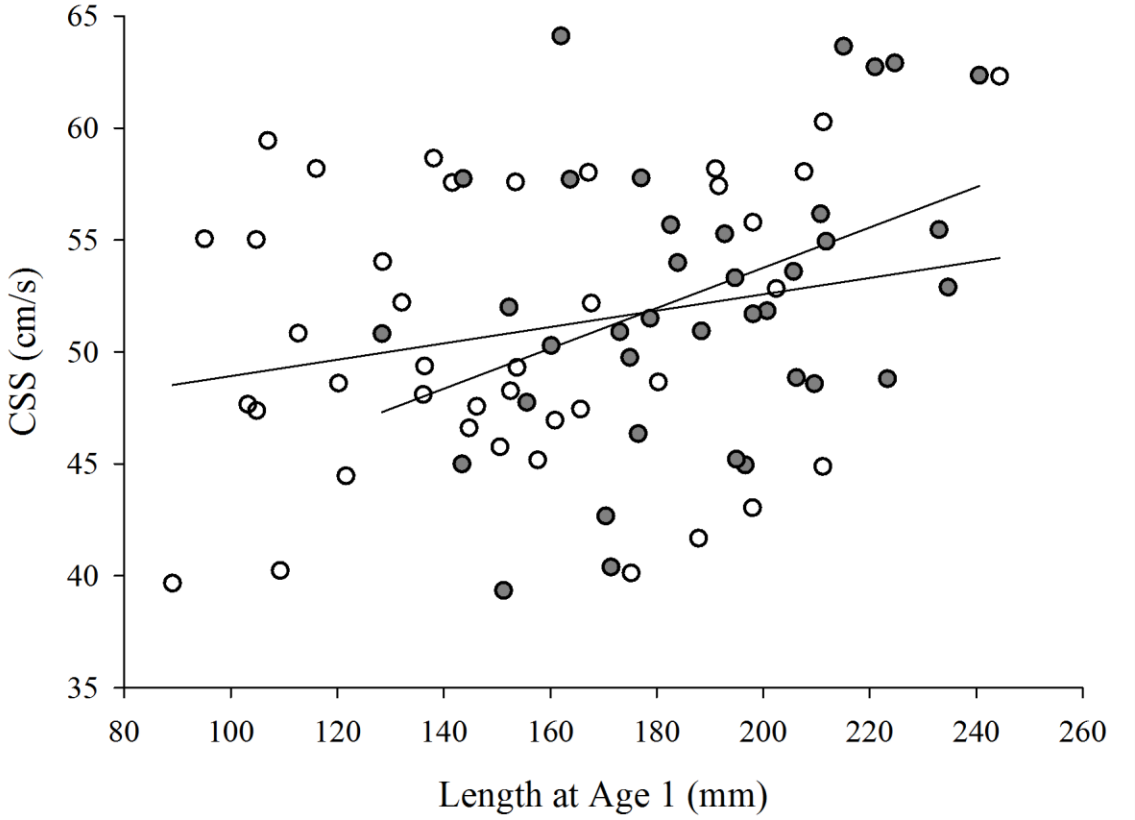

65
60
55
50
45
40
35
CSS (cm/s)
80
100
120
140
160
180
200
220
240
260
Length at Age 1 (mm)

Figure 5.

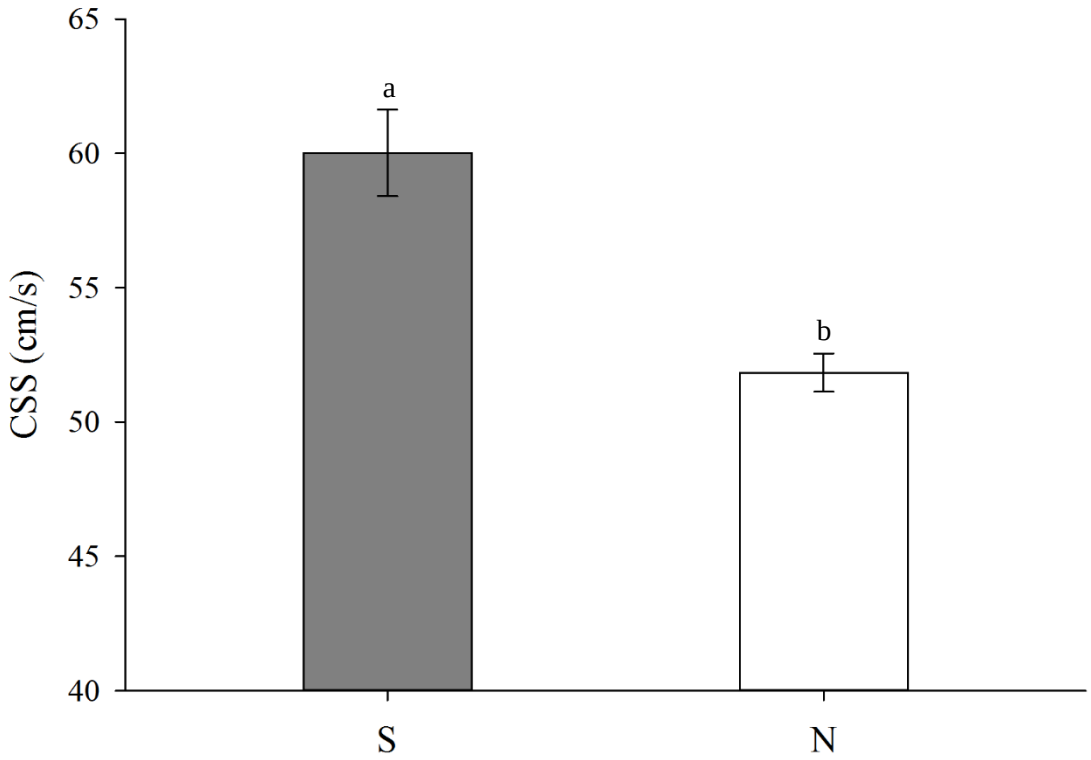
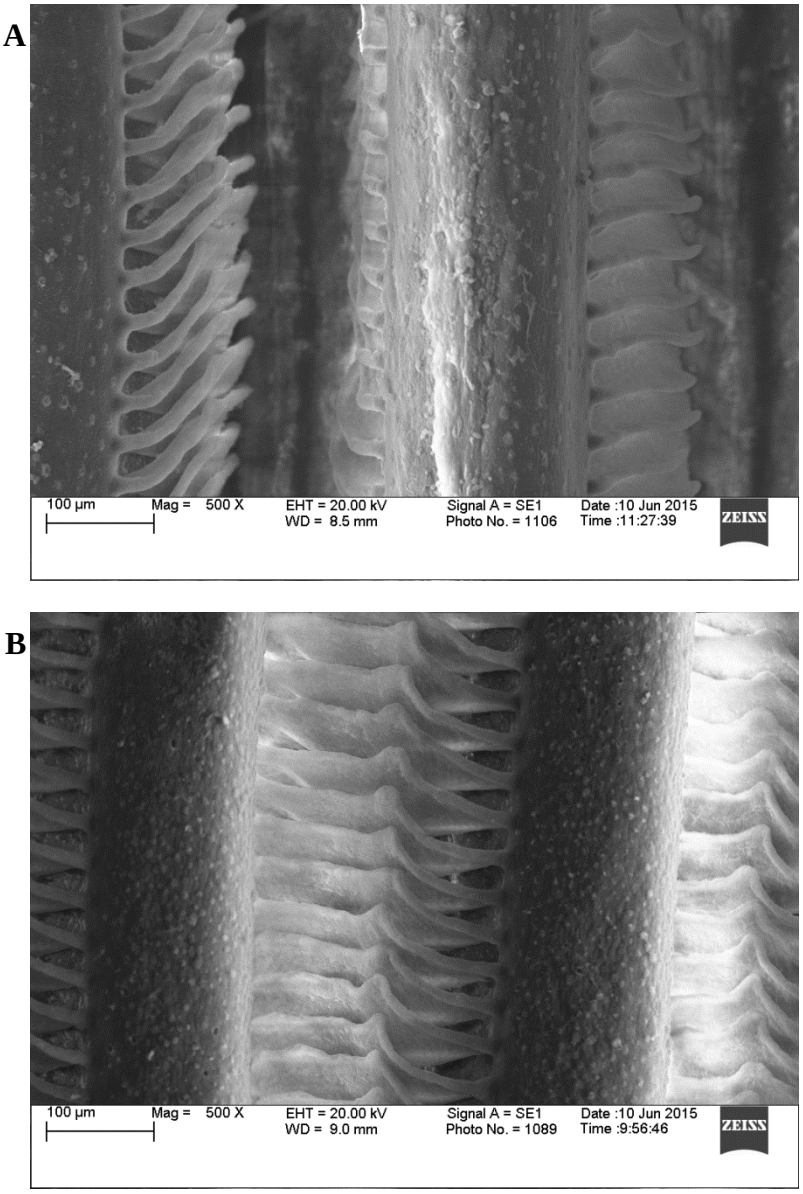


Figure 6.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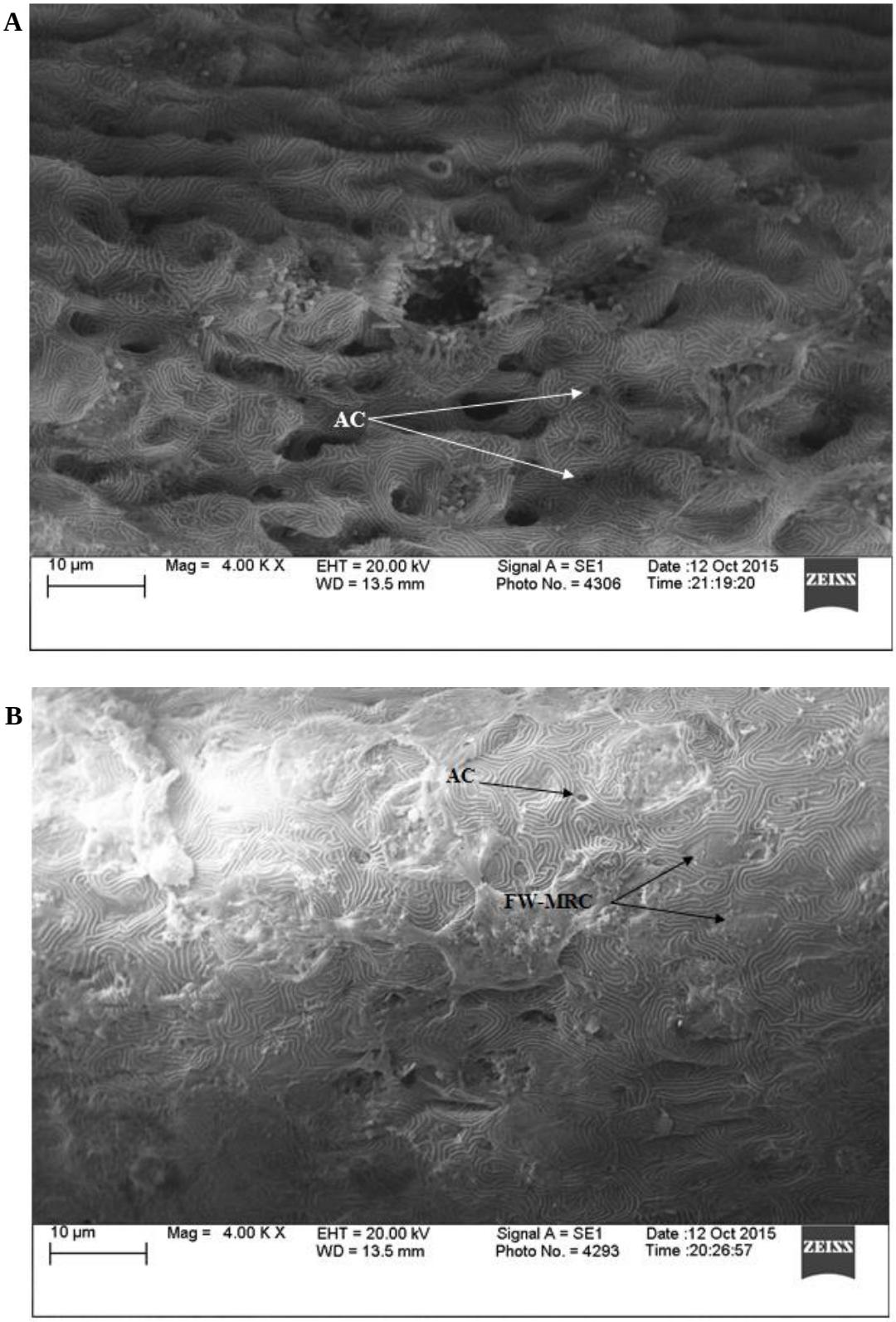
100 µm
Mag = 500 X
EHT = 20.00 kV
WD = 8.5 mm
Signal A = SE1
Photo No. = 1106
Date :10 Jun 2015
Time :11:27:39
ZEISS
B
100 µm
Mag = 500 X
EHT = 20.00 kV
WD = 9.0 mm
Signal A = SE1
Photo No. = 1089
Date :10 Jun 2015
Time :9:56:46
ZEISS

Figure 7.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AC
10 µm
Mag = 4.00 K X
EHT = 20.00 kV
WD = 13.5 mm
Signal A = SE1
Photo No. = 4306
Date :12 Oct 2015
Time :21:19:20
ZEISS
B
AC
FW-MRC
10 µm
Mag = 4.00 K X
EHT = 20.00 kV
WD = 13.5 mm
Signal A = SE1
Photo No. = 4293
Date :12 Oct 2015
Time :20:26:57
ZEISS

Figure 8.

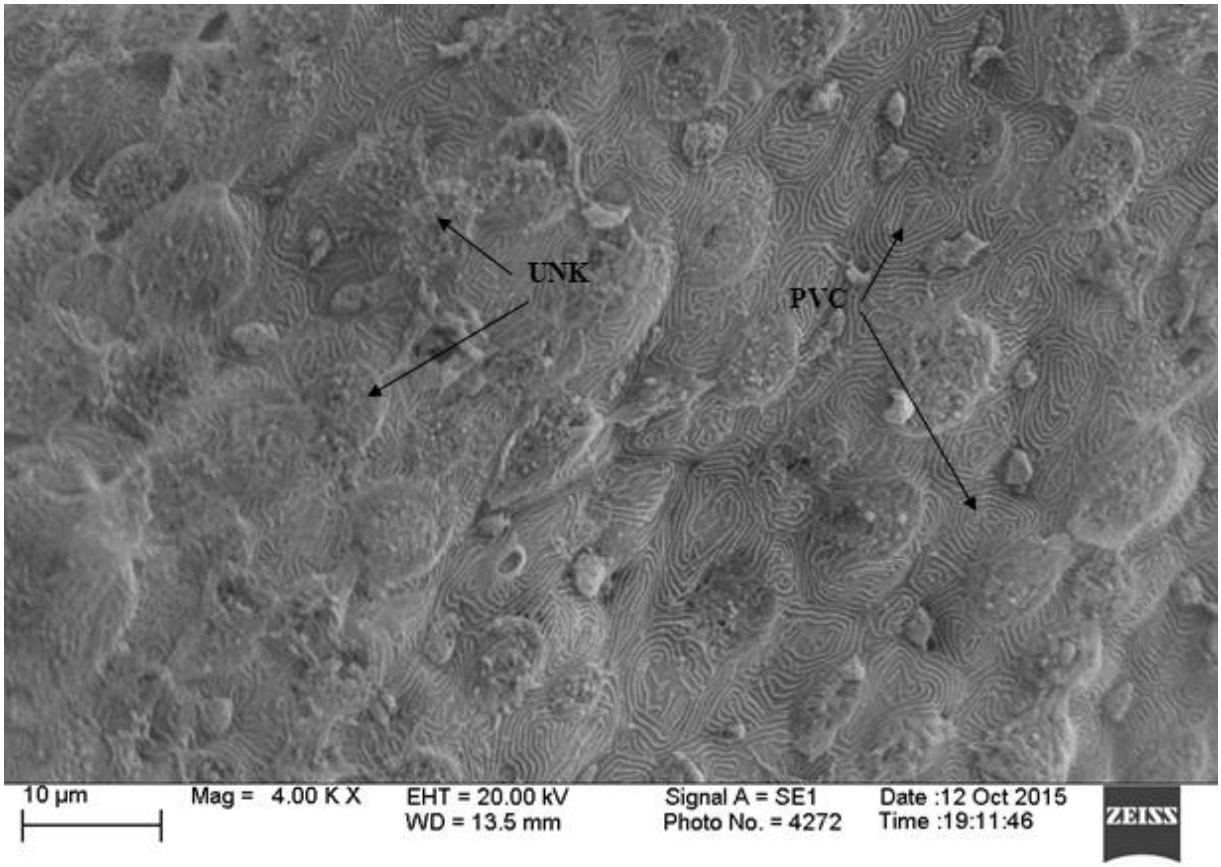
UNK
PVC
10 µm
Mag = 4.00 K X
EHT = 20.00 kV
WD = 13.5 mm
Signal A = SE1
Photo No. = 4272
Date :12 Oct 2015
Time :19:11:46
ZEISS

Figure 9.