A MESOCOSM STUDY OF THE IMPACT OF INVASIVE ARMORED CATFISH (*PTERYGOPLICHTHYS SP.*) ON ENDANGERED TEXAS WILD RICE (*ZIZANIA TEXANA*) IN THE SAN MARCOS RIVER

by

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ABSTRACT

A MESOCOSM STUDY OF THE IMPACT OF INVASIVE ARMORED CATFISH (*PTERYGOPLICHTHYS SP.*) ON ENDANGERED TEXAS WILD RICE (*ZIZANIA TEXANA*) IN THE SAN MARCOS RIVER

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Suckermouth armored catfish (Family Loricariidae) are native to rivers and streams in South America, but have invaded habitats throughout the world due to aquarium releases. The San Marcos River has an abundant population of suckermouth armored catfish (*Pterygoplichthys sp.*) that have several negative impacts on the areas they invade including nutrient alteration, increased erosion due to burrows, and threats to endemic species. Texas wild rice (*Zizania texana*) is an endemic and endangered species in the San Marcos River. When it was first discovered in 1892, it was highly abundant, but has since decreased due to anthropogenic disturbances associated with urbanization. Suckermouth armored catfish may be contributing to the decrease in Texas wild rice populations. A mesocosm experiment was conducted in raceways at Texas State University in San Marcos to determine the effects of suckermouth armored catfish on

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Texas wild rice. The influence of suckermouth armored catfish on Texas wild rice growth and biomass was examined using a 3x2 factorial design (suckermouth catfish high density, low density, and absent and wild rice present and absent) using five samples per combination of factors. The growth of the Texas wild rice leaves increased significantly as the number of catfish in the treatment increased. The nutrient concentrations in the Texas wild rice were significantly highest in treatments with one catfish, followed by cells with two catfish, and then cells lacking catfish. Texas wild rice was found in small amounts in the stomachs of two catfish, and the most abundant gut content category found in catfish stomachs was algae. Overall, the catfish seemed to have a positive impact on the growth of the Texas wild rice, likely due to them consuming algae on and around the plants. Although the catfish had a positive impact on the Texas wild rice in this experiment, there are several other factors, such as burrowing causing erosion and uprooting of plants, which could have negative impacts on the Texas wild rice in the San Marcos River.

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INTRODUCTION

Invasive Species

Invasive species are a growing problem in freshwater ecosystems due to an increase in introductions of new species (Capps et al. 2012). Invasive species are nonnative species whose introduction causes economic or environmental harm. There are many ways that invasive species can be introduced to a new environment including intentional methods such as stocking and unintentional methods such as discharge of ballast water. One way a species can be introduced into new environments is through aquarium releases. Negligent owners buy young fish that outgrow their aquarium and release them into local waterways (Padilla and Williams 2004). When multiple fish of the same species are released into the same waterway, they can reproduce and form a viable population. Suckermouth armored catfish (Loricariidae) are a popular assemblage of aquarium fish species commonly called 'plecos'. Suckermouth armored catfish have been introduced to several habitats, most likely through aquarium release (Wakida-Kusunoki et al. 2007, Cook-Hildreth 2008, Pound et al. 2011). Successful non-native populations now exist in North America, Central America, The Caribbean, and Asia (Bunkley-Williams et al. 1994, Capps et al. 2011, Sumanasinghe and Amarasinghe 2013). Within Texas several species of Loricariids have established themselves, included *Hypostomus* plecostomus, Pterygoplichthys anisitsi, P. disjunctivus, and P. multiradiatus (Hoover et al. 2004).

Characteristics of the species introduced and the habitat invaded can determine how likely a species will be at establishing a population within an area. Herbivorous and detritivorous species, such as suckermouth armored catfish, can easily invade an area since they feed at the bottom of the food web, which contains food items that are usually abundant (Gido and Franssen 2007). Species that are highly tolerable to variable environments are also more likely to be successful invaders (Nico et al. 2012). Freshwater systems are vulnerable to invasion due to the numerous ways a species can be introduced (Sala et al. 2000). Urban habitats that have been highly modified by anthropogenic activity are also easier to invade than more natural areas (Dudgeon 2006). This is due to increase in impervious material found within urban stream watersheds causing more runoff of nutrients, flashy hydrology, elevated base flow, and warmer temperatures (Paul and Meyer 2001). These biological and habitat attributes have facilitated the invasion and establishment of abundant suckermouth armored catfish populations in several freshwater systems outside their native range (Capps et al. 2011).

Once an invasive species establishes a population in a new environment, it can greatly impact the ecosystem processes and community dynamics. Invasive species can threaten locally endemic or endangered species. There is evidence that suckermouth armored catfish in Texas could be threatening the vulnerable species *Dionda diaboli* by competing with them for food (Lopez-Fernandez and Winemiller 2005). Invasive species can also alter ecosystems through top-down (grazing) and bottom-up (nutrient remineralization) mechanisms (Capps et al. 2015). Invasive species that store and process nutrients in stoichiometrically unique ratios can cause changes in the nutrient dynamics of a habitat, especially when they occur in high densities (Capps and Flecker 2013b).

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Suckermouth armored catfish store large amounts of phosphorous (P), therefore they can serve as P sinks when they are in high densities (Capps and Flecker 2013b). The aggregating behavior of suckermouth armored catfish can also create biogeochemical hotspots, areas of high nutrient remineralization rates (McClain et al. 2003, McIntyre et al. 2008, Capps and Flecker 2013b). It is necessary to fully describe the life history of an invasive species, in order to understand the extent of the potential interactions with other biota and the impact on the habitat they have invaded.

Suckermouth Armored Catfish

Fishes in the family Loricariidae, commonly known as suckermouth armored catfish, consists of over 700 species native to tropical rivers in Central and South America (Nelson 2006). They originated in the Amazon/Orinoco region and have subsequently dispersed throughout tropical South America, east of the Andes Mountains. Species invasions of new areas around the Amazon created new lineages (Silva et al. 2016). Suckermouth armored catfish can reach ages of 7-8 years in their native range (Antoniutti et al. 1985, Goulart and Verani 1992). They are characterized by an inferior mouth with lips, and a body covered in bony plates (Nelson 2006) (Figure 1) and can reach sizes up to 70 cm in length (Fuller et al. 1999). The common habitat of suckermouth armored catfish in their native range consists of areas of slow moving water including the tidal portions of rivers (Weber et al. 2012). They are found inhabiting areas with a variety of substrates, ranging from mud and detritus to stone and cobble (Burgess 1989, Weber et al. 2012). Armored catfish possess a modified vascularized stomach that functions as an accessory lung that allows them to breathe air (da Cruz et al. 2013).

Therefore, armored catfish can tolerate temporary exposure to air and survive in rivers experiencing hypoxia and anoxia. Reproductive strategies of suckermouth armored catfish include multiple-spawning, nest construction, and parental care (Tello et al. 1992). Suckermouth armored catfish exhibit an equilibrium reproductive strategy, defined by low fecundity, high survivorship due to parental care, and an even sex ratio (Gomes et al. 2015). Suckermouth armored catfish are algivores and use their sucker mouth and teeth to adhere and scrape algae and detritus from substrates (Delriva and Agostinho 2001, Lujan et al. 2012). The diet of suckermouth armored catfish make them popular aquarium fish because they are known to clean algae from surfaces in the tank; this has facilitated their introduction into new environments through aquarium release (Wakida-Kusunoki et al. 2007).



Figure 1. Photo of *Pterygoplichthys sp.* used in stream channel experiment showing body morphology.

The Sailfin Armored Catfish, *Pterygoplichthys sp.*, and the Suckermouth Catfish, *Hypostomus plecostomus* are both members of the Loricariidae family that have become established in the San Marcos River (Datri et al. 2015, Page et al. 2013, Scott et al. 2012). Both of these suckermouth armored catfish have also established non-native populations in other portions of Texas, Nevada, Florida, and possibly other states (Fuller et al. 1999, Gibbs et al 2008, Nico 2010, Pound et al 2011). The *Hypostomus* genus and *Pterygoplichthys* genus can be differentiated by the number of dorsal fin rays; *Hypostomus* have less than 9 dorsal fin rays while *Pterygoplichthys* have 10-14 (Burgess 1989). Both *Hypostomus plecostomus* and *Pterygoplichthys sp.* essentially occupy the same trophic level and have similar nitrogen and carbon content in their diets (Lujan et al. 2012). They both also construct burrows in the banks and have similar reproductive strategies (Burgess 1989).

Suckermouth catfish (*Hypostomus sp.*) were first found in Texas in the headwaters of the San Antonio River after individuals escaped the San Antonio Zoo in 1962 (Barron 1964). Established populations in the San Marcos River were reported as early as 1990 (Pound et al. 2011). Sailfin catfish (*Pterygoplichthys sp.*) were first confirmed in Texas waters in the 1970s, but earlier introduction could have gone unnoticed due to similar morphology to *Hypostomus sp.* (Edwards 2001). Nevada has had a population of *Hypostomus* in the thermal waters of Indian Spring since 1966 (Courtenay and Deacon 1982). Several water bodies in Florida have been invaded by *Hypostomus sp.* since 1958 (Burgess 1958, Rivas 1965, Courtenay et al. 1974). In Colorado, *Hypostomus sp.* were found in the geothermal waters of the Upper Rio Grande drainage (Zuckerman and Behnke 1986). A large population of *Pterygoplichthys* *multiradiatus* has become established in Wahiawa Reservoir, Oahu, Hawaii since 1986 (Devick 1991). Diet, age, and population information collected from these populations of suckermouth armored catfish in other invaded habitats may provide useful information needed to understand the effects of populations of suckermouth armored catfish on the San Marcos River ecosystem.

Pterygoplichthys multiradiatus in Florida can reach maturity at about 160 mm total length (Gestring et al. 2010). *Hypostomus plecostomus* fecundity is typically 500-700 eggs and they are considered multiple batch spawners since they typically possess several sizes of oocytes (Cook-Hildreth 2008, Gibbs et al. 2008). Similar to other suckermouth armored catfish, members of the *Pterygoplichthys* genus have a high tolerance for hypoxia and can even survive being out of the water for up to 20 hours, due to their large vascular stomach that also functions as an accessory respiratory organ (Armbruster 1998). Furthermore, the ability of members of the *Pterygoplichthys* genus to survive in salinities up to 10 ppt enhances the ability of this group to invade a wide range of habitats (Capps et al. 2011). A previous study of the gut content of *Hypostomus plecostomus* in the San Marcos River, Texas found that this species diet was dominated by detritus, filamentous red algae, and picoplankton (Pound et al. 2011). The combination of these life history traits of suckermouth armored catfish as a group has facilitated the observed negative impacts on the native species in the areas they have invaded.

The effects of suckermouth armored catfish on invaded habitats range from competition with other fishes to altering nutrient dynamics in the ecosystem. In Florida, sailfin catfish have been seen attached to the backs of manatees, eating the algae from their backs (Gibbs 2010, Nico 2010). By eating the algae off the manatees, the

suckermouth armored catfish could be making the manatees more susceptible to sun burn. Manatees have also been seen trying to rub the suckermouth armored catfish off their backs, indicating that they irritate the host animal (Gibbs 2010). When suckermouth armored catfish invade a new habitat, they can decrease the amount of algae in the ecosystem and may also ingest invertebrates and eggs attached to algae (Hoover et al. 2014). The endangered Fountain darter (*Etheostoma fonticola*) lays its eggs in algae in the San Marcos River; suckermouth armored catfish in the area may threaten Fountain darters by decreasing the spawning habitat and possibly ingesting eggs (Cook-Hildreth 2008). Suckermouth armored catfish may also directly compete for algae with the threatened *Dionda diaboli* in San Felipe Creek (Lopez-Fernandez and Winemiller 2005). Burrows created by suckermouth armored catfish can also impact the riparian environment by increasing erosion and turbidity (Lienart et al. 2013, van den Ende 2014). Large populations of suckermouth armored catfish can effectively sequester phosphorous due to their bony plated body, which can lead to a phosphorous limited system and alter nutrient cycling in invaded environments (Capps and Flecker 2013a). All of these impacts can cause long term alterations in invaded ecosystems including declines in habitat quality, alteration of water quality, a reduction in native species, and subsequent declines in biodiversity and the health of the ecosystem.

Texas Wild Rice

Texas wild rice, *Zizania texana* is a submersed macrophyte that is endemic to the upper 2.4 km of the San Marcos River (Terrell et al. 1978). It grows in areas with high to moderate current velocities, depths of less than 1 m deep, and in coarse, sandy substrate

with relatively low organic matter content (Poole and Bowles 1999) (Figure 2). When Texas wild rice was discovered in 1892, it was abundant in the San Marcos River, Spring Lake, and congruent irrigation ditches (Silveus 1933). The abundance of Texas wild rice quickly declined and by 1967 there was only one plant in Spring Lake, none in the upper 0.8 km of the San Marcos River, and only scattered plants in the next 2.4 km (Emery 1967). Texas wild rice was placed on the federal endangered species list in 1978 due to only having one small population, and because of significant threats from habitat alteration and urbanization of San Marcos (Emery 1977). Texas wild rice now grows in small fragmented clumps and reproduces asexually by tillers (Poole 2002). Sexual reproduction is limited because pollen rarely travels over 1.5m from its source, and it is rare for the next clump of Texas wild rice to be within that distance (Oxley et al. 2008). A reintroduction program was initiated in 1999 in the San Marcos River to plant Texas wild rice that was grown at the San Marcos National Fish Hatchery and Technology Center (Poole and Bowles 1999).



Figure 2. Underwater photo of Texas wild rice in shallow, clear water with gravel substrate in the San Marcos River. Photo taken October 15, 2015.

There are several documented threats that could cause Texas wild rice to become extinct. In the past, practices such as mowing aquatic vegetation, dredging, and harvesting exotic plants caused a decline in Texas wild rice populations (Emery 1977). Once the population and size of the City of San Marcos began to grow, more threats to Texas wild rice appeared such as increased urban runoff, sewage collection line leaks, competition by introduced plant species, and increased recreational use of the river. One factor that was considered most responsible for the decline of Texas wild rice was the impoundment of the river (Vaughan 1986). The building of several dams caused the water depth to increase, which reduced light levels at bottom depth that ultimately prevents Texas wild rice from receiving sufficient light to survive and produce seeds (Vaughan 1986). With Texas wild rice being such a vulnerable species, any additional threat could cause it to become extinct. Suckermouth armored catfish could be reducing the likelihood of recovery of Texas wild rice in the San Marcos River. Some effects that suckermouth armored catfish could have on Texas wild rice include increasing water turbidity, digging up roots while foraging or burrowing, directly consuming plant tissue, and decreasing necessary nutrients for plant growth.

Invasibility of the San Marcos River

The San Marcos River possesses multiple traits that increases its vulnerability to invasion by suckermouth armored catfish. The thermal regime is favorable for suckermouth armored catfish because the San Marcos River is spring fed from Edwards Aquifer and has a constant water temperature of about 23 degrees Celsius year round (Groeger et al. 1997). The San Marcos River also provides conditions favorable to the growth of primary producers. Clear shallow water and an increase in urban development and nutrient loading in to the San Marcos River supports abundant growth of attached algae and macrophytes (Groeger et al. 1997, Datri et al. 2015). The favorable environmental conditions of the San Marcos River have made it easier for suckermouth armored catfish to quickly establish abundant populations and potentially cause problems for endangered and endemic species.

Objectives and Hypothesis

The objectives of this study are to (1) estimate the potential effects that suckermouth armored catfish presence and density have on Texas wild rice using a controlled mesocosm stream channel experiment and (2) to determine the stomach contents and growth of suckermouth armored catfish kept in mesocosm cells with Texas

wild rice present and absent and (3) to determine ecosystem effects (periphyton biomass and organic matter decomposition) of Texas wild rice, suckermouth armored catfish density, and their interaction. The null hypothesis is that there will be no significant difference in the suckermouth armored catfish, Texas wild rice, or the ecosystem effects among mesocosm stream channel cells with and without suckermouth armored catfish and Texas wild rice. The alternative hypothesis is that there will be a significant difference in the suckermouth armored catfish, Texas wild rice, or the ecosystem effects among mesocosm stream channel cells with and without suckermouth armored catfish and Texas wild rice. The alternative hypothesis is that there will be a significant difference in the suckermouth armored catfish, Texas wild rice, or the ecosystem effects among mesocosm stream channel cells with and without suckermouth armored catfish and Texas wild rice. The effect of suckermouth armored catfish on Texas Wild Rice within this mesocosm will help resource managers predict and understand the impact of this invasive species on this endemic plant within the San Marcos River. Information gathered from this study can be used to better protect Texas wild rice throughout its range.

MATERIALS AND METHODS

Study Site

The San Marcos River originates at Spring Lake which is fed by about 200 ground water springs that are part of the Edwards Aquifer system. The river flows over two dams at the outlet of the lake into the San Marcos River where it continues for another 7 km before its confluence with the Blanco River (Figure 3). The upper portion of the river is characterized by clear water, an abundance of macrophytes, constant temperatures around 23°C, and a cobble/sand bottom. As the river flows downstream towards its confluences with the Blanco River, it becomes more turbid, and has a more variable temperature. Several endangered and endemic species live in the San Marcos River; including: Texas wild rice *Zizania texana*, Fountain darter *Etheostoma fonticola*, the San Marcos salamander *Eurycea nana*, and the Comal Springs riffle beetle *Heterelmis comalensis* (Groeger et al. 1997).



Figure 3. Map of the San Marcos River from its headwaters at Spring Lake, to its confluence with the Blanco River (Poole and Bowles 1999).

Stream Channel Experiment

The effects of armored catfish (*Pterygoplichthys sp.*) on Texas wild rice (*Zizania texana*) was investigated in a replicated stream channel experiment, consisting of a 3 X 2 factorial design in which presence and absence of rice was cross-classified with three

levels of catfish density. The design consisted of the following treatments; catfish absent and rice absent, catfish low density (one fish per cell) and rice absent, catfish low density and rice present, catfish absent and rice present, catfish high density (two fish per cell) and rice absent, catfish high density and rice present. A diagram showing the placement of each treatment is shown in Table 1. The high density stocking level of catfish was comparable to the densities in the upper San Marcos River while the low density treatment was comparable to densities in the lower San Marcos River (Scott et al. 2012). Cells with rice present consisted of three pots of mature Texas wild rice that were grown in open raceways at Texas State University in San Marcos. Each pot of Texas wild rice had all leaves cut to 50cm and were placed in stream channels one week before the start of the experiment (Figure 4). There were five replicates of each treatment. Cells were systematically organized so that water from cells with catfish would not run down the stream channel into cells without catfish in the most possible cases. Texas wild rice was also placed in cells containing higher sunlight, as explained later.

Table 1. Treatment set up for the ten raceways located in a covered outdoor facility at Texas State University in San Marcos, TX. C represents catfish (*Pterygoplichthys sp.*), R represents Texas wild rice (*Zizania texana*). - represents absence, + represents presence, ++ represents higher density (two fish per cell). Each column represents one stream channel. Each cell represents a treatment cell within the stream channel. The spigots are located at the top of each column and water flows downward.

#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
C+	C+	C+	C+	C-	C-	C-	C++	C++	C++
R+	R-	R-	R-	R-	R-	R-	R-	R-	R-
C+	C+	C+	C+	C-	C-	C-	C++	C++	C++
R+	R+	R-	R-	R+	R-	R-	R+	R-	R-
C+	C+	C-	C++	C-	C-	C-	C++	C++	C++
R+	R+	R+	R+	R+	R+	R+	R+	R+	R+



Figure 4. Photo of Texas wild rice plants with leaves cut to 50cm. The plants are about to be planted in pots to be placed in the stream channel cell.

Thirty total stream channel cells were created by modifying ten existing concrete raceways located in a covered outdoor facility at Texas State University (Figure 5). Dividers were created from wood and 1-cm plastic mesh to section each raceway into thirds (Figure 6). Each of the ten original raceways has a single valve that discharges water, and in order to ensure equal flow from the single valve to each of the three stream channel cells, a sump pump (Simer 2305-04 Geyser II ¼ HP Submersible Utility Pump) in a 5-gallon plastic bucket was placed in the head water of each raceway and PVC pipes were connected with ball valves opening into each cell. This ensured an equal flow over the Texas Wild Rice in each cell (Figure 6). The water pumped into the stream channel comes from an outdoor artesian well from the Edwards Aquifer, which is the same water

that is discharged into the San Marcos River. Each cell measured 1.22 X 0.8 X 0.6 m (L X W X D). A 0.61 m Fluorowing Reflector with an Agrobrite 125 Watt 6400K compact fluorescent (CFL) grow light was placed perpendicular to the water flow about 30 cm above each stream channel treatment cell (Figure 6). Lights were kept on a 14/10 h light/dark cycle, with mean light intensity immediately under the water surface at about 150umol/m²/s. This light intensity is within the range of intensities that has been observed in closed canopy sections of the San Marcos River (Scott et al. 2012).



Figure 5. Diagram of raceway set up at Texas State University in San Marcos with length, width, and depth measurements.



Figure 6. Photo of stream channel set up at raceways at Texas State University in San Marcos. The head of each stream channel has a sump pump that leads into PVC pipe and then out a ball valve in each cell. The dividers are set up to divide each channel into three different cells of equal dimensions. The lights are hung 30 cm over each stream channel to ensure equal light for each cell.

Once the stream channels were set up, Texas wild rice was planted in the designated stream channel cells, and then after one week of equilibration, the catfish were added. To ensure similar size fish were stocked in cells we purchased Sailfin armored catfish (*Pterygoplichthys sp.*) from Texas Tropical and Marine pet store located in San Antonio and then transported them to the stream channels in plastic bags filled with water and air. We selected this species because they are found in the San Marcos River and they have a similar diet to *Hypostomus plecostomus* that were used in previous studies. We purchased these fish because we were unable to capture enough suckermouth armored catfish of similar size from the San Marcos River due to time constraints and limits in which gear types could be used. The average size of catfish obtained were about 150 mm in total length, which is the average size of a mature adult suckermouth armored catfish (Grier 1980). The catfish in the bags were placed in water from the stream channels to allow them to acclimate to the temperature. The catfish were then removed from the bags and poured into a container with stream channel water. The total wet weight, standard length, and total length of each catfish was recorded before being placed in the designated stream channel cell.

To assess the response of the periphyton and decomposer community to Texas wild rice and catfish, the periphyton biomass (chlorophyll-*a* concentration) and organic matter decomposition were measured on days 14 and 28. To estimate periphyton biomass, four ceramic tiles (14.5 X 14.5 cm) were placed in each stream channel cell. Two tiles were covered by a 1 cm plastic mesh cage and therefore made inaccessible to catfish so indirect effects of the catfish on periphyton could be measured. Two tiles were placed in the open and accessible to catfish. The tiles were placed in the channel cells one

week prior to the start of the experiment. One caged and one open tile was removed during day 14 and 28.

After being pulled from the stream channel each tile was cleaned with a razor blade and a soft bristled brush and rinsed into an acid-washed HDPE bottle with DI water. A modified version of the EPA Method 445.0 was used to determine the Chlorophyll-a (Chl*a*) content of materials on each tile (Arar and Collins 1997). Part of the slurry collected from each tile was filtered onto a Whatman GF/F filter and then frozen at -80° C. Chl*a* was extracted by first grinding the filter with a RW 16 IKA mixer using a 99% HPLC grade acetone mixture and then storing in a refrigerator for 24 hrs in the dark. The samples were then centrifuged for 30 minutes and measured using a Trilogy Laboratory Fluorometer.

Organic matter decomposition was quantified by measuring leaf litter mass loss as described in Scott et al. (2012). Four pre-weighed dry leaf packs of 10 leaves of Texas Oak (*Querqus texana*), a common riparian tree species found within the San Marcos River watershed, were placed in each stream channel. Two leaf packs were enclosed in 1cm plastic mesh cages (15 X 15 cm), and two left open. Leaves were enclosed in cages to reduce the direct access of catfish to leaves, but still allow macroinvertebrates and microbes to access them. Leaves were tied together with monofilament fishing line around the petioles and weighted with metal washers to ensure submersion. One "caged" and one "open" leaf pack were removed on day 14 and 28. Leaf packs were then washed with DI water to remove organic matter and organisms and dried to a constant weight at 60° C for 48 h. Leaf litter dry mass (g) was used to calculate decomposition rate as percent change in mass over the period each pack was in the channel.

A data sonde unit (YSI 600XLM) was used to collect the following water quality variables: temperature, specific conductance, pH, and dissolved oxygen. The sonde measurements were take on days 0, 14, and 28 in each stream channel cell. A LICOR underwater quantum light sensor was used to collect PAR readings under the lights in each cell on days 0, 14, and 28. The relative chlorophyll-*a* content (Chl*a*) of leaf blades in each Texas wild rice plant was measured on days 0, 14, and 28 using a handheld SPAD meter. The length of Texas wild rice leaves was also measured on days 0, 14, and 28 to estimate somatic growth rates.

After the conclusion of the experiment, the catfish were caught using dip nets and the wet weight, standard length (SL), and total length (TL) were recorded. The catfish were then euthanized using MS-222, preserved in 70% ethanol, and transported to the lab for gut content analysis. The gut (stomach and first intestinal loop) of each fish were removed and placed under a microscope. Each gut was cut lengthwise, and the contents were removed and examined under a dissecting scope. The contents were grouped into four categories: algae, sand, detritus, and Texas wild rice. Contents grouped into each category were then placed on a Sedgwick-Rafter Counting Cell and observed under a microscope. The total area of each category was then calculated using the Olympus cellSens visual image analysis software program by drawing a polygon around each item and calculating the total area.

After the conclusion of the experiment, the Texas Wild Rice was removed from the stream channel cells, removed from their pots, and cut along the soil line to create separate above ground and below ground sections of each plant. The above ground and below ground biomass for each plant was weighed. The concentration of total nitrogen

(N) and phosphorous (P) in the Texas wild rice above ground biomass was determined by sending samples to the Texas A&M Soil, Water, and Forage Testing Laboratory.

Data Analysis

All data was analyzed using the Minitab software package. Prior to data analysis the data was tested for normality. If the data was found to fit the normal distribution, parametric tests were used. To assess the effects of suckermouth armored catfish, Texas wild rice, and their interaction on water chemistry, organic matter decomposition, and periphyton a two-way repeated measures ANOVA was performed. To assess the effects of presence, absence, and high density of catfish on Texas Wild Rice above ground and below ground biomass, and nutrient content, a one-way ANOVA was performed. Texas wild rice leaf length and relative Chla value were analyzed across time intervals using an analysis of covariance (ANCOVA) with sampling date serving as the covariate. A twoway repeated measures ANOVA was performed to assess the effects of catfish stocking rates, the presence of Texas Wild rice, and their interaction on catfish weight, catfish length, and catfish gut content. Although the experiment initially had five replicates for each treatment, one stream channel cell had to be removed due to the death of one catfish in cell 12 (C++ R+). If significant differences were found in the mean level of any variable between levels of a categorical variable, a Tukey's multiple comparison test was subsequently run to determine which treatments were significantly different.

If the distribution of data was found to be not normal, a non-parametric Kruskal-Wallis test was run in the place of the ANOVA to test for differences in the median level of a variable between levels of categorical variables. If significant differences in the

median level of a variable were detected between categories, Dunn's multiple comparison test was subsequently utilized to test for individual pair wise differences. Statistical significance for all analyses was inferred as $P \le 0.05$.

RESULTS

Prior to the start of the experiment, the San Marcos River flooded and inundated the experimental stream channel area located at Texas State University in San Marcos. This flood delayed the start of the experiment due to clean up efforts and destruction of some of the experimental components. The flood occurred before the placement of suckermouth armored catfish and Texas wild rice, however, the tiles for periphyton analysis and Texas Oak leaves were affected. The tiles were able to be cleaned of organic matter and debris and replaced before the start of the experiment. However, the Texas Oak leaves that were outside of the cages were unable to be replaced and the leaves inside of the cages likely had damage or higher accumulated organic matter due to the flood. Therefore this portion of the experiment was terminated. Any affected equipment such as lights, electrical cords, and pumps were replaced if necessary.

Water Chemistry

Some water quality variables (specific conductance, pH, and dissolved oxygen) were found to be significantly different among treatments. The actual variation of these variables among the different treatments was minimal. Cells 13, 14, and 15 had different water chemistry measurements than the other cells due to the spigot at the top of that stream channel being turned off, not allowing any new water to be cycled through the raceway initially. The spigot was turned back on when it was noticed on day 0, but the difference in water chemistry persisted throughout the experiment. However, as time

progressed, the water quality in this stream channel became more similar to all the other stream channels. Based on the tolerance of the species tested, I believe the statistical differences in water quality among stream channels were not great enough to cause a biological effect (i.e. biologically insignificant). Table 2 lists the water chemistry variables measured along with their range and p-values for catfish, rice, and the interaction of catfish and rice.

Table 2. Summary of repeated measures two-way ANOVA results for water quality variables averaged over all sample days. The significant difference, range of values, and p-value for the influence of catfish stocking levels, rice presence, and their interaction are listed for each water quality variable.

Water Quality	Significantly		Catfish	Rice	Interaction
Variable	different?	Range	p-value	p-value	p-value
Temperature (°C)	No	21.0 - 22.1	0.141	0.272	0.099
Specific					
Conductance (µS)	Yes	432 - 632	0.03	0.64	0.687
рН	Yes	7.43 - 8.43	<0.001	0.341	0.414
Dissolved Oxygen					
(mg/L)	Yes	6.48 – 9.13	<0.001	0.003	0.010
PAR (µmol/s/m²)	Yes	45.98 - 407.1	0.329	<0.001	0.121

I failed to detect a significant difference in the temperature among treatment levels for catfish (p=0.141), rice (p=0.272), and their interaction (0.099). There was a significant difference (p=0.03) in the specific conductance among cells containing different levels of catfish density (Figure 7). The treatment without catfish was significantly different from the treatment with low density of catfish (Appendix B-2). There was a significant difference (p<0.001) in the pH among the different treatments of catfish density (Figure 8). The treatment without catfish was significantly different from the treatments with low density of catfish (Appendix B-3). There was a significant difference in the dissolved oxygen among treatments for catfish (p<0.001), rice (p=0.003), and their interaction (p=0.010) (Figure 9). Cells containing one catfish per cell and rice were significantly different from all other treatments (Appendix B-4).



Figure 7. Interval plot of specific conductance with 95% Bonferroni Confidence Interval (CI) for the mean in stream channels for control (C-R-), rice (*Zizania texana*) only (C-R+), catfish (*Pterygoplichthys sp.*) only (C+R-), catfish and rice present (C+R+), catfish in high density (C++R-) and catfish in high density and rice present (C++R+) treatments.



Figure 8. Interval plot of pH with 95% Bonferroni CI for the mean among all treatments.



Figure 9. Interval plot of dissolved oxygen with 95% Bonferroni CI for the mean among all treatments.
The PAR values were significantly different (p<0.001) in cells containing rice when averaged over all sampling dates. The average PAR values in treatment cells with rice were significantly higher than the cells without rice (Figure 10). When measured on day 0 in the morning during a sunny day, the PAR measurements were highly significantly different (p<0.001) among the different treatments. This pattern in PAR measurements was likely due to the structure of the building. One wall had a chain link fence and sunlight would penetrate through unfiltered during the morning and illuminate some treatment cells more than others. During cloudy days this effect was not observed. The cells were arranged in a way so that the cells that contained Texas wild rice would receive more sunlight to facilitate growth. During cloudy sample days 14 and 28, PAR readings taken in the morning of day 14 and afternoon of day 28 showed no significant differences among stream channel treatments (Day 14 p=0.293, Day 28 p=0.992).



Figure 10. Interval plot of PAR readings with 95% Bonferroni CI for the mean among all treatments.

Organic Matter Decomposition

I failed to detect a significant difference in the decomposition of caged Texas Oak leaves due to the effect of catfish (p=0.536), Texas wild rice (p=0.957), or their potential interaction (p=0.306) based on data collected up to day 14. I was unable to analyze the open Texas Oak leaves because they were destroyed in a flood that occurred right before the start of the experiment and could not replace them (Figure 11). On day 28, some leaves were lost in an accident before I was able to weigh them. Therefore I did not have sufficient leaves to conduct a statistical analysis on the remaining leaf weights or the decomposition rate over time.



Figure 11. Photo showing flood levels in the stream channel area at Texas State University in San Marcos on September 26, 2016. All stream channels were flooded about 3ft above their normal water level.

Periphyton

There was an overall significant difference between caged and open tiles (p<0.001), with open tiles having a higher average Chla content $(0.019\mu g/cm^2)$ than caged tiles $(0.013 \ \mu g/cm^2)$. There was also a significant difference in the amount of periphyton chlorophyll-a levels (Chla) in caged tiles among different stocking densities of catfish (p=0.002). Cells containing high densities of catfish were significantly different than treatments with low densities of catfish and treatments lacking catfish (Appendix B-7). The treatments with catfish in high density had the highest average Chla content at $0.020 \ \mu g/cm^2$, the treatments with low density of catfish had the middle Chla content at $0.012 \ \mu g/cm^2$, and the treatments without catfish had the lowest Chla content at $0.012 \ \mu g/cm^2$. There was also a significant difference in the amount of periphyton Chla in caged tiles among different rice treatments (p=0.045). Treatments with rice had significantly higher periphyton Chla $(0.016 \ \mu g/cm^2)$ than treatments without rice $(0.011 \ \mu g/cm^2)$ (Figure 13). I failed to detect a significant difference in the Chla in caged tiles due to the potential interaction of catfish and rice (p=0.674).

There was also a significant difference in the Chl*a* content on open tiles for various levels of catfish stocking density (p<0.001). Treatments with high density of catfish were significantly different than treatments with low density of catfish and treatments lacking catfish (Appendix B-8). The treatments with high density of catfish had the highest average Chl*a* content at 0.029 μ g/cm², the treatments with low density of catfish had the middle Chl*a* content at 0.017 μ g/cm², and the treatments lacking catfish had the lowest Chl*a* content at 0.012 μ g/cm² (Figure 14). I failed to detect a significant difference in the Chl*a* on open tiles between Texas wild rice treatment levels (p=0.439)

or the interaction of catfish and Texas wild rice (p=0.455). Observations of the stream channels cells showed that cells without catfish appeared to have more floating algae growth than cells with high density or low density of catfish. Figure 12 shows pictures taken from sample day 14 showing more algae growth in a stream channel cell without catfish compared to a stream channel cell with catfish. The floating algae may have confounded the test results due to shading of the bottom tiles, causing lesser than expected periphyton Chl*a* concentrations in cells lacking catfish.



Figure 12. Photos taken during sample day 14 showing a cell without catfish, that is covered in algae (left) and a cell that contains catfish and does not have as much algae growth (right).



Figure 13. Interval plot of periphyton Chl*a* on caged tiles with 95% Bonferroni CI for the mean among all treatments.



Figure 14. Interval plot of Periphyton Chl*a* on open tiles with 95% Bonferroni CI for the mean among all treatments.

Texas Wild Rice

There was a significant difference (p=0.019) in the above ground biomass of Texas wild rice among the different treatments (Figure 15). The treatment lacking catfish was significantly different from the treatment with catfish in high density (Appendix C-1). The Texas wild rice in the treatments lacking catfish had the lowest calculated average above ground biomass at 22.198 g. The next highest was in treatments with low density of catfish at 27.458 g, and the highest average above ground biomass was in treatment cells with high density of catfish at 35.878 g. I failed to detect a significant difference (p=0.197) in the below ground biomass of Texas wild rice among the different catfish treatments (Figure 16). The Texas wild rice treatment lacking catfish had the lowest average below ground biomass of 13.338 g; the next highest (13.946 g) was in treatments with low density of catfish, and the highest level of below ground biomass (16.720 g) was observed in treatments with the highest density of catfish. I also failed to detect a significant difference (p=0.623) in the above ground: below ground biomass ratio among fish stocking levels (Figure 17).



Figure 15. Interval plot of above ground biomass of Texas wild rice plants with 95% Bonferroni CI for the mean among different catfish treatments.



Figure 16. Interval plot of below ground biomass of Texas wild rice plants with 95% Bonferroni CI for the mean among different catfish treatments.



Figure 17. Interval plot of above ground: below ground biomass ratio of Texas wild rice plants with 95% Bonferroni CI for the mean among different catfish treatments.

There was a significant difference (p=0.028) in Texas wild rice leaf length over time among the different treatments. The treatments lacking catfish were significantly different from the treatments with high density of catfish (Appendix D-1). The average length of Texas wild rice leaves over time in treatments lacking catfish were the shortest followed by treatments with low density of catfish, and the longest were in treatments with high density of catfish. The average length of Texas wild rice leaves in treatment cells lacking catfish were 61.2cm on day 0, 85.5cm and day 14, and 103.73cm on day 28. The average length of Texas wild rice leaves in treatment cells with low density of catfish were 63.2cm on day 0, 88.23cm on day 14, and 101.61cm on day 28. The average length of Texas wild rice leaves in treatment cells with high density of catfish were 65.25cm on day 0, 94.125cm on day 14, and 113.08cm on day 28 (Figure 18).



Figure 18. Interval plot of Texas wild rice leaf lengths measured on each sample day with 95% Bonferroni CI for mean among different catfish treatments.

There was a significant difference (p=0.006) in the N content of Texas wild rice among the different catfish stocking levels. The cells lacking catfish were significantly different than cells with low density of catfish and high density of catfish (Appendix C-4). The calculated average N content of Texas wild rice was highest (2.231%) in treatments with low density of catfish, compared to treatments with high density of catfish (2.181%), and lowest (1.857%) in treatments lacking catfish (Figure 19).

I observed a highly significant difference (p<0.001) in the P content of Texas wild rice among the different catfish treatments. There was a significant difference in the P content between treatments with catfish in high density, treatments with catfish in low density, and treatments lacking catfish (Appendix C-5). The P content of Texas wild rice was highest (7.760%) in treatments with low density of catfish, followed by treatments with high density of catfish (6.457%), and lowest (5.277%) in treatments lacking catfish (Figure 20).

There was a significant difference (p=0.006) in the N:P ratio of Texas wild rice among the different catfish treatments. The treatments lacking catfish were significantly different from the treatments with low density of catfish (Appendix C-6). The highest (0.36443) reported average of N:P occurred in the treatments lacking catfish, followed by treatments with high density of catfish (0.34464), and lowest (0.29003) in treatments with low density of catfish (Figure 21).



Figure 19. Interval plot of N concentrations in Texas wild rice plants with 95% Bonferroni CI for the mean among different catfish treatments.



Figure 20. Interval plot of P concentrations in Texas wild rice plants with 95% Bonferroni CI for the mean among different catfish treatments.



Figure 21. Interval plot of N:P ratios in Texas wild rice plants with 95% Bonferroni CI for the mean among different catfish treatments.

I observed a significant difference (p=0.010) in the relative Chl*a* content of Texas wild rice leaves over time. Treatments lacking catfish were significantly different than treatments containing high density of catfish (Appendix D-2). The Texas wild rice in the treatment lacking catfish had the lowest average relative Chl*a* content over all time periods, followed by treatments with low density of catfish, and highest in treatments with high density of catfish. The average relative Chl*a* content of Texas wild rice leaves in treatment cells lacking catfish was 14.567 on day 0, 20.560 on day 14, and 18.187 on day 28. The average relative Chl*a* content of Texas wild rice leaves in treatment cells with low density of catfish was 17.253 on day 0, 21.293 on day 14, and 21.607 on day 28. The average relative Chl*a* content of Texas wild rice leaves in treatment cells with low density of catfish was 19.233 on day 0, 23.733 on day 14, and 26.767 on day 28 (Figure 22).



Figure 22. The average SPAD meter relative Chl*a* content measured on Texas wild rice leaves for each sample day for each catfish treatment. Error bars represent Bonferroni 95% CI.

Armored Catfish

There was a significant difference (p=0.004) in catfish growth in total length (Δ cm) over the 28 day experiment between the different levels of catfish density. However, I failed to detect a significant difference in the change in catfish total length associated with the presence of rice (p=0.311) or due to any interaction between different levels of catfish density and presence of rice (p=0.857). The lowest (0.03 Δ cm) average change in total length of catfish occurred in treatments with catfish in high density that lacked rice. The next lowest (0.10 Δ cm) change in length occurred in treatments with catfish in high density of catfish but lacking rice (0.28 Δ cm). The highest (0.38 Δ cm) average change in total length of

catfish occurred in treatments with catfish in low density and containing rice (Figure 23). I failed to detect a significant difference in catfish standard length among different levels of catfish stocking (p=0.928), presence of rice (p=0.248), and potential interactions of these two factors (p=0.326) (Figure 24).



Figure 23. Interval plot of change in catfish TL among different treatments with 95% Bonferroni CI for the mean.



Figure 24. Interval plot of change in catfish SL among different treatments with 95% Bonferroni CI for the mean.

There was a significant difference in catfish weight among different levels of catfish stocking (p=0.047), presence of rice (p=0.027), and potential interactions of these two (p=0.027). The lowest (-1.2 Δ g) average change in weight of catfish occurred in treatments with catfish in low density that contained rice. The next lowest (0.5 Δ g) average change in weight occurred in treatments with catfish in high density that contained rice, and in treatments with catfish in high density that lacked rice. The highest (0.6 Δ g) average change in weight of catfish occurred in treatments containing low density of catfish and lacking rice (Figure 25).



Figure 25. Interval plot of change in catfish weight (g) among different treatments with 95% Bonferroni CI for the mean.

The most abundant category of gut content found in catfish was algae. Catfish found in all treatment cells had algae in their gut, and only one catfish (treatment cell 25 C++R-) did not have any algae in its gut. The second most abundant category of gut content found in catfish was sand. Eleven out of 19 treatment cells contained catfish with sand in their gut and 15 out of the 28 catfish examined had sand in their gut. The third most abundant category of gut content found in catfish was detritus. Eight out of 19 treatment cells contained catfish with detritus in their gut, and nine out of 28 catfish had detritus in their gut. The least abundant category of gut content found in catfish was Texas wild rice. Texas wild rice was only found in small amounts in two catfish in treatments with catfish in low density and rice present. There was a significant difference

(p=0.034) in Texas wild rice abundance in stomach contents of catfish among treatments due to the Texas wild rice only being found in two catfish in treatment cells with catfish in low density and rice present. There was not a significant difference in the amount of sand, detritus, or total gut content found in catfish among the different treatments due to catfish, rice, or their interaction (Table 3). There was a significant difference (p<0.001) in the amount of algae found in catfish guts between the two stocking levels of catfish (Figure 26). There was a significantly higher amount of algae found in catfish guts in treatment cells with catfish in low density compared to cells with catfish in high density. I failed to detect a significant difference in the amount of algae found in catfish guts between the two levels of Texas wild rice (p=0.390) or due to the potential interaction of catfish density and rice presence (p=0.430).

Table 3. Summary of repeated measures two-way ANOVA results for gut content categories. The p-value for the influence of catfish stocking levels, rice presence, and their interaction are listed for each gut content category. * denotes a significant difference.

Gut Content	Catfish p-value	Rice p-value	Interaction p-value
Category:			
Algae	<0.001*	0.390	0.430
Sand	0.545	0.862	0.333
Detritus	0.121	0.076	0.184
Texas wild rice	0.034*	0.034*	0.034*
Total gut content	0.108	0.472	0.540



Figure 26. Average area (mm²) of a Sedgwick-Rafter Counting Cell covered by algae from catfish gut content in all treatments with 95% Bonferroni CI for the mean.

DISCUSSION

Water Chemistry

The specific conductance, pH, and dissolved oxygen were significantly different among treatment cells, however, the differences were not considered great enough to cause a biological effect. The differences were likely due to the characteristics of each stream channel. Cells 13, 14, and 15 located in raceway number five had more variable water chemistry than the other stream channel cells due to the spigot being accidentally turned off before the start of the experiment. After water chemistry was measured, the spigot was turned back on to start cycling new water into the stream channel. The water chemistry in this stream channel continued to be different than the other stream channels. However, the water chemistry values did become more similar to the values of the other stream channels and were still within the range to support Texas wild rice and suckermouth armored catfish (Poole and Bowles 1999, Capps et al. 2011). The dissolved oxygen in the first stream channel (cells 1, 2, and 3) was lower than the other stream channels. This was noted at the beginning of the experiment and persisted throughout the experiment. This difference could be attributed to differences in the aeration of the water within the pipes before flowing out of the spigot at the head of each channel. The water entering the first stream channel likely had less aeration compared to other stream channels that were further down the water pipes. It is unlikely that these significant differences in water chemistry affected the catfish or the rice and it is also unlikely that

the catfish or rice caused these significant differences in water chemistry since the same stream channels were different throughout the entire experiment.

The PAR values were also significantly different among the different treatments due to the structure of the building allowing some stream channel cells to get more sunlight. To address this difference all Texas wild rice was planted in cells that had higher levels of sunlight to prevent changes in growth due to altered light availability. This placement affected the random assignment of treatments and may have introduced a systematic bias in the experimental design. However, we feel that this bias is minimal because cells with sunlight hitting them in the morning had representatives from all treatment types and light levels were similar for all cells except for about three hours on sunny mornings when sunlight would come through the east side of the building.

Organic Matter Decomposition

During this study I failed to detect a significant difference in organic matter decomposition rates among various treatments. This was likely due to the flood that occurred right before the start of the experiment. The open leaves were destroyed during the flood, and the caged leaves likely had damage or accrued additional organic matter from the flood. Previous experiments have shown that there was a significant difference in organic matter decomposition due to the effect of suckermouth armored catfish. Datri et al. (2015) and Scott et al. (2012) found that catfish significantly increased the rate of organic matter decomposition in open leaves, but did not have a significant effect on caged leaves. The accident that occurred on sample day 28 also did not allow for the

proper analysis to determine the decomposition rate. If this part of the experiment were repeated, it would likely show results similar to those found in previous experiments.

Periphyton

There was a significant difference between the Chla levels on caged and open tiles. The open tiles had significantly higher Chla concentrations than the caged tiles. This is unusual, and unlike results reported in other studies on the effects of catfish on periphyton (Datri et al. 2015, Scott et al. 2012). The lower Chla levels would be expected in open tiles due to grazing by catfish, but that is not what was found in this experiment. The Chla content was also significantly higher in caged and open tiles in treatments cells containing higher density stocking (two catfish) when compared to cells with lower catfish density and without catfish. This disagrees with what both Datri et al. (2015) and Scott et al. (2012) found. They found that catfish did not significantly impact the Chla content on caged tiles, but caused significantly lower Chla content on open tiles when compared to cells without catfish. The Chla results from this experiment also do not match what was observed in the stream channel cells. Stream channel cells without catfish appeared to have more algae growth than stream channel cells with catfish (Figure 12). The Chla results from this experiment seem to be the reverse of what has been found previously, and what would have been expected. A possible reason for this is that the tiles in treatment cells without catfish were shaded by floating algae, causing them to not receive as much light as tiles in treatment cells with catfish and resulting in a lower Chla concentration.

Texas Wild Rice

There was a highly significant difference in the above ground biomass of Texas wild rice among the different catfish treatments. The above ground biomass appeared to increase with increasing catfish density. Observations of the Texas wild rice throughout this experiment showed that plants in stream channel cells lacking catfish appeared to support more algal growth on their leaves compared to plants in cells with catfish (Figure 27). The catfish could have been grazing on the algae on or around the Texas wild rice, allowing them to grow faster since there was less competition with the attached algae for sunlight and nutrients. This same trend was also observed in the blade length of the Texas wild rice over time. Stream channel cells with catfish present had a significantly greater average blade length over time compared to those lacking catfish. This trend was even more apparent in stream channel cells with the higher densities of catfish. Suckermouth armored catfish did not have a significant effect on the below ground biomass, or the above ground: below ground biomass ratio. This could be because the Texas wild rice plants' root systems were already well established before they were planted in the stream channels. The roots may have not needed to grow anymore to support the growth of the leaves.



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Figure 27. Photos of Texas wild rice on day 28 in stream channel cell lacking catfish (left) and stream channel cell with catfish (right) showing the difference in the amount of algae growth on the Texas wild rice plants.

The relative Chl*a* concentration of Texas wild rice plant tissue increased significantly as catfish density increased. Texas wild rice plants in stream channel cells lacking catfish had the lowest Chl*a* concentration and treatment cells with higher density of catfish had the highest Chl*a* concentration. This could have been another effect of the catfish grazing the algae off of the Texas wild rice plants, allowing them to absorb more sunlight, resulting in higher Chl*a* concentrations in the leaves, increased photosynthesis rates, and consequently higher growth rates. Suckermouth armored catfish could have also positively impacted the Texas wild rice by indirectly influencing nutrient levels in plants.

Based on the results of this study it appears that selected nutrient levels in Texas wild rice were influenced by the presence of suckermouth armored catfish. Nitrogen levels in treatment cells lacking catfish were significantly less than treatment cells containing either low density or high density of catfish. There was also a significant difference in the P levels among all stocking levels of catfish. The treatment level containing low density of catfish had the highest P levels in the Texas wild rice, while plants in treatments lacking catfish had the lowest. Therefore, the concentration of P in Texas wild rice did not increase with the increasing number of catfish, but rather was highest in treatments with only one catfish. The N:P ratios were significantly lower in treatments with low density of catfish than in treatments lacking catfish.

Suckermouth armored catfish are known to sequester P (Capps and Flecker 2013a). This process could cause the Texas wild rice to get less P, changing their N:P ratio and resulting in a lower growth rate. The results found in this experiment show that Texas wild rice had the lowest N:P ratios in treatments with low density of catfish, followed by treatments with high density of catfish, and then treatments without catfish. Datri et al. (2015) found that catfish did not significantly affect N:P ratios of periphyton on open tiles, which does not match with the results found in this experiment. Both outcomes do not agree with stoichiometric theory that predicts that catfish sequester P and excrete nutrients at relatively high N:P ratios (Capps and Flecker 2013a). Hillebrand et al. (2008) performed a meta-analysis of current literature and also found that grazers that sequester P typically increased P content in vascular plants. They hypothesized that this was due to poorly understood interactions between grazer growth rates, flexibility of body P content, and P requirements for growth. The catfish in this experiment could have

been experiencing different levels of P sequestration, for example, the catfish in cells with two catfish present could have been using more P to rebuild lost fin tissue due to nipping from the other catfish. This would explain why the P levels were lower in treatment cells with two catfish present than in cells containing one catfish.

Armored Catfish

The change in weight, SL, and TL of a catfish during the time of the experiment could indicate if the catfish were growing from consuming Texas wild rice. The change in weight and TL of the catfish were significantly different among the different treatments. There was a smaller change in TL for treatments with two catfish than in treatments with one catfish, but this effect was caused by the catfish density, not Texas wild rice presence. Since there was not a significant difference in SL, the difference in TL could have been due to aggression between the two catfish. The competition for food and space in the small stream channel cell could have caused aggression or nipping towards the other catfish. Observing some of the catfish fins did show some tears that could have resulted from the aggression. Treatments with catfish in low density and rice present had significantly lower change in catfish weight than all other treatments. The change in weight for all treatments was small (-1.2g to 0.6g), so differences could have been due to how full the stomach of the catfish was at the time that each weight was taken.

Texas wild rice was only found in small amounts in two of the catfish dissected. The Texas wild rice could have been consumed accidentally while grazing on algae, or it could have been dead Texas wild rice that was consumed on the bottom of the stream

channel cell. It is unlikely that the catfish were purposefully grazing on the Texas wild rice. This is similar to results found in a previous study of the trophic ecology of invasive *Hypostomus plecostomus* in the San Marcos River. Pound et al. (2011) found that plant material only had a 5.6% occurrence rate and made up less than 1% of the total volume of gut content found in invasive *Hypostomus plecostomus*. The catfish in this experiment (*Pterygoplichthys sp.*) have been found to have similar diets to *Hypostomus plecostomus* in the San Marcos River also had algae, detritus, and sand in their stomachs (Pound et al. 2011), showing that *Pterygoplichthys sp.* and *Hypostomus plecostomus* also have similar diets in this invaded habitat.

The two catfish that had Texas wild rice in their stomach were in treatment cells that only contained one catfish, so even high densities of catfish did not have enough competition for food for them to consume the Texas wild rice. The only category of gut content that was significantly different among the different treatments was algae. Catfish in treatments with low density of catfish consumed more algae than catfish in treatments with two catfish. This suggests that some competition for food in the treatment cell was occurring. However, algae levels apparently did not decline to critical levels that would have induced consuming Texas wild rice. Instead, it appears that catfish consumed detritus and sand instead. These items were apparently ingested in an attempt to locate epibenthic algae.

Conclusions

The suckermouth armored catfish had an overall positive impact on the growth of the Texas wild rice during the short duration of this mesocosm experiment. The catfish's diet of mostly algae likely reduced epiphytic layers that allowed more sunlight to reach Texas wild rice plant tissue. This was reflected in increased levels of Chla and higher growth rates. Only two catfish had ingested small amounts of Texas wild rice, but not enough to affect the growth of the plant as a whole. The catfish did impact nutrient levels in the Texas wild rice, but in a positive way which enhanced the growth of the Texas wild rice. Although the catfish appeared to have positively impacted the Texas wild rice during this experiment, there are other negative impacts they may have on the environment that could impact the Texas wild rice in the San Marcos River. Suckermouth armored catfish are known to dig burrows in river banks (Lienart et al. 2013), which could cause erosion and instability that could increase turbidity in the water and cause less sunlight to reach the Texas wild rice. The catfish foraging on the bottom of the river could also cause the uprooting of some Texas wild rice plants. Further research should be done to determine the effects of armored catfish burrows on Texas wild rice and the San Marcos River.

If this project were repeated, some aspects could have been improved to gain more understanding of how suckermouth armored catfish affect Texas wild rice in the San Marcos River. The decomposition rate of organic matter could have been determined if the leaves would have been able to be replaced with new ones after the flood. A substrate similar to the San Marcos River could have been placed on the bottom of the stream channel cells to determine how suckermouth armored catfish could cause the

uprooting of Texas wild rice plants. Replicates of each treatment type could have been placed in the same stream channel to remove bias from incomplete blocks. If we had more time, suckermouth armored catfish could have been caught from the San Marcos River to ensure that they were the same fish that had invaded the system. The experiment also could have taken place in outdoor stream channels to avoid the use of lights to simulate the sun, and ensure that light levels in channels matched those in the San Marcos River at all times, causing a more accurate representation of the amount of periphyton that would grow in the river and potentially cover Texas wild rice plants.

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APPENDICES

Appendix A: Supplementary Photos



Figure A-1. Underwater photo of suckermouth armored catfish burrows with torn Texas wild rice found in the San Marcos River on October 15, 2015.

Appendix B: Two-way ANOVA Results

Appendix B-1. Temperature Two-way ANOVA Factor Information

Factor Catfish Rice	Type Fixed Fixed	Lev	els Val 3 C-, 2 R-,	ues C+, C++ R+		
Analysis	of Var	ianc	e			
Source Catfish Rice Catfish Error Total	n ∗Rice	DF 2 1 23 28	Adj SS 0.07600 0.02253 0.09118 0.40878 0.59856	Adj MS 0.03800 0.02253 0.04559 0.01777	F-Value 2.14 1.27 2.57	P-Value 0.141 0.272 0.099
Model Sum	mary					
S 0.133316	R-s 31.71	qR %	-sq(adj) 16.86%	R-sq(pre 0.0	d) 0%	

Appendix B-2. Specific Conductance Two- way ANOVA

Factor Information

Type Levels Values Fixed 3 C-, C+, C++ Fixed 2 R-, R+ Factor Catfish Fixed Rice Fixed Analysis of Variance ourceDFAdj SSAdj MSF-ValueP-ValueCatfish24748.92374.44.120.030Rice1129.7129.70.220.640Catfish*Rice2440.3220.10.380.687 Source Error 23 13261.7 576.6 Total 28 18596.8 Model Summary S R-sq R-sq(adj) R-sq(pred) 24.0124 28.69% 13.19% 0.00%

Tukey Pairwise Comparisons: Response = Cond., Term = Catfish

Grouping Information Using the Tukey Method and 95% Confidence

Catfish	Ν	Mean	Grouping
C+	10	624.533	A
C++	9	624.067	A B
C-	10	597.367	В

Means that do not share a letter are significantly different.

Appendix B-3. pH Two- way ANOVA

Factor Information

Factor Type Levels Values Catfish Fixed 3 C-, C+, C++ Rice Fixed 2 R-, R+

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Catfish	2	0.28343	0.14171	11.60	0.000
Rice	1	0.01155	0.01155	0.95	0.341
Catfish*Rice	2	0.02237	0.01119	0.92	0.414
Error	23	0.28092	0.01221		
Total	28	0.59796			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.110516	53.02%	42.81%	26.19%
Tukey Pairwise Comparisons: Response = pH, Term = Catfish

Grouping Information Using the Tukey Method and 95% Confidence

Catfish	Ν	Mean	Grouping	
C-	10	7.86100	А	
C++	9	7.71925		В
C+	10	7.62433		В

Means that do not share a letter are significantly different.

Appendix B-4. Dissolved Oxygen Two-way ANOVA

Factor Information

Factor Type Levels Values Catfish Fixed 3 C-, C+, C++ Rice Fixed 2 R-, R+

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Catfish	2	3.209	1.60468	17.34	0.000
Rice	1	1.002	1.00203	10.83	0.003
Catfish*Rice	2	1.060	0.53010	5.73	0.010
Error	23	2.129	0.09255		
Total	28	7.450			

Model Summary

S R-sq R-sq(adj) R-sq(pred) 0.304216 71.43% 65.22% 55.05%

Tukey Pairwise Comparisons: Response = DO mg/L, Term = Catfish

Grouping Information Using the Tukey Method and 95% Confidence

Catfish	Ν	Mean	Grouping
C-	10	8.66967	A
C++	9	8.24375	В
C+	10	7.86900	С

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Response = DO mg/L, Term = Catfish*Rice

Grouping Information Using the Tukey Method and 95% Confidence

Catfish*Rice	Ν	Mean	Grouping	g
C- R-	5	8.70600	A	
C- R+	5	8.63333	A	
C+ R-	5	8.32267	A	
C++ R-	5	8.31333	A	
C++ R+	4	8.17417	A	
C+ R+	5	7.41533	1	В

Appendix B-5. PAR Two-way ANOVA

Factor Information

FactorTypeLevelsValuesCatfishFixed3C-, C+, C++RiceFixed2R-, R+

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Catfish	2	2166	1083.0	1.17	0.329
Rice	1	22284	22284.3	24.04	0.000
Catfish*Rice	2	4308	2153.8	2.32	0.121
Error	23	21324	927.1		
Total	28	49906			

Model Summary

S R-sq R-sq(adj) R-sq(pred) 30.4486 57.27% 47.98% 31.10%

Appendix B-6. Organic Matter Decomposition Two-way ANOVA Factor Information

Factor	Туре	Leve	ls	Valu	les				
Catfish	Fixed		3	C-,	C+,	C++			
Rice	Fixed		2	R-,	R+				
Analysis	of Vari	ance							
Source		DF	Ad	j SS	A	dj MS	F-V	Value	P-Value
Catfish	ı	2	5.	.628	2.	81397		0.64	0.536
Rice		1	0.	.013	Ο.	01279		0.00	0.957
Catfish	n*Rice	2	10.	.966	5.	48310		1.25	0.306
Error		23	100.	964	4.	38974			
Total		28	117.	364					
Model Sum	nmary								

S R-sq R-sq(adj) R-sq(pred) 2.09517 13.97% 0.00% 0.00%

Appendix B-7. Periphyton Chla on Caged Tiles Two-way ANOVA Factor Information

Factor Type Levels Values Catfish Fixed 3 C-, C+, C++ Rice Fixed 2 R-, R+

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Catfish	2	0.001007	0.000503	7.20	0.002
Rice	1	0.000296	0.000296	4.24	0.045
Catfish*Rice	2	0.000055	0.000028	0.40	0.674
Error	52	0.003635	0.000070		
Total	57	0.004940			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0083606	26.43%	19.35%	8.78%

Grouping Information Using the Tukey Method and 95% Confidence

Catfish	Ν	Mean	Grouping
C++	18	0.0195944	A
C+	20	0.0115688	В
C-	20	0.0098100	В

Means that do not share a letter are significantly different.

Appendix B-8. Periphyton Chla on Open Tiles Two-way ANOVA Factor Information

Factor Type Levels Values Catfish Fixed 3 C-, C+, C++ Rice Fixed 2 R-, R+

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Catfish	2	0.002753	0.001377	14.95	0.000
Rice	1	0.000056	0.000056	0.61	0.439
Catfish*Rice	2	0.000147	0.000074	0.80	0.455
Error	52	0.004787	0.000092		
Total	57	0.007787			
Rice Catfish*Rice Error Total	1 2 52 57	0.000056 0.000147 0.004787 0.007787	0.000056 0.000074 0.000092	0.61 0.80	0.439 0.455

Model Summary

S R-sq R-sq(adj) R-sq(pred) 0.0095949 38.52% 32.61% 23.86%

Tukey Pairwise Comparisons: Response = open chla (ug/cm2), Term = Catfish

Grouping Information Using the Tukey Method and 95% Confidence

Catfish	Ν	Mean	Grouping
C++	18	0.0285167	A
C+	20	0.0167658	В
C-	20	0.0117525	В

Appendix B-9. Catfish TL Two-way ANOVA

Factor Information

FactorTypeLevelsValuesCatfishFixed2C+, C++RiceFixed2R-, R+

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Catfish	1	0.44944	0.449440	10.40	0.004
Rice	1	0.04624	0.046240	1.07	0.311
Catfish*Rice	1	0.00144	0.001440	0.03	0.857
Error	24	1.03700	0.043208		
Total	27	1.54857			

Model Summary

S R-sq R-sq(adj) R-sq(pred) 0.207866 33.04% 24.66% 6.79% 6.79%

Appendix B-10. Catfish SL Two- way ANOVA Factor Information

Factor	Туре	Levels	Val	ues
Catfish	Fixed	2	C+,	C++
Rice	Fixed	2	R-,	R+

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Catfish	1	0.00064	0.000640	0.01	0.928
Rice	1	0.10816	0.108160	1.40	0.248
Catfish*Rice	1	0.07744	0.077440	1.01	0.326
Error	24	1.84800	0.077000		
Total	27	1,99429			

Model Summary

S R-sq R-sq(adj) R-sq(pred) 0.277489 7.34% 0.00% 0.00%

Appendix B-11. Catfish weight Two-way ANOVA Factor Information

Factor	Туре	Levels	Value	S
Catfish	Fixed	2	C+, C	++
Rice	Fixed	2	R-, R	+

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Catfish	1	4.096	4.0960	4.37	0.047
Rice	1	5.184	5.1840	5.53	0.027
Catfish*Rice	1	5.184	5.1840	5.53	0.027
Error	24	22.500	0.9375		
Total	27	34.714			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.968246	35.19%	27.08%	5.93%

Tukey Pairwise Comparisons: Response = change wt, Term = Catfish*Rice

Grouping Information Using the Tukey Method and 95% Confidence

Catfish*Rice	Ν	Mean	Grouping
C+ R-	5	0.6	A
C++ R-	10	0.5	A
C++ R+	8	0.5	A
C+ R+	5	-1.2	В

Means that do not share a letter are significantly different.

Appendix B-12. Catfish total gut content Two-way ANOVA

Factor Information

Factor Type Levels Values Catfish Fixed 2 C+, C++ Rice Fixed 2 R-, R+ Analysis of Variance Source DF Adj SS Adj MS F-Value P-Value Catfish 1 3893.8 3893.8 2.79 0.108 Rice 1 745.7 745.7 0.53 0.472 Catfish*Rice 1 538.6 538.6 0.39 0.540 Error 24 33511.9 1396.3 Total 27 38460.1 Model Summary S R-sq R-sq(adj) R-sq(pred) 37.3675 12.87% 1.97% 0.00%

Appendix B-13. Algae in catfish gut content Two-way ANOVA

Factor Information

Factor Catfish Rice	Type Fixed Fixed	Leve	els 2 2	Valı C+, R-,	ues C++ R+		
Analysis	of Var	iance	e				
Source Catfis Rice Catfis Error Total	h h*Rice	DF 1 1 24 27	Ad 65 2 2 90 166	j SS 56.0 90.0 43.6 69.2 93.1	Adj MS 6556.0 290.0 243.6 377.9	F-Value 17.35 0.77 0.64	P-Value 0.000 0.390 0.430
Model Sur	mmary						
s 19.4392	R-sq 45.67%	R-s	sq(a 38.	dj) 88%	R-sq(pre 17.5	ed) 56%	

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Appendix B-14. Texas wild rice in catfish gut content Two-way ANOVA

Factor Information

Factor	Туре	Lev	els	Valı	les		
Catfish	Fixed		2	C+,	C++		
Rice	Fixed		2	R-,	R+		
Analysis	of Var	ianc	е				
Source		DF	Adj	SS	Adj MS	F-Value	P-Value
Catfish	n	1	1.5	537	1.5366	5.05	0.034
Rice		1	1.5	537	1.5366	5.05	0.034
Catfish	n*Rice	1	1.5	537	1.5366	5.05	0.034
Error		24	7.3	309	0.3045		
Total		27	11.2	253			
Model Sur	nmary						
s 0.551845	R-s 35.05	qR %	-sq(a 26.	adj) 93%	R-sq(p 0	ored) .00%	

Tukey Pairwise Comparisons: Response = TWR, Term = Catfish*Rice

Grouping Information Using the Tukey Method and 95% Confidence

Catfish*Rice	Ν	Mean	Grouping
C+ R+	5	0.98	A
C+ R-	5	0.00	В
C++ R-	10	0.00	В
C++ R+	8	-0.00	В

Means that do not share a letter are significantly different.

Appendix B-15. Sand in catfish gut content Two-way ANOVA

Factor Information

Factor Catfish Rice	Type Fixed Fixed	Leve	ls 2 2	Valu C+, R-,	les C++ R+		
Analysis	of Var:	iance					
Source Catfish Rice Catfish Error Total	n n*Rice	DF 1 1 24 27	Ad 49 120 3118 3303	j SS 90.5 40.2 66.5 38.5 36.9	Adj MS 490.55 40.22 1266.46 1299.52	F-Value 0.38 0.03 0.97	P-Value 0.545 0.862 0.333
Model Sur	nmary						
S 36.0489	R-sq 5.59%	R-sq	(ad <u>-</u> 0.00	j) F 2응	R-sq(pred) 0.00%		

Appendix B-16. Detritus in catfish gut content Two-way ANOVA

Factor Information

Factor Catfish Rice	Type Fixed Fixed	Leve	els 2 2	Valu C+, R-,	les C++ R+				
Analysis	of Vari	ance	e						
Source Catfish Rice Catfish Error Total	n*Rice	DF 1 1 24 27	Adj 5.4 7.2 3.9 50.7 65.3	SS 176 273 954 712 322	Adj MS 5.476 7.273 3.954 2.113	E-1	Value 2.59 3.44 1.87	P-V 0 0	alue .121 .076 .184
Model Sun	nmary								
s 1.45361	R-sq 22.37%	R-	sq(ad 12.0	dj) 56%	R-sq(p 0	red) .00%			

Appendix C: One-Way ANOVA and Kruskal-Wallis Results

Appendix C-1. Texas wild rice above ground biomass Kruskal-Wallis

Catfish	Ν	Median	Ave	Rank	Z
C-	15	20.38		15.4	-2.40
C+	15	24.70		21.8	0.12
C++	12	35.41		28.8	2.42
Overall	42			21.5	

 $H = 7.91 \quad DF = 2 \quad P = 0.019$

Dunn's Pairwise Comparisons: Response = above ground biomass, Term = Catfish

The following groups showed significant differences:

 Groups
 Z vs. Critical value
 P-value

 C- vs. C++
 2.80975 >= 1.834
 0.005

Appendix C-2. Texas wild rice below ground biomass Kruskal-Wallis

Catfish	Ν	Median	Ave Rank	Z
C-	15	11.37	18.3	-1.27
C+	15	12.32	20.6	-0.35
C++	12	15.68	26.7	1.73
Overall	42		21.5	
H = 3.25	DF	= 2 P :	= 0.197	

Appendix C-3. Texas wild rice above ground: below ground biomass ANOVA Factor Information

Factor Type Levels Values Catfish Fixed 3 C-, C+, C++ Analysis of Variance Source DF Adj SS Adj MS F-Value P-Value Catfish 2 0.8115 0.4057 0.48 0.623 Error 39 33.0067 0.8463 Total 41 33.8182 Model Summary S R-sq R-sq(adj) R-sq(pred) 0.919960 2.40% 0.00% 0.00%

Appendix C-4. Texas wild rice N-content ANOVA

Factor Information Factor Type Levels Values Catfish Fixed 3 C-, C+, C++ Analysis of Variance Source DF Adj SS Adj MS F-Value P-Value Catfish 2 1.206 0.60291 8.85 0.001 Error 39 2.656 0.06811 Total 41 3.862 Model Summary S R-sq R-sq(adj) R-sq(pred) 0.260988 31.22% 27.69% 20.88%

Tukey Pairwise Comparisons: Response = N, Term = Catfish

Grouping Information Using the Tukey Method and 95% Confidence

Catfish	Ν	Mean	Grouping
C+	15	2.23067	A
C++	12	2.18083	A
C-	15	1.85733	В

Appendix C-5. Texas wild rice P-content ANOVA

Factor Information Factor Type Levels Values Catfish Fixed 3 C-, C+, C++ Analysis of Variance Source DF Adj SS Adj MS F-Value P-Value Catfish 2 46.29 23.147 19.95 0.000 Error 39 45.25 1.160 Total 41 91.55 Model Summary S R-sq R-sq(adj) R-sq(pred) 1.07720 50.57% 48.03% 42.66%

Tukey Pairwise Comparisons: Response = P, Term = Catfish

Grouping Information Using the Tukey Method and 95% Confidence

Catfish	Ν	Mean	Grouping
C+	15	7.76013	A
C++	12	6.45732	В
C-	15	5.27653	С

Means that do not share a letter are significantly different.

Appendix C-6. Texas wild rice N:P ratio Kruskal-Wallis

Catfish	Ν	Median	Ave	Rank	Z
C-	15	0.3554		26.5	1.96
C+	15	0.2977		13.4	-3.19
C++	12	0.3368		25.4	1.31
Overall	42			21.5	

H = 10.22 DF = 2 P = 0.006

Dunn's Pairwise Comparisons: Response = N:P, Term = Catfish

The following groups showed significant differences:

Groups	Z vs. Critical value	P-value
C- vs. C+	2.91694 >= 1.834	0.0035
C+ vs. C++	2.52912 >= 1.834	0.0114

Appendix D: ANCOVA Results

Appendix D-1. Texas wild rice leaf length ANCOVA Factor Information

Factor Type Levels Values Catfish Fixed 3 C-, C+, C++ Analysis of Variance Adj SS Source DF Adj MS F-Value P-Value 1 38063.1 38063.1 224.48 2 1243.8 621.9 3.67 0.000 Day Catfish rror 0.028 Error 122 20686.4 169.6 Lack-of-Fit 5 924.8 185.0 1.10 0.367 Pure Error 117 19761.5 168.9 Total 125 59993.3 Model Summary S R-sq R-sq(adj) R-sq(pred) 13.0215 65.52% 64.67% 63.25%

Tukey Pairwise Comparisons: Response = blade length, Term = Catfish

Grouping Information Using the Tukey Method and 95% Confidence

Catfish	Ν	Mean	Grouping
C++	36	90.8194	A
C+	45	84.3467	A B
C-	45	83.4778	В

Means that do not share a letter are significantly different.

Appendix D-2. Texas wild rice relative SPAD Chla ANCOVA Factor Information

Factor Type Levels Values Catfish Fixed 3 C-, C+, C++ Analysis of Variance Adj SS Adj MS F-Value P-Value 525.0 525.00 7.12 0.009 Source DF 1 525.0 525.00 2 599.8 299.91 Day 0.020 4.07 Catfish Error 122 8998.7 73.76 Lack-of-Fit 5 53.99 0.72 0.607 270.0 Pure Error 117 8728.7 74.60 125 10123.5 Total Model Summary S R-sq R-sq(adj) R-sq(pred) 8.58835 11.11% 8.93% 5.26%

Tukey Pairwise Comparisons: Response = Chla, Term = Catfish

Grouping Information Using the Tukey Method and 95% Confidence

Catfish	Ν	Mean	Grouping
C++	36	23.2444	A
C+	45	20.0511	A B
C-	45	17.7711	В