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# Growth Rate of Mugil cephalus from Two Isolated Ponds in Huntington Beach State Park, South Carolina

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BY

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**ABSTRACT:**

Measurement of growth of the euryhaline teleost *Mugil cephalus* is quite common in open ocean settings, but growth rates of *M. cephalus* in isolated areas is non-existent. Two isolated ponds in Huntington Beach State, Horry County, South Carolina were selected to study the growth rate of *M. cephalus*; Sandpiper Pond with virtual no hydrologic connection to the ocean and Jetty Pond with adequate hydrologic connection to the ocean were the sites of sample collection. Scales from *M. cephalus* in both ponds were collected then aged by two readers, where agreeing ages were kept in the dataset. A von Bertalanffy growth model was fit individually for the two study sites. Length-at-age data and von Bertalanffy growth curves for Sandpiper Pond and Jetty Pond were compared to similar data from the Atlantic Ocean near Charleston, South Carolina and the Gulf of Mexico near Vera Cruz, Mexico. Sandpiper Pond was observed to be the most stressful of the environments and resulted in the slowest growth rate. Jetty Pond specimens on the other hand exhibited the fastest growth rate, possibly due to regeneration of eggs and optimal water circulation. Length-at-age data for *M. cephalus* in the Atlantic Ocean near Charleston fitted a von Bertalanffy growth curve that reached an asymptotic length at age 3, the same age as maturity. The von Bertalanffy growth model for *M. cephalus* in the Gulf of Mexico showed a slower growth than those in the Atlantic Ocean but greater than that of Sandpiper Pond. Each environment influenced the growth of the fish within it and caused significantly different growth rates between the four locations.



*Keywords: Mugil cephalus*, growth rate, isolated ponds, von Bertalanffy growth models

**INTRODUCTION:**

Growth is a fundamental function of all living things. Many organisms grow rapidly as juveniles during initial development but slow or stop when adulthood is reached. In fish, growth is continuous, so they become larger as they age (Boeuf and Payan 2001). Although fish continue to grow throughout their lifetime, once maturity is reached, the majority of energy consumed is used for maintenance and gamete production rather than for growth. This results in the common perception that fish will grow until they reach an asymptotic length around the age of maturity and then expend little energy toward growth and remain about the same size. Though exceptions do exist, an asymptotic length is commonly reached but rarely exceeded. Even with the possibility of becoming extremely large, fish are particularly dependent upon environmental conditions for survival and growth.

The most important abiotic factors that influence fish growth are temperature, dissolved oxygen concentration, salinity, and pH. Optimum temperature ranges are species and population specific, but it has been observed that higher temperatures generally result in higher growth rates (Nordlie 2006). This is true except when the high temperatures cause low dissolved oxygen content in the water. Dissolved oxygen in the water is very important abiotic factor for survival, but it also impacts behavior and therefore energy expenditure. Many estuarine species have developed the ability of aquatic surface respiration which allows them to survive in nearly anoxic conditions for extended periods of time (Nordlie 2006). Species that are not tolerant of even slightly hypoxic conditions will leave an area if possible, otherwise they will soon perish. Both methods of survival require additional energy expenditure above similar individuals living in adequately oxygenated conditions. This increased energy expenditure will likely result in a slower growing and possibly smaller fish.

Most fish species show optimal growth rates when the salinity is similar to that of their internal osmotic conditions, though exceptions do exist (Boeuf and Payan 2001; Nordlie 2006). The pH of the water is important for maintaining osmoregulation within fish and can cause stress that will affect the growth rate if not within an acceptable range. The pH is not a major factor in marine systems due to the buffering capacity of $HCO_3^-$ but is extremely important for fresh and low salinity waters. These factors along with biotic factors such as competition, food availability and predation can greatly affect the growth rate of all fish.

To understand the growth rate of fish, it is necessary to determine the age of the fish using reliable techniques. The age of an individual fish is found by determining the number of growth rings or annuli present on some body structure such as scales, otoliths, vertebrae or some other hard part of the fish. Interpretation of scales has long been the standard for aging fish because scales does not require sophisticated preparation, they are easy to collect and can be collected without sacrificing lives of fish (Hawkins et al 2004). Scales are often hard to interpret because they are subject to calcium reabsorption and regeneration, false annuli and misreading. An experiment with the Colorado pikeminnow (*Ptychocheilus lucius*), a long-lived freshwater fish, showed that vertebrae and sectioned otoliths provided more reliable aging when compared to scales and whole otoliths (Hawkins et al 2004). On the other hand, studies with *Mugil cephalus*, a relatively short lived marine species, have shown that aging by both scales and whole otoliths are valid for this species (Broadhead 1958; Ibanez-Aguirre and Gallardo-Cabello 1996). Other aging techniques exist for some species such as the fin ray technique, but each method must first be proved valid for the species in question. If not proved valid, the calculated growth rate will unknowingly be incorrect due to erroneous aging.

Growth equations for a species are created by fitting a collection of length-at-age data to an appropriate mathematical function. These models are used to generalize the growth process, predict the growth trend of fish and to compare the growth patterns between populations of a species (Chen et al 1992). The most commonly used growth model is the von Bertalanffy growth function. This function is given by the equation:

$$\mathbf{L_t = L_\infty \, (1\text{-}exp^{(-K(t\text{-}to))}).}$$

In this equation $L_t$ is the length at age t, $L_\infty$ is the asymptotic length that is not exceeded by the model, K is the Brody growth coefficient, t is the current age and $t_o$ is the age at which the fish is zero length. When compared against other growth equations, the von Bertalanffy equation performed the best in goodness-of-fit, reliability of coefficients estimates and growth rate in length as dL/dt (Chen et al 1992). When fitted to the data using nonlinear optimization methods, the von Bertalanffy equation gives reliable and easily comparable coefficients for many different species. It is also easy to check reasonability of calculated coefficients, for example $L_\infty$ should be similar to the largest length observed in the data, K the Brody growth coefficient should be between 0 and 1, and $t_o$ should be less than 0. This equation is preferential for modeling fish growth because it is thought to accurately model the growth of long-lived fish. Although fish growth is continuous, the majority of the energy in mature fish is used in reproduction and little is used for growth. For this reason, growth rates or increases in length are believed to be near asymptotic to zero in reproductively active fish. This trend is accurately described by the von Bertalanffy equation, but is missing in most others. The von Bertalanffy equation is the most adaptable and preeminent equation to use when modeling fish growth, regardless of species.

*Mugil cephalus* is a euryhaline marine teleost that has worldwide economic importance. *Mugil cephalus* is known by many different common names in the USA such as grey mullet, striped mullet, black mullet, common mullet and flathead mullet. This species has a worldwide distribution in topical, subtropical and temperate climates from 42$^{o}$N to 42$^{o}$S (McDonough and Wenner 2003; McDonough et al 2003; Nordlie 2003). *M. cephalus* is commonly harvested in North Carolina and Florida during the fall spawning migration as a target for roe and throughout the rest of the year is fished commercially for human consumption and use as bait. This species is economically important worldwide and research on the species is extensive (Broadhead 1958; Griffiths 1999; McDonough et al 2005). *M. cephalus* is also cultured in brackish and freshwater commercial ponds as a secondary species to improve sediment quality (Torras et al 2000). *M. cephalus* is known to feed primarily upon bottom detritus in shallow water areas but recent studies have shown this species also feeds upon zooplankton and large phytoplankton (Broadhead 1958; Torras et at 2000).

*M. cephalus* is reproductively active from October through April and spawns offshore. Adult females that are found inshore during this time do not have completely developed eggs and are thought to require the offshore migration to become reproductively active. Males and females of this species become sexually differentiated at a length of between 170 mm and 200 mm, which is approximately the size range where the first annuli is deposited (McDonough et al, 2005). Female *M. cephalus* become sexually mature at a length of 230 mm to 350 mm between the ages of two and five (McDonough et al, 2003). Male *M. cephalus* become sexually mature as early as 250 mm of length within the first year, but most commonly between 275mm and 350mm between ages two and four (McDonough et al

2005). Both sexes of *M. cephalus* most commonly mature around age three. Larvae of the species have been observed to immigrate into estuaries during the spring in large numbers (Bozeman and Dean 1980; McDonough et al 2003). For this reason, *M. cephalus* is considered a marine nursery species in estuarine systems, despite the fact that young of this species successfully develop in coastal marine environments that are devoid of estuaries (Nordlie 2003).

The growth rate of *Mugil cephalus* in enclosed areas has been previously studied but was not fitted to any growth models. A study by Griffiths (1999) in Australia of an intermittently closed lagoon, which remained closed for the entirety of the study, conducted a length versus time analysis of *M. cephalus* from March through July. This study found that the closed lagoon had an adverse effect upon the species. Catch frequency during the months of May and July were observed to be significantly lower than at the start of the study. The decrease in catches during these months coincided with a decrease in salinity and water temperature and thus an increase in physiological stress on the fish. The closed lagoon also prevented the recruitment of juvenile fish to the area as indicated by the presence of few relatively large fish and lack of juveniles in the closed lagoon. The open lagoons to which the study site was compared showed recruitment upon opening in May. Though this study computed a length at time frequency chart, no length-age growth curves were calculated (Griffiths 1999). Another study found that *M. cephalus* in a closed freshwater system began reproductive development but did not spawn. Instead the fish reabsorbed the developing gametes back into its body. This reabsorption undoubtedly would have a positive impact upon growth rate of the fish (Shireman 1975; Tamaru et al 1994). On the other hand, gamete production is greatest when gonads develop in salinities ranging from 13 to 35 ppt (Brusle 1981; Tamaru et al 1994).

Two ponds in Huntington Beach State Park were selected to investigate the growth rate of *Mugil cephalus* in isolated ponds: Sandpiper Pond and Jetty Pond. Sandpiper Pond is a shallow serpentine pond that is 1 kilometer long and .1 kilometer at its widest point, which runs parallel to the Atlantic Ocean. Historically, Sandpiper Pond was irregularly connected to the Atlantic Ocean. With the instillation of jetties at Murrells Inlet in 1981 the pond received saltwater on a less frequent basis. With the passing of hurricane Hugo in 1989, the connection to the ocean was increased in size (Tosso et al 2004). Yet by 1992, Sandpiper Pond had become completely isolated from the Atlantic Ocean. As a result of this separation, Sandpiper Pond became a freshwater ecosystem. The connection to the ocean was opened twice since 1989 by the digging of culverts but neither attempt had a lasting effect. Though not connected to the ocean, a twelve-inch diameter pipe connects Sandpiper Pond to a tidal creek of the nearby salt marsh. Only a small amount of sea water is transferred through this connection and is not enough to circulate the entire pond. This long-term disconnection from the ocean has caused marine organisms in Sandpiper Pond to be secluded from their natural habitat. This environmental stress will undoubtedly have an impact upon the *M. cephalus* that became trapped in Sandpiper Pond after its last closure.

Jetty Pond lies 1.4 kilometers northeast of Sandpiper Pond and is more ovular with a length of .5 kilometers and width of .15 kilometers. Jetty Pond was created by the installation of the jetties on the northwest edge of the pond. These jetties allow the pond to be hydrologically attached to the ocean but serves as a potential barrier to the translocation of fish. Jetty Pond has a depth that is more uniform and deeper than Sandpiper Pond. The volume of water in Sandpiper Pond and Jetty Pond is thought to be fairly similar, though their hydrology is quiet different.

This study will collect growth data for *M. cephalus* from the two isolated ponds Sandpiper Pond and Jetty Pond. The growth data will then be fitted to a von Bertalanffy growth equation for each location. The impact of living in an isolated area on the growth of *Mugil cephalus* will be assessed by comparing coefficients of these models with previous growth equations from other studies. *M. cephalus* have been present since the pond's closing in 1992 but it is unlikely that the fish present are older than thirteen years of age. Whether reproduction occurs in the pond or fish are recruited to the pond during infrequent connections will determined. It is predicted that spawning does not occur within Sandpiper Pond because an offshore migration is impossible. Therefore, the reabsorption of gametes is feasible and may play an important part in the growth rate of the fish. It is hypothesized that due to higher physiological stresses and less availability of food, the growth rate of *Mugil cephalus* in the enclosed Sandpiper Pond will be less than those observed by studies in the open ocean. It is also hypothesized that the growth rate of *Mugil cephalus* in the isolated but hydrologically connected Jetty Pond will be more similar to the ocean and greater than that of Sandpiper Pond.

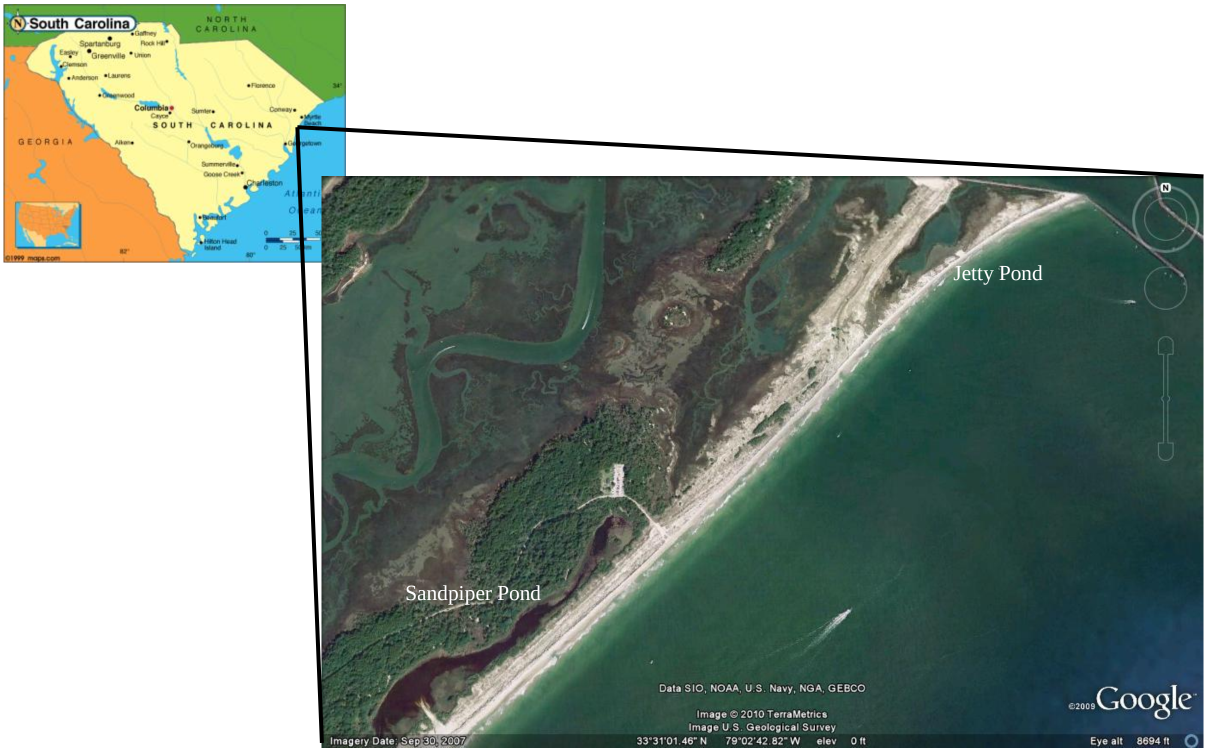


Figure 1. Sample of *Mugil cephalus* were collected from the center of Sandpiper Pond on the eastern side and from the northeast side of Jetty Pond in Huntington Beach State Park, South Carolina.

## **METHODS:**

*Mugil cephalus* samples were collected from Sandpiper Pond and Jetty Pond, Huntington Beach State Park, South Carolina. Samples of *Mugil cephalus* were caught with a 250 foot long, 4 foot deep experimental gill net with graded mesh ranging from 1 inch to 2 inches. Seining occurred biweekly from March 29, 2005 through October 25, 2005, though samples of *Mugil cephalus* were not caught on all occasions. Data from each fish were collected including total length, fork length, standard length, wet weight and gonad development. Environmental conditions such as temperature, salinity, water temperature were measured and recorded. Multiple scales were taken from the left side of the mullet from behind the pectoral fin in advance of the dorsal fin and above the lateral line. All scales collected were cleaned and stored on a microscope slide with the corresponding data or some other identifying number. Pictures of each scale were taken and corresponding data was kept separate from the pictures to avoid bias when reading the scales (Hawkins et al 2004). Pictures were magnified and printed with only an identifying picture number, but individual fish were kept separate from one another by an indicator picture. Interpretation of pictures was conducted individually by two readers. The number of annuli and distance from center on the scales was marked and counted using the criteria for recognition of annulus as established by Broadhead (1958). The interpreted current age of each scale was compared between the two readers. Fish with at least one scale with matching age between the two readers were selected. From each selected fish the modal age interpreted by both readers was used. Some pictures were reprinted and interpreted by both readers a second time because of poor picture quality and disagreement. If no similar age was agreed upon for individual scales on the second attempt, the fish was not used. A dataset of the marked annuli from selected scales with modal age for individual fish was compiled. Pictures were then matched to the corresponding total length, standard length, weight and collection location. Lengths of fish at each previous age were back-calculated based upon position of the interpreted annuli. A final dataset of length-at-age was compiled from the back-calculated lengths for each of the two ponds. The two datasets were then fitted to von Bertalanffy equations using standard nonlinear optimization techniques. The von Bertalanffy models for Sandpiper Pond and Jetty Pond were compared to models of *M. cephalus* growth presented by Ibanez-Aguirre and Galiardo-Cabello (1996) and McDonough (personal communication, November 30, 2009). A dataset of length-at-age for Ibanez-Aguirre and Galiardo-Cabello (1996) was reconstructed from a frequency table presented in the paper. One-way ANOVA was used to compare the

Jetty Pond, Sandpiper Pond, McDonough and Ibanez-Aguirre and Galiardo-Cabello datasets at individual ages. For ages that did not have sufficient samples to fulfill the ANOVA assumption of large numbers or did not appear to be normally distributed, a Kruskal-Wallis Rank Sum Test was used. A Wilcoxon Rank Signed Test was used to compare the median of age eight fish from McDonough (personal communication, November 30, 2009) and Jetty Pond. All statistical tests (6 - one-sample ANOVAs, 1 - two-sample ANOVA, 1 - Wilcoxon Ranked Sign Test, and 1 - Kruskal-Wallis Rank Sum Test) were preformed using the statistical program R.

## RESULTS:

A total of thirty-three specimens of *M. cephalus* were collected from Sandpiper Pond and Jetty Pond with twenty-seven from the former and six from the latter. From these specimens, seventeen from Sandpiper Pond and three from Jetty Pond were selected to be used in growth rate analysis. There was much disagreement between ages interpreted from different scales of the same fish and also between readers. There were three fish that had the same interpreted age for each scale collected by both readers, all of age five. Multiple scales were removed from the growth analysis due to regeneration. The oldest fish of eight years old was found in Jetty Pond, but two seven year old fish were found in Sandpiper Pond. The average age of fish collected from Sandpiper Pond was 4.2 and 5.6 from Jetty Pond. Age four fish were most commonly found from Sandpiper Pond. The longest total length measure in Sandpiper Pond and Jetty Pond were 406 mm and 560 mm, respectively. Through back-calculation of the length at age using all interpretations of scales with modal age, 263 length-at-age data points were established for Sandpiper Pond while fifty-one were created for Jetty

Pond. The back-calculated lengths were used to virtually increase the sample size to make more accurate predictions about growth rates.

Growth approximation by the von Bertalanffy growth model was successful for Sandpiper Pond and Jetty Pond. Sandpiper Pond yielded a growth equation of: $L_t$= 605.8287 ($1\text{-}e^{-0.09814(t-(-1.4239))}$) with a sum of squared differences ($SD^2$) of log normal difference in predicted and actual length of 13.11328 (Figure 2, Table 1). Jetty Pond yielded a growth equation of: $L_t$=630.7733($1\text{-}e^{(-0.22626(t-(-0.64986)}$) with a $SD^2$ of 2.099406 (Figure 3, Table 1). By looking at the growth equations, *M. cephalus* in Sandpiper Pond are expected to have an asymptotic growth of 605 mm, while in Jetty Pond the species is expected to reach 630 mm. Both estimates are well above the maximum lengths collected from the ponds. Yet the von Bertalanffy growth approximation for both locations falls within the range of measured length for each age (Figure 2 and Figure 3). This along with a relatively low sum of log normal residuals squared, $SD^2$, of 13.11328 for Sandpiper Pond and 2.099406 for Jetty Pond an appropriate approximation of growth for the two locations is presented by the von Bertalanffy curve. Brody growth coefficient (K) for the Sandpiper Pond is 0.09814 and for Jetty Pond is 0.22626. The expected age at length zero ($t_o$) is less than zero for both locations, indicating that the model was correctly calculated to fit the data (Table 1). The approximation by the von Bertalanffy growth curve for Sandpiper Pond was less than that predicted for Jetty Pond at all ages, except birth (Figure 4).

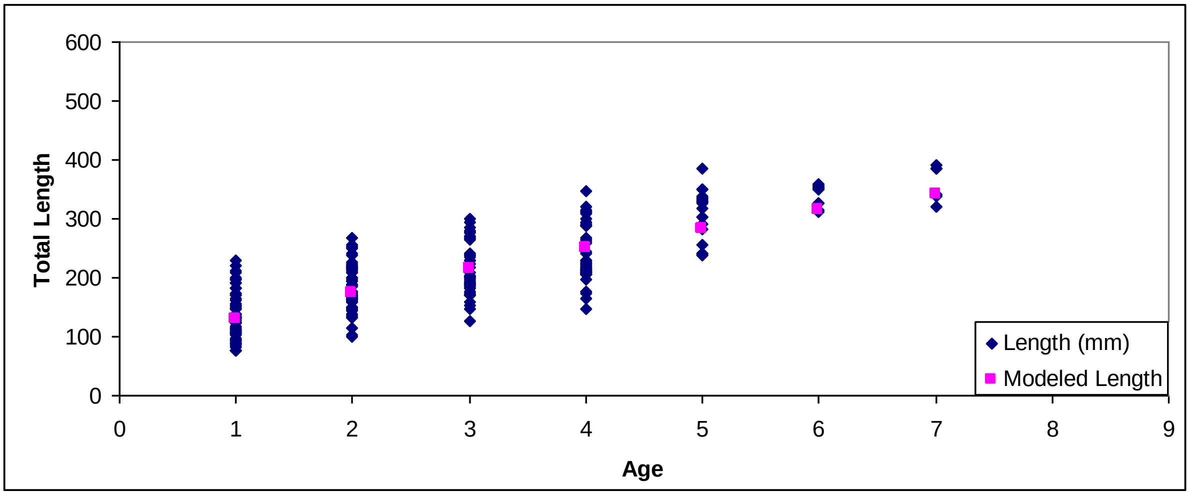


Figure 2. Von Bertalanffy growth approximation of *M. cephalus* in Sandpiper Pond fitted to back-calculated lengths at age interpreted from scales of sixteen fish.

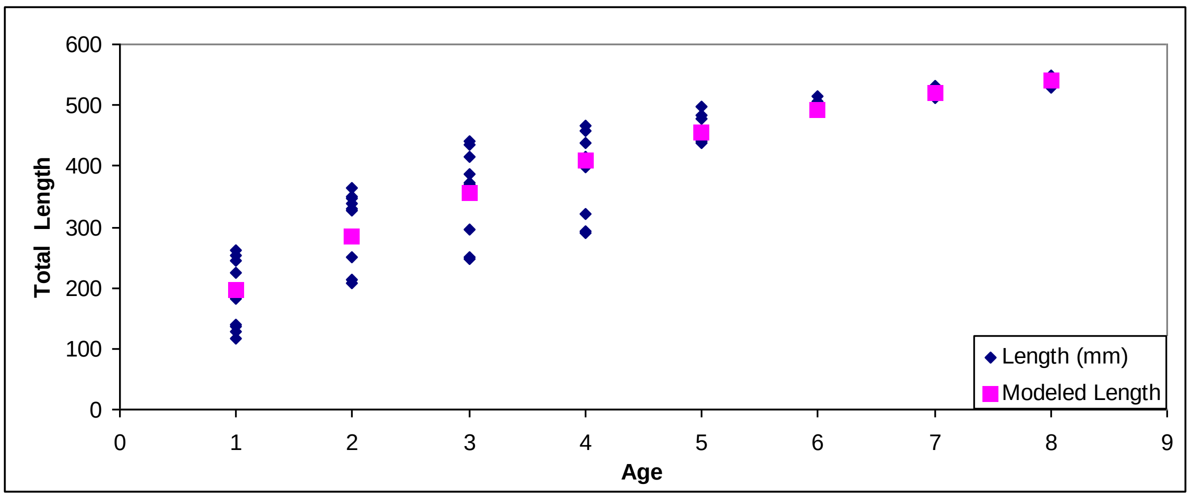


Figure 3. Von Bertalanffy growth approximation of *M. cephalus* in Jetty Pond fitted to back-calculated lengths at age interpreted from scales of three fish.

| | Jetty Pond | Sandpiper Pond | Ibanez | McDonough |
|---|---|---|---|---|
| $L_\infty$ | 630.7733 | 605.8287 | 477.7 | 381.3 |
| K | 0.22626 | 0.09814 | 0.17 | 0.818 |
| $t_o$ | -0.64986 | -1.4239 | -2.367 | -0.392 |
| $SD^2$ | 2.099406 | 13.11328 | 6.21490 | 455.709 |

Table 1. Comparison of von Bertalanffy growth constants and sum of squared of difference of log normal difference in predicted and actual length, for four models of *M. cephalus* growth in Sandpiper Pond, Jetty Pond, Atlantic Ocean off the coast of Charleston, South Carolina (McDonough Pooled) and Gulf of Mexico off the coast of Vera Cruz, Mexico (Ibanez Pooled).

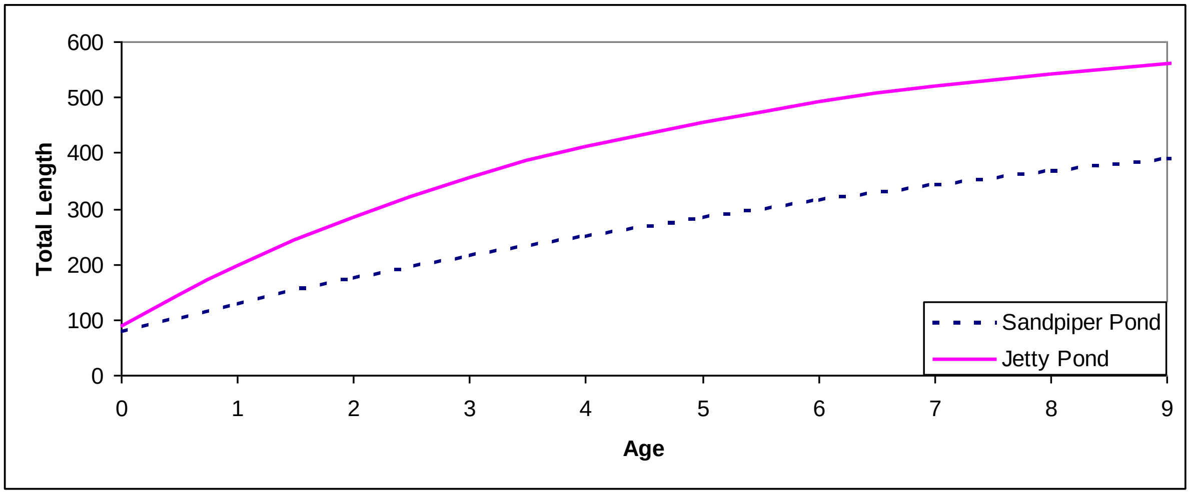


Figure 4. Comparison of von Bertalanffy growth models of *M. cephalus* in Sandpiper Pond and Jetty Pond.

A reconstructed dataset was assembled using a length-at-age chart with 10 mm increments from Ibanez-Aguirre and Galiardo-Cabello (1996). A von Bertalanffy growth equation was fitted to the reconstructed data and compared with the published coefficients. No difference was observed between the newly calculated and published coefficients so the reconstructed dataset was used for statistical analysis. A von Bertalanffy model was obtained for *M. cephalus* from off the coast of Charleston, South Carolina (McDonough, personal communication, November 30, 2009). The von Bertalanffy growth models for *M. cephalus* in the four locations were plotted against one another for comparison. The estimated length at age zero for Sandpiper Pond, Jetty Pond and McDonough's data were all similar, but Ibanez appeared slightly higher than all three. The curvature of the McDonough data is different compared to the other three models and reaches its asymptotic length around age three or four, whereas none of the other three curves appear to reach an asymptotic length. Despite this, McDonough's, Ibanez's and Sandpiper Pond's von Bertalanffy models approach similar lengths at age nine and ten. The growth model for Sandpiper Pond is less than McDonough and Ibanez for all ages up to nine, while Ibanez is only less than McDonough between ages one and seven. Jetty Pond shows an initial growth rate at age zero slightly less than McDonough, but exceeds the latter by age three and is larger than all others after this age. Each location had a unique growth curve.

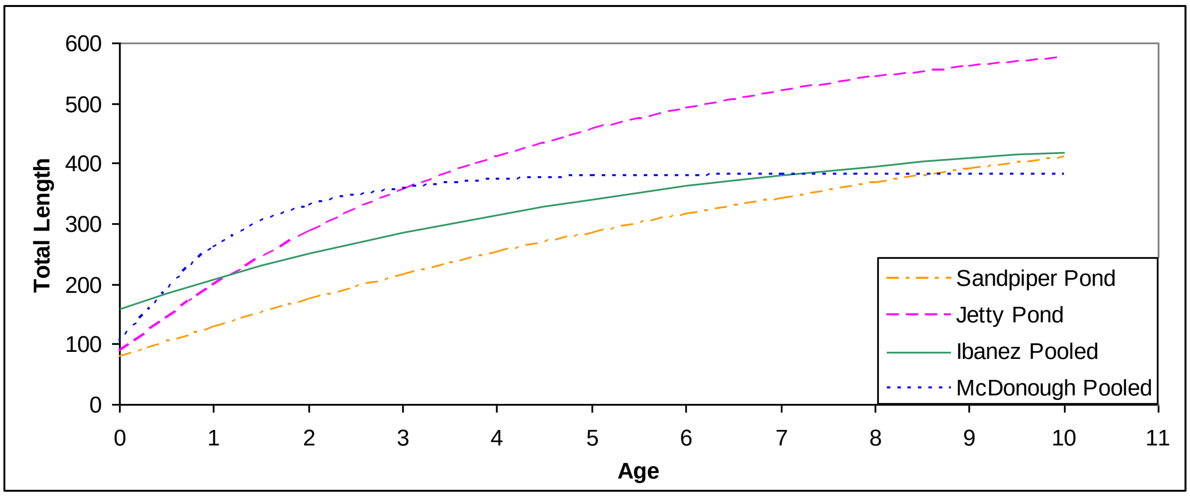


Figure 5. A comparison of four von Bertalanffy growth models for *M. cephalus* in Sandpiper Pond, Jetty Pond, Atlantic Ocean off the coast of Charleston, South Carolina (McDonough Pooled) and off the coast of Vera Cruz, Mexico (Ibanez Pooled).

Comparison of length between locations occurred through multiple tests. Ages represented in the four dataset are as follows: McDonough observed ages zero through ten, Ibanez reported ages two through six, ages one through eight were back-calculated for Jetty Pond and ages one through seven were back-calculated for Sandpiper Pond. Age one fish were present in McDonough, Jetty Pond and Sandpiper Pond datasets and were compared using a one-way ANOVA. Ages two through six were compared individually between all four samples using one-way ANOVAs. Age seven fish were present in McDonough, Jetty Pond and Sandpiper Pond datasets but only twenty, three and six length-at-age were reported from the respective locations, so a Kruskal-Wallis Rank Sum Test was used to compare the medians. A Wilcoxon Ranked Sign test was used to compare the medians of age eight between McDonough and Jetty Pond because only twenty-three and three length-at-age were recorded, respectively. ANOVA of each age one through five resulted in extremely significant results ($P<2.2e^{-16}$), age six was also significantly different ($P=1.088e^{-11}$). The Kruskal-Wallis test showed there was a significant difference (P= 0.005328) between the medians of McDonough, Jetty Pond and Sandpiper Pond datasets at age seven. A significant difference between the median of McDonough and Jetty Pond at age eight was determined by the Wilcoxon Signed Rank Test (P=0.0007692). A two-way ANOVA of location and age against standard length showed significant differences between age, location and combined ($P<2.2e^{-16}$). All comparisons of total lengths between locations were significant regardless of test.

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Author1 | 2 | 1110284 | 555142 | 322.02 | < 2.2e-16 |
| Residuals | 1526 | 2630693 | 1724 | | |

Table 2. Summary Table of Analysis of Variance of length of age one *M. cephalus* from Sandpiper Pond, Jetty Pond and McDonough.

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Author2 | 3 | 1086004 | 362001 | 193.12 | < 2.2e-16 |
| Residuals | 1169 | 2191308 | 1875 | | |

Table 3. Summary Table of one way Analysis of Variance of length of age two fish from Sandpiper Pond, Jetty Pond, Ibanez and McDonough.

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Author3 | 3 | 1216967 | 405656 | 150.25 | < 2.2e-16 |
| Residuals | 477 | 1287862 | 2700 | | |

Table 4. Summary Table of one way Analysis of Variance of length of age three *M. cephalus* from Sandpiper Pond, Jetty Pond, Ibanez and McDonough.

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Author4 | 3 | 927376 | 309125 | 157.59 | < 2.2e-16 |
| Residuals | 284 | 557080 | 1962 | | |

Table 5. Summary Table of one way Analysis of Variance of length of age four fish from Sandpiper Pond, Jetty Pond, Ibanez and McDonough.

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Author5 | 3 | 176801 | 58934 | 41.324 | < 2.2e-16 |
| Residuals | 174 | 248147 | 1426 | | |

Table 6. Summary Table of one way Analysis of Variance of length of age five *M. cephalus* from Sandpiper Pond, Jetty Pond, Ibanez and McDonough.

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Author6 | 3 | 69439 | 23146.3 | 26.174 | 1.088e-11 |
| Residuals | 75 | 66323 | 884.3 | | |

Table 7. Summary Table of one way Analysis of Variance of length of age six *M. cephalus* from Sandpiper Pond, Jetty Pond, Ibanez and McDonough.

| Kruskal-Wallis chi-squared | Df | p-value |
|---|---|---|
| 10.4694 | 2 | 0.005328 |

Table 8. Summary of Kruskal-Wallis Test of median length of age seven *M. cephalus* from Sandpiper Pond, Jetty Pond, and McDonough

| W | p-value |
|---|---|
| 69 | .0007692 |

Table 9. Summary table of Wilcoxon Test on median length of age eight *M. cephalus* from Jetty Pond and McDonough.

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Location | 3 | 3384966 | 1128322 | 282.241 | < 2.2e-16 |
| Age | 1 | 53875040 | 53875040 | 13476.425 | < 2.2e-16 |
| Location x Age | 3 | 966933 | 322311 | 80.624 | < 2.2e-16 |
| Residuals | 6786 | 27128561 | 3998 | | |

Table 10. Summary table of two way Analysis of Variance of all available data points from Sandpiper Pond, Jetty Pond, Ibanez and McDonough.

## **DISCUSSION:**

This study investigated whether reproduction by *Mugil cephalus* was occurring in Sandpiper Pond or if all of the fish present had become trapped in 1992 or had been recruited to the pond since then. The oldest age interpreted and agreed upon by both readers was eight years of age. Yet no fish less than two years old was encountered and the mode age interpreted by the readers was four years of age. It was also interesting to find that no fish of age three were encountered. No reproductive activities were expected due to the necessity of offshore migration to allow eggs to develop in females. During the study no juveniles or eggs were observed in Sandpiper Pond. Furthermore, no advanced stages of gonad maturation were observed in the collected specimens. Therefore, it can be concluded that reproduction is not occurring within Sandpiper Pond. Since no reproduction is occurring within Sandpiper Pond and there are no fish older than age eight, *Mugil cephalus* is recruited to the pond during high storm surges on irregular occasions. On the other hand, young of year were observed in Jetty Pond, though not included in data analysis. Like Sandpiper Pond, *M. cephalus* in Jetty Pond did not show any advanced maturation of gonads. Therefore, *M. cephalus* is entering Jetty Pond at a young age by swimming through the cracks in the rocks of the jetty and into the isolated pond. Whether *M. cephalus* is capable of exiting Jetty Pond after it has grown is unlikely, but cannot be excluded until further experiments are conducted.

Growth rates of fish can be indicative of the environment in which they grow. Stressful environments that put a physiological strain upon the animal will result in a depressed growth rate and possibly lowered reproductive capacity. In Sandpiper Pond the Brody growth coefficient of *Mugil cephalus* was at least half as small compared to other coefficients present in this study. This is likely due to the stressful environment in Sandpiper

Pond. This isolated pond has an extremely low salinity that is classified as freshwater. This increased difference from internal salinity compared to seawater could cause increased osmoregulatory energy expenditure by the fish to maintain homeostasis. This results in less partitioning of energy toward growth in these fish and therefore a slower growth rate. In Sandpiper Pond the fish do not expend energy toward reproductive endeavors or gonad development and possibly use this energy for maintenance and growth. Yet the growth rate of *Mugil cephalus* in Sandpiper Pond was comparatively less than the others. Despite this slower growth rate, the modeled length by the von Bertalanffy equation in Sandpiper Pond eventually reached the predicted length for McDonough and Ibanez at age nine, though no specimens of this age were collected. The McDonough von Bertalanffy growth curve shows that *Mugil cephalus* reaches it asymptotic length around age three or four. This is the same age at which sexual maturity is reached, showing that the majority of energy is going toward gamete production instead of growth. Since the *M. cephalus* in Sandpiper Pond are not producing gametes they most likely are reallocating energy toward survival and growth, yet this does not happen until they have reached maturity around age three or four. *Mugil cephalus* in Sandpiper Pond have a lower growth rate for the first three or four years of life compared to McDonough, but continue to grow after this age. It is probable that because *M. cephalus* do not reproduce in Sandpiper Pond, they continue growing after sexual maturity is reached. The von Bertalanffy model for Sandpiper Pond predicts an asymptotic length of 630 mm which is not reached within *M. cephalus*'s lifetime. The isolation in Sandpiper Pond that prevents *Mugil cephalus* from reproducing allows the fish to experience continued growth past sexual maturity though at a slower rate compared to the fish in the ocean (McDonough, personal communication, November 30, 2009). The combination of environmental stress and reallocation of energy may explain the modeled growth predicted by the von Bertalanffy equation for *Mugil cephalus* in Sandpiper Pond.

The von Bertalanffy growth equation for *Mugil cephalus* in Jetty Pond showed the second highest Brody growth coefficient; double that of Ibanez but only a quarter of McDonough. This coefficient relates closely to the curvature of the model with higher numbers reaching asymptotic lengths at younger ages. The slope of the line is also determined by this coefficient with lower numbers have a flatter slope and higher numbers having a steeper slope. Both these attributes of the Brody growth coefficient help explain the nature of the growth curve for Jetty Pond; what explains the nature of the calculated Brody growth coefficient? Jetty Pond is still connected to the ocean via a small stream that flows under and through the large rocks of the jetty system. This stream allows for fresh seawater to enter Jetty Pond, increasing the circulation of water and decreasing the likelihood of anoxic conditions. The water that enters Jetty Pond via this connection is crucial in maintaining the salinity, pH and temperature near that of the ocean and may also contain food resources for *Mugil cephalus*. This increased stability in environmental factors may lessen the osmoregulatory expenditures by *Mugil cephalus*. This connection to the ocean is also a probable source of the immigration of *M. cephalus* into Jetty Pond. The conditions for growth during the first three to four years of life are as optimal as those observed for *M. cephalus* off the coast of Charleston in the Atlantic Ocean. The fish in Jetty Pond do not reach asymptotic lengths at three years of age as do the fish in the Atlantic Ocean. For this reason *M. cephalus* in Jetty Pond experience prolonged growth that results in the longest predicted lengths in this study. *Mugil cephalus* in Jetty Pond like those in Sandpiper Pond do not participate in reproductive activities, but whether these fish reabsorb their gametes is unknown. Since no advanced maturation of gametes was observed in Jetty Pond it is likely that *M. cephalus* in this environment do not allocate energy toward gamete production and therefore are not reabsorbing their gamete. This process is probable due to the lack of asymptotic length reached at age three and continued growth after. More stable environmental conditions and the reallocation of energy commonly used during gamete production for *M. cephalus* in Jetty Pond explains the prolonged steady growth.

The appearances and overall shape of the von Bertalanffy growth curves are unique for the four locations. Since these curves are an illustration of the growth rate of the fish, it would be assumed that there is a high degree of difference in growth between the four locations. Yet a qualitative comparison of the curves is not enough to support a difference so multiple statistical analysis were carried out to compare length at each age between groups. The length of *M. cephalus* was shown to be different between the sample locations at each age one through eight, where data was available. A two-way ANOVA confirmed the individual tests of length-at-age and showed that there was an overall difference between the four locations, not just differences at individual ages. These tests support the assumption made by comparison of the von Bertalanffy growth curves that the growth of *M. cephalus* is different between the four locations. This difference in growth is likely due to the vast difference between environmental conditions. The environmental conditions of Sandpiper Pond have a limiting effect upon the growth of *M. cephalus*, while Jetty Pond promotes the prolonged growth of *M. cephalus*. The Gulf of Mexico near Vera Cruz where Ibanez-Aguirre and Gallardo-Cabello (1996) obtained samples is a tropical ocean, while the Atlantic Ocean off the coast of Charleston is a sub-tropical ocean. If the only environmental factor different between the two populations was the temperature, then *M. cephalus* from the Gulf of Mexico would be predicted as longer than those from the Atlantic Ocean. This is not the case; therefore other differences in the two environments must be present. *M. cephalus* living in the Atlantic Ocean were observed to spend time in the salt marsh system of the area. Salt marshes are areas of very high biological productivity that may provide greater food resources compared to those living in the Gulf of Mexico. This is a possible explanation for the difference in growth between the two populations but the food resources of the two locations must first be quantified and compared for this explanation to be conclusive.

The difference in the shape of the four von Bertalanffy may be due to the sampling technique of the studies. The data from near Charleston was composed of thousands of age zero, one and two *M. cephalus*, hundreds of age three, four and five and less than fifty age six through ten. This weighted data for younger ages is possibly responsible for the large curvature and following asymptotic growth around age three. Though this is possibly what actually occurs in nature, it likely that *M. cephalus* older than six years of age have a larger length than that predicted by the von Bertalanffy growth model. If the young-of-year were removed from the dataset, a larger $L_\infty$ and therefore larger older fish would be predicted, but the predicted length for younger aged fish would be less accurate (McDonough, personal communication, November 30, 2009). To receive a more accurate growth curve, sample sizes of each age should be similar to one another, though this may be near impossible. The data for the Gulf of Mexico was composed of only ages 2 through 6 and was obtained from scales of fish in a local fish market. This is by no means a fair representation of the entire population of *M. cephalus* in the Gulf of Mexico near Vera Cruz. Even the ages present are likely to only be represented by the largest and fastest growing in the population that have already reached sexual maturity. Therefore, the von Bertalanffy growth model does not show an asymptotic length because younger fish that would be experiencing the greatest growth rate are not sampled. This may contribute to the difference in shape and lack of an asymptotic length reached within the first ten years. Therefore, the statistical analysis of the lengths at each age is a more accurate comparison than a visual inspection of the von Bertalanffy growth models.

The von Bertalanffy growth curves for Sandpiper Pond and Jetty Pond both showed a slow curvature and did not reach an asymptotic length within the first ten years. This may be attributed to the extremely small sample sizes. It is important to keep in mind that the von Bertalanffy growth models were fitted to data from three specimens for Jetty Pond and sixteen specimens for Sandpiper Pond. Back-calculation of these fish and use of multiple interpretations per fish were used to increase the number of data points. These strategies were used as a proxy for larger sample size, though not remotely as accurate. A large portion of the variation of length at age is due to differences in reader interpretation rather than differences in growth rate. Another unaccountable downfall of using back-calculation is the often overestimation of previous length-at-age. This overestimation of young fish may have caused the von Bertalanffy to have a slow curvature and not reach an asymptotic length within the first ten years of age for both Jetty and Sandpiper Pond. All these dilemmas are associated with the extremely small sample sizes of collected specimens. If the sample size of the study were increased it is likely there would be no more *M. cephalus* in Sandpiper Pond or Jetty Pond, though this isolated extinction will likely occur in the near future if recruitment to the area ceases.

## CONCLUSION:

*Mugil cephalus* in Sandpiper Pond and Jetty Pond are incapable of reproducing due to isolation and lack of offshore migration required for egg maturation. The growth rate of both isolated ponds were affected by environmental conditions though in opposite directions. Sandpiper Pond *M. cephalus* showed reduced growth rates due to strenuous osmoregulatory demands, while Jetty Pond *M. cephalus* exhibited increased growth due to favorable water circulation and reallocation of energy away from gamete production. Environmental factors may play an important role in determining growth rate of *Mugil cephalus*, but sampling technique and sample size may influence the von Bertalanffy growth approximation equation.


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