

INTERANNUAL VARIABILITY IN MORPHOMETRIC CONDITION OF LARVAL FISH  
AS A FUNCTION OF ENVIRONMENTAL VARIABLES IN BEAUFORT, NC

By

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Recruitment of marine fishes can vary by several orders of magnitude and can be unpredictable from year-to-year, with its variability influenced by oceanic conditions experienced by fish larvae. Variations in larval abundance and condition have not been consistently related to juvenile abundance and recruitment for many fisheries species. By studying how environmental factors affect larval fish condition, we may be able to better anticipate when a high or low recruitment year will occur since condition affects larval fish growth and survival. This project aims to expand our understanding of the relationship between morphometric condition, environmental conditions, and prey abundance among four North Carolinian fishes: Atlantic Croaker (*Micropogonias undulatus*), Mojarra (*Eucinostomus* sp.), White Mullet (*Mugil curema*), and Broad Striped Anchovy (*Anchoa hepsetus*). All species had some aspect of their morphometric condition influenced by an environmental or ecological variable. When reviewing the dominant model of variability in morphometric condition, temperature and density dependent effects were widespread, affecting the condition of three out of four species. Zooplankton effects were less common when examining the dominant mode of

variability, affecting two out of four species. Interactions between temperature and zooplankton, while individually important for multiple species, was only significant for one species. The relationship between environmental variables and morphometric condition can lead to insight about the species dynamics that was not previously known. Future findings like these could potentially lead to the development of better recruitment models.



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By

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## CHAPTER 1: Literature Review

### 1.1 *Recruitment and Larval Fishes*

In the field of marine science, recruitment has several definitions. In fisheries, the definition used most often to describe recruitment is the number of young fish from a single year-class that enter a fishery, which is affected by the gear type used to sample a species (Ferron & Leggett 1994). Another definition occasionally used among benthic ecologists is when a fish or benthic organism metamorphosizes from its planktonic larval stage and settles into its benthic habitat for its next life history stage (Allen & Barker 1990).

Why study recruitment and larval fishes? Key ideas related to fisheries recruitment were developed in the early 20<sup>th</sup> century. In early fisheries biology and oceanography, it was thought that variations in fish stock abundance were caused by fish migration along the coasts (Sinclair et al. 1997, Llopiz et al. 2014). This changed when Johan Hjort, a Norwegian scientist, began to research variations in fish stocks around northern Europe by examining larval fishes (Hjort 1926, Houde 2008). While Hjort was studying these fish stocks and their larvae, he developed two major hypotheses that could help explain some of the variations in year class size. The first hypothesis was entitled the “critical period” hypothesis. This hypothesis states that the first-feeding stage, when larvae must first capture their own food and do not rely solely on endogenous yolk energy, is one of the most important times in a fish’s life cycle that can be a major bottleneck in the fish’s survival to the next life history stage. The second hypothesis is called the “aberrant drift” hypothesis, which addresses how larvae disperse to new habitats (Hjort 1926, Llopiz et al. 2014). For many species, this planktonic larval dispersal is the primary agent that connects populations, supplies recruits to specific regions, and through which fish colonize new habitats (Bashevkin et al. 2020). Thus, these two hypotheses provide a foundation to

examine how fish larval dynamics affect recruitment to the adult fish stock, since their survival directly affects the abundance and distribution of adult fishes. Although there may be other processes that can contribute to the survival of larval fishes, these hypotheses about larval recruitment provide a framework by which scientists can examine fluctuations in fish stocks (Pepin, 2004).

Food availability related to the critical period hypothesis and environmental conditions experienced during larval dispersal related to the aberrant drift hypothesis are both likely to influence the morphological condition of larvae in North Carolina and elsewhere. As a result, this set of hypotheses forms a framework that I use to assess common themes that can affect larval survival, abundance, and geographic distribution. These are food availability, environmental variables, growth rate, ocean retention zones, density dependent mortality, and predation. These themes will be described in further detail below. I will also touch on indices of larval fish condition that were used in several of these papers to describe one or more of the variables influencing recruitment. The background information presented in this literature review provides a foundation for understanding the research presented in Chapter 2 of my thesis.

### *1.2 Environmental and Biological Variables that Affect Fish Condition*

It can be complicated to make predictions about fisheries recruitment to inform estimates of fish stock size for future generations. What makes the process of recruitment so hard to predict? Many biological and physical factors can interact to affect the growth and mortality of larval fishes during the first year of life (Fogarty et al. 1991). Starvation, predation, and environmental variables have been identified as the major causes of larval mortality (Hjort 1926, Blaxter & Ehrlich 1974, Hoey & McCormick 2004). Predation is most likely the ultimate cause



of mortality but it is sometimes left out of many recruitment analyses (Bailey & Houde 1989). This is due to the difficulty of measuring predation in field experiments (Litvak & Leggett, 1992). Predation experiments can be mimicked in the lab or in mesocosms to control environmental variability (Cowan & Houde 1990). Even with these predation studies, adding accurate information on spatial and temporal variations in potential larval fish predators that influence natural mortality rates to a recruitment model is extremely hard.

One of the most prevalent topics in this literature review pertains to food availability to larvae. I hypothesize that larvae that experience high food concentrations will consume a greater volume of prey, thus increasing their condition and chances of survival. For instance, Frank and Leggett (1986) looked at how food size and abundance affected larval capelin in a mesocosm experiment. In this experiment, they noticed that first feeding larvae fed on zooplankton from the 30-200- $\mu\text{m}$  size range. Other conclusions derived from this study were that the average prey size was about 13-38% of the larvae's gape and zooplankton were consumed in proportion to availability (Frank & Leggett 1986). This might inform us that larvae feed more when more food is available. Secondly, we learn from this study that larvae have food size preferences, which can encompass several different prey items (Mayer & Wahl 1997). Thus, care must be taken in identifying and quantifying the potential prey field for fish larvae from planktonic sampling.

With greater feeding success, there is generally an expected increase of condition (Knutsen & Tilseth, 1985). Availability of prey to larvae seemed to play an important role. When prey availability was high, prey preferences start to emerge (Knutsen & Tilseth, 1985). Preferences may be for the more nutritional prey item, but larvae may also be limited by gape size and ability to capture prey (Makrakis et al. 2008). It is important to restate that abundant prey of the right size and species is hypothesized to lead to greater measures of condition of fish

larvae, which might be used to predict survival reliably under a given food regime and ultimately used as an early predictor of recruitment (Frank & McRuer, 1989).

Food availability experiments can inform us on how phytoplankton and zooplankton blooms may influence food concentration in the ocean. Phytoplankton serve as the primary food source for zooplankton. Their abundance can influence zooplankton growth and reproduction (Behrenfeld and Boss 2014). In nutrient-rich regions with favorable conditions, higher phytoplankton productivity can provide more food resources, leading to increased zooplankton abundance and potentially to increases in organisms higher on the food chain. This relationship is often described as the bottom-up control of planktonic ecosystems, where phytoplankton biomass influences higher trophic levels (Hirst and Bunker 2003).

A fundamental hypothesis related to this topic was developed by D.H. Cushing, an English scientist. This hypothesis, which is referred to as the match/mismatch hypothesis, states that the degree of synchronicity in the seasonal timing of larval production and the production of their food will explain part of the variability in recruitment of fish to the adult stock (Cushing et al. 1990). Without the larvae and the prey occurring at the same time in a particular area, then there can be repercussions related to fish reproduction. If prey availability occurs too early or too late relative to larval abundance, then this would lead to poor fish condition. This is an example of phenological variation and can be affected by many environmental variables to which the larvae are exposed and react while they are progressing throughout larval stages (Portner & Peck. 2010, Gotceitas et al. 1996).

An example of this is a study on haddock in Nova Scotia conducted by Platt et al. (2003). They found that larval production during earlier spring blooms would yield higher recruitment among haddock. By using remote sensing and a long-term fisheries dataset, they determined that

phytoplankton blooms were able to account for 89% of the variation in larval survival between years (Platt et al. 2003). Further, spring phytoplankton blooms that occur earlier in the year increased survival of larval haddock. You can also see this phenological effect in work by Drolet et al. (1991). In the sub-arctic, algae forms on the bottom of sea ice. When the ice melts and cracks, there are phytoplankton blooms due to increased light availability for photosynthesis. Larvae that can feed on ice-associated algae and phytoplankton had higher chances of survival than those species that fed on phytoplankton alone (Drolet et al. 1991). However, not all studies have shown that the spring phytoplankton bloom are correlated to larval survival. Friedland et al. (2008) found that the fall phytoplankton bloom had a higher impact on parental condition, which indirectly affected larval survival through reproductive investment. This gives adults more nutrients to pass to larvae to increase survival during the yolk sac stage (Friedland et al. 2008). In all these field studies and experiments described above, the biggest take away is that the concentration and availability of prey is important to larval fish survival, but studies must also incorporate information on prey taxa, size, and timing to fully grasp how food availability affects fish larvae as accurately as possible.

Larval survival has also been shown to be dependent on growth rate. Growth rate is interrelated with food availability and other environmental factors. This gave rise to the “bigger is better” hypothesis, stating that the faster and bigger a larva can grow, the more advantageous it is for survival (Litvak & Leggett 1992). The longer a larva stays in each early life history stage, the more susceptible it is to predation when mortality is analyzed cumulatively across life history stages. Fontes et al. (2011) looked at how fish growth rate in rainbow wrasse was correlated with size at settlement, size at age, and pelagic larval duration. Pelagic larval duration explained most of the variation in size at settlement in rainbow wrasse (Fontes et al. 2011).

Pelagic larval duration was also slightly correlated with recruitment magnitude. This research showed that early growth in larvae should have a large effect on survival. Houde (1997) described the influences of mortality and growth on larval survival across several life stages. His studies showed how size can be important for avoiding predation in different life stages and how rapid growth can be advantageous (Houde 1997). Feeding success was seen to be the reason for faster growth rates due to environmental factors and behavior of larvae (Robert et al. 2014). However, growth rates can be biased when rates are only taken from larvae that are captured. Scientists can only measure what they collect, and healthy larvae may be able to avoid capture, leaving less healthy larvae to be analyzed.

Environmental variables, such as wind speed and direction, salinity, and temperature in ocean systems, also affect larval recruitment (Miller et al. 1988, Cushing et al. 1990, Sponaugle et al. 2006). From field observations, Frank and Leggett (1986) noted that onshore winds at Bryant's Cove, a major spawning site for capelin in Conception Bay, Newfoundland, advected smaller zooplankton. In contrast, offshore winds transported larger zooplankton. Thus, onshore winds resulted in reduced fish growth because smaller zooplankton occurred during those times (Frank & Leggett 1986). Allen & Barker (1990) showed that, among Atlantic croaker and pinfish, salinity plays a part in larval recruitment in estuaries where upstream, fresher water had a higher abundance of juveniles than downstream waters (Allen & Barker 1990). Depending on their tolerance range for salinity, these results probably vary among species.

All species of larvae have an optimal thermal range for survival (Koenker et al. 2018). Unless greenhouse gas emissions are drastically reduced, winter bottom temperatures in the Southeast US region may rise by up to 2-3°C by the end of the century (Grieve et al. 2016). Analyzing potential climate change consequences requires an understanding of probable links

between water temperature and the life cycles of fish in the area. In several studies, temperature has been shown to affect recruitment. There is a high degree of variability in temperature at the study site but the regularity in arrival time of these fishes to estuaries as juveniles was quite surprising; result suggests temperature-driven seasonality has an influence on estuarine ingress (Allen & Barker 1990, Thaxton et al. 2020). Temperature explained 60% of the variation in spring and winter arrival time of spawning fishes in the Plymouth Sound in the Western English Channel (Genner et al. 2010). Temperature also significantly affected larval occurrence causing variations of several days or even weeks in seasonal occurrence (Genner et al. 2010).

The environmental variables previously described are some of the more important ones when it comes to determining fish body condition, survival, and recruitment. Nonetheless, several studies examined other unique variables that are worth describing. For example, retention zones are areas of mixing waters or bodies of waters that hold larvae for an extended period of time within a favorable area. Retention zones can be studied with biophysical models, remote sensing, and field data (Cury et al 2008). Mesoscale eddies and larval retention zones have been shown to be positively correlated to larval recruitment (Cury et al. 2008). Shoji et al. (2005) conducted a field experiment in Chesapeake Bay which examined estuarine turbidity zones (ETZ) and the feeding success of larval fish. Outcomes from this experiment show that the ETZ influenced retention of larvae within optimal feeding habitat. A decrease in larval feeding success was influenced by the overlap between larval distribution and temperature and salinity ranges tolerated by larvae in this area of the Chesapeake. This lead to good prey concentration and refuge from predators. Highly mixed areas allow for organic matter to be dispersed through the ETZ giving rise to more planktonic prey for larval fishes (Shoji et al 2005). Shoji et al. (2005) also noticed that, since the water was turbid, larval fishes that reside in this area fed as

well during the night as during the day, probably due to a lack of vision in this zone at all times (Shoji et al. 2005).

If larval mortality is high and recruitment is low, the population of a fish stock is likely to decline. Poor recruitment success coupled with overfishing can also lead to stock collapse. Low stock size can influence the recruitment of larval fish because there will be less fecund adults that spawn each year leading to less eggs produced (Shepard & Cushing 1980). Mean weight of larval fish has been shown to increase with decreasing stock size likely due to density-dependent effects; in other words, a reduction in intraspecific competition can improve fish condition (Shepard & Cushing 1980). With less larvae to compete with in a year class for resources, then the more food there will be for others. Predation on larval fish can also be density-dependent, with increased larval survival occurring at low larval densities (Shepard & Cushing 1980). Generally, as larvae grow the likelihood of being captured by a predator decreases while the availability to food increases with less competition and stronger prey capture abilities.

### *1.3 Pros and Cons of Different Ways to Measure Morphometric Condition*

Fish condition has been studied for several decades. This has led to multiple types of measurements that describe fish body condition. Some of the biological characteristics that describe body condition are robustness, health, seafood nutritional composition, meat yield rates, evolutionary fitness, and reproductive potential (Bolin et al. 2021). Traditionally, fish condition has been measured by assessing their robustness via morphological measurements, often using a length-verses-width or a length-verses-weight index. In more recent decades, there have been histological and biochemical measurements of condition, such as lipid percentage and RNA/DNA ratios (Ferron & Leggett 1994). In the latter measurements, the quantity of DNA per

cell is believed to be a species constant. In contrast, the quantity of RNA varies with the rate of protein synthesis. Since growth in fish is mainly accomplished through protein synthesis, the RNA/DNA ratio has been proposed as an index of growth rate in adult fishes (Bulow 1970, Haines 1973). While these measurements are more sophisticated techniques for determining the fish condition, they also require a lot of time, preservation, special handling and equipment, and funds for sample processing. Therefore, there continues to be a need for less demanding methodology using body measurements to analyze fish condition (Koslow et al. 1985).

Alternatively, use of multiple methods can be beneficial since they are often complementary. Sometimes the choice of method might depend on sample size and available research funding.

Many studies examining condition consist of measurements of length verses weight, which is known as Fulton's K index (Fulton 1926, Nash et al. 2006). This index shows that, when fish weigh more at a particular length, they are generally in better condition (Blaxter 1971, Neilson et al. 1986, McCormick & Molony 1995, Hoey & McCormick 2004). However, a critique of Fulton's index is that during starvation some fish species, such as plaice (*Clupea harengus*), simultaneously lost both length and weight. This implies that Fulton's K may not always fully capture deterioration in body condition (Blaxter & Ehrlich 1974, Ehrlich 1974, Ferron & Leggett 1994).

There have been several studies that examine other ratios between body parts of larval fish to see if there is any relationship to condition. In one morphometric condition study examining length, height, and length/height ratios in lab experiments, the larval fish condition was shown to vary compared to the ration of food given to the fish larvae (Wyatt 1972). Another example of alternate condition measurements is the ratio of gut height and myotome (a group or segment of muscles in the body that is innervated by a specific spinal nerve) height, which was

useful for determining differences between fed and starved specimens before the point of irreversible starvation (Yin & Blaxter 1986). Among larvae in poor condition, the digestive tract is often incompletely developed. Later, as the yolk was resorbed by larvae in this study, the body height decreased, whereas the gut height increased rapidly. This is because the further development of the digestive tract indicated increased condition (Yin & Blaxter 1986). These authors also reported the total length/body height and eye height/head height ratios to be effective for categorizing nutritional status (Yin & Blaxter 1986).

Theilacker (1978) was one of the first studies to use body condition ratios in which the weight, width, or body height of an individual was compared to a standardized body measurement, usually related to length. Here the numerator is a body measurement that may be affected by starvation and the denominator is a given length measurement. As fish grow and proceed through larval development, their relative girth will be affected. Thus, you must standardize width or body height-based measurements using a measurement of length. Theilacker (1978) also used multivariate stepwise discriminant analysis (SDA) to look at several variables that describe nutritional status. Over time, other multivariate techniques have also gained traction for analyzing multiple indicators of variations in larval condition simultaneously.

Several studies followed the Theilacker (1978) approach and showed that body depth at the anal and pectoral fins were highly important when determining condition (Powell & Chester 1985, McGurk 1985). Around the same time, Koslow (1985) used residuals from linear regression as input into a principal component analysis (PCA) to examine body condition in cod. At the time, this analysis showed no difference from the alternative approach of using stepwise discriminant analysis.



Morphometric condition has also been cross-checked with other physiological indicators of fish condition, such as triacylglycerol content (Lochmann & Ludwig, 2003). In this case, these two indicators of condition were comparable, suggesting that morphometric condition can provide accurate proxies for energy content in cases where it is not possible to conduct detailed physiological assays.

More recently, morphometric condition has been used to determine condition of larvae from the Deep Water Horizon Oil Spill (DWHOS) in the Gulf of Mexico. Ransom et al. (2016) studied the condition of Spanish mackerel and noticed that this species was resilient to the oil spill in terms of abundance. However, before the spill, morphometric condition of mackerel was much greater compared to condition after the spill (Ransom et al. 2016). In another example of recent use of morphometric condition, Axler et al. (2020) looked at similar condition indices and otolith microstructure of sand seatrout and striped anchovy. Larvae captured in the Mississippi River plume were in poorer morphometric condition (skinnier at length) than their counterparts caught on the continental shelf, even though there were higher concentrations of zooplankton prey in plume water masses. Taken together, these results suggest that elevated prey concentrations do not necessarily translate to higher growth and condition all the time. In the above example, high turbulence and turbidity within the plume may have physically inhibited the prey capture ability and feeding success of fish larvae (Axler et al. 2020).

#### *1.4 Thesis Study Site and the Surrounding Estuary*

In this study, I will be focusing on Beaufort Inlet, North Carolina for my thesis research. Beaufort Inlet is located near the northern boundary of the South Atlantic Bight (SAB), which encompasses the continental shelf from West Palm Beach, Florida to Cape Hatteras, North

Carolina. (Epifanio and Garvine 2001; Figure 1). This inlet connects the Newport River and North River Estuaries to the ocean. The majority of the ichthyoplankton community found in the Newport River Estuary originates from spawning in and around Onslow Bay, an embayment bounded by Cape Fear, Cape Lookout, and the shelf break (Epifanio and Garvine 2001). Beaufort Inlet contains the longest-running, continuous time series of ichthyoplankton collections on the East Coast of the United States (Ortner et al. 1999; Thaxton et al. 2020).

Beaufort Inlet is the main pathway for larval fish to reach the estuarine nurseries of Newport River, Bogue Sound, North River, and Back Sound, which are connected to the much larger Albemarle-Pamlico Estuarine System via Core Sound. The inlet is connected to the estuarine systems lying west of the inlet by a regularly dredged navigation canal that begins at the Morehead City port and runs 3.5 kilometers offshore (Churchill et al. 1999a). Shackleford Channel, Bird Shoal Channel, and Bulkhead Channel (also called Radio Island Channel) are three additional channels that branch off the Morehead City Channel and direct water north and east from the inlet, while the Morehead City Channel transports water west of Beaufort Inlet (Churchill et al. 1999a). The tidal exchange of the system's estuaries is controlled by these channels. The majority of flow entering the inlet on flood tide flows to the east through Shackleford Channel, while the majority of flow exiting the inlet passes to the west via Morehead City Channel, according to studies of the inlet's water circulation (Churchill et al. 1999a). As a result, the largest populations of larval fish pass through the entrance to the inlet from the east, with concentrations diminishing westward (Hettler & Hare 1998, Churchill et al. 1999b). Since the species composition of larval fishes is mostly consistent across the inlet, sampling in any of the channels is expected to be representative of the community that entered the inlet (Hettler & Hare 1998). The larvae numbers inside and outside the inlet were shown to

be correlated with each other, suggesting that sampling within the inlet may also provide information on the ichthyoplankton community retained at the inlet mouth (Hettler & Hare 1998). Most larvae that are spawned offshore in Onslow Bay are thought to enter estuaries by the process of selective tidal stream transport (Forward et al. 1999), which is when plankton ascend in the water column during flood tides and descend during ebb tides. The majority of fish larvae at Beaufort Inlet are collected during nighttime flood tides (Hettler et al. 1997, Forward et al. 1999). Night-time ichthyoplankton collection is important because the larvae come to the surface to feed on zooplankton at night and use the cover of darkness to avoid predators. Ichthyoplankton sampling is most effective when done at night at the flood tide's peak flow, since this increases the chances of catching larvae that use selective tidal stream transport.

The Newport River estuary is a dynamic environment influenced by various factors, including tidal cycles, temperature variations, and nutrient inputs (Hall et al. 2003). In the early spring (March to May), water temperatures begin to rise as the weather warms. Increased solar radiation promotes the growth of phytoplankton and other primary producers. Tidal dynamics and mixing contribute to the transport of nutrients and organic matter within the estuary (Hall et al. 2003). As the year progresses into summer (June to August), water temperatures are at their highest and phytoplankton blooms continue, leading to periodic blooms in primary production. Increased temperature leads to increased metabolic rates in aquatic, ectothermic organisms. Tidal exchanges continue to influence water circulation and nutrient transport. As fall (September to November) begins, water temperatures gradually decrease. Phytoplankton populations start to decline as sunlight decreases and nutrient availability may be reduced (Morse et al 2014). Some species of zooplankton peak in abundance and certain species of fish may still be active and reproducing (Mallin, 1991). This is until winter (December to February) when water

temperatures are at their lowest during the winter months. Reduced sunlight and lower temperatures lead to decreased primary production. Zooplankton populations may be reduced as a result of lower food availability (Vanni, 1987). Therefore, some fish species may move from estuarine habitats to deeper waters with more stable conditions due to warmer offshore water.

### 1.5 Life History Characteristic of Target Species

Species selected for this study were those that occurred at high abundances as larvae during the period of May through October at Beaufort Inlet. These include Atlantic Croaker (*Micropogonias undulatus*), Mojarra (*Eucinostomus* spp.), White Mullet (*Mugil curema*), and Broad-Striped Anchovy (*Anchoa hepsetus*). Table 1 summarizes key characteristics of these species, which are elaborated upon below.

Atlantic Croaker (*Micropogonias undulatus*) is a species of fish found across the western Atlantic Ocean, ranging from Massachusetts in the United States to the Gulf of Mexico and south to Brazil (Chao & Musick, 1977). It belongs to the family Sciaenidae, which is known for its drumming or croaking sounds produced by specialized muscles against their swim bladder. The Atlantic Croaker is a commercially important species, supporting both recreational and commercial fisheries. Atlantic Croaker has an elongated and slightly compressed body with a silver-white belly and a dusky to silvery-blue coloration on its upper sides. The species exhibits sexual dimorphism, with males generally being larger than females (Hill et al, 1987). The average length of an adult Atlantic Croaker is between 12 and 18 inches (30 to 46 centimeters). The Atlantic Croaker is primarily a coastal species and is commonly found in estuaries, bays, and nearshore waters with sandy or muddy bottoms (Hansen, 1969). Atlantic Croakers are opportunistic benthic feeders, consuming a diverse diet that includes crustaceans, mollusks,

small fish, and polychaete worms (Hansen, 1969). They use their specialized chin barbels, which are sensitive to touch, to locate and capture prey buried in the sediment. They prefer areas with submerged vegetation, oyster reefs, and rocky structures. Eggs are primarily spawned offshore during the migration. Eggs hatch into yolk-sac larvae in about two days; yolk-sac larvae initiate exogenous feeding and become feeding larva after about 4–5 days. These larvae feed on zooplankton (Govoni et al. 1986) and depend mostly on passive transport mechanisms to carry them inshore to their estuarine nursery areas (Miller et al. 1984). After 30–60 days, the larvae attain 7–12 mm in body length and arrive at the estuary (Warlen & Burke 1982, Nixon & Jones 1997), where they become estuarine larvae. Estuarine larvae transit through the estuaries to reach the primary nursery areas (shallow, brackish creeks and marshes). At about 20 mm in length estuarine larvae become early juveniles.

Hare and Able (2007) identified a correlation between Atlantic Croaker abundance and winter temperatures across many locations along the East Coast, and have studied thermal tolerances of juveniles. Their hypothesis was that winter temperature variability controls Atlantic Croaker population dynamics. Hare and Able (2007) suggest that year-class strength is decoupled from larval supply and is determined by temperature-linked, overwintering survival of juveniles. Morley et al. (2017) also observed that landings were positively related to winter temperatures for Atlantic Croaker and other important commercial species, showing there is a lower abundance following winters that approached their lower thermal tolerance range. Searcy et al. (2007) found juvenile and larval croaker growth decreased in association with high levels of freshwater runoff that displaced juvenile croaker from nurseries to downstream habitats, in which they had reduced feeding success. Therefore, larval and juvenile croakers seem to be

sensitive to low winter temperatures and riverine flow rates within nursery habitats. Under these conditions, it might be more difficult for Atlantic Croaker to capture food.

The genus *Eucinostomus* is commonly known as Mojarra or Mojarritas. This genus belongs to the family Gerreidae and is found in coastal and estuarine waters of the western Atlantic Ocean and the Caribbean Sea. The genus *Eucinostomus* is a group of fish primarily found in tropical and subtropical coastal waters. They are known for their slender bodies and silvery coloration. *Eucinostomus* spp. are commonly found in coastal and estuarine habitats, including sandy or muddy bottoms, seagrass beds, and mangrove areas. They prefer shallow, warm waters and are often encountered in estuaries and nearshore environments. Mojarra in the genus *Eucinostomus* are omnivorous, meaning they have a varied diet that principally includes small, benthic invertebrates, such as isopods, copepods, and polychaetes (Robins & Ray 1986, Gning et al. 2010).

Previous studies in Brazil have shown that *Eucinostomus* spp. exhibit environmental preferences, with the larvae and juveniles of *E. argenteus* and *E. gula* positively correlated with smaller waves and smaller morphodynamism (Herrera-Reveles et al. 2012). Morphodynamics is the process by which morphology affects hydrodynamics in such a way as to influence the further evolution of the morphology itself (Friedrichs 2011). Other Brazilian species prefer periods with lower temperatures and larger morphodynamism (Herrera-Reveles et al. 2012). In another study, the range in the critical thermal minimum was low (1°C in most species); only *Thalassoma lucasanum* and *Eucinostomus gracilis* presented ranges over 4°C (Mora & Ospina 2002). There was another study examining the critical thermal maximum that shows *Eucinostomus* spp. has a thermal temperature mean of about 38°C (Mora & Ospina 2001). Another environmental influence on Mojarra is that their egg densities decreased from the

estuarine mouth to the upstream areas following a salinity increase. This pattern was also found when examining the density of larvae, which decreased in estuarine regions with hypersalinity (Junior et al. 2023). The low diversity group consisted of only nine fish species, which included two of my study species, *Anchoa hepsetus* and *Eucinostomus* spp. Junior et al. (2023) observed within this assemblage that stress-tolerant *Eucinostomus* spp. and Broad Striped Anchovies can withstand this hypersaline estuarine ecosystem and allow for them to make up a large part of the assemblage. Along with tolerance to a wide temperature range, the ability to withstand hypersaline conditions makes these Mojarra capable of greater dispersal and survival.

*Anchoa hepsetus*, commonly known as the Scaled Anchovy or Broad-Striped Anchovy, is a species of small forage fish. *Anchoa hepsetus* is a member of the Engraulidae family, which includes small, slender, and silvery fishes commonly known as Anchovies, and falls under the order Clupeiformes, which also includes herring, sardines, and other related fishes. The maximum reported size of *Anchoa hepsetus* is around 12 centimeters in length.

The Broad-Striped Anchovy is widely distributed in the western Atlantic Ocean, ranging from Massachusetts, USA, to southern Brazil, including the Gulf of Mexico and the Caribbean Sea. It inhabits nearshore and inshore waters, often found in estuaries, lagoons, and shallow coastal areas with sandy or muddy bottoms. *Anchoa hepsetus* is primarily a planktivorous species, feeding on small zooplankton and phytoplankton. They use their specialized gill rakers to filter tiny prey from the water column. Anchovies play an essential role in marine ecosystems as an important prey species for larger predators, such as larger fish, seabirds, and marine mammals (Pikitch et al. 2014). They are also commercially important for fisheries in some regions, where they are caught for use as bait or for processing into fishmeal and fish oil.

Previous studies done on Anchovies showed that larvae are tolerant of a wide range of salinity conditions (Junior et al. 2023). In the Mid-Atlantic Bight, it was shown that this subtropical species of Anchovy was the most abundant in a fish assemblage that was studied (Levesque 2019). Assemblage studies have shown that *Anchoa hepsetus* is abundant during the summer months, while *Anchoa mitchilli* tends to be more frequently observed in the spring and fall (Hagan & Able 2003). A similar distinction between the seasonal abundance of *A. hepsetus* and *A. mitchilli* larvae is also observed at my study in Beaufort Inlet (Hettler and Chester 1990).

*Mugil curema*, commonly known as the White Mullet, is a species of fish in the Mugilidae family. The Mugilidae family includes fish known for their distinctive body shape, with a slender, elongated body, and a deeply forked tail. This species can grow up to approximately 38-46 centimeters in length. *Mugil curema* is commonly found in both marine and estuarine environments along the western Atlantic Ocean, ranging from the southeastern coast of the United States (including Florida) to South America, including the Gulf of Mexico and the Caribbean Sea. They are euryhaline, meaning they can tolerate a wide range of salinities, allowing them to move between freshwater, brackish, and marine habitats. White Mullet are primarily herbivorous, feeding on algae, detritus, and other plant material. They are well-adapted to grazing on submerged vegetation and play an essential role in the coastal ecosystem by helping control algal growth and nutrient cycling. A number of environmental factors influence the spatio-temporal distribution of White Mullet. Critical thermal minimum of *Mugil curema* is 10.8°C on average across individuals (Mora & Ospina 2002). The critical thermal maximum of *Mugil curema* is 40.8 °C (Mora & Ospina 2001). White Mullet occur during all seasons in the South Atlantic Bight (Collins et al 1989). In Venezuela, the relationship between the timing of successful spawning and coastal recruitment in White Mullet was likely due to variations in food



availability for first-feeding larvae, as well as to variations in the duration of the transport of larvae shoreward as a result of currents associated with upwelling (Marin et al. 2003).

### *1.6 Overview of Research Objectives*

Following the literature review presented in this chapter, the second chapter of this thesis examines larval morphometric condition of the four species described above that were caught between May and October of 2017-2019 in Beaufort Inlet, North Carolina. In this chapter, I will compare body condition to potential environmental factors. The following research hypotheses are addressed:

- There will be interannual and seasonal differences in body condition of fish larvae.
- If there is a larger zooplankton displacement volume, then there is more food for fish larvae leading to better body condition.
- If temperatures are outside of a species' thermal tolerance zone, there will be a decline in fish body condition.
- With higher larval density of a given species, there will be lower morphometric condition due to intra-specific competition.

## CHAPTER 2: FISH CONDITION OF ATLANTIC CROAKER, WHITE MULLET, BROAD STRIPED ANCHOVY, AND MOJARRA IN BEAUFORT, NORTH CAROLINA AS A FUNCTION OF ENVIRONMENTAL VARIABLES

### 2.1 *Introduction*

Fisheries recruitment is influenced by a variety of factors, including environmental conditions, stock size, and the biology of the fish species in question. Understanding recruitment is essential for fisheries managers to set sustainable catch limits and to conserve fish populations (Houde, 1987). Stock-recruitment models have been developed to help predict and understand recruitment patterns and how they influence fluctuations in population size. Some of these models also attempt to identify the factors that influence recruitment and how they interact. Environmental variables can have a significant impact on the recruitment of larval fish. One of the most important factors affecting recruitment is oceanographic conditions, which can influence the dispersal and survival of larval fish. For example, ocean currents can transport larvae away from their spawning grounds or into areas with unfavorable conditions (Houde 1987; Cowen and Sponaugle 2009; Planes et al. 2009). In addition, temperature can affect the rate of development and growth of larval fish (Pepin 1991; Herbing 2002; Green & Fisher 2004; Llopiz et al. 2014). Other environmental variables that can affect larval fish recruitment include prey availability and predation. Prey availability is important for larval fish survival and growth, and changes in prey availability can affect recruitment success (Munk et al. 2010). Predation can also be a significant source of mortality for larval fish, and predator abundance and behavior can influence recruitment patterns (Shulman 1985; Houde 1987; Anderson 1988; Bailey 1994; Robert et al. 2023). Competition for resources in a changing environment has also been known to effect recruitment dynamics through density dependent processes (Taylor et al. 2009).

The morphometric condition of larval fish can also influence recruitment success. Morphometric condition refers to the overall health and physical condition of the fish as reflected by their body size and shape. Fish in better morphometric condition are more likely to survive and grow and, therefore, have higher recruitment success (Auer et al. 2015). However, the factors that influence morphometric condition can vary between fish species and populations.

In this chapter, I will explore how variations in morphometric condition among four fish species from Beaufort Inlet, NC are affected by environmental factors including temperature, zooplankton concentration, and density dependence. I hypothesize that during periods of the year when temperatures are increasing, there will be a decrease in morphometric condition in each species, especially if temperature exceeds a species' thermal tolerance range. It is expected that, with high zooplankton displacement volume, larval fishes may be in better body condition since more food is desirable for these larval fishes to progress through life history stages. Also, if there is a high density of fish in a cohort, this may have a negative effect on condition, that is potentially due to competition. Testing these hypotheses will provide information that can potentially be used in models of fisheries recruitment. Ideally, models that include information on the morphometric condition of a larval cohort and environmental influences will be more effective at predicting the future of the adult stock. This research is novel because it occurred at a long-term sampling station. Further, I examined larval body condition over multiple years and with multiple species.

## 2.2 Methods

### Sample Collection

Samples were collected from May to October in 2017-2019 by members of the Asch Fisheries Oceanography Lab at East Carolina University (ECU) and the National Oceanic and Atmospheric Administration (NOAA) lab in Beaufort, NC through the Beaufort Inlet Ichthyoplankton Sampling Program (BIISP). Each week members of these labs deployed a 200-micron mesh net with a 0.196 m<sup>2</sup> mouth size, twice for three minutes to collect mesozooplankton. Also, a 1,000-micron mesh neuston net with a 1.76 m<sup>2</sup> mouth size was deployed four times each for a five-minute duration to collect ichthyoplankton. Samples were collected from a bridge-platform suspended over the water surface sampling the surface of the water column. Sampling was timed to coincide with nighttime flood tides, starting 2.5 hours prior to the peak of the flood tide. General Oceanics low flow meters were used to quantify the volume of seawater sampled. The zooplankton samples that were collected were stored in 5% formalin and ichthyoplankton samples were placed in  $\geq 90\%$  ethanol to preserve the fishes.

Several environmental variables were collected using a YSI EXO1 water quality sensor while the nets were deployed. For this study, only temperature and chlorophyll *a* data from the YSI will be used to test hypotheses. YSI measurements were taken at the surface and every meter down to a depth of six meters. Samples were then brought back to the lab and sorted by Asch lab members to separate zooplankton and ichthyoplankton. After sorting, the fishes were identified to the lowest possible taxonomic level, mainly using information from Richards (2006) and Fahay (2007).

Larval fish species examined to assess morphometric condition were selected based on the species composition of the summer 2017 samples described above. We focused on the most

frequently caught species to ensure a sufficient sample size for each species and to guarantee that fishes were caught across a diversity of environmental conditions observed. The four target larval fish species that were selected for this study were Atlantic Croaker (*Micropogonias undulatus*), Mojarra (*Eucinostomus* sp.), White Mullet (*Mugil curema*), and Broad-Striped Anchovy (*Anchoa hepsetus*).

### Zooplankton Measurement

Once the ichthyoplankton samples were processed and each species of larval fish was identified, the volume of zooplankton was measured in each sample that contained one of the target species. All the zooplankton samples that were preserved in 5% formalin were transferred to 70% ethanol prior to taking measurements. Samples were also washed with 1,000 mL of water to remove any remaining formalin.

Mesozooplankton abundance is often characterized as the number of individuals in a sample but, for this analysis, zooplankton displacement volume measurements were used. This measurement was used because of its ability to assess a large quantity of zooplankton quickly. Also, zooplankton volume is related to the amount of zooplankton available to a larvae on a given date, assuming that the larvae could potentially prey on all types of zooplankton. Zooplankton displacement volume was measured by pouring samples into a graduated cylinder, recording the sample volume in milliliters, straining off the zooplankton through a 212-micron sieve into another graduated cylinder, and recording the remaining volume of ethanol in milliliters (Kramer et al. 1972). The difference in the two volume measurements recorded is the zooplankton volume in each sample. This volume of zooplankton was then standardized by the volume of seawater filtered during each plankton tow, which was calculated based on data from

the General Oceanics low flow meter. Since there were two zooplankton samples for each day of data collection, an average was taken of each sample for a particular day.

### Photographing and Measuring Larval Morphometric Features

To measure morphometric condition, photographs of individual larval fishes were taken on a Zeiss 508 dissecting microscope with a Lumenera Infinity 4 camera, which was positioned for a full view of a larval fish on a stage micrometer. During this process, a live image is presented on the computer screen connected to the Infinity camera. The slide was secured with tape to the microscope stage. Calibration photos of the stage micrometer were taken for each larvae to keep calibration consistent on each date when measurements were made. Infinity Capture software allowed adjustments to be made to the brightness and contrast to obtain the best image possible. In the majority of the photos, it was found that brightfield produced the best photo at 4x magnification. If the lateral view of the larvae exceeds the microscope's field of view, then multiple pictures were taken of the fish's side and stitched together in Image Premier Pro software. A dorsal view and lateral view image were taken of each fish to determine standard length, total length, eye diameter, head diameter, interorbital distance, and body height at the pectoral fin and anus (Koslow et al. 1985). Pins were used to larvae in place while imaging.

Several fish were damaged during collection with the plankton net, which was noted on the data sheet. The protocol for measuring interorbital distance in damaged fish who are missing one eye was to take a measurement of the whole head width, then subtract out the measurement of one eye's diameter twice. The remaining length is the interorbital distance. This method implicitly assumed that both eyes on each fish are symmetrical and the same size. This approach

for filling gaps in our dataset only worked with fish that are missing one eye. Those missing both eyes were excluded from the measurements.

We developed a methodology for making morphometric measurements for *Micropogonias undulatus* larvae when the pectoral or anal fin could not be seen due to damage during capture or preservation. This method can be applied to other species if needed. We performed calculations to see if there was a high variability in the position of the pectoral and anal fins relative to a fish's total length. We set these measurements up as a proportion of pectoral fin (or anal fin) position relative to total length to determine where to take measurements with more precision in damaged fish with missing fins. The pectoral fin's position was found to be located at 0.2748 of a fish's body length, with a standard deviation of 0.0312. There was 95% confidence the pectoral fin would be between ~26% and ~28% of a fish's body length. The anal fin origin had a mean position of 0.5864 of a fish's body length, with a standard deviation of 0.0507. There was 95% confidence that the anal fin would be located between ~57% and ~60% of a fish's body length. The anal length ratio had a slightly greater variation in its placement than the pectoral fin. However, the variation was considered acceptable and the means of the ratios were used to calculate the location of the pectoral and anal fins that were not visible on the other larvae. This was used on a very small amount of fish in this dataset (about 13 croaker larvae out of 499 total).

### Data Analysis

With all the measurements taken, I was able to develop morphometric indices based on residuals from linear regression models calculated using MATLAB as described below. The following measurement indices and methodology was adapted from Koslow et al. (1985). The

first step entailed regressing log transformed morphometric measurements related to fish length [i.e., standard length (SL), total length (TL), head length (HEAD), eye diameter (ED)] against another log transformed morphometric measurement related to body thickness or width [i.e., body height at the pectoral (BHP) and anal fin origin? (BHA), interorbital distance (IOD)]. The length and body thickness were compared because these indices may be indicative of whether a larval fish is malnourished or is thriving with plenty to eat (Gisbert et al. 2004; Diaz et al. 2013; Yusoff et al 2023). Since thickness is often proportional with length, the goal of this analysis is to look at deviations from these relations to use as a measure of condition. In these regressions, length measurements were treated as the independent variable, whereas body thickness was treated as the dependent variable. The anomalies were calculated based on linear regression residuals following a log transformation of the data. A positive anomaly indicates that body thickness is greater than expected based on fish a given length metric, whereas a negative anomaly indicates the opposite. Anomalies were calculated for all combinations of measurements described in Table 2.

Once all 12 indices of morphometric condition described in Table 2 were calculated, I used principal component analysis (PCA) to reduce the dimensionality in the dataset. PCA is a statistical technique used to identify patterns in high-dimensional data. PCA identifies the main axes of variability in a dataset to see which variables have the greatest contribution to variation in the data (Clarke and Warwick 2001). To better understand patterns related to morphometric condition in this study, the dimensionality of the data must be reduced. This involves using the original variables to create a new set of uncorrelated variables, called principal components, that are ordered such that the first principal component in a series explains the maximum amount of variance in the data (Jolliffe 2002). PCA is commonly used in data analysis where large amounts



of data need to be analyzed and variables exhibit some degree of covariation. It can be used to reduce the dimensionality of a data set, identify underlying patterns or relationships among variables, and visualize high-dimensional data in a lower-dimensional space.

All PCA analysis was done in R2021a version of MATLAB. Prior to performing the PCA, residuals from the previously described linear regressions were standardized, meaning that each variable was transformed to have a mean of zero and a standard deviation of one. Then all the positive and negative anomalies were input into MATLAB and run through PCA. The number of principal components to analyze can be determined by examining a scree plot, which shows the amount of variance explained by each component and then selecting the components that explained a large portion of the variance (i.e., typically the top 2-3 components). The number of principal components to use are determined by looking at the percent variation explained by each component shown in these scree plots (Figure 2). The principal components retained were those that had a cumulative variance that explained at least 70% of the variance in the dataset (Holland 2008). Thus, the first two principal components were examined and used as variables in subsequent analyses. For White Mullet, the first principal component explained more than 70% of the variance (Figure 2), but I still examined the first two principal components to be consistent with the treatment of other species. After performing the PCA, the loadings of each variable (i.e., the morphometric condition indices) on each principal component selected were examined to see which variables were most strongly associated with each component. The first two principal components were plotted to visualize the relationships among the variables.

The first two principal components were then used as new dependent variables to represent holistic morphometric indices in analyses examining the effects of environmental variables on condition. These dependent variables were modeled with Generalized Additive

Mixed Models (GAMMs). GAMMs are a type of statistical model that can be used to analyze complex relationships in data (Wood 2006). They are an extension of Generalized Linear Mixed Models (GLMMs) and can be fitted to examine non-linear relationships and interactions in datasets (Hastie & Tibshirani 1990). One of the key features of GAMMs is the ability to include smooth functions to model covariates, which allow for the modeling of non-linear relationships between the response and predictor variables (Wood 2006). GAMMs allow for the capability to use fixed and random variables (Zuur et al. 2009). Fixed effects like environmental variables are useful for explaining variation due to specific conditions of interest, while random effects are valuable for accounting for unexplained variability within and between groups, like years or months. Often, a combination of both fixed and random effects is necessary for a comprehensive analysis of complex datasets. In this study, the response variables (i.e., each of the two principal components) and the predictor variables (i.e., a set of environmental variables) were loaded into the GAMM. The GAMM residuals were checked to ensure that they are normal distributed with a constant variance (Wood 2006). The concurvity function in the mgcv package in R was also checked to see if there was any non-linear correlation between predictor variables. Results from this function showed no nonlinear correlation. A test of autocorrelation was performed to make sure that data from subsequent dates of sampling were not closely related. Results from this function were as expected meaning that the concurrent sampling dates in this dataset are not significantly correlated with each other. I also checked for collinearity between each environmental variable. The only correlation seen in the data was between the day of year, which was originally used as an independent variable, and temperature. This was taken into account by making month and year random intercept effects in lieu of using day of year as an independent

variable. The following formula describes the model that incorporated all variables used in this analysis:

Equation 1:  $PC_i \sim te(\text{temperature}) + te(\log(\text{zooplankton})) + te(\text{density dependence}),$   
random=list(year=~1, month=~1),

where  $PC_i$  is referring to the principal component,  $i$  has a value of either 1 or 2 for PC1 and PC2, respectively, and density dependence was approximated based on the average standardized abundance of a species of larval fish during a given week of sampling.

Seven models with different combinations of the variables temperature, zooplankton displacement volume, density dependence, and interactions between temperature and zooplankton displacement volume were performed for each species using R 4.0.2 GUI 1.72 Catalina build. The coding package used was mgcv (Wood 2006). The interaction between temperature and zooplankton volume was included in the GAMM because these two variables may affect each other due to the relation between metabolic rate and prey consumption (Czaja et al. 2023). In other words, increases in temperature heightens the metabolic rate of ectothermic fish, inducing a need to potentially consume a greater volume of zooplankton prey. Once all the models were run, the model fit was checked using Akaike Information Criterion (AIC). AIC is a statistical measure used for model selection, particularly in the context of regression models (Akaike 1974). The principle behind AIC is finding the model that provides the best trade-off between goodness of fit and complexity (Burnham and Anderson 2004). AIC is based on the calculation of the relative likelihood of the model given the data, penalized for the number of parameters in the model (Hurvich and Tsai 1989). Specifically, AIC is calculated with the equation below (Akaike 1974):

Equation 2:  $AIC = 2k - 2\ln(\hat{L})$

The model with the lowest AIC is considered the best model, although models with a  $\Delta AIC$  differing by less than two are often considered to have undistinguishable predictive power from each other. In such cases, I examined and presented results from multiple candidate models.

### 2.3 Results

Since body thickness will generally increase proportionally with length, the goal of this analysis was to look at deviations from these relationships to use as a measure of condition. A positive anomaly indicated that body thickness or width was greater than expected based on fish length, whereas a negative anomaly indicated the opposite (Figure S1). These anomalies are used as a measurement of larval condition.

#### Broad Striped Anchovy

Broad-Striped Anchovy (*Anchoa hepsetus*) had the smallest sample size of the fish species examined (N = 199 observations). Principal component 1 (PC1) accounted for 57% of the variance explained in the data for this species while PC2 explained 23% (Figure 2). Based on the eigenvalues from the PCA, it was determined that PC1 of anchovy is related more to the width observations (IOD) and PC2 was related to the body depth observations (BHP, BHA) (Figure 3A). When looking at the principal components plot, each year increased in condition when examining PC1, based on the observation that fish condition on PC1 was separated out by year (Figure 3B). However, the 95% confidence intervals for the years 2017 and 2018 overlap

indicating that condition during these years is not significantly different from each other, although both years differ from condition in 2019. There was very little change in PC2 condition across years (Figure 3B). Based on PC1, anchovy larvae that occurred in earlier and later months (May and September) had worse condition than those that occurred in midsummer (June and July; Figure 3C). There was relatively no change in PC2 across months, except that May had an overall worse condition and fish had a narrower body depth at a given length.

After assessing the PCA, the GAMM models that were selected based on the AIC criterion were examined. For PC1 in Broad-Striped Anchovy, the null model, a model with zooplankton only, and a model with a zooplankton and temperature interaction effect were selected (Table 3). Since the null was selected for PC1 using the model selection approach, we cannot rule out that these relationships with environmental variables were weak and explained little variation in morphometric condition. When assessing the zooplankton model output, Broad-Striped Anchovy body condition remained relatively stable at low zooplankton volume and then increased when the zooplankton volume increased (Figure 4). In the zooplankton and temperature interaction model, the data were separated into quadrants (Figure 5). When anchovy experienced low temperature and low food, then this species' condition worsened. Condition became slightly better when one variable began to increase. This was especially true for temperature, which had less data gaps. Surprisingly, worse condition was also observed with higher zooplankton volume and higher temperatures occurring simultaneously.

For PC2, the null model, model with temperature only, and a model with zooplankton and temperature interaction effects were selected for Broad-Striped Anchovy (Table 4). Since the null model was selected for PC2 (which is related to the body height), it cannot be ruled out that environmental relationships with anchovy condition may be weak or even non-existent. The

temperature model showed that, with an increase of temperature, anchovy larvae experienced an increase in condition (Figure 6). Anchovies were in better condition when in water over 27°C. This model had broad confidence intervals that overlap with zero. When confidence intervals overlap with zero, this serves as another indicator that the relationship with temperature may be weak. The zooplankton and temperature interaction model for PC2 exhibited a quadrant-like pattern similar to that seen for PC1 (Figure 7). There were a lot of data available when food is scarce, thus allowing for better inferences on how anchovies reacted to the summer temperatures in low food conditions than when zooplankton volume was high. These data had different gaps because not all possible combinations of temperature and zooplankton volume values were observed. Therefore, there is incomplete information when it comes to assessing food and temperature interactions. Current data suggested that maximal condition for PC2 occurred at either low zooplankton volume/high temperature or high zooplankton volume/low temperature, but the nature of this relationship could change if additional data under extreme conditions were available.

### Atlantic Croaker

Atlantic Croaker (*Micropogonias undulatus*) had a sample size of 499 observations. PC1 accounted for 45% of the variance explained in the data while PC2 explained 25% (Figure 2). When looking at the eigenvalues outputted by the PCA, it was determined that PC1 in Atlantic Croaker was positively related to the overall condition (IOD, BHA, BHP) and PC2 was positively related to the body width (IOD) and negatively related to body depth (BHP, BHA) (Figure 8A). When PC1 and PC2 were graphed against each other, it was noted that 2017 had overall better condition than 2018 and 2019 when looking at PC1 (Figure 8B). There was very

little change in PC2 in condition across years. PC1 showed that Atlantic Croaker larvae were in worse condition at the start and finish of the season of larval occurrence (i.e., May, September, and October) compared to Atlantic Croaker caught in the month of August (Figure 8C). There was an absence of larval Atlantic Croaker caught in June and July in this dataset. There was relatively no variation in PC2 when it came to month.

After assessing the PCA, the GAMM models that were selected by AIC scores were examined. For PC1, the density dependence model and the model that included both temperature and density dependence were selected for Atlantic Croaker (Table 5). When Atlantic Croaker density was low, there was a general decrease in body condition in the model that utilized density dependence as its only selected environmental variable (Figure 9). This pattern is consistent with the hypothesized influence of density dependence on larval condition. When density was high, overall better condition was observed, but this finding is based on a single outlier and, hence, unreliable (Figure 9). There is a lack of data at higher densities of Atlantic Croaker larvae, which adds uncertainty to this relationship. In the model that included both temperature and density dependence, the relationship between Atlantic Croaker condition and density had a similar shape to that seen in the model including density dependence only. Temperature and condition had a positive linear relationship in this model (Figure 10). Since the  $\Delta AIC$  differed by less than 2 between these models, both are equally plausible. For PC2 for Atlantic Croaker, the null model was the only model selected based on the AIC (Table 6).

### Mojarra

Mojarra (*Eucinostomus* sp.) had a sample size of 707 observations. PC1 accounted for 53% of the variance explained in the data while PC2 explained 31% (Figure 2). When looking at

the eigenvalues outputted by the PCA, PC1 data for Mojarra were positively related to the overall condition (IOD, BHP, BHA), while PC2 was positively related to the body width (IOD) and negatively related to body depth (BHP, BHA) (Figure 11A). When PC1 and PC2 were graphed, it was noted that 2019 has a better overall condition than 2017 or 2018, which were similar to each other (Figure 11B). There was very little change in PC2 in condition across years. PC1 distinguished by month showing that Mojarra that occur in earlier and later months (May and October) had worse condition than those occurring in mid-summer (June and July) (Figure 11C). May had an overall much better width condition than other months, but otherwise months were similar to each with regard to PC2.

For PC1 in Mojarra, the full model including all environmental variables was the best fit GAMM model (Table 7). For this model, when zooplankton volume was low, morphometric condition initially was invariant and then increased when the volume increased (Figure 12). Mojarra body condition increased with temperature. Also, when Mojarra density increased, there was an increase in condition that eventually leveled off at densities of 0.1 fish/m<sup>3</sup>.

For PC2 in Mojarra, the density dependence model and the model that included both temperature and density dependence were selected (Table 8). When Mojarra density was low, there was initially an increase in condition After a peak of 0.06 fish/m<sup>3</sup> in the density dependence model, body condition declined with increasing density (Figure 13). In the temperature and density dependence model, Mojarra density and temperature both explained variation in morphometric condition (Figure 14). For this model, the relationship between body condition and density dependence was similar to that seen in Figure 13, while there was a negative relationship between temperature and the condition of larval Mojarra.



## White Mullet

White Mullet (*Mugil curema*) had a sample size of 344 observations. PC1 accounted for 75% of the variance explained in the data while PC2 explained 18% (Figure 2). PC1 was positively related to body width (IOD), while PC2 was positively related to body depth (BHA, BHP) (Figure 15A). When PC1 and PC2 were graphed, 2018 had better condition than 2019, while 2019 had better condition than 2017 when examining PC1 (Figure 15B). There was very little change in condition between years based on PC2. When analyzing patterns by month, PC1 showed a large degree of variation, indicating a lack of a strong seasonal pattern (Figure 15C). May, June and July did stand out as months that were different from each other, with greater condition in in June and July compared to May. PC2 showed that White Mullet collected during the first three months (May, June, and July) were in better condition than those collected during the last three months (August, September, and October).

For PC1 in White Mullet, the null model and the density dependence model were selected (Table 9). Since the null model was selected when examining PC1, we cannot rule out that no relationships existed between body condition and environmental variables. Based on the density dependence model output, when White Mullet density was low, body condition was high and declines at higher densities. When density was high, then decreasing body condition was observed potentially due interspecific competition (Figure 16).

For PC2 in White Mullet, a model with zooplankton and temperature interaction effects was selected (Table 10). The zooplankton and temperature interaction model for PC2 had a quadrant-like pattern similar to that seen for the Broad-Striped Anchovy (Figure 17). Here maximal body condition related to PC2 occurred at either low zooplankton volume combined with high temperature, or high zooplankton volume combined with low temperature. In this

model, temperature observations were well distributed across the full range, but zooplankton volume data were scarce, thus producing uncertainty in how White Mullet reacted to temperatures in low food conditions.

## *2.4 Discussion*

PC1 accounted for >50% of the variation in the dataset for three of the target species. As a result, patterns seen in PC1 with environmental variables are more prominent than those seen in PC2. Year and month differences in condition were seen for every species. This suggests that interannual and intraseasonal variations are very important. It looks like these effects were more common than environmental effects, influencing a greater number of species. The impact of month seems to be that the larvae spawned early or late are in relatively poor condition. This supports the tenets of the match/mismatch hypothesis, indicating that the mean spawning time was adapted to the environmental conditions when resources for larvae are greatest. Year-related patterns were highly species specific.

All species had some aspect of their morphometric condition influenced by an environmental or ecological variable, but in two cases patterns were weak and could not be differentiated from a null model.

Temperature and density dependent effects were fairly widespread, affecting the condition of three out of four species. Zooplankton effects were less widespread, affecting two out of four species. This might or might not be because zooplankton volume is an imperfect indicator of prey availability. Interactions between temperature and zooplankton, while individually important, were less widespread, affecting only one species. Mojarra, Croaker, and Anchovy were the three species that had significant temperature effects for PC1. These species

generally experienced better morphometric condition as temperatures warmed. This makes sense for species that have their peak occurrence during summer months. The thermal range at which I observed these species was 25-30 °C for Anchovy, 20-29°C for Croaker, and 26-30°C for Mojarra. The table on species characteristics (Table 1) indicates that the maximum thermal range for many of these species is 32-33°C. Therefore, this indicates that temperature is within the thermal range for each of these species and has not reached the point where they are experiencing stress, causing their condition to be affected. This is noteworthy since early life history stages are often more sensitive to temperature than later life history stages (Dahlke et al. 2020). However, this doesn't mean that stressful temperatures will not be reached in the future.

Of species where zooplankton were important, there seems to be an increase in zooplankton condition for PC1 under the regions of graphs where data were most abundance. Density dependent effects were mixed and varied in direction between species. Two species primarily had decreasing condition at higher densities. The third species, Mojarra, reacted to density dependence by initially increasing and then having a stabilization in condition. This could indicate that time periods with low density might coincide with periods when some unknown oceanic factor is constraining mojarra larval production, growth, and survival.

When food availability was measured by zooplankton volume interactions with temperature, there was a quadrant-like development that occurred. The quadrants represent the four states a larvae might find itself in: high temperature and high zooplankton, high temperature and low zooplankton, low temperature and high zooplankton, and low temperature and low zooplankton. The patterns among these quadrants would be accurate with the addition of more sampling to fill in the data gaps of patchy zooplankton blooms. However, based on current results, body condition was often maximal at lower temperatures with high food availability,

with good condition also seen at warm temperatures and low zooplankton volume. A major difficulty in assessing these patterns is that zooplankton abundance is patchy in space and time and may not simultaneously occur in areas with high larval fish densities. Another interesting finding from these data was the relationship of larval fish density and body condition. Generally, it is expected that there is an increase in body condition at low density, but when density is high enough it leads to a decrease in body condition of the larvae. This seems to be true for Mojarra in this study. Although sometimes there was an increase in body condition with density for Atlantic Croaker, but only small amounts of data supported this finding. Previous studies have noticed similar relationships to density like that of Mojarra giving rise to the assumption of intraspecific competition.

In the principal components analysis, the PC1 eigenvalues of the Broad-Striped Anchovies and White Mullet are both related more to the width observations and PC2 is related to the body depth observations. This pattern is more pronounced in White Mullet than it is in the Broad-Striped Anchovy body condition based on the magnitude of eigenvalues. When it comes to highlighting interannual variations in body condition, the PCAs showed the most distinct body condition separations when distinguished by year. It is assumed that this is due to PC1 explaining the majority of the data that related to width measurements.

Results of the PCAs performed on Atlantic Croaker and Mojarra were similar to one another, showing that PC1 was more related to overall body height while PC2 was more related to width. These two species did not have distinct interannual variations in morphometric condition like Broad-Striped Anchovy and White Mullet did. Altogether, it seems that all species had worse body condition at the beginning of the season of larval occurrence, which is to be

expected. The warmer months later in this early life stage allow for a more optimal body condition.

It is believed that if this work was expanded upon with increased supply of funding and researchers that there will be greater insight into species thermal tolerance using the influence of temperature on body condition as the indicator. Additionally, with increased frequency of sampling to observe sporadic large zooplankton blooms, I would be able to better determine if the impact of zooplankton abundance is as important as temperature when it comes to influencing morphometric condition.

It is concluded that during months of warmer temperatures, there is a better overall body condition seen with an increased width of larval fish. It is difficult to determine the effects on larval fish of rapidly changing temperatures on a daily occurrence. This was not considered during the creation of this study but is a topic that should be studied further as the climate becomes more unpredictable. These findings on temperature can be important when trying to understand larval fish life history stages and when they are at their strongest or most fragile point. Zooplankton when abundant seems to allow for increased condition but due to the lack of data on how larval fish body condition results on days of high zooplankton volume, it is concluded that these results are inconclusive but plausible. This work could elucidate recruitment dynamics by providing data on which environmental variables have a direct effect on body condition. These data and models could be used to understand the source of variability observed when fitting a stock-recruitment curve to spawning stock abundance and recruitment observations. This methodology can also give insight on species thermal tolerance and species spawning phenology. This can give rise to how larvae are going to respond to their ever so changing environment.

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Table 1. This table shows the distribution, prey, temperature range and spawning months of target species in this study.

<b>Species</b>	<b>Distribution</b>	<b>Prey</b>	<b>Temperature Range</b>	<b>Spawning</b>
Atlantic Croaker ( <i>Micropogonias undulatus</i> )	Massachusetts to the Gulf of Mexico	Crustaceans, with a frequent occurrence of annelids, mollusks, and sometimes detritus	11°C to 32°C but prefer temperatures over 24°C	July-October, but can occur sooner in the year
Mojarra ( <i>Eucinostomus</i> sp.)	New Jersey, USA to southeastern Brazil	Microcrustaceans, mainly copepods	13°C to 32°C but prefer temperatures over 27°C	May-September
White Mullet ( <i>Mugil curema</i> )	Western Atlantic from Nova Scotia to Argentina; uncommon north of Cape Cod	Microscopic or filamentous algae, detritus, and potentially other small juveniles of planktonic organisms	11°C to 33°C but prefer temperatures over 28°C	March- August
Broad Striped Anchovy ( <i>Anchoa hepsetus</i> )	Atlantic coast of North and South America from Uruguay to Nova Scotia	Zooplankton and other small crustaceans	10°C to 32°C but prefer temperatures over 28°C	May-September



Table 2. Combinations of measurements used to create multivariate morphometric indices. Body measurements of fish length were standard length (SL), total length (TL), head length (HEAD), and eye diameter (ED). Body measurement related to body thickness are body height at the pectoral fins (BHP), body height at the anal fin origin (BHA), and interorbital distance (IOD).

BHA vs SL	BHA vs.TL	BHA vs. HEAD	BHA vs. ED	IOD vs.TL	IOD vs. SL
BHP vs.SL	BHP vs.TL	BHP vs. HEAD	BHP vs. ED	IOD vs. ED	IOD vs. HEAD

Table 3. Estimated degrees of freedom, AIC,  $\Delta$  AIC, and AIC weights for GAMM model selection for Broad Striped Anchovy's first principal component.

Anchovy PC1				
Model	DF	AIC	$\Delta$ AIC	AIC weights
<b>Null Model</b>	<b>4</b>	<b>135.32</b>	<b>0</b>	<b>0.3639</b>
Temperature	6	139.27	3.957	0.0503
<b>Log Zooplankton</b>	<b>6</b>	<b>136.61</b>	<b>1.291</b>	<b>0.1908</b>
Density Dependence of Anchovy	6	139.32	3.998	0.0492
<b>Temperature and Log Zooplankton Interaction</b>	<b>9</b>	<b>135.89</b>	<b>0.570</b>	<b>0.2736</b>
Temperature and Log Zooplankton	8	140.24	4.921	0.0310
Temperature and Density Dependence of Anchovy	8	143.27	7.951	0.0068
Log Zooplankton and Density Dependence of Anchovy	8	140.37	5.051	0.0291
Temperature, Log Zooplankton, and Density Dependence of Anchovy	10	143.88	8.561	0.0050

Table 4. Estimated degrees of freedom, AIC,  $\Delta$  AIC, and AIC weights for GAMM model selection for Broad Striped Anchovy's second principal component.

Anchovy PC2				
Fixed Effects Variables	DF	AIC	$\Delta$ AIC	AIC weights
<b>Null Model</b>	<b>4</b>	<b>73.09</b>	<b>0</b>	<b>0.3710</b>
<b>Temperature</b>	<b>6</b>	<b>74.37</b>	<b>1.282</b>	<b>0.1950</b>
Log Zooplankton	6	76.68	3.585	0.0618
Density Dependence of Anchovy	6	76.58	3.492	0.0647
<b>Temperature and Log Zooplankton Interaction</b>	<b>9</b>	<b>74.43</b>	<b>1.335</b>	<b>0.1900</b>
Temperature and Log Zooplankton	8	77.66	4.571	0.0377
Temperature and Density Dependence of Anchovy	8	77.09	3.994	0.0503
Log Zooplankton and Density Dependence of Anchovy	8	79.76	6.672	0.0132
Temperature, Log Zooplankton, and Density Dependence of Anchovy	10	79.49	6.401	0.0151

Table 5. This table shows the Estimated Degrees of Freedom, AIC,  $\Delta$  AIC, and AIC Weights for model selection for the first principal component for Atlantic Croaker body condition.

Atlantic Croaker PC1				
Model	DF	AIC	$\Delta$ AIC	AIC weights
Null Model	4	396.23	4.406	0.0593
Temperature	6	397.76	5.941	0.0275
Log Zooplankton	6	396.92	5.103	0.0418
<b>Density Dependence of Croaker</b>	<b>6</b>	<b>391.82</b>	<b>0</b>	<b>0.5371</b>
Temperature and Log Zooplankton Interaction	9	401.92	10.103	0.0034
Temperature and Log Zooplankton	8	400.63	8.812	0.0065
<b>Temperature and Density Dependence of Croaker</b>	<b>8</b>	<b>393.78</b>	<b>1.956</b>	<b>0.2019</b>
Log Zooplankton and Density Dependence of Croaker	8	395.31	3.494	0.0936
Temperature, Log Zooplankton, and Density Dependence of Croaker	10	397.69	5.870	0.0285

Table 6. This table shows the Estimated Degrees of Freedom, AIC,  $\Delta$  AIC, and AIC Weights for model selection for the second principal component for Atlantic Croaker body condition.

Croaker PC2				
Model	DF	AIC	$\Delta$ AIC	AIC weights
<b>Null Model</b>	<b>4</b>	<b>260.10</b>	<b>0</b>	<b>0.4499</b>
Temperature	6	262.83	2.732	0.1147
Log Zooplankton	6	262.12	2.015	0.1642
Density Dependence of Croaker	6	263.32	3.224	0.0897
Temperature and Log Zooplankton Interaction	9	264.84	4.735	0.0421
Temperature and Log Zooplankton	8	263.43	3.326	0.0852
Temperature and Density Dependence of Croaker	8	266.30	6.198	0.0202
Log Zooplankton and Density Dependence of Croaker	8	266.18	6.080	0.0215
Temperature, Log Zooplankton, and Density Dependence of Croaker	10	267.34	7.239	0.0120

Table 7. This table shows the Estimated Degrees of Freedom, AIC,  $\Delta$  AIC, and AIC Weights for model selection for Mojarras the first principal component for Mojarra body condition.

Mojarra PC1				
Model	DF	AIC	$\Delta$ AIC	AIC weights
Null Model	4	106.34	9.356	0.0067
Temperature	6	109.67	12.680	0.0012
Log Zooplankton	6	109.67	12.677	0.0012
Density Dependence of Mojarra	6	104.27	7.278	0.0189
Temperature and Log Zooplankton Interaction	9	99.43	2.445	0.2127
Temperature and Log Zooplankton	8	110.58	13.586	0.0008
Temperature and Density Dependence of Mojarra	8	103.85	6.866	0.0233
Log Zooplankton and Density Dependence of Mojarra	8	105.14	8.154	0.0122
<b>Temperature, Log Zooplankton, and Density Dependence of Mojarra</b>	<b>10</b>	<b>96.99</b>	<b>0</b>	<b>0.7226</b>

Table 8. This table shows the Estimated Degrees of Freedom, AIC,  $\Delta$  AIC, and AIC Weights for model selection for the second principal component of Mojarra body condition.

Mojarra PC2				
Model	DF	AIC	$\Delta$ AIC	AIC weights
Null Model	4	-338.28	3.343	0.0888
Temperature	6	-334.32	7.303	0.0122
Log Zooplankton	6	-334.55	7.069	0.0137
<b>Density Dependence of Mojarra</b>	<b>6</b>	<b>-341.62</b>	<b>0</b>	<b>0.472</b>
Temperature and Log Zooplankton Interaction	9	-337.90	3.725	0.0734
Temperature and Log Zooplankton	8	-330.65	10.973	0.0019
<b>Temperature and Density Dependence of Mojarra</b>	<b>8</b>	<b>-340.14</b>	<b>1.484</b>	<b>0.2251</b>
Log Zooplankton and Density Dependence of Mojarra	8	-338.06	3.565	0.0795
Temperature, Log Zooplankton, and Density Dependence of Mojarra	10	-336.24	5.385	0.0320

Table 9. This table shows the Estimated Degrees of Freedom, AIC,  $\Delta$  AIC, and AIC Weights for model selection of GAMMS examining White Mullet's first principal component.

White Mullet PC1				
Model	DF	AIC	$\Delta$ AIC	AIC weights
<b>Null Model</b>	<b>4</b>	<b>187.70</b>	<b>0.0418</b>	<b>0.3588</b>
Temperature	6	191.36	3.7030	0.0575
Log Zooplankton	6	191.65	3.9950	0.0497
<b>Density Dependence of Mullet</b>	<b>6</b>	<b>187.66</b>	<b>0</b>	<b>0.3664</b>
Temperature and Log Zooplankton Interaction	9	197.48	9.8196	0.0027
Temperature and Log Zooplankton	8	195.34	7.6841	0.0078
Temperature and Density Dependence of Mullet	8	190.42	2.7595	0.0922
Log Zooplankton and Density Dependence of Mullet	8	191.56	3.9047	0.0520
Temperature, Log Zooplankton, and Density Dependence of Mullet	10	194.38	6.7241	0.0127



Table 10. This table shows the Estimated Degrees of Freedom, AIC,  $\Delta$  AIC, and AIC Weights for model selection for White Mullet's second principal component.

White Mullet PC2				
Model	DF	AIC	$\Delta$ AIC	AIC weights
Null Model	4	-143.74	10.937	0.0041
Temperature	6	-142.11	12.559	0.0018
Log Zooplankton	6	-140.80	13.875	0.0009
Density Dependence of Mullet	6	-143.04	11.638	0.0029
<b>Temperature and Log Zooplankton Interaction</b>	<b>9</b>	<b>-154.67</b>	<b>0</b>	<b>0.9852</b>
Temperature and Log Zooplankton	8	-138.24	16.432	0.0002
Temperature and Density Dependence of Mullet	8	-142.79	11.888	0.0025
Log Zooplankton and Density Dependence of Mullet	8	-141.46	13.215	0.0013
Temperature, Log Zooplankton, and Density Dependence of Mullet	10	-140.01	14.665	0.0006

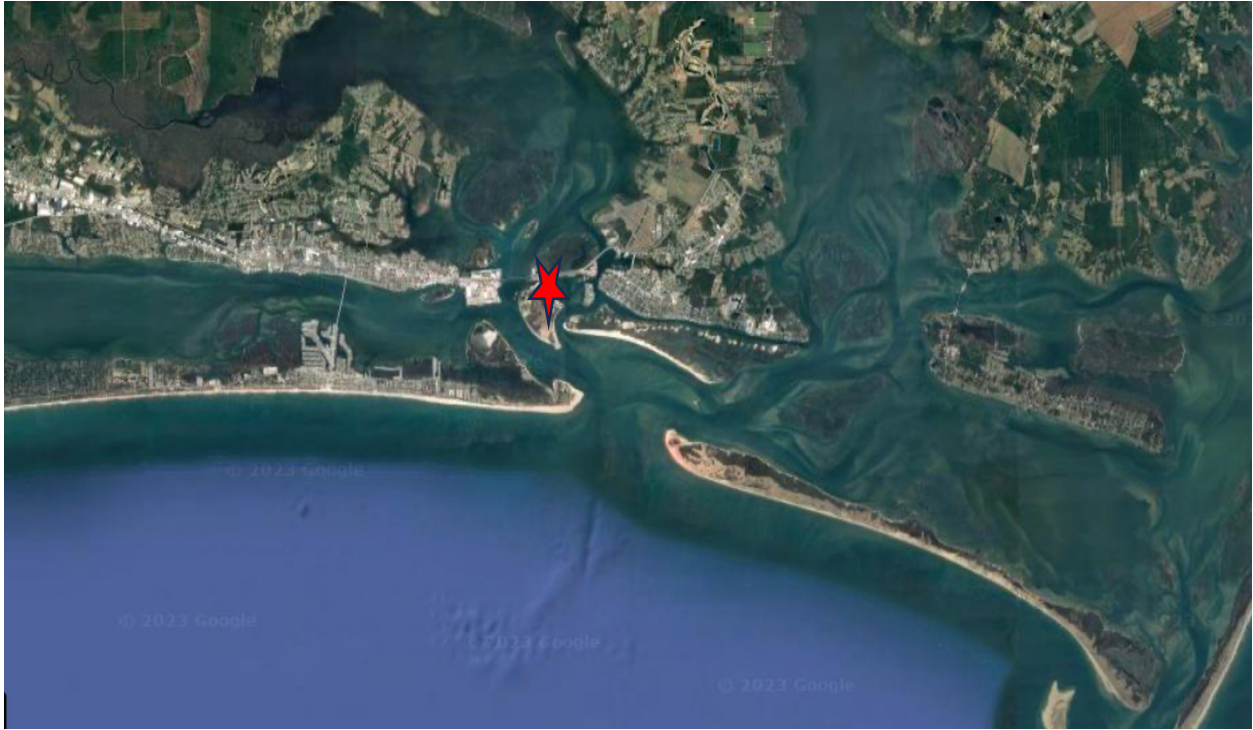


Figure 1. Satellite photo of Beaufort Inlet and the Newport River Estuary. The red star marks the sampling location at the Pivers Island Bridge platform by the NOAA Research Laboratory.

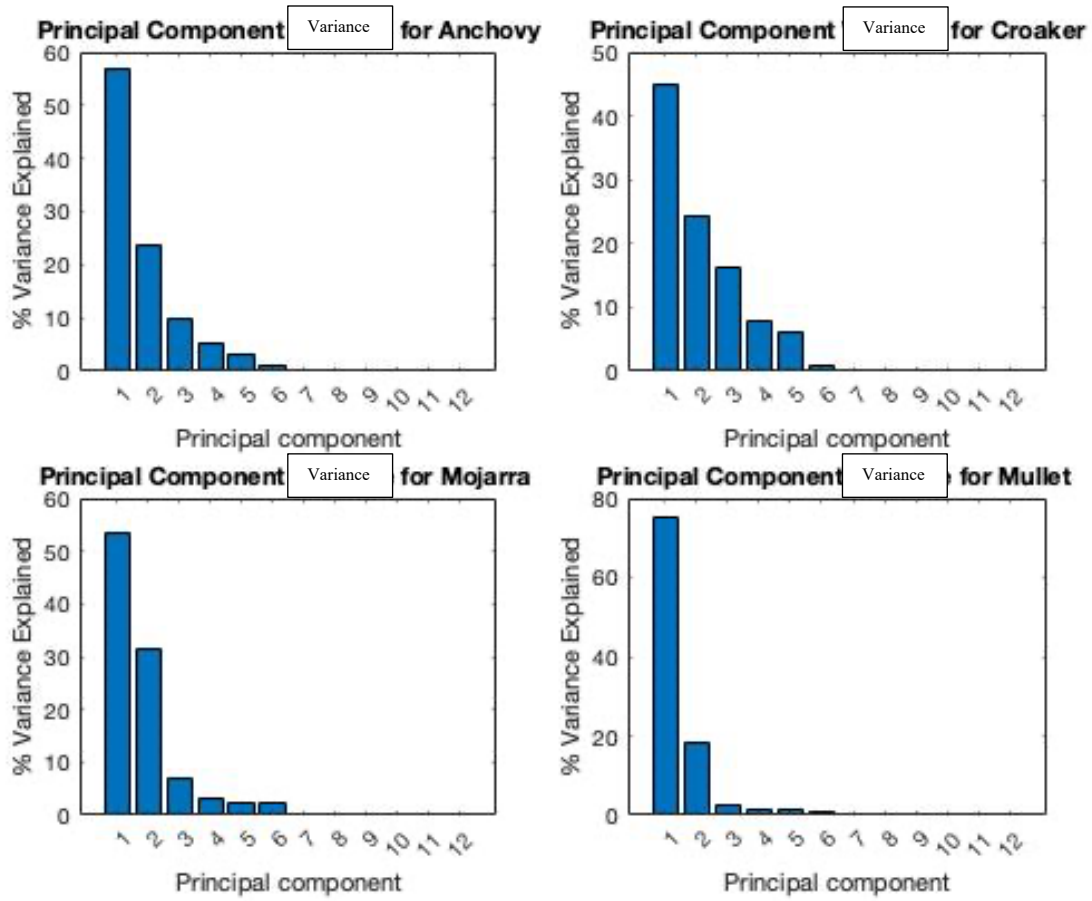


Figure 2. Scree plots from the PCA run on the morphometric anomaly data. The first two principal components were selected for every species to be examined and used as new variables in subsequent analyses.

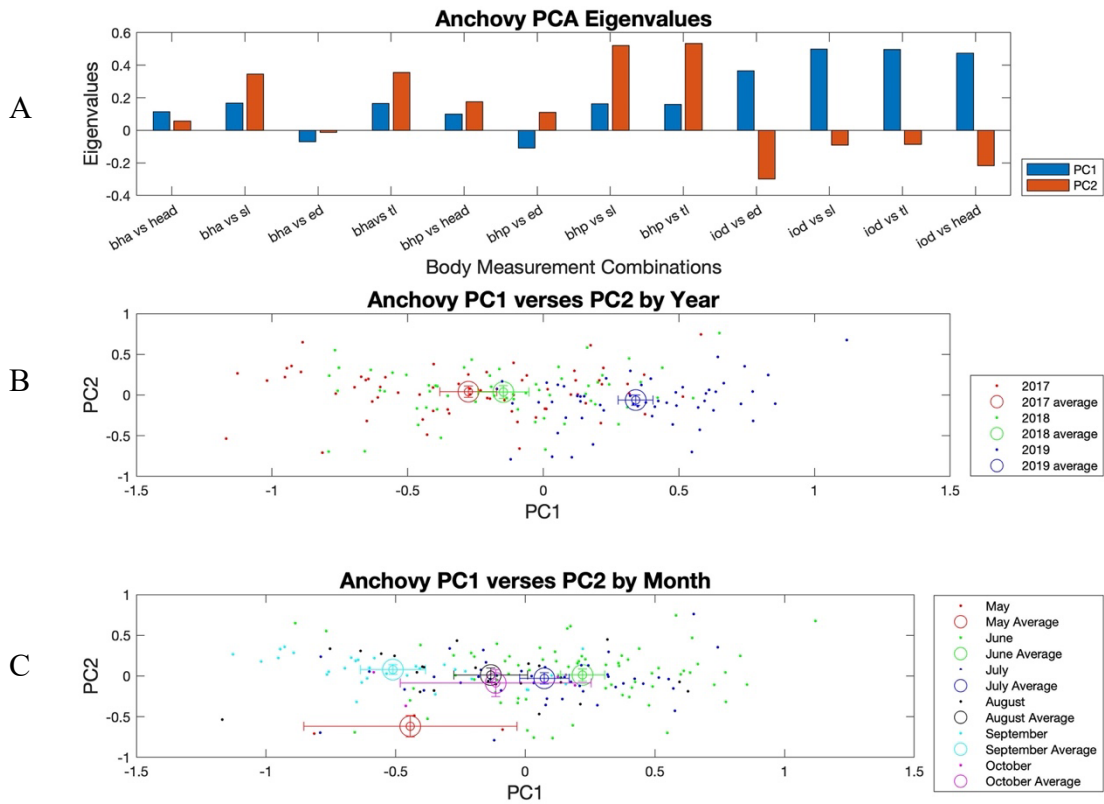


Figure 3. (A) Eigenvalues from principal components related to body width or depth for Broad-Striped Anchovy. Relationships between principal components and mean patterns related to year (B) and month (C). This allows insight to what year or month are related to better or worse condition. Circular markers indicate the mean of each principal component in a given month or year, while also showing 95% standard error. Dots indicate PC1 and PC2 values for particular larval fish specimens.

## PC1 Model 2 Condition vs. Log Zooplankton

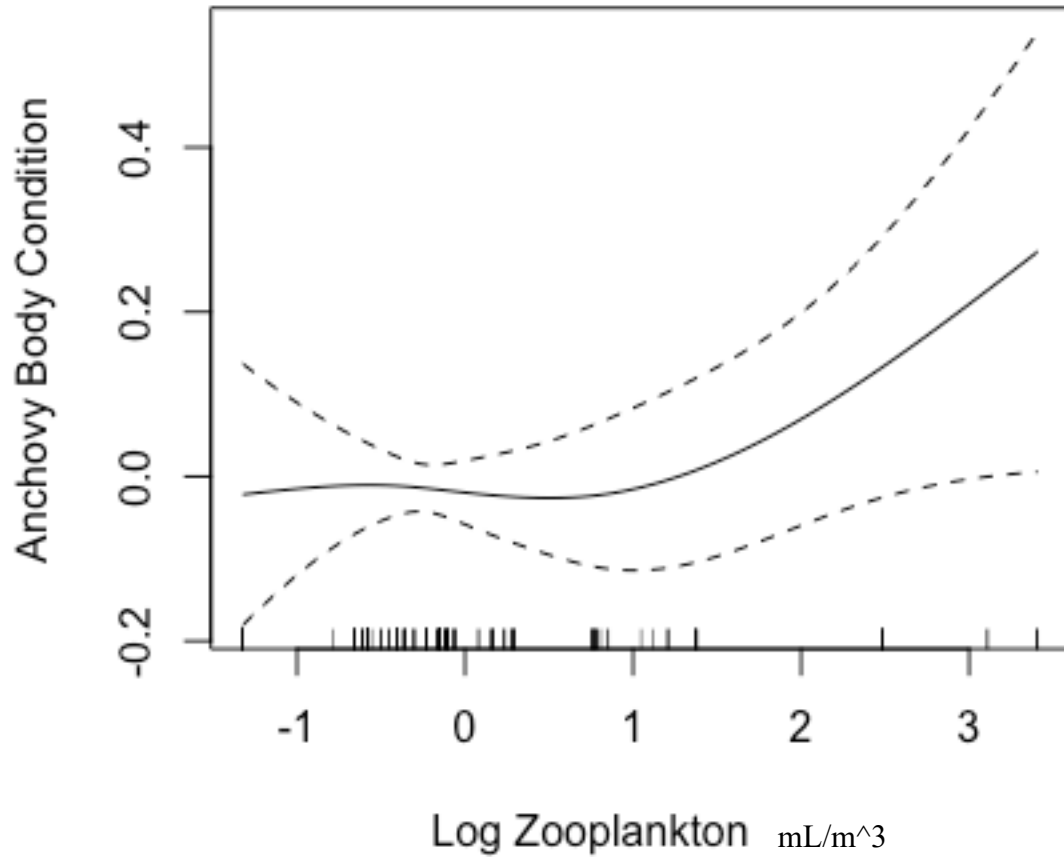


Figure 4. GAMM response curve showing the relationship between log-transformed zooplankton volume and Broad-Striped Anchovy Body condition based on PC1. A rug plot showing the sampled range of zooplankton volume is included on the x-axis. The solid line is the mean response, while the dashed lines indicated 95% confidence intervals.

### Anchovy PC1 Model 4

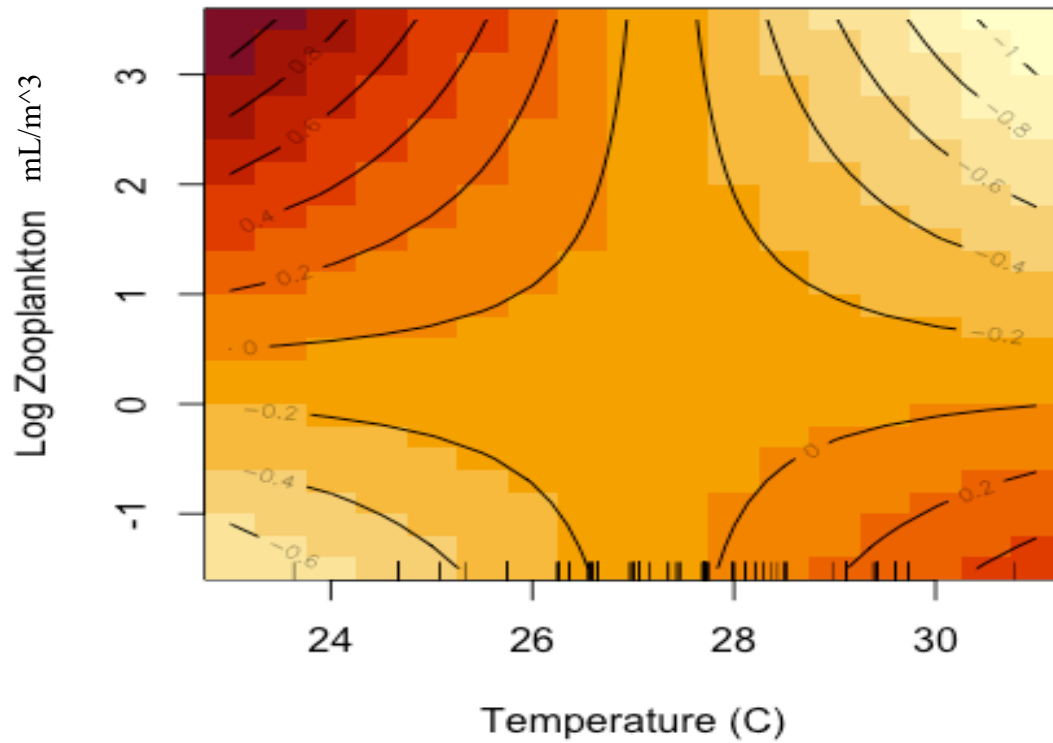


Figure 5. GAMM response curve showing the interaction between temperature and log-transformed zooplankton volume that affected body condition of Broad Striped Anchovy for PC1. A rug plot showing the sampled range of temperature is included on the x-axis. Variations in PC2 are shown as contour lines.

## PC1 Model 2 Condition vs. Temperature

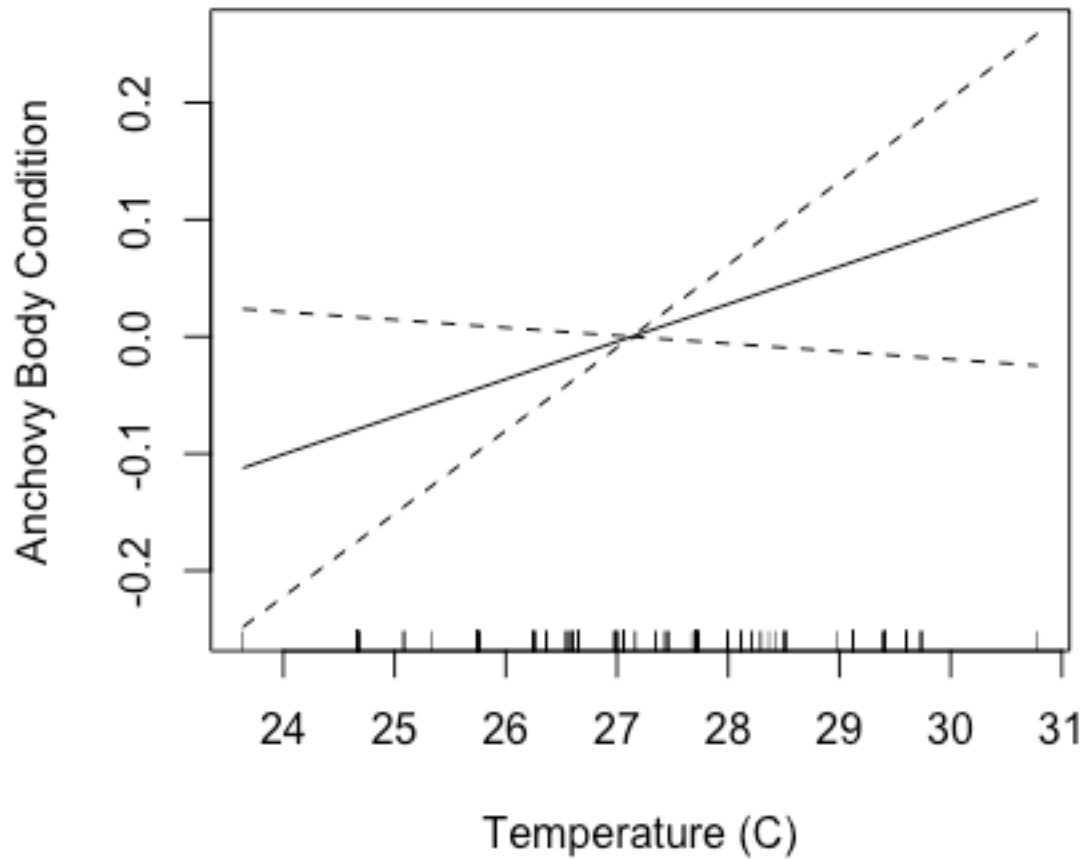


Figure 6. GAMM response curve showing the relationship between log-transformed zooplankton volume and Broad-Striped Anchovy Body condition based on PC1. A rug plot showing the sampled range of zooplankton volume is included on the x-axis. The solid line is the mean response, while the dashed lines indicated 95% confidence intervals.

## Anchovy PC2 Model 4

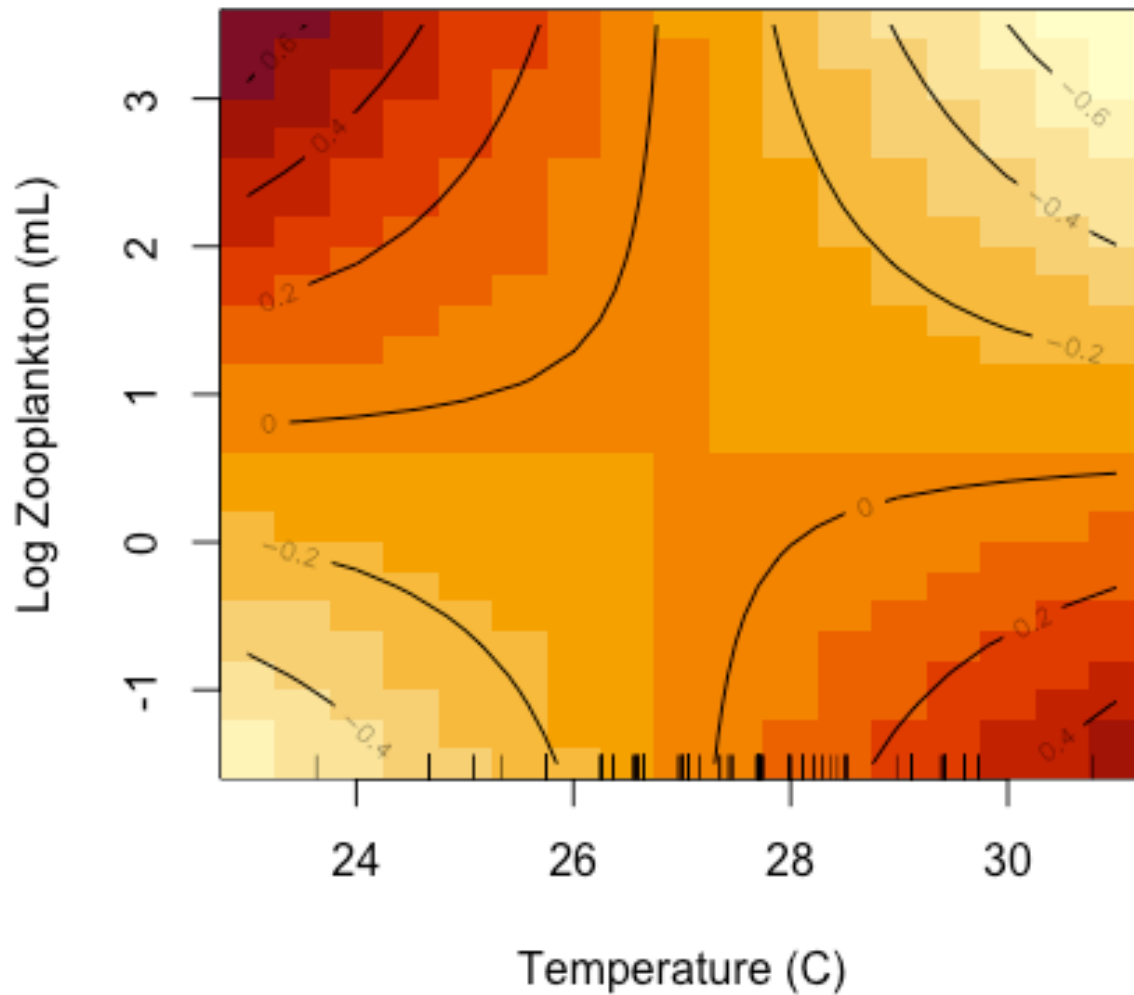


Figure 7. GAMM response curve showing the interaction between temperature and log-transformed zooplankton volume that affected body condition of Broad Striped Anchovy for PC2. A rug plot showing the sampled range of temperature is included on the x-axis. Variations in PC1 are shown as contour lines.



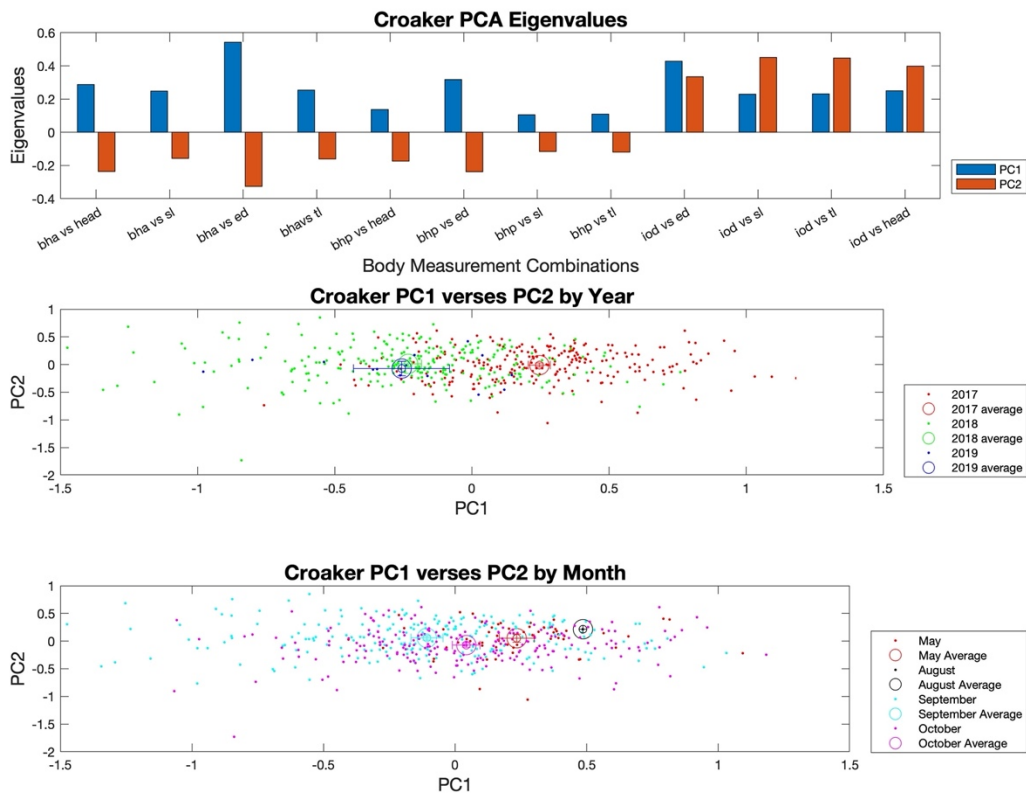


Figure 8. (A) Eigenvalues from principal components related to body width or depth for Atlantic Croaker. Relationships between principal components and mean patterns related to year (B) and month (C). This allows insight to what year or month are related to better or worse condition. Circular markers indicate the mean of each principal component in a given month or year, while also showing 95% standard error. Dots indicate PC1 and PC2 values for particular larval fish specimens.

### PC1 Model 3 Condition vs. Density

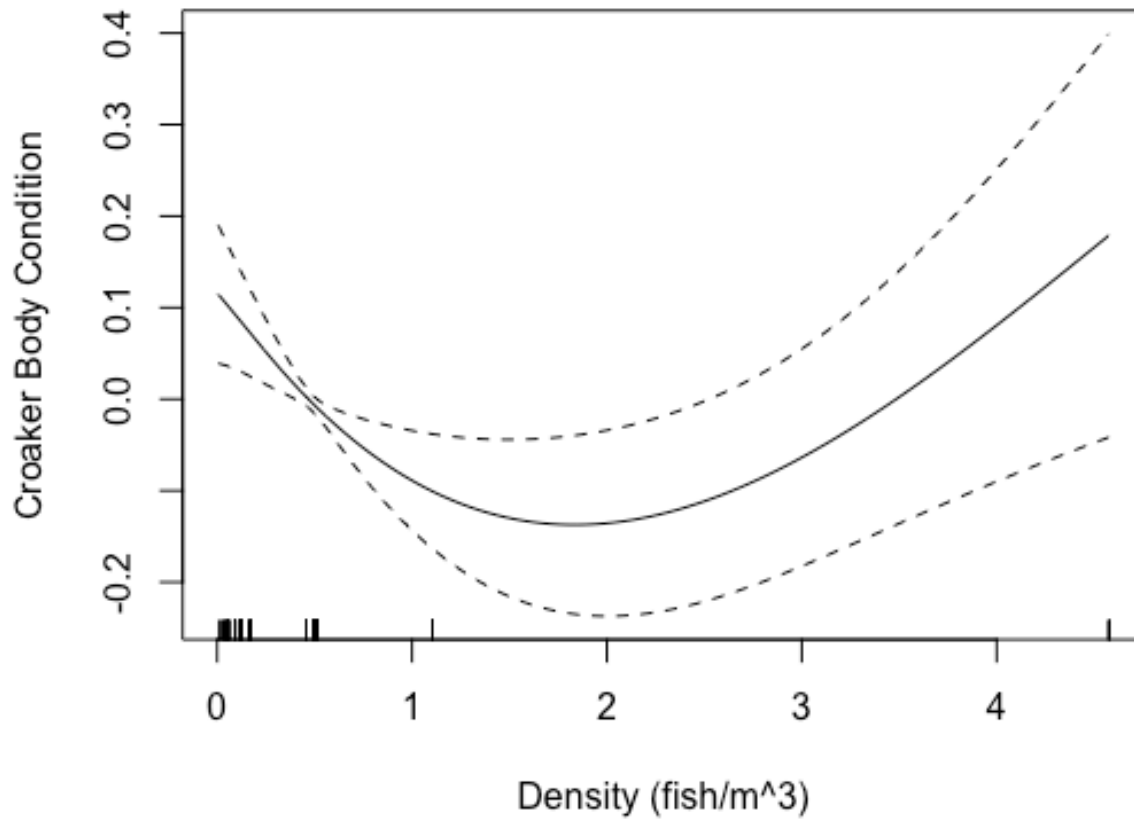


Figure 9. GAMM response curve showing the effect of density dependence on the body condition of Atlantic Croaker for PC1. Units for density dependence are fish  $m^{-3}$  of water. A rug plot showing the sampled range of density is included on the x-axis. 95% confidence intervals are shown as dashed lines.

### Croaker PC1 Model 6 Temperature and Density

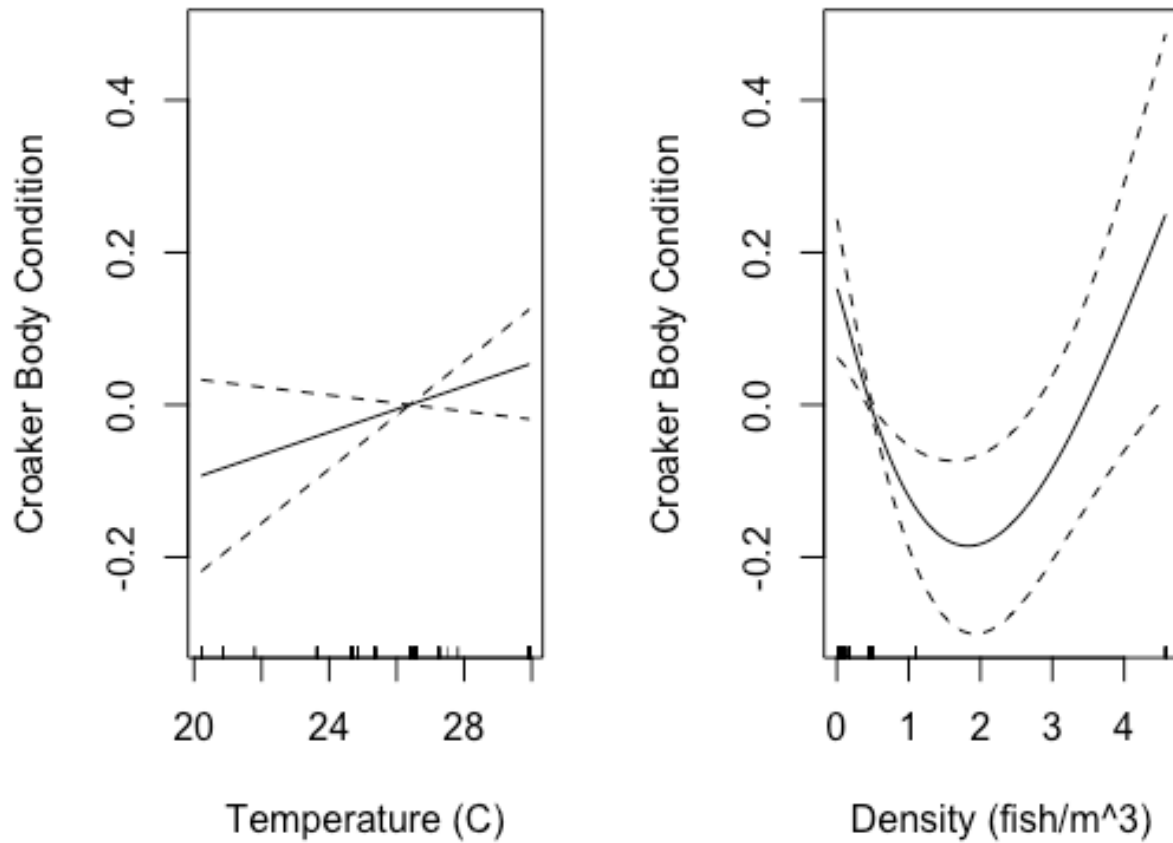


Figure 10. GAMM response curve showing the effect of temperature and density dependence on the body condition of Atlantic Croaker for PC1. Rug plots showing the sampled ranges of temperature and density are included on the x-axes of plots. 95% confidence intervals are shown as dashed lines.

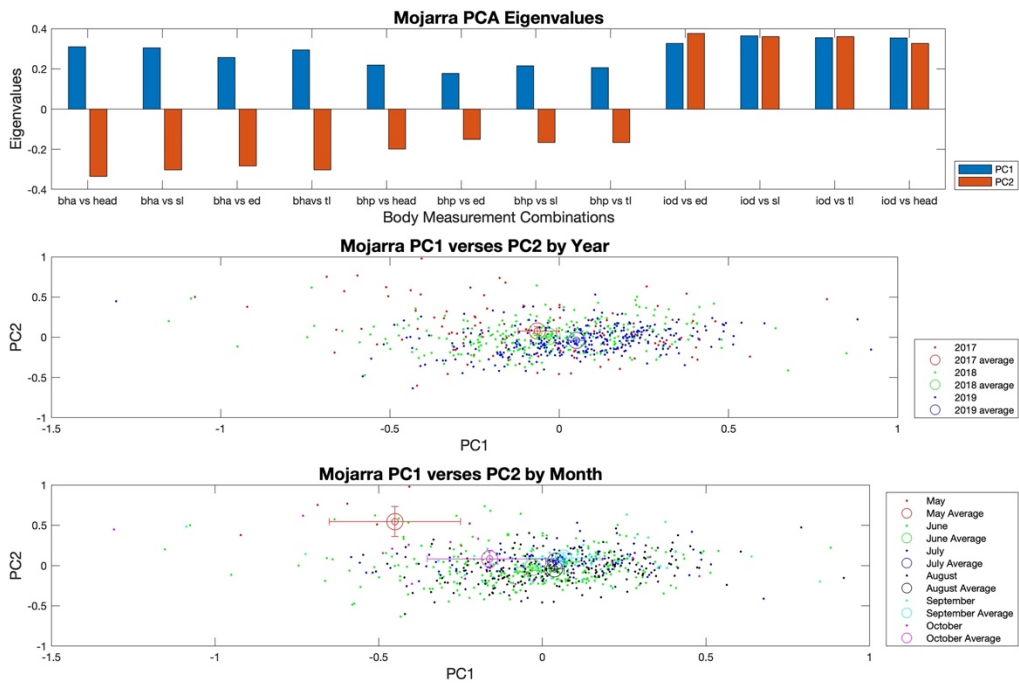


Figure 11. (A) Eigenvalues from principal components related to body width or depth for Mojarra. Relationships between principal components and mean patterns related to year (B) and month (C). This allows insight to what year or month are related to better or worse condition. Circular markers indicate the mean of each principal component in a given month or year, while also showing 95% standard error. Dots indicate PC1 and PC2 values for particular larval fish specimens.

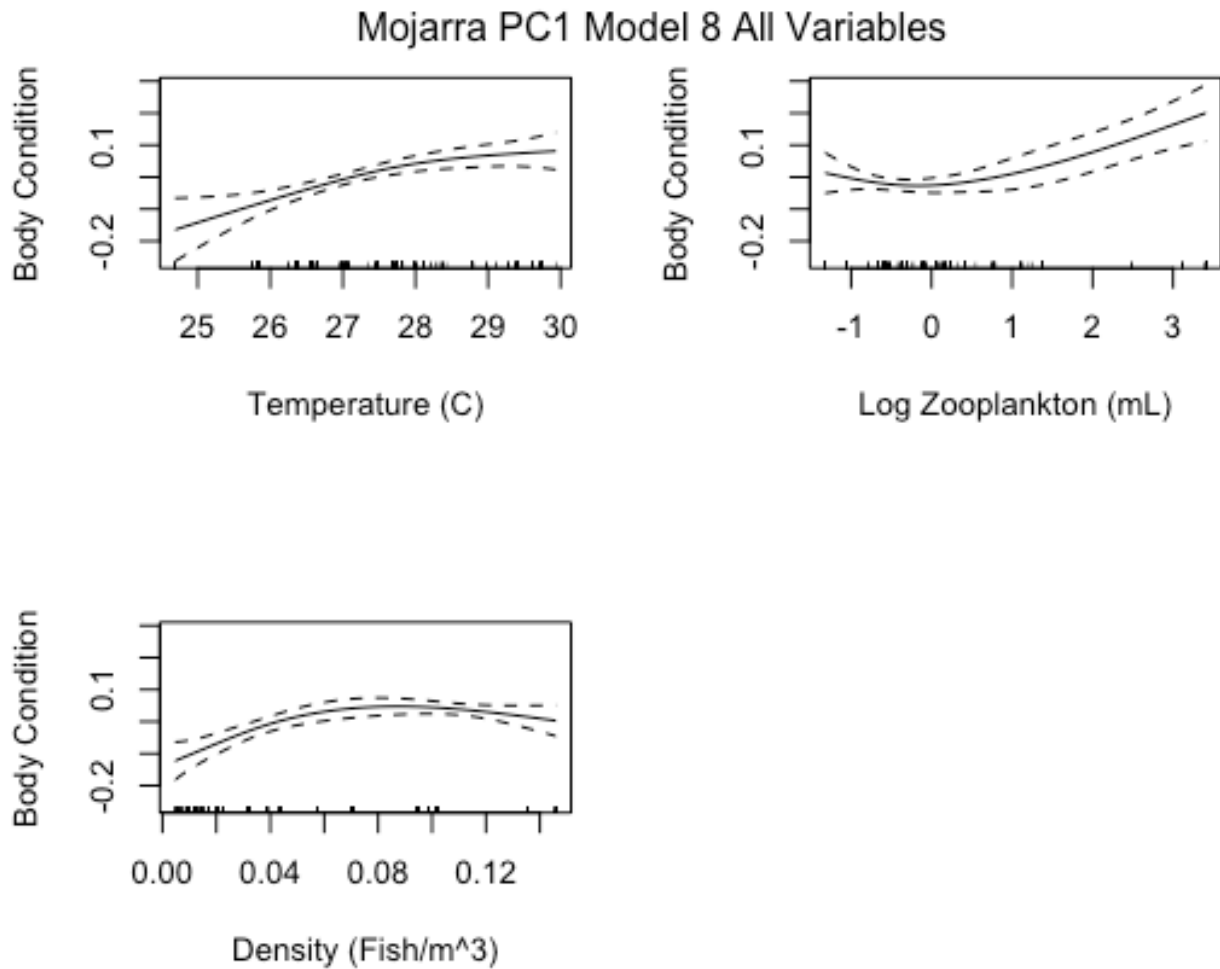


Figure 12. GAMM response curve showing the effect of temperature, log zooplankton volume, and density dependence on the body condition of Mojarra for PC1. Rug plots showing the sampled ranges of temperature, log zooplankton volume, and density are included on the x-axes of plots. 95% confidence intervals are shown as dashed lines.

### Mojarra PC2 Model 3 Condition vs. Density

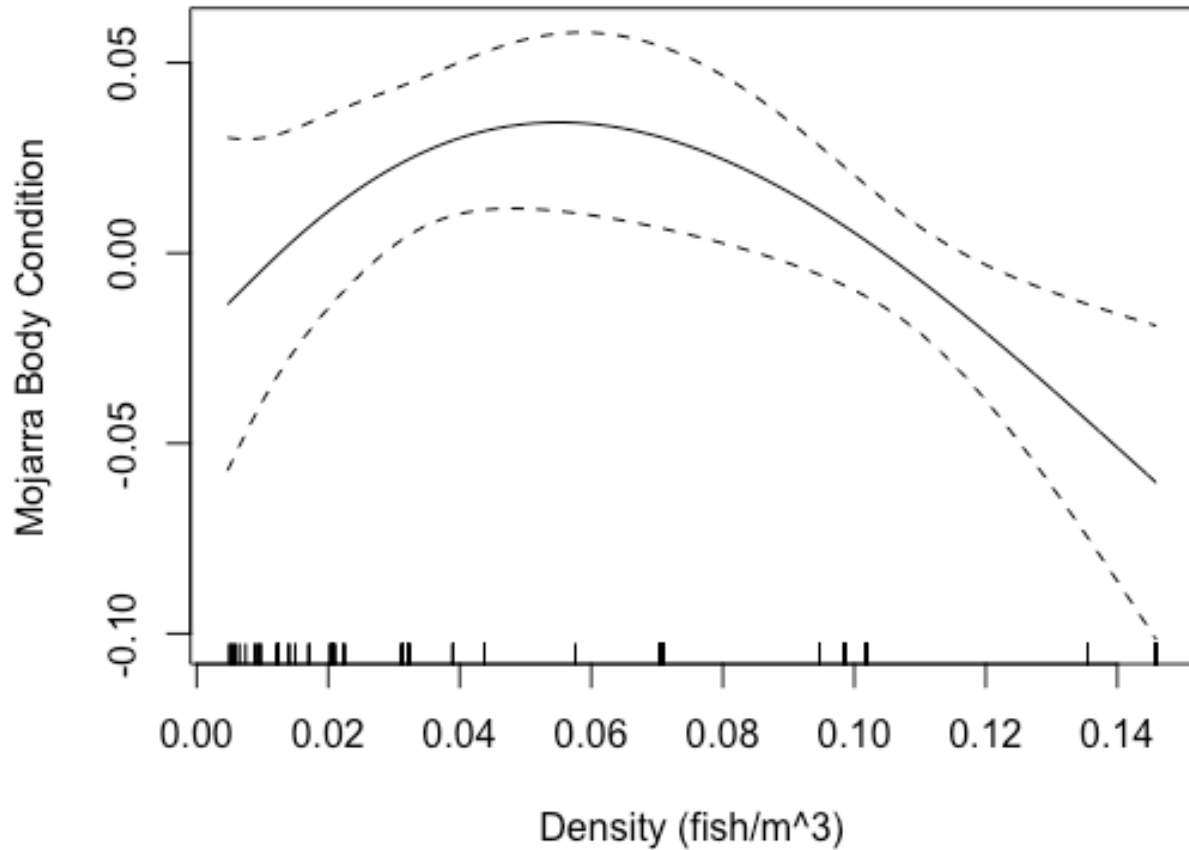


Figure 13. GAMM response curve showing the effect of density dependence on the body condition of Mojarra for PC2. A rug plot showing the sampled ranges of density are included on the x-axes of plots. 95% confidence intervals are shown as dashed lines.

### Mojarra PC2 Model 6

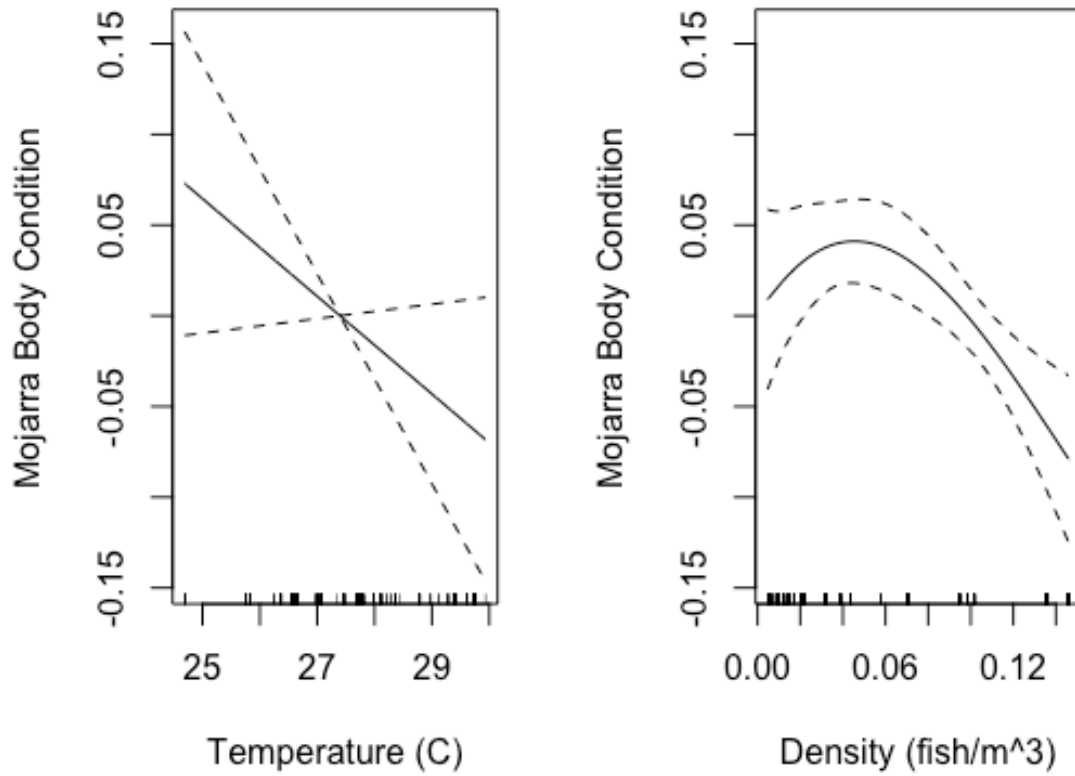


Figure 14. GAMM response curve showing the effect of temperature and density dependence on the body condition of Mojarra for PC2. Rug plots showing the sampled ranges of temperature and density are included on the x-axes of plots. 95% confidence intervals are shown as dashed lines.

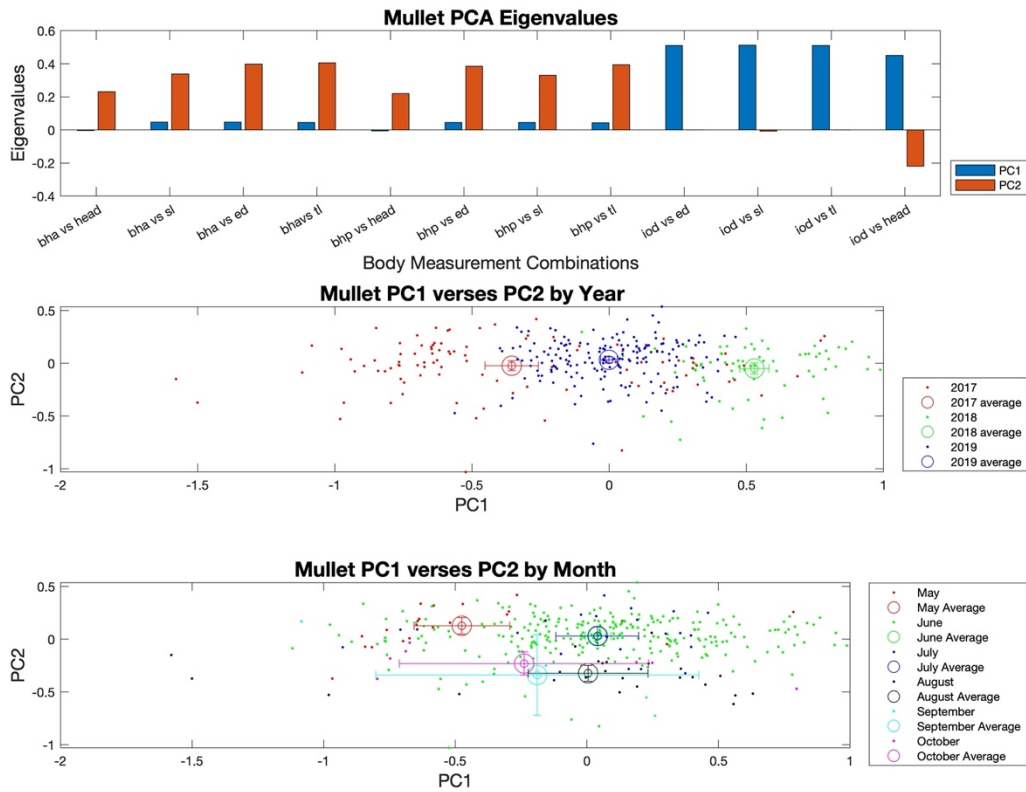


Figure 15. (A) Eigenvalues from principal components related to body width or depth for Mullet. Relationships between principal components and mean patterns related to year (B) and month (C). This allows insight to what year or month are related to better or worse condition. Circular markers indicate the mean of each principal component in a given month or year, while also showing 95% standard error. Dots indicate PC1 and PC2 values for particular larval fish specimens.



### GAM 3.1 Mullet Output

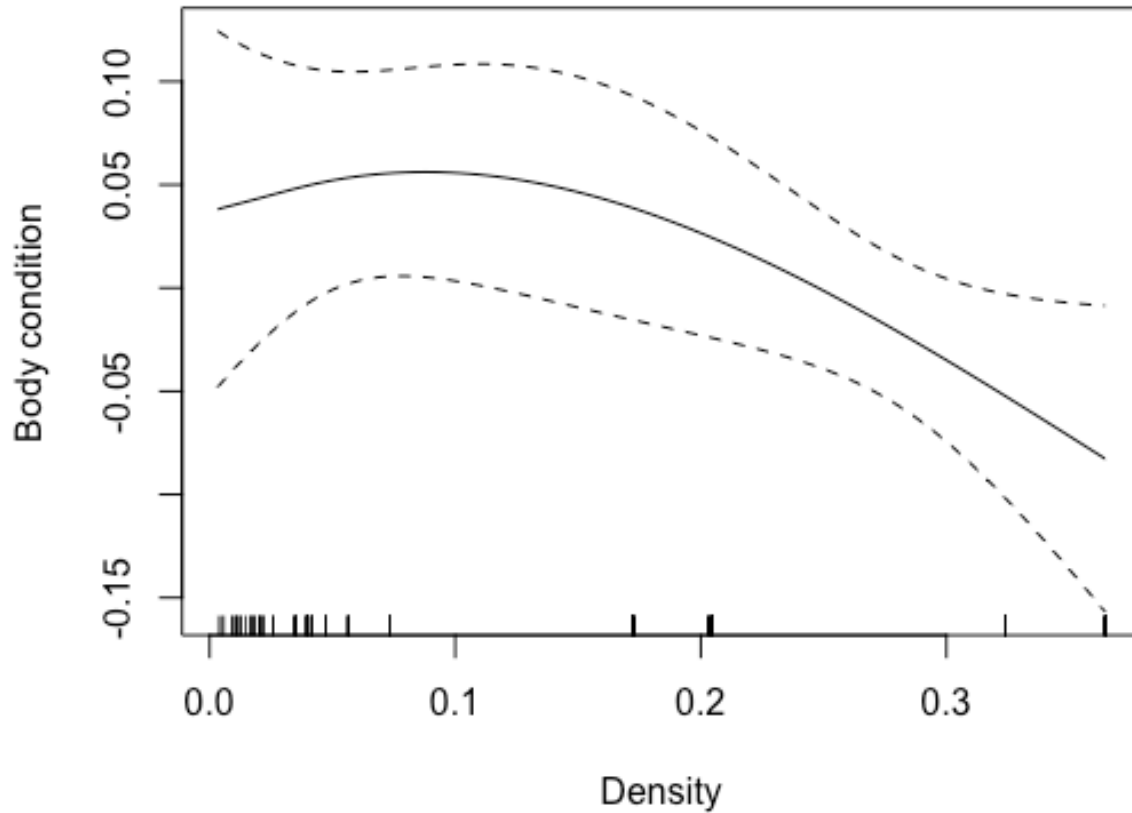


Figure 16. GAMM response curve showing the effect of density dependence on the body condition of White Mullet for PC1. Units for density dependence are fish  $\text{m}^{-3}$  of water. A rug plot showing the sampled range of density is included on the x-axis. 95% confidence intervals are shown as dashed lines.

### Mullet PC2 Model 4

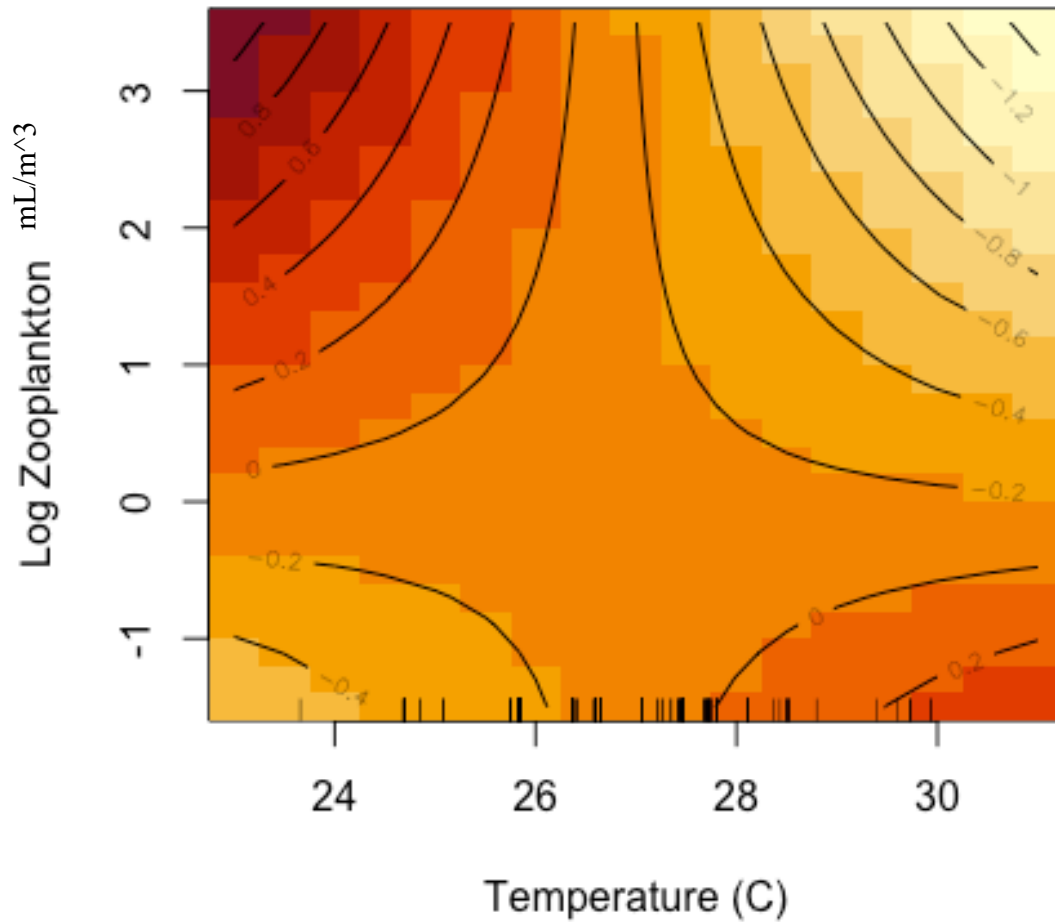


Figure 17. GAMM response curve showing the interaction between temperature and log-transformed zooplankton volume that affected body condition of White Mullet for PC2. A rug plot showing the sampled range of temperature is included on the x-axis. Variations in PC1 are shown as contour lines.

## Supplemental Figures

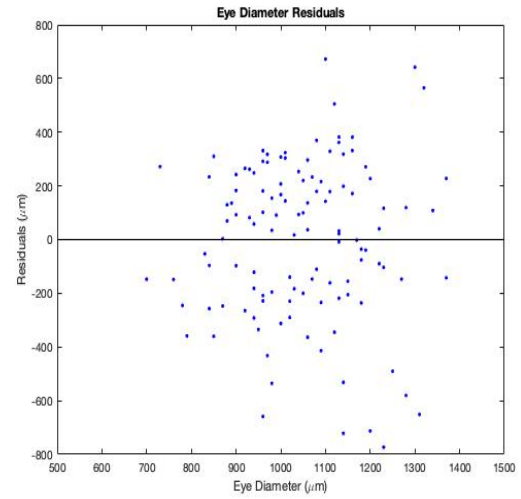
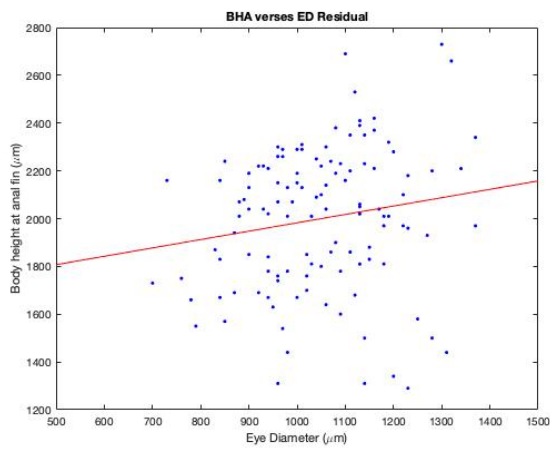


Figure S1. Example of some of the many linear regressions done on length and width measurements taken from larval fish.

## APPENDIX I: ECU IACUC APPROVAL MEMO



Animal Care and Use Committee  
003 Ed Warren Life Sciences Building | East Carolina University | Greenville NC 27354 - 4354  
252-744-2436 office | 252-744-2355 fax

April 5, 2023

Rebecca Asch, Ph.D.  
Department of Biology, ECU

Dear Dr. Asch:

Your Animal Use Protocol entitled, "Climate-related shifts in larval fish and zooplankton phenology of Beaufort Inlet, NC" (AUP #D361a) was reviewed by this institution's Animal Care and Use Committee on 4/4/2023. The following action was taken by the Committee:

"Approved as submitted"

**\*\*Please contact Aaron Hinkle prior to any hazard use\*\***

A copy of the protocols is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP/Amendment and are familiar with its contents.**

Sincerely yours,

A handwritten signature in black ink that reads "S. McRae".

Susan McRae, Ph.D.  
Chair, Animal Care and Use Committee

SM/GD

enclosure

[www.ecu.edu](http://www.ecu.edu)