Abstract

EARLY LIFE HISTORY OF LARVAL RIVER HERRING

IN A COASTAL WATERSHED:

ABUNDANCE, GROWTH, AND MORTALITY

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River herring are two closely-related, anadromous fish species, Alewife (*Alosa aestivalis*) and Blueback Herring (*A. pseudoharengus*), which have been historically, commercially, and ecologically important along the North American Atlantic coast for hundreds of years. However, recent decades have been marked by their dramatic population declines and a collapse of the fishery. Historical records show that the coastal watershed of North Carolina's Chowan River was an epicenter for river herring harvest and spawning from pre-1700 through the late 1980s. I spatiotemporally characterized the early life history of river herring larvae in the Chowan River and its tributaries in the spring spawning season of 2011 by calculating larval abundance, growth, mortality, and diet relative to water quality and chemistry. Results show that the Chowan River and its tributaries supported relatively high numbers of river herring larvae in 2011 compared to an early 1980s study, with mean catches per unit effort (CPUEs) ranging from 52.87 $\pm$ 71.68 larvae/100 m$^3$ to 1583.53 $\pm$ 2698.18 larvae/100 m$^3$ compared to a similar and

neighboring riverine system – the Roanoke River – with mean CPUEs ranging from $4.1 \pm$ 20.9 larvae/100 m$^3$ in 2008 to $30.8 \pm 149.8$ larvae/100 m$^3$ from a study in 2009. A concurrent study to my research indicated that larval river herring diets are very similar between the adjacent systems, consisting primarily of copepods and rotifers in both the lower Chowan and the lower Roanoke River. Also, analyses of abundance, growth rates, and mortality rates suggest that density-dependent mechanisms likely control larval river herring trends throughout the Chowan system. Although all nursery habitats are worthy of research and conservation efforts, the Chowan River has continually proved to be a regional epicenter for successful reproduction and early life history of river herring and, therefore, merits special attention as a Strategic Habitat Area (SHA) by the State of North Carolina.

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ABUNDANCE, GROWTH AND MORTALITY


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by

Anne Dowling Ezzard

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DEDICATION

This is for my father, Robert Dowling, the first fisherman I ever knew; for my mother, Nancy Dowling, the first academic and free spirit I ever knew; for my husband, Meredeth Jared Ezzard, my rock and the love of my life; and to our sweet son, Ira Flynn Ezzard, may you always know the power of completion.  I cannot imagine completing any of this without the love, loyalty, and unwavering belief and positivity from each of you. Thank you all for all the inspiration, encouragement, and support that has made the completion of this degree possible. It has been a trying, but hugely educational and rewarding journey.

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# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

Introduction

When an important fishery collapses, one challenge to facilitating its recovery is understanding the criteria for successful reproduction and subsequent survival during the early life development of the fish within the watershed in question. If the general location and habitat for reproduction are already known, then delving into survival in the nursery habitats and during early life history become priorities. One approach to characterizing these habitats is to investigate the early life history attributes, including larval distribution, abundance, growth, mortality, and diet. This approach helps fisheries scientists and managers understand how a given area functions as nursery habitat for the larvae of important food web predators and consumers like river herring.

"River herring" is a term that collectively represents two ecologically and morphologically similar species of Atlantic alosine fishes, Alewife *Alosa aestivalis* and Blueback Herring *A. pseudoharengus*, which are usually managed together as a single entity. Alewife range from Nova Scotia to South Carolina, and Blueback Herring range from Labrador to Florida (Rulifson et al. 1994; Haas-Castro 2006). Like most alosines, including the closely related American Shad *A. sapidissima*, river herring are anadromous, making them ecologically uncommon (Hall et al. 2011). Anadromous fishes are predominantly marine, spending most of their adult lives at sea, but they migrate briefly back into coastal freshwater rivers for spawning. Unlike some other anadromous species, such as salmon, river herring can be iteroparous; they can spawn multiple times throughout life (Hall et al. 2011).

North Carolina's Chowan River historically supported one of the USA's most productive river herring fisheries and nursery habitats (Kosa and Mather 2001). However, since the late 1950s, river herring populations in the Chowan River, as well as the U.S. portion of their ranges, have suffered dramatic population declines (Figure 1) to the point of moratoria being instated in many Atlantic coastal states (Table 1). The decline of river herring populations has been attributed to three main causal factors: habitat alteration and loss (e.g., damming), pollution, and overfishing (Boreman 1981; Schmidt et al. 2003; Waldman and Limburg 2003; Hall et al. 2011); however, current data are insufficient to conclude that any one of these factors is primarily at fault (Winslow 1990; Hightower et al. 1996).

The 1996 Magnuson-Stevens Act mandated fisheries managers to identify and describe essential fish habitats (EFHs) of federally managed species with consideration to conserve and enhance such habitats (Magnuson-Stevens Fishery Conservation and Management Act 1996). As a result of their fishery collapse, river herring became a primary interest of fisheries managers for nursery habitat characterization, conservation, and restoration work (Boreman 1981; Rulifson et al. 1994; Hightower et al. 1996; Waldman and Limburg 2003; Savoy and Crecco 2004; Haas-Castro 2006).

One aspect of EFH use and survival during early life history is the feeding ecology of a species. Limited information has been published characterizing larval river herring diets throughout the Chowan River, NC. However, prey availability and abundance is essential to larval survival (Jepsen et al. 2014), and identifying prey species that are critical to larval fish diets is considered necessary in trying to identify and describe essential habitat and in understanding and predicting recruitment patterns (Cowan Jr and Shaw 2002). Therefore, there is

a need to evaluate how Blueback Herring and Alewife consume and use food web resources in the Chowan River nursery habitats.

There are economic, ecological and cultural rationales for conserving and restoring river herring populations. From an economic perspective, it would be in the best interest of fishermen, restaurants (who now import herring from foreign countries), and other local constituents to restore the populations to harvestable numbers. The harvest of river herring was once a source of income and way of life for many fishing communities, provided food to thousands of people, and supported rich cultural traditions and ways of life in North Carolina (Bowden 2013). For example, for many people along the Chowan River the spring spawning runs of river herring equated to village-wide fish fries and a traditional fish stew on Easter Sundays (T. Pratt 2011, Albemarle Fishermen's Association, personal communication). Now that moratoria on fishing for river herring have been in effect for many years, newer generations are not able to work as river herring fishermen, so many of these rich cultural traditions and ways of life, which were passed down generation to generation, are at risk of being lost forever.

Ecologically, the two river herring species are important predators on zooplankton and important as prey for larger fishes and variety of other animals in the coastal food web; even their eggs and larvae provide an excellent source of nutrition for a variety of freshwater organisms (Crowder 1980). Their anadromous behavior connects them to riverine and marine food webs. In lakes and rivers, the various life stages of river herring can become prey to animals like river otters, raccoons, double-crested cormorants, ospreys, snakes, frogs, ctenophores, and commercially and recreationally important fish like Striped Bass *Morone saxatilis* and Largemouth Bass *Micropterus salmoides* (Manooch 1973; Boreman 1981; Klauda et al. 1991;

3

Yako et al. 2002; Dalton et al. 2009). Bottlenose dolphins, sea gulls, Bluefish *Pomatomus saltatrix* (Boreman 1981), and commercially and recreationally important fish species may also prey on adult river herring in marine environments. As economically and ecologically significant fish, the results of my research will be a source of information for ecosystem planning, habitat restoration, and fisheries management efforts to conserve river herring.

My research targeted areas of Chowan River that are deemed to be critical for successful reproduction and larval survival; such areas would be considered EFH (Rosenberg et al. 2000; Bilkovic et al. 2002). The EFHs are important because they allow for the future propagation of fish populations by providing a place for larval fish to hatch, feed, grow, and dwell until they are larger and able to move into more open waters (Rosenberg et al. 2000). In North Carolina, the Department of Environmental Quality (NCDEQ) has a similar classification known as Strategic Habitat Areas (SHAs; Street et al. 2005). Studying larval mortality in these habitats is an important aspect to determine relative contributions of a given habitat to the surviving larvae. Consequently, the absence and degradation of EFHs can compromise the long-term integrity and elasticity of populations.

### River Herring Life History

Blueback Herring and Alewife have similar spawning patterns. Both spawn in the running waters of rivers and streams with no significant difference between mid-river and shoreline environments, but they can also spawn in a wider range of habitats from flooded woodlands to lentic areas (Kissil 1974; Loesch and Lund Jr 1977; O'Rear 1983). They both spawn in the spring (Klauda et al. 1991; Rulifson et al. 1994), with Alewife generally spawning

from mid-March to early April before Blueback Herring, which spawn from mid-April to late May (Klauda et al. 1991).

When water temperatures are appropriate and the river herring spawn, their fertilized eggs are initially demersal and adhesive for a maximum of 24 hours (Fay et al. 1983). Initially, the fertilized eggs range in diameter from 0.80-1.27 mm (Mansueti and Hardy 1967; Norden 1967). After hardening, the eggs are semi-buoyant and drift with the current along the benthic substrate becoming susceptible to capture. Within 3-6 days, the eggs hatch and become yolk-sac larvae ranging in size from 2.5-5.0 mm in total length (TL) (Mansueti and Hardy 1967; Norden 1967; Jones et al. 1978). This stage can last from 2-5 days depending on water temperature, dissolved oxygen, and water acidity (Mansueti and Hardy 1967; Jones et al. 1978; Klauda 1989). During this phase, the yolk-sac larvae are planktonic and are still susceptible to dispersal by passive transport (Overton et al. 2012).

### *Larval Ecology*

Studies of early life history for any fish species *in situ* always hold potential for examining the effect of fluctuating external variables. These external (and environmental) variables can shape the critical larval developmental period by controlling growth and mortality rates. For example, warmer post-hatching temperatures can allow for faster growth rates (Houde and Zastrow 1993; Houde 2002); fluctuations in storm patterns or human activities (e.g., agriculture, pollution, fishing, etc.) potentially affect prey and/or predator abundances. The growth of larval fish depends on their prey availability and abundance, and their survival also depends on their size (growth) and the abundance of their predators; thus, early life history

studies often demonstrate the presence of density-dependent control, whereby density of larvae depends on the adult spawning stock density (Shepherd and Cushing 1980).

Annual recruitment to a fishery is heavily connected to the survivorship of given year classes or cohorts (Hjort 1926; Kondo 1980; Bailey and Houde 1989; Dippner 1997; Houde 2002). The rate of survival is a function of the difference between increase in biomass (growth rate) and decrease in abundance (mortality rate) (Houde 2002). The mortality rates are highest during the larval phase (Hjort 1914; Hjort 1926; Houde 2002), and larval mortality is mainly a result of predation, starvation, and distribution to unfavorable habitats (Hjort 1914; Hunter 1981; Bailey and Houde 1989; Houde and Zastrow 1993; Letcher and Rice 1997; Houde 2002). Studies have estimated that for most species, 95% to >99% of larvae will not survive to the juvenile stage (Houde and Zastrow 1993; Houde 2002).

Dietary resource use and availability are important to consider while examining natural larval mortality and EFH (Shepherd and Cushing 1980; Jepsen et al. 2014). Food availability factors, in addition to predation, directly impact the maximum instantaneous mortality rates during initial life phases (Lasker and Zweifel 1978; Shepherd and Cushing 1980; Bailey and Houde 1989; Houde 2002; Jepsen et al. 2014). The significance of food to larval survival is echoed by the fact that EFH is defined by waters where fish larvae feed and grow to maturity (Magnuson-Stevens Fishery Conservation and Management Act 1996). Previous studies in Connecticut and Virginia (Marcy 1976; Loesch and Kriete Jr 1980) demonstrated that larval river herring diets consisted mostly of phytoplankton and zooplankton, especially rotifers. A recent study showed that copepod nauplii, bosminids, and rotifers accounted for over 85% of larval river herring diets in Roanoke River, NC (Riley 2012). A prior study from the 1980s

6

reported bosminids, other cladocerans, rotifers, dipteran larvae, and copepodite copepods as the dominant prey of young clupeids (including river herring) in Roanoke River, NC (Rulifson et al. 1994). This suggests a steady trend in rotifers and crustacean zooplankton (i.e. bosminids and copepods) dominating larval river herring diets across various riverine systems.

More research has been conducted on juvenile diets. For example, in Hamilton Reservoir, Rhode Island, juvenile river herring diets consisted primarily of zooplankton belonging to Tendipedidae, Cladocera, Ostracoda, and Copepoda (Vigerstad and Cobb 1978) compared to larval American Shad, which have been shown to consume copepods, cladocerans, chironomids, and various stages of aquatic and terrestrial insects (Maxfield 1953; Levesque and Reed 1972; Crecco and Blake 1983; Johnson and Dropkin 1995). In Chowan River, a study of juvenile river herring diets and zooplankton found that, while nauplii and bosminids were the most commonly occurring zooplankton from March through May of 1983, juvenile river herring positively selected for cladocerans, ostracods, and chironomid larvae, as well as rotifers, which were abundant in their stomachs regardless of the individual larval river herring size (SL mm) (Winslow et al. 1985). Additionally, Winslow et al. (1985) reported that the Chowan River zooplankton compositions in 1982 and 1983 were not strongly influenced by total algal biomass or cyanobacteria, which previously had been thought to negatively impact prey densities. However, it should be noted that the study results were limited due to inadequate numbers of captured fish and due to possible malfunctions in the plankton traps that were used, which may have resulted in under sampling of the zooplankton (Winslow et al. 1985).

Rulifson et al. (1994) examined food web structure from 1982 to 1988 of a neighboring river system – the Roanoke River – that also supports river herring populations. The study had a

broad scope and was intended to investigate the poor recruitment of larval Striped Bass *Morone saxatilis*, another anadromous fish species that co-habits many of the same river systems as river herring. This research found variation in water quality relative to annual and seasonal fluctuations. Results showed that overall low and patchy zooplankton abundances were not phytoplankton limited, but were affected by seasonal environmental factors; the zooplankton community composition of the river region of the study exhibited high abundances of copepods and cladocerans (with special emphasis on bosminids and daphnids). Clupeids (including river herring) exhibited variable feeding success rates between years and sites, with a comprehensive average of around 43%, with primary prey consisting of cladocerans (mainly bosminids) and rotifers. It should be noted that these results included fish from larval to juvenile stages (Rulifson et al. 1994).

*Historical and Management Context*

Historically, adult river herring populations supported extensive fisheries along the Atlantic coast (Hightower et al. 1996). In the mid-1700s, river herring were of particular economic value because, as a protein source with high oil content, they could be salted and preserved without refrigeration (Hightower et al. 1996).

Albemarle Sound historically supported one of the largest and most valuable river herring fisheries through the 1950s, especially the Greenfield Fishery along Chowan River (Hightower et al. 1996). While the fishery peaked in the 1950s, river herring stocks collapsed all along the East Coast of the United States from 1970 through the 1990s (Figure 1[this paper]; Hightower et al. 1996; Haas-Castro 2006). Since the early 1900s, management attempts to prevent the population declines were unsuccessful (Hightower et al. 1996). In September of 2007, North Carolina joined

the growing list of eastern states implementing both recreational and commercial moratoria on river herring (Table 1 [this paper]; Haas-Castro 2006). Since the imposition of the moratoria, river herring have been designated as species of concern, according to the National Oceanic and Atmospheric Administration (NOAA) and are a focus of fisheries managers throughout the East Coast (Haas-Castro 2006).

North Carolina's Greenfield Fishery, located at the mouth of Chowan River, formerly produced record high catches of river herring (Hightower et al. 1996). Historically high catches within and near the Chowan River suggest that the Chowan River and the environmental conditions present provided propitious spawning and nursery habitats for the river herring, but there has been a lack of data characterizing early life history of river herring from the Chowan River system. As of 2011, larval abundance (O'Rear 1983; A. Kenyon 2011, North Carolina Division of Marine Fisheries, personal communication), average larval and juvenile size (Kenyon 2011, personal communication), and juvenile diet (Creed Jr 1985) represent the primary attempts to document and investigate the early development of river herring in the Chowan River. Expanding the breadth of available data would contribute essential information for fisheries managers on a broad scale and add to the growing body of literature on larval fish ecology. This type of research could support developing management plans to identify, describe, and conserve EFH for a variety of fish species, particularly alosines.

Despite the river herring population declines and the significance of the Chowan River watershed as a nursery, the only published survey of Chowan River evaluating larval abundance and distribution was from 1980-1982 (O'Rear 1983). O'Rear measured larval river herring abundance and water quality at approximately 15 sites throughout Chowan River and its

tributaries for two spawning seasons (of O'Rear's original 15 sites, nine of the same sites were used as the sample sites for this study) (Figure 2). The decade preceding his study was marked by poor water quality in Chowan that was associated with problem algal blooms. Therefore, O'Rear's (1983) data provide unique historical records of river herring nursery habitats along Chowan River, which can provide a basis for comparison with findings of the current study.

The existence of suitable river herring spawning and nursery habitats is essential to their successful reproduction, larval survival and growth, and population integrity and recovery. It is beneficial to identify and describe such locations that support fast growth and low mortality rates during early life stages. Identification and characterization of those nursery habitats that support flourishing abundances of larvae can guide future management, monitoring, and conservation efforts necessary for the rehabilitation of river herring stocks.

### *Current context*

In the last several years, river herring have become an even higher conservation priority to the extent that there has been discussion of whether to elevate them to the endangered species list level (Cournane et al. 2013). Also, there have been several recent papers specifically detailing early life history, water quality and chemistry, and genetics of river herring throughout their ranges, and specifically throughout their North Carolina habitats (Binion 2011; Riley 2012; Butler 2012; Overton et al. 2012; Palkovacs et al. 2013; Hasselman et al. 2014).

Many recent studies investigated larval alosine (including river herring) prey requirements, availability, suitability, and overlap. Binion (2011; Binion et al. 2012) reported that American Shad, Alewife, and Blueback Herring larvae in Roanoke River and Albemarle Sound were not limited by prey availability, and that first feedings were most likely on copepod

nauplii and rotifers because of prey size and mouth gape limitations. Additionally, the zooplankton quantification and composition results showed that Daphiniidae, Bosminidae, calanoid and cyclopoid copepods, copepod nauplii, and rotifers were the most prevalent groups of zooplankton present in the sound, delta and riverine areas of Roanoke River and Albemarle Sound, with the highest densities occurring in the sounds areas (16,546 $\pm$ 14,678 [number/m$^3$ $\pm$ SD]) versus the riverine (4,934 $\pm$ 3,806) and delta (4,647$\pm$ 2,864) areas. The pattern of zooplankton abundance was negatively correlated with larval alosine abundance (number/100 m$^3$), which was highest in the riverine areas (21.0 $\pm$ 127.6) versus the delta (7.5 $\pm$ 35.5) and sound (4.6 $\pm$ 24.8) areas. Despite the opposing abundance patterns, Binion et al. (2012) noted a large amount of overlap between larval alosines and size-appropriate prey across space and time (Binion 2011; Binion et al. 2012).

Riley (2012; Riley et al. 2012) echoed Binion's findings in a diet study that demonstrated a significant degree of dietary overlap for larval alosines in Roanoke River and Albemarle Sound, reporting that 85% of their diets consisted of copepod nauplii and rotifers. Riley's research also found significant differences in larval river herring abundances (expressed as number/100 m$^3$) between the 2008 and 2009 spawning seasons (4.1 $\pm$ 20.9 and 30.8 $\pm$ 149.8, respectively). Additionally, Riley calculated growth rates of larval Alewiife (0.30 $\pm$ 0.14 mm/d) and Blueback Herring (0.29 $\pm$ 0.16 mm/d), and these rates were used as a proxy for habitat quality. Mortality rates of the larval Alewife and Blueback Herring were also estimated (0.64 $\pm$ 0.17 per day and 0.76 $\pm$ 0.23 per day, respectively) (Riley 2012; Riley et al. 2012).

Lichti (2014) investigated fatty acid profiles of zooplankton in the Chowan River, which can be considered a proxy for larval fish habitat quality. This research found evidence of

extensive zooplankton composition fluctuations relative to saltwater intrusion vs freshwater influx events in the Chowan River, suggesting considerable annual temporal and spatial variability in zooplankton communities throughout the watershed as a function of seasonal salt vs freshwater mixing patterns in any given year. The study also showed that zooplankton composition across all three sampling regions of the Chowan River were dominated by copepods and rotifers. The findings also indicated that the zooplankton community was adequate both in density and fatty acid content to sustain larval fish (Lichti 2014).

And while the zooplankton has been deemed adequate to support both Alewife and Blueback Herring larvae, it seems that conditions in the Chowan River are also such that the two species are interbreeding. In a seminal hybridization study by Hasselman et al. (2014), genetic analyses of over 29 Atlantic coastal watersheds with anadromous Alewife and Blueback Herring populations showed frequent hybridization rates ranging from 0-8%. However, landlocked sites proved to be outliers in the dataset with dramatically higher rates of hybridization, as high as 100%. Chowan River was one of the sites sampled and did not exhibit high rates of hybridization; only 7 out of 126 river herring were genetic hybrids, which is a rate of 0.048% (Hasselman et al. 2014). Thus, while hybridization was present in Chowan River, it was rare.

Also, it should also be noted that in a study parallel to mine (i.e., Butler 2012), water samples were taken by a different researcher at the same locations and times. Butler (2012) examined physicochemical factors (chlorophyll α, ammonium, nitrate/nitrite, inorganic and organic dissolved nitrogen, chloride, phosphate, calcium, potassium, magnesium, and sodium) to group the sample sites into like-clusters based on the degree of dissimilarity. Clusters with 90% similarity were grouped together for further comparisons (Butler 2012); the resulting

physicochemically-similar clusters (Figure 3) were used in my research to compare larval river herring growth and mortality rates and diet composition. Butler's (2012) Cluster 1 comprised site 9 (South Chowan), Cluster 2 comprised site 8 (Rockyhock Creek), Cluster 3 comprised sites 1 and 7 (North Chowan and Holiday Island, respectively), and Cluster 4 comprised sites 2, 3, 4, 5, and 6 (Meherrin River, Sarem Creek, Wiccacon Creek, Bennett's Creek, and Catherine's Creek, respectively).

These groupings by Butler (2012) showed the two most easterly sites (sites 8 and 9) forming two single-site clusters (Clusters 1 and 2), which is reasonable considering their proximity to the waters of Albemarle Sound where water chemistry would be more dramatically different than further upstream. Cluster 3 was formed by the only other two sites (sites 1 and 7 – North Chowan and Holiday Island, respectively) that were in the main stem of the river, where water chemistry would have been better mixed. Thus, it is not surprising that the largest cluster – Cluster 4 – consisted of sites that were all in adjacent tributaries, and that these sites held the highest abundances of larvae.

Butler (2012) used the clusters to investigate the effect of water chemistry on abundance of river herring larvae in Chowan River. The results showed a relationship between levels of chlorophyll a, phosphate, and organic nitrogen and habitats with higher abundances of larvae (Catherine's Creek and Wiccacon Creek) (Butler 2012). He noted that the sites with higher abundances were in the same cluster – Cluster 4 – and that those sampling sites had higher concentrations of chlorophyll a, phosphate, and potassium.

While current and historical literature have examined many aspects of river herring, a gap in data still exists specifically regarding the spatiotemporal patterns of their larval stage in the

Chowan River. Therefore, my research is intended to contribute to the current body of literature by providing insight into the spatiotemporal abundances, growth and mortality rates, and dietary composition of river herring larvae in the coastal watershed of the Chowan River.

Goals and Objectives

The goal of my research was to investigate how Chowan River system functions as a nursery habitat for early life stages of river herring throughout the spring spawning season. The specific objectives were: (1) to determine the spatiotemporal distribution and abundance of river herring larvae and compare them to historical records; (2) to determine spatiotemporal patterns of larval growth and mortality rates among sites with unique water quality and chemistry; and (3) to identify dietary composition of larval river herring among sites with unique water quality and chemistry.

I used studies by O'Rear (1983) and Riley (2012) for historical and contemporary abundance comparisons (Table 2). Data by Butler (2012) provided a physicochemical characterization of the Chowan River sites that I used to compare growth and mortality rates, along with dietary differences (Figure 3).

Methods

## Site Description

The Chowan River is a well-mixed, highly turbid, freshwater coastal river that flows into the Northwestern portion of North Carolina's Albemarle Sound (Stanley and Hobbie 1981). The flow is weakest in the summer, reaching lows of $<30$ $m^3s^{-1}$, and generally peaks in the winter near 400 $m^3s^{-1}$. The average depth is 4 m and temperature usually ranges from 4° C to 30° C (Stanley and Hobbie 1981). The Chowan River basin drains roughly 3,569 $km^2$ of land and consists of approximately 1,260 stream kilometers (Giese et al. 1985), and integrates numerous tributaries. The surrounding land use is characterized by agriculture (including swine farms) and forestry, which have contributed significantly to decreased water quality, especially due to nutrient runoff containing phosphorus (Paerl 1982). In the late 1970s the Chowan River was noted for problematic blue-green algal blooms, but water quality has improved since then (Paerl 1982; Giese et al. 1985) though noxious blooms still appear occasionally under the right conditions.

## Field Sampling and Processing

We used push nets to collect larval river herring, and a drift net to sample eggs. Concurrent water quality measurements were taken at the surface (0.5 m below) and off the bottom of the river (1.0 m above) at each sampling location. I removed otoliths from a subsample of post-yolk sac larvae and aged them, so that growth and mortality rates could be calculated. I identified the stomach contents of a subsample of larvae and enumerated them to the lowest taxon possible to characterize dietary composition.

Samples collected from nine sites were used to determine water quality and
spatiotemporal patterns of larval fish distribution and abundance. These sites were within the
Chowan River system and tributaries, and included Wiccacon, Catherine's, Bennett's, and
Rockyhock creeks (Figure 2). I chose these sampling locations based both on the previous
sample sites of O'Rear (1983) and in consultation with knowledgeable North Carolina Division
of Marine Fisheries (NCDMF) personnel, North Carolina Wildlife Resource Commission
(NCWRC) personnel, and commercial fishermen from the Chowan River who are familiar with
historical and current spawning patterns of river herring. Each location was sampled once each
week for 11 weeks from March 18 to May 26, 2011. This sampling schedule spanned the
predicted spawning peaks with additional time added on both ends to account for annual
variation (Street et al. 1975; Klauda et al. 1991; Walsh et al. 2005).

Sites were sampled during daylight hours from sunrise until late afternoon, as it has been
shown that there is no significant difference between river herring larval abundance during the
day and night (Overton and Rulifson 2007). Because of the distances between sites, and the
number of sites involved, it was necessary to sample in a linear fashion; however, the potential
time-location issue was partially "unlocked" by alternating the direction of sampling each week:
starting either from the most upstream (northerly) station or from the most downstream
(southerly) station. However, it should be noted that the linearity of the study area (i.e. upstream
to downstream) may have unavoidably biased the data based on the natural distribution of river
herring larvae. Most river herring larvae are spawned toward the headwaters of tributaries, so
convergence of these tributaries at the most downstream main stem sites will aggregate the

16

larvae; results could reflect natural abundance loss due to mortality, and surviving larvae may be larger after having time to grow.

Samples of river herring larvae were obtained by using paired conical, bow-mounted plankton push nets approximately 0.5 meter below the surface. Each net was constructed of 500-μm nitex mesh with a 0.5-m diameter mouth and 5:1, tail: mouth ratio (Overton and Rulifson 2007; Overton et al. 2012) and fitted with a General Oceanics (model 2030) flowmeter. Based on the successful use of this method in previous studies (Overton and Rulifson 2007; Riley 2012), it was assumed that the sub-surface, bow-mounted plankton push nets would effectively sample the ichthyoplankton present. Based on O'Rear's (1982) findings that there was no significant difference (chi-square analysis; $p > 0.1$) between the distribution of larval catches from center and edge transects, sampling transects for my study were run in the center of the river and the creek sites. Sampling was conducted against the prevailing current once at each site per sampling day for a duration of two minutes at approximately 1200 rpms (Overton and Rulifson 2007). Readings from the flowmeters were used to calculate the theoretical volume of water that passed through each net during each sampling event. These volumetric data, in concert with the numbers of clupeid larvae, allowed us to quantify clupeid larval densities and enabled more accurate comparisons.

The push net design allowed for simultaneous replicate samples to be collected. The contents of one sample were fixed and preserved in 5% formalin for preserving the pigmentation and body structure of the larvae to facilitate morphometric measurements and identification (Tucker Jr and Chester 1984; Fox 1996; Murphy and Willis 1996). The other replicate sample

was fixed and preserved in 70-80% ethanol to protect the integrity of the otoliths for daily growth ring aging (Fox 1996; Murphy and Willis 1996).

### *Water Quality and Abundance*

Conductivity-temperature-depth (CTD) casts were taken 0.5 m below the surface and 1.0 m off the bottom of each site per sampling event to profile conductivity (μS), temperature (°C), salinity (ppt), pH, and dissolved oxygen (percent and mg/l). Some parameters, particularly temperature and dissolved oxygen, have been significantly correlated with the hatching success of river herring eggs (Bozeman Jr and Avyle 1989; Sismour 1994).

The fish larvae were separated from other contents in the rest of the push net samples (e.g., algae) and initially sorted into two categories – "clupeids" and "other." Then the alosine larvae within the clupeids were identified to species by using taxonomic keys.

An extensive literature review of river herring identification and differentiation was conducted to ensure proper speciation between larvae from the samples. A blind study of the taxonomic keys was conducted to validate identification of *A. aestivalis* and *A. pseudoharengus* because of the similarities present between the species. However, inconsistencies between the keys were frequently found in the descriptions and counts of distinguishing morphometric characteristics critical to taxonomic classification. Six larval identification manuals and around twenty peer-reviewed articles were included in the literature review (Appendix 1). The outcome of the review revealed overlapping and unclear morphometric descriptions and evidence of recurring, inaccurate citations for myomere counts and morphometric ratios (Mansueti and Hardy 1967; Cianci 1965; Lippson and Moran 1974; Chambers et al. 1976; Lam and Roff 1977; Jones et al. 1978; Auer 1982; Bulak 1985; Sismour 1994; Wallus and Kay 1990; Fahay 2007).

Additionally, throughout academia there is mounting discussion and evidence of hybridization occurring in some river herring populations (Hasselman et al. 2014). Considering the lack of clarity and accuracy in the literature, the difficulty in differentiating between the two species, and the recent documentation of genetic hybridization in some river herring (Hasselman et al. 2014), I grouped Blueback Herring and Alewife larvae as "river herring" for data analysis and the duration of this study. Furthermore, O'Rear (1983) grouped the two species as "river herring" in his investigation of river herring larval abundance in Chowan River, so combining the species allowed for direct comparisons to O'Rear's (1983) study.

When a sample contained $\leq$100 larvae, all larvae in the sample were identified and measured as described above. However, if a sample contained >100 larvae, the larvae were subsampled using the ichthyoplankton protocols of Dauphin Island Sea Lab to produce accurate data in a timely, efficient manner (Appendix 3). The goal was to subsample a minimum of 10% of the whole sample, with an upper cap of around 200 larvae; however, the original sample was never split more than five times (i.e., a sample may have been split five times maximum to produce an aliquot 1/32 of the whole sample). Larval fish abundances were volumetrically standardized as the number of fish per 100 $m^3$, and a maximum of 200 river herring larvae per sample were measured for standard length (SL; from the snout to the caudal peduncle) to the nearest 0.1 mm (Walsh et al. 2005). Note that any yolk sac larvae were measured for notochord length (NL; from the snout to the end of the notochord).

***Growth and Mortality***

Accurate aging of individual fish can improve the estimation of population growth rates, age-specific growth, and individual variation in growth and survival. For this study, I assumed

that otolith increments are formed daily and that they provide a historical record of growth (Sismour 1994; Fox 1996; Murphy and Willis 1996). The larval otolith analysis followed the protocols given by Secor et al. (1995) and Walsh et al. (2005).

With considerations for numeric, resource, and time constraints, not all sites were represented in the subsampling used to calculate daily growth (G) and mortality (Z) rates, but larvae from each of four physicochemically similar clusters *were* represented. Larval otolith analysis was completed for site 9 (South Chowan), which represented Cluster 1; for site 8 (Rockyhock Creek), which represented Cluster 2; sites 1 and 7 (North Chowan and Holiday Island, respectively), which represented Cluster 3; and sites 2, 6, and 4 (Meherrin River, Catherine's Creek, and Wiccacon Creek, respectively), which represented Cluster 4.

A subsample of at least two ethanol-preserved larvae from each available length class (i.e., $\geq 3.0$ to $\leq 6.0$ mm, $>6.0$ to $\leq 9.0$ mm, $>9.0$ to $\leq 12$ mm) per physicochemically similar Cluster (Butler 2012), and for each sampling event, were randomly selected for otolith analysis for a maximum total of 550 otoliths (Rilling and Houde 1999). After each fish was measured, sagittal otoliths were removed from larvae by using tweezers and fine dissecting needles. The otoliths were washed in ethanol and cleaned of adherent tissues, then air-dried and mounted on a glass microslide using low-viscosity epoxy resin (DePeX mounting medium, Electron Microscopy Sciences, Fort Washington, PA). All otoliths were mounted prior to independent age determination and marginal increment analysis.

Otoliths were analyzed at 1000X magnification by using a compound microscope (Olympus BH-2 microscope) and oil immersion. The microscope was equipped with a high-resolution video camera and computer with image analysis software (Image Pro Discovery).

20

Polarizing light and filters were used to improve the contrast of otolith microstructure in digital photographs and allowed increments to be measured precisely. Image analysis was used to measure the radius and diameter of each otolith and its nucleus. Increment widths were measured along the longest axis from the center of the nucleus to the outer edge (Stevenson and Campana 1992). The distance between each pair of consecutive rings was used to estimate daily growth.

To estimate age, increments were counted from the nucleus, beginning at the first clearly defined mark encircling the primordium, to the outer edge of the otolith. Each growth increment showed a common bipartite structure consisting of an incremental zone that appeared light and translucent, and a discontinuous zone that appeared dark and opaque (Secor et al. 1995). Otolith increments were counted blind (i.e., no sample information available) on two separate occasions by a single reader. If the difference in increment counts was two increments or less, the average of the two counts was used to estimate age; otherwise, the sample was discarded. A correction factor of two days was added to all age estimates to account for the number of days between spawning and first increment formation (Essig and Cole 1986; Sismour 1994).

The aged larvae were measured, and their ages and lengths were used to calculate growth for seven of the sites. Because of time and funding constraints growth was calculated for seven out of the nine total sample sites. However, the seven sites were chosen with the goal of representing three aspects: (1) the physicochemically unique clusters; (2) sites with historically high abundances of larval river herring (e.g., Wiccacon Creek and Catherine's Creek); and (3) the range of habitat types across the sites (e.g., mainstream river vs a creek or tributary). Therefore, growth was calculated for site 9 (South Chowan River, the only site within that

cluster) to represent Cluster 1, site 8 (Rockyhock Creek, the only site within that cluster) to represent Cluster 2, and sites 1 and 7 (North Chowan River and Holiday Island, the only two sites within that cluster) to represent Cluster 3. Cluster 4 was represented by site 2 (Meherrin River), site 4 (Wiccacon Creek), and site 6 (Catherine's Creek). Wiccacon Creek and Catherine's Creek historically had the highest abundances of river herring larvae (O'Rear 1983), and site 2 (Meherrin River) was the only other mainstream river site in the study that was not within the Chowan River. Sites 3 and 5 (Sarem Creek and Bennett's Creek, respectively) were excluded from growth analysis.

 Only river herring larvae between 5 and 15 mm SL were used to calculate growth and mortality; this size range was used to represent larval fish that were post-endogenous phase (i.e., no longer reliant on the yolk sac for nutrition; >5 mm SL) and vulnerable to the ichthyoplankton push nets (<15 mm SL). Therefore, age was determined only for river larvae within this size range. Larval age determined the distribution of hatch dates, which defined cohorts; these data were also used to calculate growth and mortality rates. Growth rate was then used to conduct a linear regression of growth rate in length of larvae per day (Rilling and Houde 1999).

Somatic larval growth by length (mm/d) was calculated from the slopes of the linear regressions of otolith increment analyses (Sismour 1994; Rilling and Houde 1999):

$$L_t = a + gt; \text{ where}$$

$L_t$ = the standard length (mm) at age $t$ (d);

$a$ = the estimated length (mm) at hatch, axis-intercept of regression; and

g = somatic growth rate (mm/d).

Coefficients in growth-model regressions from the four physicochemically similar clusters were compared among sites in analysis of covariance (ANCOVA). The ANCOVA tested for significant differences in slopes (growth rates) and intercepts in the growth equations.

Catch curve-based analysis was used to calculate instantaneous mortality (Z) using Age in days estimated from otolith-increment analysis and age-specific abundance derived from age-length keys. A regression of $\log_e$ transformed age and abundance data was used to establish the daily instantaneous mortality rates at each site analyzed by using Ricker's (1975) exponential model of abundance decrease with age:

$$N_t = N_0 \cdot e^{-Zt};$$

where:

$N_t$ = predicted abundance of larvae at age $t$ (larvae/m$^3$);

$N_0$ = estimated initial abundance (larvae/m$^3$), represented by the age zero (i.e., the axis-intercept);

$Z$ = instantaneous daily mortality coefficient (larvae/d); and

$t$ = age in days (d) (i.e., days since hatching).

This catch-curve analysis for calculating instantaneous mortality (Z) assumes that there is no emigration or immigration throughout the time period being analyzed (i.e. March – May), which relies on calculating the mean abundance of fish for each age group across the entire sampling period, whereby the y-axis intercepts represent the estimated initial abundance of larval river herring per site (Ricker 1975). Thus, this model provides instantaneous mortality estimates

across the sampling period per site based on the theoretical trend that there are higher abundances of younger-aged fish than older-aged fish because some of the younger-aged fish are lost to mortality, so there are lower abundances of the older-aged fish. Certain data points were excluded from the calculations (e.g., the mortality regressions) because there were fish from those age groups that were not fully recruited to the gear. And due to natural variation in larval length at age (i.e., hypothetically, an age 8 larvae at a site with slower growth rates could be 4 mm in SL, but an age 8 larvae at a site with faster growth rates could be 7 mm in SL), some data points per site were excluded from the analysis to ensure that only fish 5 – 15 mm SL were used to calculate mortality. After the abundance data were log transformed, the data were fit to the model's log-linear form. Standard regression analysis was used to calculate 95% confidence intervals (e.g., $P > 0.05$) for the mortality estimates.

### *Diet Composition*

The diets of the larval river herring were analyzed by randomly sub-sampling a maximum of five larvae from a random site within each physicochemically similar cluster per sampling event for a total maximum of 200 larvae per cluster. For example, Cluster 3 consisted of sites 1 and 7, so for the sampling event on March 31, 2011, five larvae from Site 7 had stomach contents analyzed to represent Cluster 3 for that sampling date. The stomach contents were then removed, examined under a dissecting microscope, identified to the lowest possible taxonomic level (usually to family), and enumerated.

Stomach content data were analyzed by using a numerical index of composition (percent composition) and a percent frequency of occurrence index. Percent composition was estimated by recording the number of prey items within a specific prey type as a percentage of the total

number of prey items; frequency of occurrence index was used to estimate the percentage of all stomachs that contained at least one item of a given prey type (Hyslop 1980). Stomach content data were also used to determine feeding incidence and feeding ratio. The feeding incidence was determined by calculating the percentage of larvae whose stomachs contained at least one prey item, and the feeding ratio was determined by calculating the mean number of prey items (of any type) per stomach. In line with standard method for these calculations, stomachs containing no prey items were included in the frequency of occurrence, feeding incidence, and feeding ratio analyses, which also allowed for comparison to previous studies. These methods provided a qualitative evaluation of the prey assemblage in a timely manner that was feasible within the time and funding limitations of the study (Hyslop 1980).

*Environmental characteristics and water quality*

There were no major storm events (i.e., hurricanes or tropical storms) during the sampling period, and air temperatures were, on average, between 16.32 and 18.23 °C, with a maximum of 34.30 °C on May 26th and a minimum falling to 5.40 °C on March 25th. Mean water temperatures ($\pm$ SD) ranged from 13.0 $\pm$2.1 °C in March to 22.7 $\pm$ 2.2 °C in May. During the peak abundances of sampled larvae (on April 29th and May 5th), the mean water temperature was 21 $\pm$ 0.7 °C.

Larval abundances increased on April 20th, peaked during the weeks of April 29th and May 5th, and declined the week of May 13th before decreasing to near absence during the week of May 19th.

Water temperature varied temporally, but was consistent with patterns from previous years (Figure 4). Dissolved oxygen (mean $\pm$ SD) levels decreased throughout the sampling season, with values for March, April, and May of 7.32 $\pm$ 1.67 mg/L, 5.95 $\pm$ 1.99 mg/L, and 5.21 $\pm$ 1.98 mg/L, respectively. Dissolved oxygen levels were not above 70% saturation throughout the sampling period, and hypoxic events (< 3.0 mg/L) occurred infrequently, accounting for only 8% of readings. Anoxic conditions (< 0.5 mg/L) were not observed during sampling. Salinity was < 0.10 ppt 17% of the time, with samples greater > 0.16 ppt occurring only 3% of the time. The highest salinity reading (0.57 ppt) occurred on the last day of sampling (May 26, 2011). The water flow at all of the sampling sites flowed predominantly downstream, and surface currents were similar among sites, ranging from 0.01 − 0.12 m/s, with a mean velocity ($\pm$SD) of 0.05 $\pm$ 0.03 m/s.

At the same sampling times and locations, Butler's (2012) analysis of water chemistry showed that site 8 (Rockyhock Creek) had the highest concentrations of nitrate/nitrites, chloride, calcium, and potassium, and the second highest, site 9 (South Chowan River), had the highest sodium and magnesium levels among all sites. Site 2 (Meherrin River) had the highest ammonium, the lowest chloride and sodium, and second lowest phosphate and potassium concentrations (Figures 5 and 6). Other sites seemed to fall more within the median ranges of the detected parameters (Figures 5 and 6).

*Abundance*

Over 98% of larvae collected were clupeids, and river herring larvae were the most abundant clupeids at every site sampled along Chowan River, accounting for approximately 96% of all clupeid larvae sampled (Table 3). The river herring larvae ranged in standard length (SL) from 2.5 to 19.50 mm, with an overall mean SL of 7.6$\pm$2.7 mm (Table 4). River herring larvae were present in nearly 99% of all 98 total samples collected. American Shad, Gizzard Shad *Dorosoma cepedianum*, and Hickory Shad *A. mediocris* were uncommon and never accounted for > 0.5% of any larval sample.

A total of 46,612 fish larvae was collected, and subsampling protocols were used to identify, count, and measure representative larvae when collected samples contained >99 larvae/tow. The highest number of river herring larvae collected in a single sample was 4,933 (or a CPUE of 10,500 larvae/100 m$^3$), which occurred at the site 6 (Catherine's Creek) on the 17$^{th}$ week of the year (Figure 7). The lowest abundance of river herring larvae sampled was 0 (or a CPUE of 0 larvae/100 m$^3$), which occurred at several sites near the end of the sampling season sampling (Figure 7). The mean number of river herring larvae sampled per site was 214.26 (or a

CPUE of 473.0 larvae/100 m$^3$). Sites 6 (Catherine's Creek) and 4 (Wiccacon Creek) had the highest mean CPUEs of river herring larvae at 1,583.53$\pm$2,698.18 larvae/100 m$^3$ (SD) and 1,316.69$\pm$3026.96 larvae/100 m$^3$, respectively (Tables 4 and 5; Figures 7 and 8). Sites 9 (South Chowan River) and 7 (Holiday Island) had the lowest mean larval CPUEs at 52.87$\pm$71.68 river herring larvae/100 m$^3$ and 69.61$\pm$127.31 larvae/100 m$^3$, respectively (Tables 4 and 5; Figures 7 and 8).

O'Rear (1983) calculated larval river herring densities from 1982 at five of the same sites that I sampled:  Wiccacon Creek, Bennett's Creek, Catherine's Creek, Rockyhock Creek, and South Chowan River. For the spring of 1982, O'Rear (1983) reported the two highest mean densities of river herring larvae from Catherine's Creek and Wiccacon Creek at 0.34$\pm$0.59 larvae/m (SD) and 03.1$\pm$0.44 larvae/m, respectively (Table 2). My data (from the spring of 2011) also showed the highest mean densities of river herring larvae at Catherine's Creek (site 6) and Wiccacon Creek (site 4) at 3.78$\pm$5.09 larvae/m (SD) and 2.89$\pm$48.69 larvae/m, respectively. Additionally, O'Rear (1983) reported the lowest mean density from Rockyhock Creek (site 8) 0$\pm$0 larvae/m (SD) (Table 2).

### *Growth and Mortality*

All slopes of linear growth rate regressions from each site were significant ($P<0.0001$) (Table 6). The highest instantaneous larval growth rate was $0.71 \pm 0.04$ mm/d and occurred in Site 8 (Rockyhock Creek). The lowest instantaneous larval growth rate was $0.36 \pm 0.05$ mm/d and occurred in Site 2 (Meherrin River) (Table 6; Figure 10). The *y*-intercepts of the linear regressions (i.e., the mean estimated length at hatch) by site ranged from $1.96 \pm 0.39$ to $4.43 \pm 0.51$ mm (Table 6; Figure 11).

28

Four clusters of sites were identified based on similarities in water chemistry parameters (Figure 3). The growth patterns of river herring larvae in each of the clusters was significant and followed the linearized equation within the 95% confidence intervals (P<0.0001) (Table 7; Figure 12, 13). Cluster 2 (represented by larvae from site 8 – Rockyhock Creek) had the highest mean instantaneous growth rate (0.71 ± 0.04 mm/d), and Cluster 4 (represented by larvae from sites 2, 4, and 6 – Meherrin River, Wiccacon Creek, and Catherine's Creek, respectively) had the lowest growth rate (0.48 ± 0.03 mm/d) (Table 7). The *y*-intercepts of the linear regressions (i.e., the mean estimated lengths at hatch) by cluster ranged from 1.96 ± 0.39 to 4.10 ± 0.32 mm and varied among clusters (Table 7; Figure 12, 13).

The mean instantaneous growth rates of river herring larvae differed significantly among all clusters except between Clusters 2 and 3 (ANCOVA P<0.0001, F=239.69) (Table 7; Figure 12, 13). Cluster 1 had the highest mortality rate (0.60 ± 0.06 larvae/d, P<0.0001), and Cluster 3 had the lowest mortality rate (0.26 ± 0.06 larvae/d, P=0.0003) (Table 9; Figure ). The *y*-intercepts (i.e., the estimated initial abundances of larvae) of the significant linear regressions for mean mortality by cluster ranged from 3.44 ± 0.46 to 4.79 ± 0.66 larvae/m$^3$ (Table 9; Figure ). The sites representing the maximum (site 8 – North Chowan River) and minimum (site 2 – Meherrin River) larval growth rates supported relatively similar mean CPUEs, 93.18 ± 125.27 and 133.40 ± 308.84 larvae/100 m$^3$, respectively (Table 6).

Larval mortality rates varied among sites, and only three of the seven total sites included in the mortality regressions had slopes that were significantly different from zero (linear regression, P<0.05) (Table 8; Figure ). Of the significant results, the maximum instantaneous mortality rate (± SD) was 0.28 ± 0.05 larvae/d (P=0.0015) and occurred at site 1 (North Chowan

River). The lowest significant instantaneous mortality rate was 0.17 ± 0.05 larvae/d (P=0.0247) and occurred in site 2 (Meherrin River) (Table 8; Figures 14 and 15). The *y*-intercepts (± SD) of the significant linear regressions (i.e., the estimated initial larval abundances) by site ranged from 3.98 ± 0.5 to 5.04 ± 0.66 larvae/m³ (Table 8; Figures 14 and 15).

*Diet Composition*

All diet analyses reported here were summarized as quantitative descriptions. River herring larvae from Cluster 4 had the maximum observed feeding incidence (the percentage of larvae sampled for gut analysis that had food present in their gut) at 53.1%, and Cluster 2 had the minimum feeding incidence at 28.6% (Table 10). Cluster 4 river herring larvae also represented the highest feeding ratio (the mean number of prey items present in the gut) with a ratio of 0.72, and Cluster 2 again represented the minimum with a feeding ratio of 0.17 (Table 10). A regression of feeding ratio as a function of standard length was not significant (P>0.05) (Figure ).

Percent composition of the river herring larval diets generally differed among clusters and areas throughout the river. Copepods represented the highest percentage of the larval river herring diet composition from both Clusters 1 and 2 (Figure 13). Cluster 3 larval diets consisted primarily of rotifers and bosminids (Figure 18). Diets of Cluster 4 larvae consisted primarily of eggs, but this resulted from one day at one site, which may have represented an anomalous feeding opportunity. Eggs were excluded from the percent prey composition calculations, but were included in the frequency of occurrence (FO) calculations (Figures 18 and 19). Excluding an apparent anomalous feeding incidence on eggs, cladocerans were the dominant prey type of Cluster 4 larvae (Figure 18).

For each prey type, the FO showed similar results to that of the percent composition (Figure 18 and 19). Based on FO, copepods were the prey type that occurred in stomach contents most frequently in Clusters 1 and 2 (Figure 19). River herring larvae from Cluster 3 primarily consumed rotifers, and river herring larvae from Cluster 4 preyed primarily on cladocerans (Figure 14).

Discussion

This research characterized basic water quality parameters throughout the Chowan River in relation to larval abundance and growth and mortality rates. I also identified two distinct nursery habitats of the Chowan River that historically and currently support relatively high larval abundances. Additionally, my study characterized trends in growth and mortality rates and diets of river herring among similar habitats in the river system.

The goal of this research was to investigate how the Chowan River system functions as a nursery habitat for early life stages of river herring throughout the spring. The results showed that all sample sites, both those within the main stem of the river and within various tributaries, contained river herring larvae. This demonstrates that the Chowan River system as a whole is functioning as a widespread and productive nursery habitat for early life stages of river herring. However, what variations in larval development do exist throughout the system and across time? The specific objectives of my research provide some answers to this question.

The first objective of my study was to determine the spatio-temporal distribution of river herring larvae and compare it to historical data gathered by similar methods. My data show that larval abundances in the Chowan River have increased in number and expanded in area during the last several decades. Also, the Chowan River currently supports much higher densities of river herring larvae in comparison to historical numbers, and in comparison to other systems in neighboring regions. In 1982, O'Rear's study found a maximum mean number of larvae/m ($\pm$SD) of 0.34 $\pm$ 0.59 (n = 5; n represents number of sampling events) in Catherine's Creek; in 2011, my research found the highest mean number of larvae/m ($\pm$SD) to be 3.78 $\pm$ 5.09 (n=8) in the same system. The highest mean number of larvae found in my study was more than ten times that from O'Rear's study, but in both studies Catherine's Creek had the highest abundances of

river herring larvae compared to other river and tributary sites throughout the Chowan River system; these results indicate that the Chowan has been and continues to be a critical spawning and nursery habitat for river herring. Furthermore, O'Rear (1983) reported the lowest mean number of larvae/m ($\pm$SD) to be $0.0 \pm 0.0$ (n = 5) at Rockyhock Creek. None of my research sites had a mean larval density of zero, but I did report the lowest mean number of larvae/m also at Rockyhock Creek, with $0.18 \pm 0.22$ mean larvae/m $\pm$ SD (n=8) from sampling during the spring spawning season of 2011 (Table 2).

Spatial differences in the distribution and abundance of larval river herring throughout Chowan River occurred during the spring of 2011. The highest mean catches were found in tributary sites: the highest three being Catherine's Creek, Wiccacon Creek, and Sarem Creek, with respective mean CPUEs of $1583.53 \pm 2698.18$, $1316.69 \pm 3026.96$, and $567.91 \pm 1496.23$ larvae/100m$^3$. The lowest mean catches were found in the main stem river sites: South Chowan, Holiday Island, and North Chowan ($52.87 \pm 71.68$, $69.61 \pm 127.31$, $93.18 \pm 125.27$ larvae/100m$^3$, respectively). This suggests that river herring spawning areas are within the backwater tributaries, which is consistent with patterns in the Tar-Pamlico River (Overton et al. 2012). Water flow was consistently higher in the main-stem river sites than the tributaries; lower flows in the tributaries may create more suitable environments for spawning and support higher egg and larval retention, which is also consistent with patterns in the Tar-Pamlico River (Overton et al. 2012).

Of the tributary sites, Rockyhock Creek had the lowest mean densities with a CPUE of $94.05 \pm 131.43$ larvae/100m$^3$. The low CPUEs in the main-stem river sites are to be expected as larvae are not as concentrated in the main stem of the river. The low CPUEs observed at

Rockyhock Creek, however, are interesting because Butler's (2012) physicochemical cluster analysis of water quality (throughout these same Chowan sites) showed that Rockyhock Creek has significantly poorer water quality than any other sites. Butler's (Butler 2012) data show that, compared to the other eight sites, Rockyhock had the highest median concentrations of nitrate/nitrites, potassium, calcium, and chlorophyll α. Furthermore, during several sampling events, dense filamentous algal blooms were observed only at the Rockyhock Creek site, to the point that it partially clogged the push nets, which may have also contributed to the low CPUEs. The unique physicochemical cluster analysis grouping and the high nutrient and chlorophyll α levels from Butler (2012) data were clearly reflected by the observed algal blooms at Rockyhock Creek, all of which suggest that this site has poorer water quality than the other sampling sites. Conversely, algal blooms documented in the lower Chowan River in 1983 were found to be positively associated with higher levels of zooplankton abundance and higher feeding success in juvenile river herring (Winslow et al. 1985). Unfortunately, the dataset was too limited to draw strong explanatory conclusions, but it seemed that river flow was associated with controlling the concentration of the algal blooms and the density of the zooplankton forage base (Winslow et al. 1985).

Sites 8 (Rockyhock Creek) and 2 (Meherrin River) represented the maximum and minimum larval growth rates (Table 6; Figures 10 and 11) and supported similar mean CPUEs, $94.05 \pm 131.43$ and $133.40 \pm 308.84$, respectively (Table 5; Figures 7-9). They also generally exhibited maximums and minimums of water chemistry parameters among the sampled sites (Figures 5 and 6). Site 8 (Rockyhock Creek), which had the highest mean larval growth rate, was the only site with visible, mass amounts of filamentous algae present regularly observed in

samples, and exhibited the highest mean chlorophyll α concentration of all sites. Water analysis showed that site 8 also had the highest concentrations of nitrate/nitrites, chloride, calcium, and potassium, and the second highest sodium and magnesium levels among all sites (site 9 – South Chowan River – had the highest). Site 2 (Meherrin River), which had the lowest mean larval growth rate, had the highest ammonium, the lowest chloride and sodium, and second lowest phosphate and potassium concentrations (Figures 5 and 6).

Temporally, there were differences in the distribution and occurrence of larval river herring throughout the Chowan River system during the spring of 2011. Although larvae were present during the first sampling event on the 11th week of the year (March 18, 2011) through the 20th week (May 19, 2011), there was a lull (relatively low densities of larval river herring) from the 11th week to the 15th week, a marked peak from the 16th week to the 19th week, a lull the 20th week, and then none were collected during the sampling event of the 21st week (Figure 7). The mean CPUE reached the maximum on the 17th week of the year (April 29, 2011), followed by a secondary peak the next week (May 5, 2011). This spawning period and bell-shaped four-week peak was consistent with temporal patterns reported by similar studies of larval river herring abundance throughout North Carolina (Walsh et al. 2005; Binion 2011; Overton et al. 2012; Riley 2012).

Additionally, I found that the Chowan River supports much higher densities of River herring larvae in comparison to other riverine systems in the same region such as the Roanoke River (O'Rear 1983; Hightower et al. 1996; Overton and Rulifson 2007; Binion 2011; Overton et al. 2012; Riley et al. 2012). After collecting over 1,000 ichthyoplankton samples from 2001-2003, Rulifson and Overton reported a mean larval river herring density of $0.045 \pm 0.56$ (SD)

larvae/m$^3$ throughout the Roanoke River (Rulifson and Overton 2005). In a separate study, Rulifson and Overton (2006) reported a total mean density of larval river herring of 0.26/m$^3$ based on 245 samples collected during Spring 2002 and 2003 from the lower Roanoke River. My research found densities more than 100 times higher than the densities reported in the Roanoke from the 2001-2003 spring seasons and slightly more than 18 times the densities found in the lower Roanoke from the 2002-2003 spring seasons. The same sampling equipment and methods were used in all three studies. This suggests that the Chowan River supports nursery habitat that is some of the most widely used by river herring in the region, and therefore should be a priority for conservation.

The second objective of my research was to determine spatiotemporal patterns of larval growth and mortality rates among physicochemically unique sites. "Physicochemically unique sites" were defined by Butler (2012), which sampled the Chowan River system during spring 2011 on the same 11 days, at the same times, and at the same nine sites as my study. Butler's (2012) physicochemical cluster analysis grouped the nine sites into four clusters based on >90% similarity (Figure 3). These four clusters were used to analyze and compare growth (G) and mortality (Z) rates of larval river herring throughout the Chowan River.

There were differences in the growth and mortality rate estimates of larval river herring throughout the Chowan River during the 2011 spring spawning season. The highest growth was from Cluster 2 (represented by site 8 – Rockyhock Creek) with a mean daily growth rate of 0.71 $\pm$ 0.04 mm/d, followed by Cluster 1 (represented by site 9 –South Chowan) with a mean daily growth rate of 0.60 $\pm$ 0.06 mm/d. The lowest mortality estimate was from Cluster 4 (represented by sites 2, 4, and 6 – Meherrin River, Wiccacon Creek, and Catherine's Creek, respectively) with

a mean daily mortality rate of 0.01 ± 0.06 larvae/d, followed by Cluster 2 (represented by site 8 –
Rockyhock Creek) with a mean daily growth rate of 0.12 ± 0.05 mm/d.

Site 8 (Rockyhock Creek) had the highest growth rate (Table 6; Figure 10, 11). Site 8
(Rockyhock Creek) also had the highest mean water temperature (Figure 4). Additionally, sites 2
(Meherrin River) and 6 (Catherine's Creek) had the lowest growth rates, respectively (Table 6;
Figure 10, 11). Although site 2 (Meherrin River) did not have a notably high or low mean water
temperature, site 6 (Catherine's Creek) did have the lowest mean water temperature (Figure 4).
This suggests a growth pattern being favorably associated with water temperature; additional
study and analysis will be required to test for this specifically. Previous research has
demonstrated a positive correlation between temperature and larval growth within species-
specific ideal temperature ranges (Houde 1989; 2002). However, it is important to consider that
Rockyhock Creek is the most (non-main stem) downstream site, which could present a source of
age bias as a factor in the analyses.

Overall, there do not appear to be any conclusive correlations between abundance and
growth or mortality rates at the various site clusters, but there are a few subtle patterns. Cluster 2
(site 9 – Rockyhock Creek) supported the lowest CPUE of the tributaries, the highest growth rate
overall, and the second lowest mortality rate overall. One possible explanation of this pattern
could be density dependent growth; i.e., less intraspecific competition, and therefore more
habitat and resources available relative to each individual larva allowing for higher growth and
lower mortality rates. However, additional research on prey availability, distribution, and
density would be critical to demonstrate more conclusive evidence of this phenomenon. Another
possible explanation for the pattern could be the correlation of the higher nutrient levels found in

Cluster 2 (site 8 – Rockyhock Creek) by Butler (2012). Cluster 1 (site 9 – South Chowan) data echoed this pattern, as it supported the lowest larval river herring CPUE and the second highest growth rate. The pattern might be associated with to more favorable larvae-to-resource ratios, but would require further study of prey availability and larval diet. However, Cluster 1 (site 9 – South Chowan) also exhibited the third highest mortality rate, perhaps a function of increased predation. Again, further study involving food web dynamics in the Chowan River system would be necessary to support or refute this possibility.

The final objective of my research was to identify dietary composition of larval River herring across physicochemically-similar sites. I found that there were clear differences in dietary patterns among the four Clusters. Each Cluster typically exhibited larval diets dominated by a different family of prey type, and each of these prey types were consistent with findings of other studies (Marcy 1976; Vigerstad and Cobb 1978; Loesch and Kriete Jr 1980; Winslow et al. 1985; Rulifson et al. 1994; Riley et al. 2012; Binion et al. 2012).

Across all clusters, there was a positive association between feeding incidence (the percent of larvae with food in their gut) and feeding ratio (the mean number of prey items/gut). Cluster 4 (represented by sites 2, 4, and 6 – Meherrin River, Wiccacon Creek, and Catherine's Creek, respectively) had both the highest feeding incidence at 53.1% and the highest feeding ratio at 0.52; Cluster 2 (site 8 -Rockyhock Creek) had the lowest feeding incidence at 28.6% and the lowest feeding ratio at 0.17. This pattern is consistent with the feeding incidences ranging from 40 to 54% as reported by Rulifson et al. (1994) in larval and juvenile clupeids (including river herring) between 1922 and 1988 from the neighboring Roanoke River. Additionally, Winslow et al. (1985) reported feeding ratios ranging from 0.30 to 83.0 (mean number of

prey/gut) for year-of-young Blueback Herring that ranged in size (SL$\pm$SE) from 24.6$\pm$0.7 to 37.6$\pm$0.9 mm from the Chowan River in 1982. One explanation of this association is that areas exhibiting higher percentages of larvae with food in their guts also had higher relative concentrations of available prey (e.g., more phytoplankton and zooplankton), which likely accounts for the higher mean number of prey items per gut. This explanation is supported by Winslow et al.'s (1985) reporting increased zooplankton prey fields in years with algal blooms at sites in the Chowan River, and I did document a strong algal bloom at Cluster 2 (site 8 - Rockyhock Creek) during the 2011 spring. Furthermore, if this was the case, then it supports the previous supposition that high growth and low mortality rates at Cluster 2 (site 8 – Rockyhock Creek) were possibly a result of increased prey availability. Alternatively, higher concentrations of nutrients and/or higher water temperatures may have influentially contributed to the high growth rate, and perhaps the low mortality rate was a result of fewer predators in Cluster 2. However, there are many other factors, including flow and dissolved oxygen levels, that could affect these relationships, and further research would be necessary to identify any conclusive cause-and-effect relationships.

At all sites the actual percentages and values of feeding incidences and feeding ratios were reasonable, but relatively lower in comparison to comparable systems in North Carolina. For example, Riley (2012) reported a range of daytime feeding incidences for non-yolk sac larvae of 71.4-78.0%; however, nighttime feeding incidences for non-yolk sac larvae were lower with a range of 28.2 - 40.0%. Rulifson et al. (1994) reported that clupeid daytime feeding incidence was 43.1%. My research showed a range of daytime feeding incidences between 28.6% and 53.1%. I did not specifically distinguish between non-yolk sac larvae and

intermediate phase larvae with yolk-sac and feeding present simultaneously, but Riley (2012) did record larval phase. My research did sample a vast majority of non-yolk-sac larvae, but I did not report exact numbers because that was not recorded reliably during laboratory analyses; if the study was repeated I would recommend recording the numbers of yolk-sac and post yolk-sac larvae, which could possibly help explain the lower mean feeding incidence percentages.

Another possible explanation for the lower feeding incidence is that the Chowan River may have had lower densities of suitable plankton, or that there may be more competition for resources like plankton in the Chowan River. However, this explanation presumably can be ruled out according to research that monitored Chowan River water quality, phytoplankton abundance, and zooplankton abundance over a 30-year period (Ensign et al. 2014). That study found that, while phytoplankton densities had decreased, nutrients had not, and zooplankton densities had increased. The investigators inferred that the marked historical decline in river herring abundance may have positively impacted zooplankton densities due to lower predation, while the decline of river herring negatively impacted phytoplankton densities indirectly as a result of the increased grazing by the positively impacted zooplankton densities.

Furthermore, Riley (2012) reported larval river herring feeding ratios between 2.3 and 2.9 in the Roanoke River, while my research estimated these ratios to be between 0.17 and 0.72 in the Chowan River. This suggests that larval river herring in the Chowan have, on average, less mean numbers of prey items in their gut. Again, this could be indicative of lower plankton abundance in the Chowan River or relatively higher competition among larvae for prey. However, it is important to consider that abundance of larval river herring was found to be higher in the Chowan River relative to neighboring river systems, and the relative higher larval

abundance may have an overall reducing effect on the zooplankton prey field of the Chowan River from larval predation compared to neighboring river systems (e.g., the Roanoke River). Further research examining phytoplankton, zooplankton, and river herring larvae in neighboring river systems could elucidate these relationships.

The prey types most consumed by larval river herring, as indicated by both percent composition (the percent of food items of each prey type) and by frequency of occurrence (the percent of fish in which at least one item of each prey type was found), were rotifers, cladocerans, bosminids, and copepods. These findings echoed previous findings in similar river systems (Winslow et al. 1985; Rulifson et al. 1994; Binion 2011; Riley et al. 2012; Binion et al. 2012); Winslow et al. (1985) even found that larval river herring in the Chowan River were positively selecting for cladocerans. Also, an unidentified small egg was the most consumed prey item in Cluster 4, but I did not consider this to be a significant prey item because the prey type "egg" was represented almost entirely by four individual larvae from one site in Cluster 4 (Catherine's Creek) on a single day (May 15, 2011). The stomach contents of these larvae were extreme outliers compared to the other 145 larval guts that were examined. These four individual larvae (ranging in size from 11.00-13.0 mm SL) had 30, 65, 67, and 70 prey items (respectively) in each of their guts, all of which were unidentified small eggs. Therefore, these occurrences may have been more of opportunistic chance events, where these larvae happened to be in an area where a spawning event of an unknown species occurred, and therefore eggs were abundant in a brief and concentrated area.

Important research questions generated by my study include: What are the survival rates for the eggs through spawning adult life stages? What are the survival rates for the larvae to

juvenile stage? What is the availability of plankton in the Chowan River compared to other rivers? Do adult river herring exhibit fidelity; i.e., return to spawn in their natal tributary? How does adult river herring abundance relate to larval density (i.e., density-dependence)?

The question of density-dependence and the larval recruitment relationship to adult spawning stock biomass is currently difficult to answer because there are no longer commercial adult landings as a result of the moratoria on river herring across the East Coast. Historical datasets of adult river herring abundances were fisheries dependent and based on commercial landings. Even more recent datasets that could provide information on incidental river herring landings from inshore and oceanic recreational and commercial fisheries are sparse and unreliable and have been deemed of limited use by the South Atlantic States Marine Fisheries Commission's River Herring Stock Assessment Subcommittee. However, recent anecdotal appraisals from local fishermen who have lived and fished (including commercially for river herring prior to the 1970s population collapse) the Chowan River for decades and continue to currently, report that river herring spawning runs still have not rebounded from the collapse (Terry Pratt 2001, personal communication). The implication of this in concert with the present increased larval abundances emphasizes the specific need for further research to examine larval survival to juvenile and adult stages.

Although many questions remain and further research is needed, my research provided a snapshot of how the Chowan River functions as nursery habitat for larval river herring. Undoubtedly, the Chowan River's nursery habitat supports a relatively high density of larval river herring compared to other neighboring rivers, which emphasizes the need to protect, restore, and conserve areas supporting high larval abundances and feeding incidence/ratios like

Catherine's Creek and Wiccacon Creek if North Carolina river herring stocks are to have a chance for recovery.

Fish nursery habitat is a term that actually has many definitions that are important to consider in discussing the results of my study. Historically, nursery habitat was simply defined as any area that supports high densities of larval or juvenile fish, but recently has been more specifically characterized by Dahlgren et al. (2006) as habitat that contribute abnormally large numbers of individuals to the adult population.

Other important habitat terms, such as "strategic habitat areas," "essential fish habitat," and "critical habitat" should also be considered. What all these terms have in common is the notion that certain areas and environments have physical, chemical, and biological properties necessary to the survival and propagation of a given species. Whether it be on a management, conservation, or ecological basis, these phrases begin to differ in terms of perspective. Regardless of perspective, habitats that support the very young life stages of individuals of a species are of utmost importance because without adequate conditions for growth and survival they cannot contribute significantly to the adult population. My research provides information that helps characterize where, when, and how river herring larvae are using parts of the Chowan River. Therefore, it contributes to understanding larval river herring nursery habitat by demonstrating implied evidence of successful spawning, successful survival from egg to larval stage, and by documenting abundant larval individuals with varying rates of growth and mortality across the different sites.

Not all parts of the Chowan River system functioned equally as suitable nursery habitats for river herring. My results show that water quality, abundance and distribution, growth and

mortality rates, feeding ratio, diet composition, and prey frequency of occurrence all varied across sites and throughout time. However, Catherine's Creek specifically seems to be one of the most important areas of nursery habitat due to its high larval densities, both past and present. This is significant because Catherine's Creek could be a useful area for ecosystem planning, management, and conservation efforts. Also characterizing productive areas, such as Catherine's Creek, can provide useful insight for restoration efforts by providing a current example of nursery habitats that are functioning well (Hasselman et al. 2014).

Additionally, my research contributes to larval ecology by illustrating resource use. It characterizes the daily growth and mortality rates of larval river herring in a region that once supported one of the largest river herring fisheries in the world. This type of research generally clarifies our understanding of early life history patterns and ecosystem interactions. The results help identify uniquely productive sites that can focus future management, restoration, and conservation efforts relevant to fisheries professionals who are working to assess population dynamics.

This research is particularly relevant because the North Carolina River Herring Plan Development Team and the River Herring Advisory Council have made recommendations for the prompt update of river herring spawning and nursery area surveys. They have also advocated for an assessment of river herring restoration strategies, particularly within the Chowan River system (Yako et al. 2002). My research addresses these concerns by surveying spatiotemporal larval abundance, by providing current data for historical comparisons, and by documenting spatiotemporal resource use during the early life history of river herring.

Successful reproduction and appropriate habitat to support early life history are essential for recruitment and restoration. The decline of fish stocks that rely on estuaries as spawning and nursery habitats is not necessarily doomed by overfishing, but in part because of a lack of recruitment (Boreman and Friedland 2003). Recruitment is certainly affected by the availability and condition of spawning and nursery habitats, as well as the abundance of spawning stock biomass. Thus, by characterizing early life stages and their relationship to their environment, this research provides important ecological information about river herring that could be used by fisheries managers.

Findings from my study will be particularly informative if North Carolina fisheries managers decide to stock river herring to increase recruitment to the population, like North Carolina Wildlife Resources Commission (NCWRC) does with other regional anadromous species that have struggled with population declines. Such stocked species include American Shad and Striped Bass, the latter of which has become an example of successful population recovery through concerted fisheries management and stocking efforts. Striped Bass populations declined dramatically from 1970 to the early 1980s (Boreman and Austin 1985). Strict harvest regulations and the creation of a state-run hatchery that release young striped bass helped the population recover. Understanding the Chowan River as a significant nursery for young river herring would be essential in executing similar efforts for river herring population recovery. Furthermore, this information will be useful to fisheries managers across the eastern United States trying to manage and rehabilitate the declined populations of river herring.

In conclusion, this research found that there has been an increase in larval abundance and distribution over the last 30 years in the Chowan River system, which provides fisheries

managers with positive feedback on current management measures that have prohibited

commercial and recreational harvest of river herring. This study provides critical data to support

the ongoing efforts to protect preferred nursery habitats and provides incentive to maintain

current regulations.

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*Tables*

Table 1. States from the Eastern United States that have established river herring moratoria listed in chronological order. Details provided summarize basic moratoria measures.

| State | Establishment of Moratorium | Type of Moratorium | Notes |
|---|---|---|---|
| Connecticut | April 2002 to present | -No taking of alewives and Blueback Herring from inland and marine state waters | -Has been extended each successive year by the commissioner |
| Massachusetts | January 2006 to present | -No harvest, possession, and sale of river herring in the state | -Extended initial moratorium for additional years in 2008 |
| North Carolina | September 2007 to present | -No possession of river herring over six inches in length from inland waters of coastal river systems up to the first impoundment dam on the main river | |
| Maryland | December 2011 to present | -An individual may not possess river herring within the jurisdiction of Maryland | |
| New Jersey | January (marine) and February (freshwater) 2012 | -No possession, take, attempt to take, sale or purchase of river herring | |
| New York | September 2012 | -No possession except in Hudson River and tributaries | |

Table 2. Estimated densities of larval river herring collected in the spring of 1982 compared to those collected in the spring of 2011 from five sites on the Chowan River.

| Sampling Location | Mean # of larvae/m ± S.D. | | # of samples | |
| --- | --- | --- | --- | --- |
| | Year | | | |
| | 1982 (O'Rear) | 2011 (Ezzard) | 1982 (O'Rear) | 2011 (Ezzard) |
| S. Chowan River | 0.12 ± 0.24 | 0.11± 0.11 | 5 | 8 |
| Catherine's Creek | 0.34 ± 0.59 | 3.78 ± 5.09 | 10 | 9 |
| Wiccacon Creek | 0.31 ± 0.44 | 2.89 ± 48.69 | 6 | 9 |
| Bennett's Creek | 0.004 ± 0.009 | 0.52 ± 0.64 | 6 | 9 |
| Rockyhock Creek | 0 ± 0 | 0.18 ± 0.22 | 3 | 9 |

Table 3. The mean standard lengths and total percentages of Clupeid species caught in 98 total samples from nine Chowan River sites sampled once weekly over 11 weeks in spring 2011. Note: R. = River, H. =Hickory, G.= Gizzard, and A. = American.

| Species | % of All Larvae | Mean SL (mm) | Std Dev (mm) | % of Samples in which the Species was Present |
| --- | --- | --- | --- | --- |
| R. Herring | 95.88% | 8.0 | 2.93 | 98.98% |
| H. Shad | 2.90% | 9.0 | 1.57 | 23.86% |
| G. Shad | 0.91% | 5.6 | 1.44 | 22.44% |
| A. Shad | 0.31% | 8.1 | 3.17 | 20.48% |

Table 4. The mean concentration (larvae/100 m³), minimum, maximum, and mean (SD) standard length (mm) of river herring caught from nine Chowan River sites sampled once weekly over 11 weeks in the spring of 2011. N = number of river herring larvae measured per site.

| Site | N | Mean CPUE (larvae/100 m³) (SD) | Standard length (mm) | |
| | | | Range | Mean (SD) |
| --- | --- | --- | --- | --- |
| 1-N. Chowan R. | 299 | 93.18 (125.27) | 3.00 – 16.50 | 6.46 (2.50) |
| 2-Meherrin R. | 241 | 133.40 (308.84) | 3.40 – 19.50 | 6.50 (3.05) |
| 3-Sarem C. | 504 | 567.91 (1496.23) | 3.00 -17.50 | 7.43 (2.26) |
| 4-Wiccacon C. | 409 | 1316.69 (3026.96) | 3.30 -18.00 | 8.37 (2.36) |
| 5-Bennett's C. | 389 | 261.44 (423.91) | 3.10 - 15.20 | 9.86 (3.04) |
| 6-Catherine's C. | 608 | 1583.53 (2698.18) | 3.90 – 14.90 | 9.40 (2.65) |
| 7-Holiday I. | 205 | 69.61 (127.31) | 2.50-17.50 | 6.74 (3.22) |
| 8-Rockyhock C. | 239 | 94.05 (131.43) | 3.00-18.00 | 7.50(3.24) |
| 9-S. Chowan R. | 241 | 52.87 (71.68) | 3.50-13.50 | 6.22(1.98) |

Table 5. Mean catch per unit effort (CPUE) for river herring larvae sampled during the spring of 2011 in Chowan River, NC. The grey highlights indicate the two lowest (dark grey) and the two highest (light grey) CPUEs.

| Site | Mean CPUE (larvae/100 m$^3$) |
|---|---|
| 1-N. Chowan R. | 93.18 $\pm$ 125.27 |
| 2-Meherrin R. | 133.40 $\pm$ 308.84 |
| 3-Sarem C. | 567.91 $\pm$ 1496.23 |
| 4-Wiccacon C. | 1316.69 $\pm$ 3026.96 |
| 5-Bennett's C. | 261.44 $\pm$ 423.91 |
| 6-Catherine's C. | 1583.53 $\pm$ 2698.18 |
| 7-Holiday I. | 69.61 $\pm$ 127.31 |
| 8-Rockyhock C. | 94.05 $\pm$ 131.43 |
| 9-S. Chowan R. | 52.87 $\pm$ 71.68 |

Table 6. Statistics for growth equations by site derived from standard linear regressions of increasing weekly mean SL of river herring larvae in the Chowan River, NC, spring 2011. G = instantaneous growth; linear regression analysis values: P= P value (calculated probability of overall statistical significance of model), F = F value (if the mean is significantly different from zero), r2= r-squared value (also called the coefficient of determination).

| Site | Name | n | y-intercept (mm) | G (mm/d) | P | F | $r^2$ |
|---|---|---|---|---|---|---|---|
| 1 | N. Chowan | 65 | 3.56 $\pm$ 0.48 | 0.53 $\pm$ 0.05 | <0.0001 | 103.27 | 0.62 |
| 2 | Meherrin R. | 48 | 4.29 $\pm$ 0.42 | 0.36 $\pm$ 0.05 | <0.0001 | 53.28 | 0.53 |
| 4 | Wiccacon C. | 145 | 3.51 $\pm$ 0.50 | 0.60 $\pm$ 0.05 | <0.0001 | 172.07 | 0.54 |
| 6 | Catherine's C. | 80 | 4.43 $\pm$ 0.51 | 0.40 $\pm$ 0.04 | <0.0001 | 90.30 | 0.53 |
| 7 | Holiday Island | 34 | 3.93 $\pm$ 0.62 | 0.53 $\pm$ 0.07 | <0.0001 | 57.80 | 0.63 |
| 8 | Rockyhock C. | 61 | 1.96 $\pm$ 0.39 | 0.71 $\pm$ 0.04 | <0.0001 | 299.66 | 0.83 |
| 9 | S. Chowan | 56 | 3.44 $\pm$ 0.46 | 0.60 $\pm$ 0.06 | <0.0001 | 86.29 | 0.61 |

Table 7. Statistics for growth equations by cluster derived from increase in weekly mean SL of river herring larvae in the Chowan River, NC, spring 2011. "G" represents growth; "$\pm$" precedes standard error values; linear regression analysis values: P= P value (calculated probability of overall statistical significance of model), F = F value (if the mean is significantly different from zero), r2= r-squared value (also called the coefficient of determination).

| Cluster | Sites | n | y-intercept (mm) | G (mm/d) | P | F | $r^2$ |
|---------|-------|-----|-------------------|-----------------|---------|--------|-------|
| 1 | 9 | 56 | $3.44 \pm 0.46$ | $0.60 \pm 0.06$ | <0.0001 | 86.29 | 0.61 |
| 2 | 8 | 61 | $1.96 \pm 0.39$ | $0.71 \pm 0.04$ | <0.0001 | 299.66 | 0.83 |
| 3 | 1, 7 | 99 | $3.72 \pm 0.37$ | $0.52 \pm 0.04$ | <0.0001 | 160.23 | 0.62 |
| 4 | 2, 4, 6 | 273 | $4.10 \pm 0.32$ | $0.48 \pm 0.03$ | <0.0001 | 283.08 | 0.51 |

Table 8. Statistics for mortality equations by site. Mortality was derived from decreases in estimated weekly age-specific abundance of river herring larvae in the Chowan River, NC, spring 2011. Z = instantaneous mortality; n = the number of larvae whose SL was used to calculate mortality; linear regression analysis values: P= P value (calculated probability of overall statistical significance of model), F = F value (if the mean is significantly different from zero), r2= r-squared value (also called the coefficient of determination).

| Site | Name | n | y-intercept (larvae/m$^3$) | Z (larvae/d) | P | F | $r^2$ |
|------|------|-----|-----------------------------|-----------------|--------|-------|-------|
| 1 | N. Chowan | 218 | $5.04 \pm 0.64$ | $0.28 \pm 0.05$ | 0.0015 | 25.51 | 0.75 |
| 2 | Meherrin R. | 149 | $3.98 \pm 0.52$ | $0.17 \pm 0.05$ | 0.0247 | 10.09 | 0.60 |
| 4 | Wiccacon C. | 377 | $15.13 \pm 2.82$ | $0.92 \pm 0.21$ | 0.0478 | 19.42 | 0.86 |
| 6 | Catherine's C. | 518 | $4.96 \pm 1.21$ | $0.11 \pm 0.08$ | 0.2221 | 1.75 | 0.08 |
| 7 | Holiday Island | 124 | $4.11 \pm 1.61$ | $0.21 \pm 0.13$ | 0.1636 | 2.66 | 0.22 |
| 8 | Rockyhock C. | 152 | $3.78 \pm 0.63$ | $0.12 \pm 0.05$ | 0.0629 | 5.19 | 0.37 |
| 9 | S. Chowan | 178 | $4.68 \pm 0.43$ | $0.25 \pm 0.04$ | 0.0005 | 37.98 | 0.82 |

Table 9. Statistics for mortality equations by cluster derived from increase in weekly mean SL of river herring larvae in the Chowan River, NC, spring 2011. Z = instantaneous mortality; "$\pm$" precedes standard error values; n = the number of data points used in the regression; linear regression analysis values: P= P value (calculated probability of overall statistical significance of

model), F = F value (if the mean is significantly different from zero), r2= r-squared value (also called the coefficient of determination).

| Cluster | Sites | n | y-intercept (larvae/m$^3$) | Z (larvae/d) | P | F | $r^2$ |
|---|---|---|---|---|---|---|---|
| 1 | 9 | 10 | $3.44 \pm 0.46$ | $0.60 \pm 0.06$ | <0.0001 | 86.29 | 0.61 |
| 2 | 8 | 9 | $3.78 \pm 0.63$ | $0.12 \pm 0.05$ | 0.0629 | 5.19 | 0.37 |
| 3 | 1, 7 | 18 | $4.79 \pm 0.66$ | $0.26 \pm 0.06$ | 0.0003 | 22.53 | 0.59 |
| 4 | 2, 4, 6 | 24 | $3.08 \pm 0.77$ | $0.01 \pm 0.06$ | 0.8392 | 0.04 | -0.05 |

Table 10. Feeding incidence and feeding ratio percentages for larval river herring collected in spring 2011 from the Chowan River, NC. n = the number of larvae used in determining feeding success.

| Cluster | n | Feeding Incidence (% of larvae with food in gut) | Feeding Ratio (mean # of items of prey/gut) |
|---|---|---|---|
| 1 | 27 | 40.8 | 0.34 |
| 2 | 28 | 28.6 | 0.17 |
| 3 | 45 | 44.4 | 0.52 |
| 4 | 49 | 53.1 | 0.72 |
| Combined | 149 | 43.6 | 0.44 |
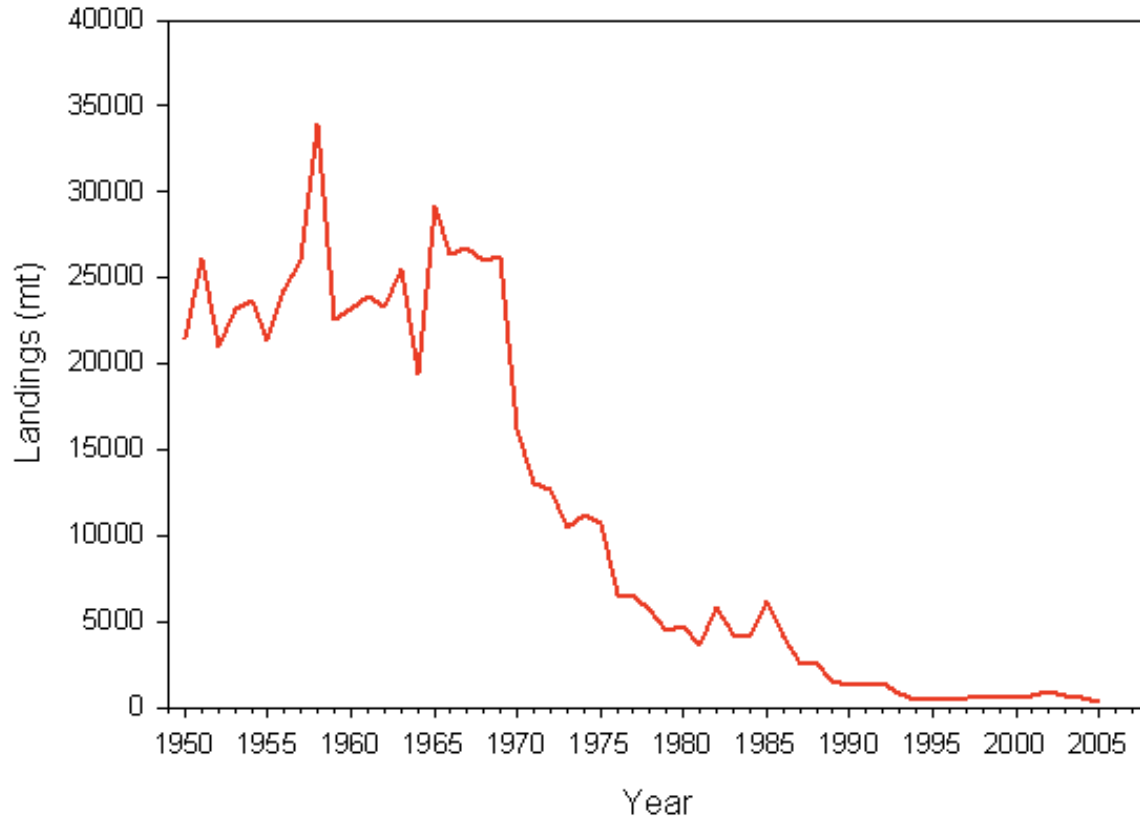
Figure 1. Commercial landings of river herring in the United States from 1950-2005. (Source: Haas-Castro 2006).
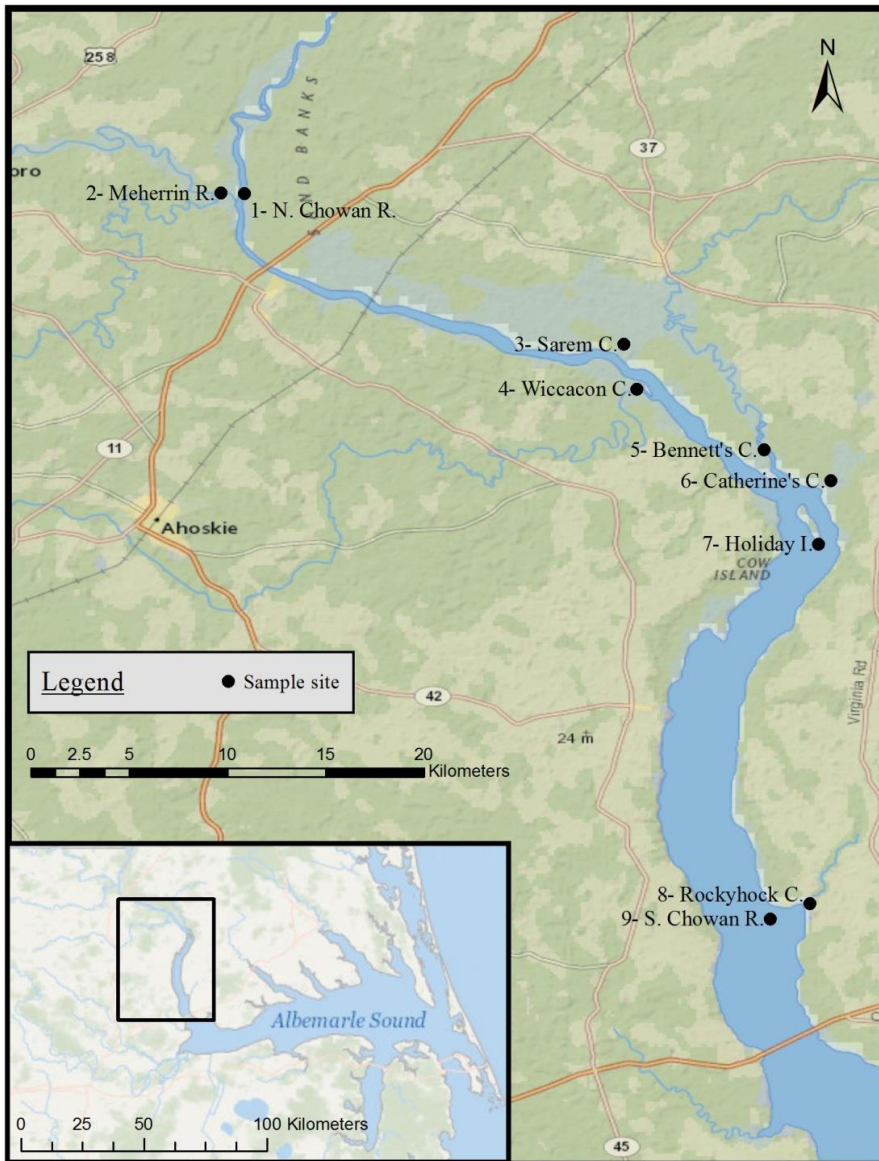
Figure 2. Map of the Chowan River, located in the Northwestern portion of Albemarle Sound, North Carolina. Black dots represent sampling sites including (from North to South): North Chowan River (1), Meherrin River (2), Sarem Creek (3), Wiccacon Creek (4), Bennett's Creek (5), Catherine's Creek (6), Holiday Island (7), Rockyhock Creek (8), and South Chowan River (9). Sites were sampled for larval river herring in spring 2011 (March 18 – May 26).

60
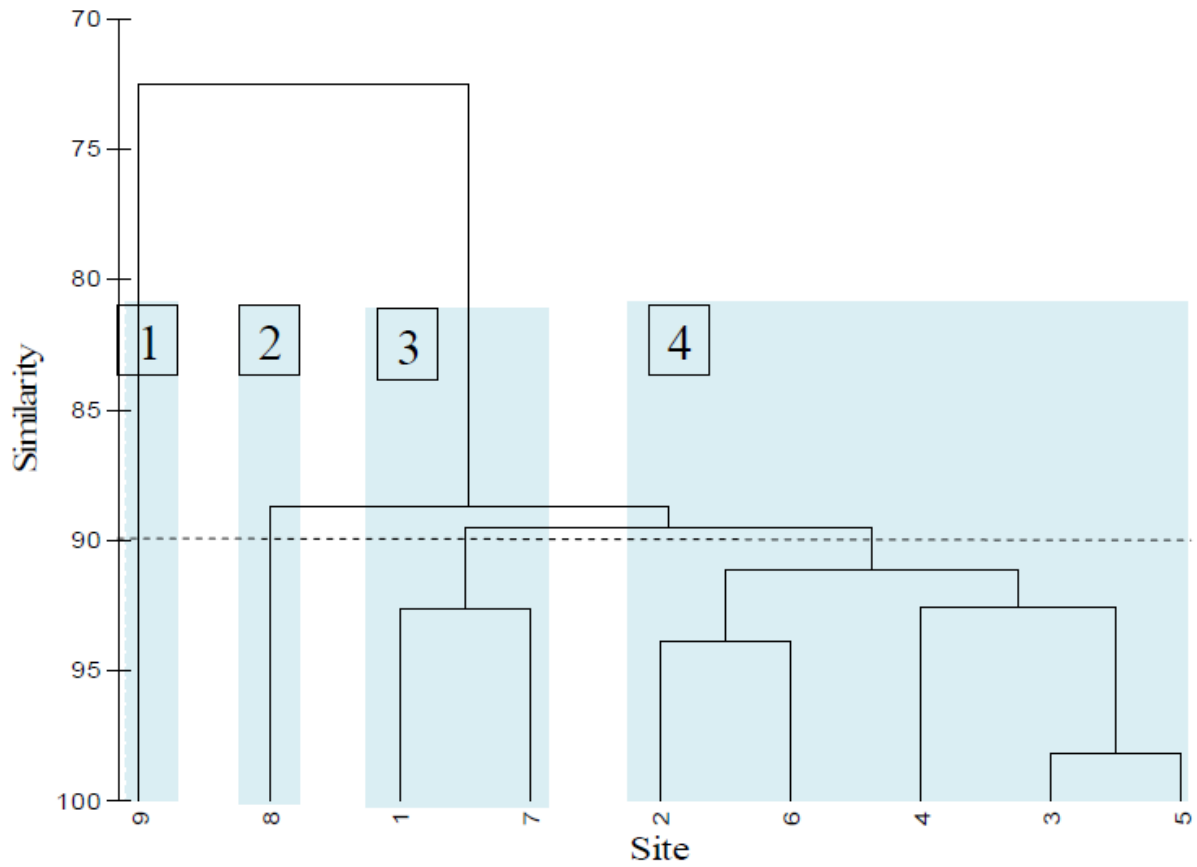
Figure 3. Cluster analysis using physicochemical factors to group similar sites from samples collected from the Chowan River, North Carolina (March 18 – May 26, 2011). Dotted line indicates 0% similarity. Numbered boxes indicate cluster number. (Source: Butler 2012).
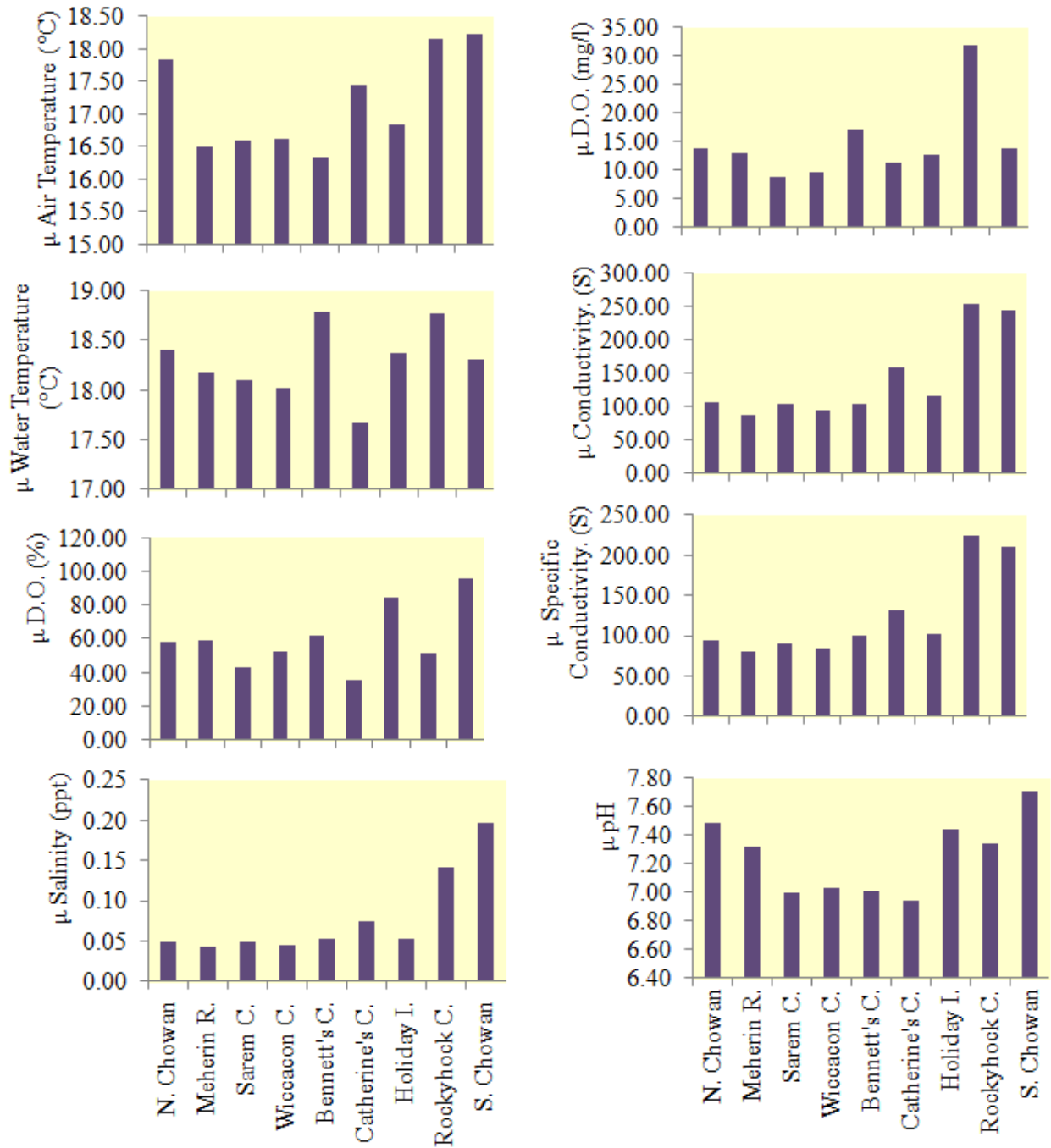
Figure 4. Mean environmental and water quality parameters from nine Chowan River sites over 11 weeks in the spring of 2011 from March 18 to May 26. Note that the means represent combined data from both bottom and surface readings when available.
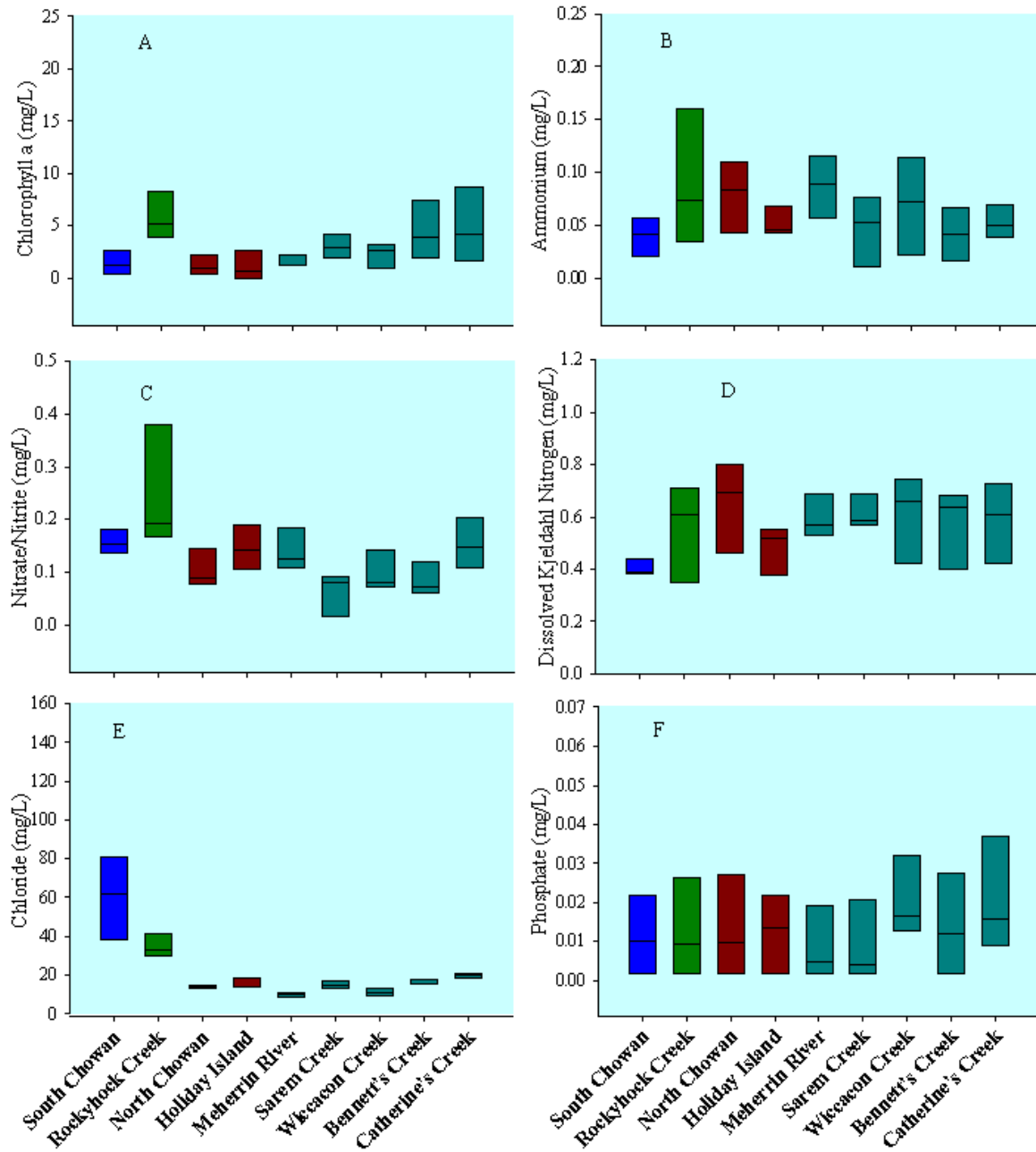
Figure 5. Boxplots of the upper and lower quartiles and median of six physicochemical parameters (A – Chlorophyll α, B – Ammonia, C – Nitrate/Nitrite, D – Dissolved Kjeldhal Nitrogen, E – Chloride, F – Phosphate) from all sample sites along the Chowan River, NC spring 2011. Colors of boxes indicate groupings from Cluster Analysis. (Source: Butler 2012).
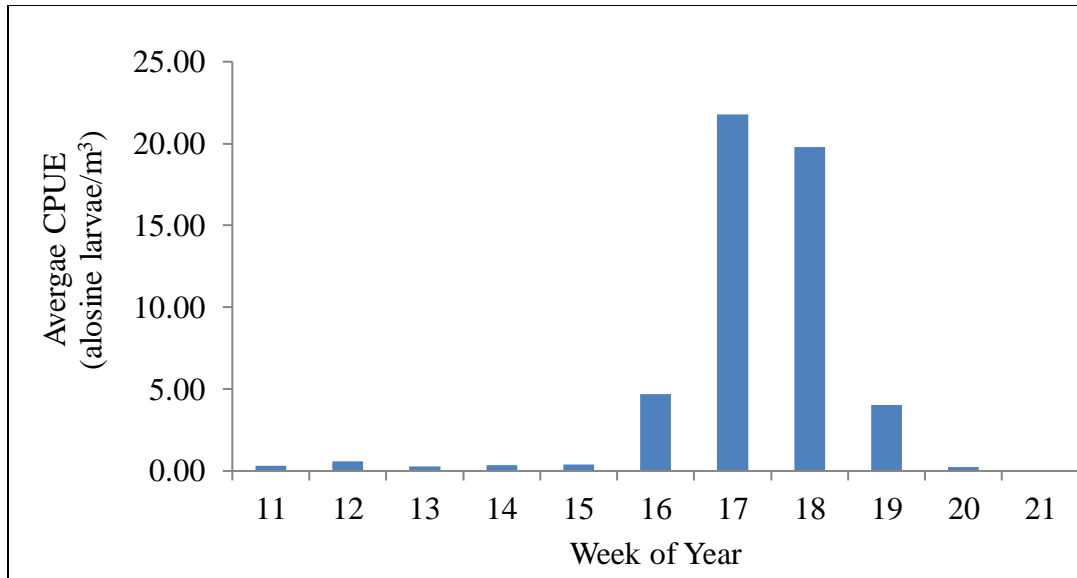
63
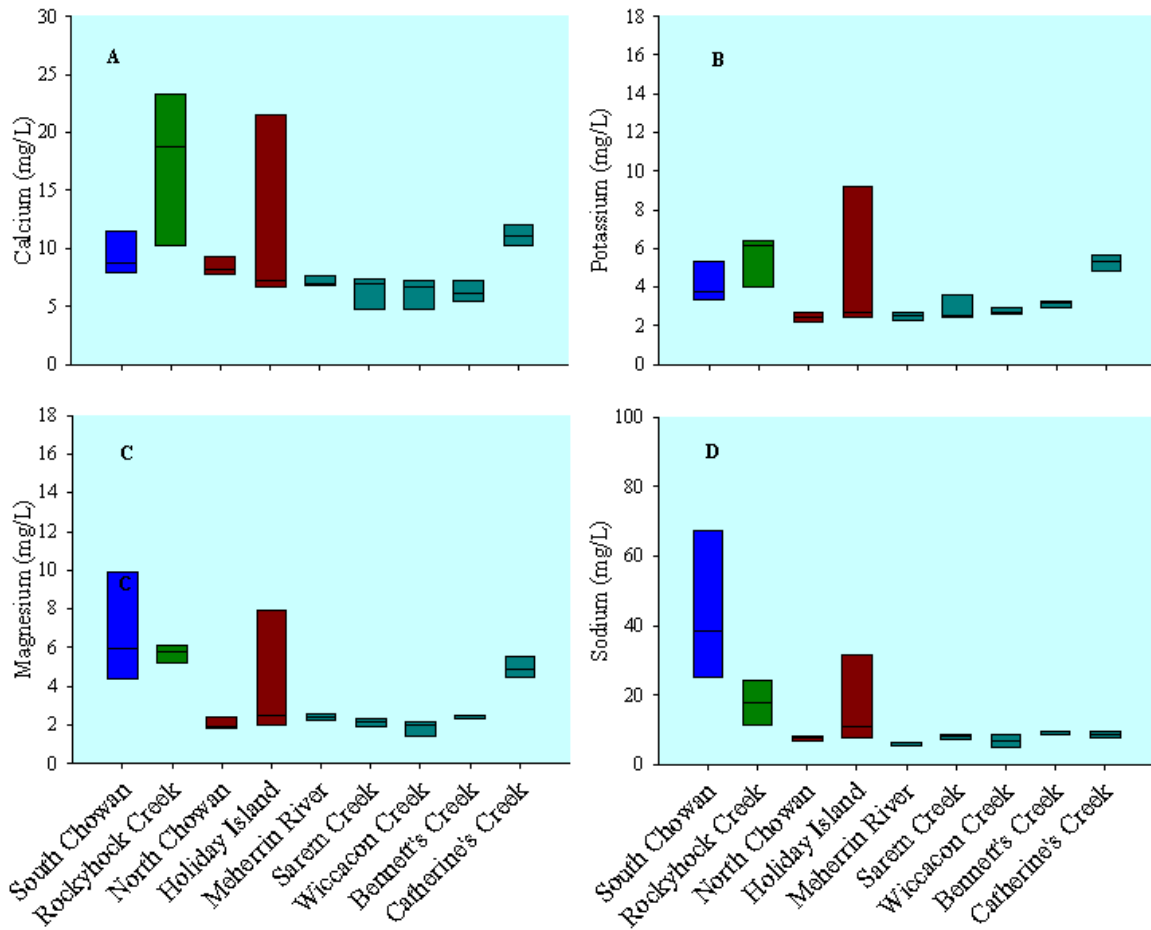
Figure 6. Boxplots of the upper and lower quartiles and median of four cation parameters (A – Calcium, B – Potassium, C – Magnesium, D – Sodium) from all sample sites along the Chowan River, NC spring 2011. Colors of boxes indicate groupings from Cluster Analysis. (Source: Butler 2012).

Figure 7. The mean catch per unit effort (CPUE) of alosine larvae from all Chowan River sites combined per date for the spring of 2011 from March 18 (week 11) to May 26 (week 2). Note that CPUE were calculated based on samples from the starboard side of the push net.
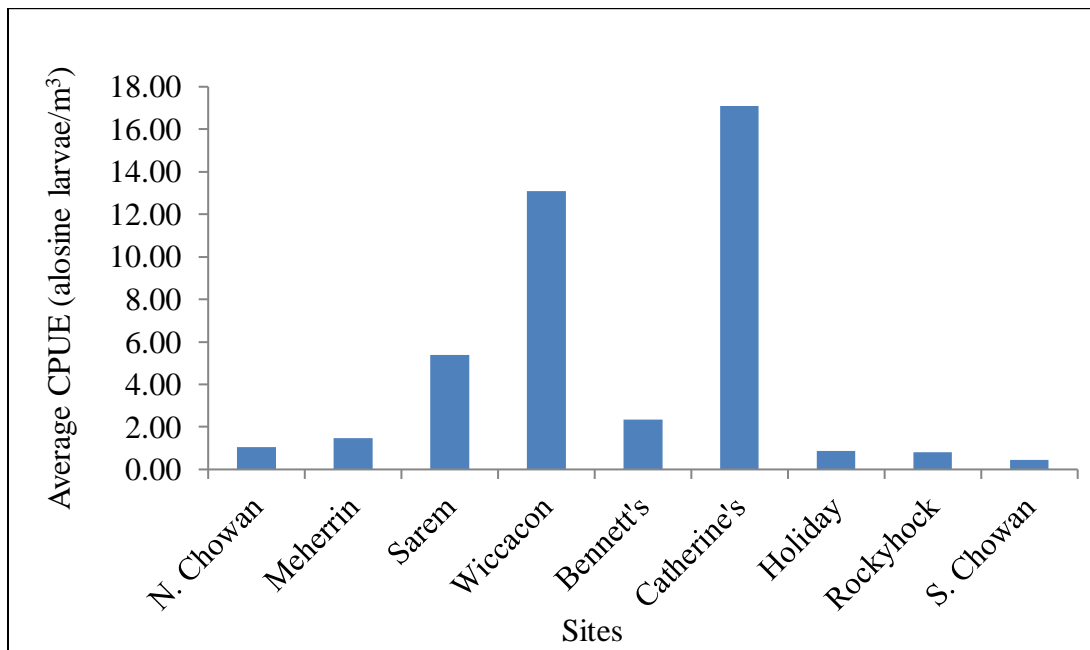


Figure 8. The mean catch per unit effort (CPUE) of alosine larvae per Chowan River site over 11 weeks in the spring of 2011 from March 18 to May 26. Note that CPUE were calculated based on samples from the starboard side of the push net.
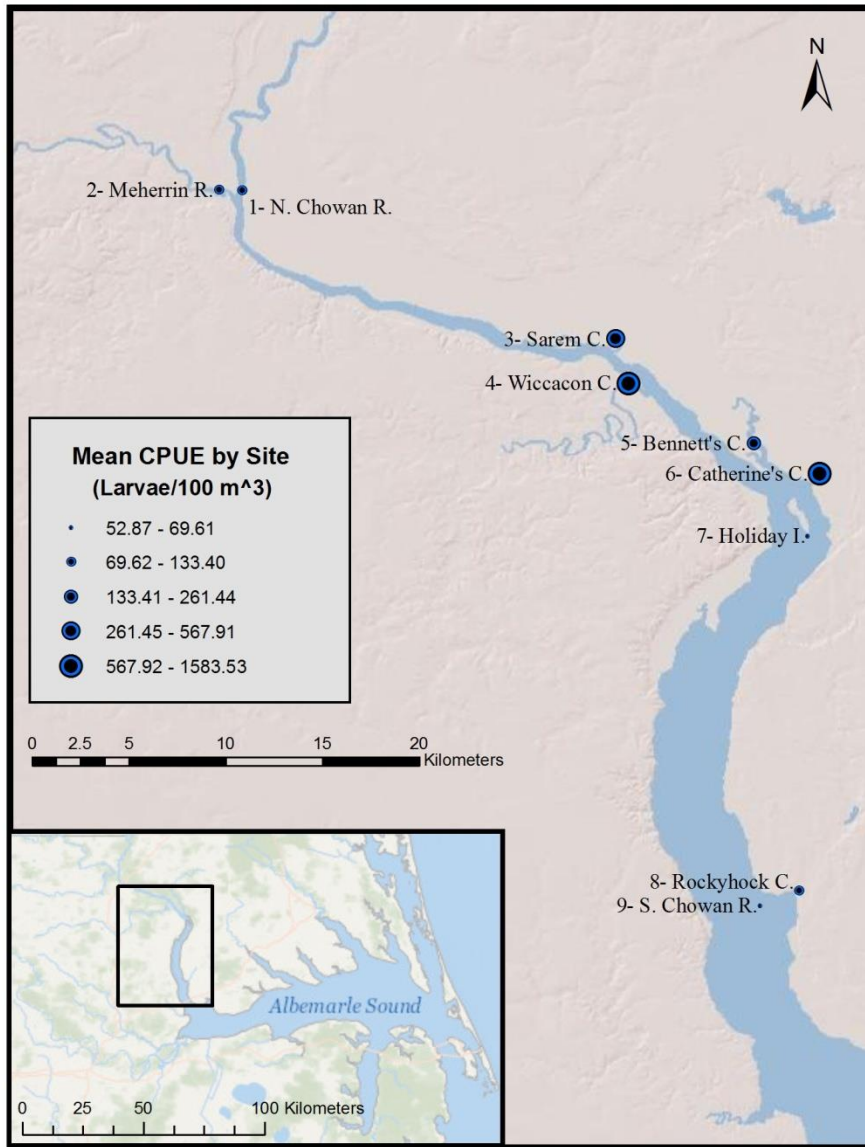
Figure 9. Map of the Chowan River, located in the Northwestern portion of Albemarle Sound, NC showing distribution of larval river herring CPUE (larvae/100 m³) across sampling sites. Black and blue bull's eye circles represent sampling sites including (from North to South): North Chowan River (1), Meherrin River (2), Sarem Creek (3), Wiccacon Creek (4), Bennett's Creek (5), Catherine's Creek (6), Holiday Island (7), Rockyhock Creek (8), and South Chowan River (9). The size of the black and blue bull's eye circles is relative to the CPUE of larvae per site, where the largest circles indicate the highest values and the smallest circles indicate the lowest values. Sites were sampled for larval river herring in spring 2011 (March 18 – May 26).
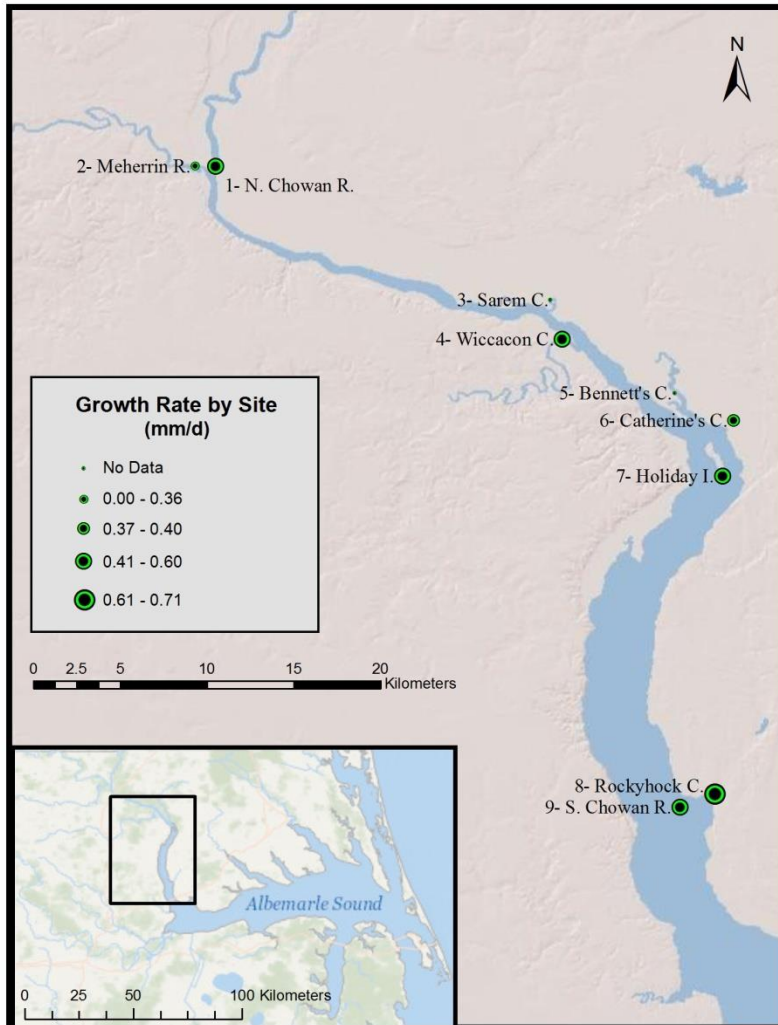
Figure 10. Map of the Chowan River, located in the Northwestern portion of Albemarle Sound, NC showing distribution of larval river herring growth rates (mm/d) across sampling sites. Black and green bull's eye circles represent sample sites including (from North to South): North Chowan river (1), Meherrin River (2), Sarem Creek (3), Wiccacon Creek (4), Bennett's Creek (5), Catherine's Creek (6), Holiday Island (7), Rockyhock Creek (8), and South Chowan River (9). The size of the black and green bull's eye circles is relative to the larval growth rate (mm/d) per site, where the largest circles indicate the highest rates and the smallest circles indicate the lowest rates. Growth models using age and length were used to determine growth rates at each sampling site. Sites were sampled for larval river herring in spring 2011 (March 18 – May 26).
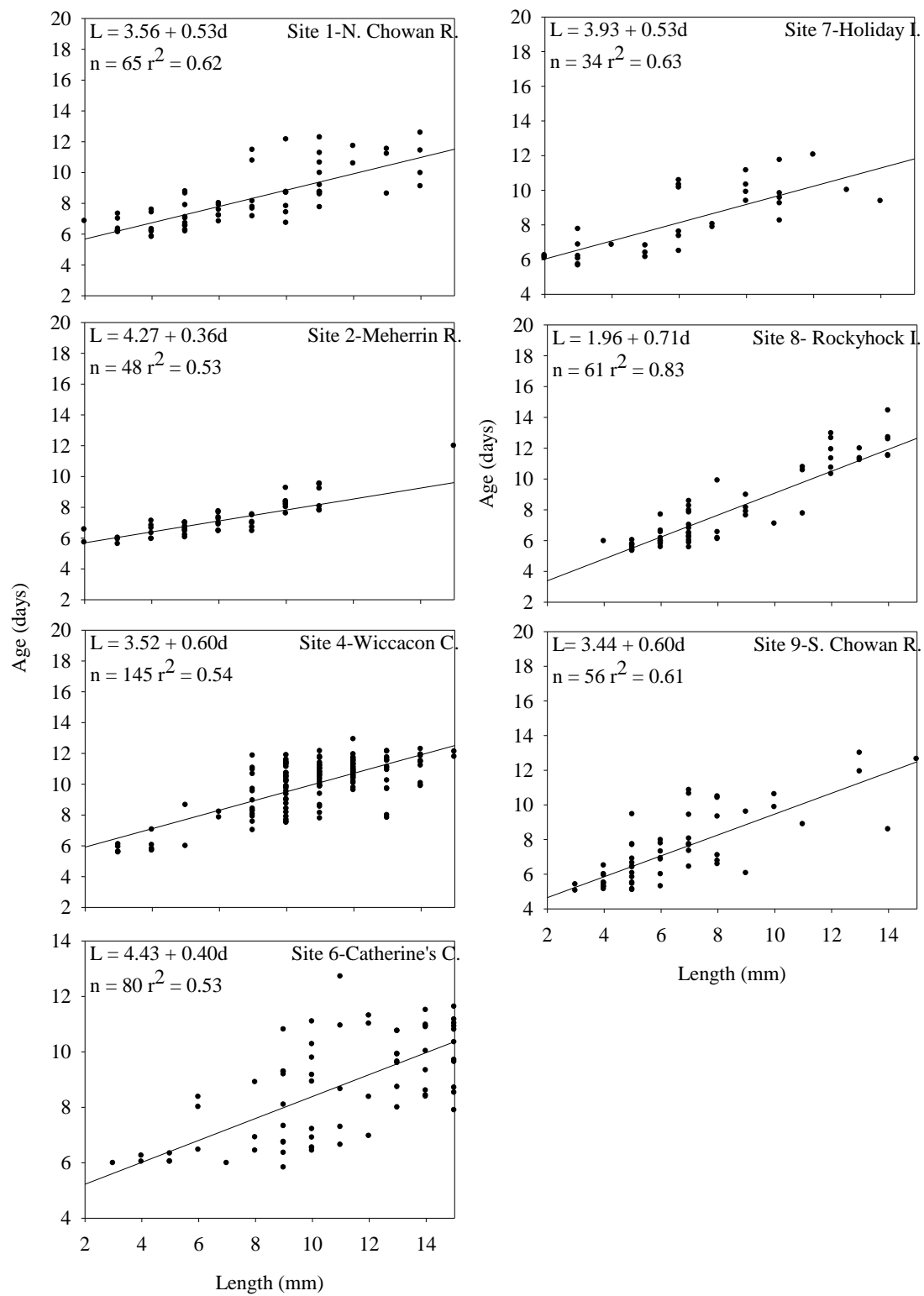
L = 3.56 + 0.53d      Site 1-N. Chowan R.
n = 65 $r^2$ = 0.62

L = 3.93 + 0.53d      Site 7-Holiday I.
n = 34 $r^2$ = 0.63

L = 4.27 + 0.36d      Site 2-Meherrin R.
n = 48 $r^2$ = 0.53

L = 1.96 + 0.71d      Site 8- Rockyhock I.
n = 61 $r^2$ = 0.83

L = 3.52 + 0.60d      Site 4-Wiccacon C.
n = 145 $r^2$ = 0.54

L= 3.44 + 0.60d      Site 9-S. Chowan R.
n = 56 $r^2$ = 0.61

L = 4.43 + 0.40d      Site 6-Catherine's C.
n = 80 $r^2$ = 0.53

Age (days)

Length (mm)

Figure 11. Growth models for river herring larvae in the Chowan River, NC, spring 2011. L = standard length (mm); d = age in days, estimated from otolith-increment analysis.
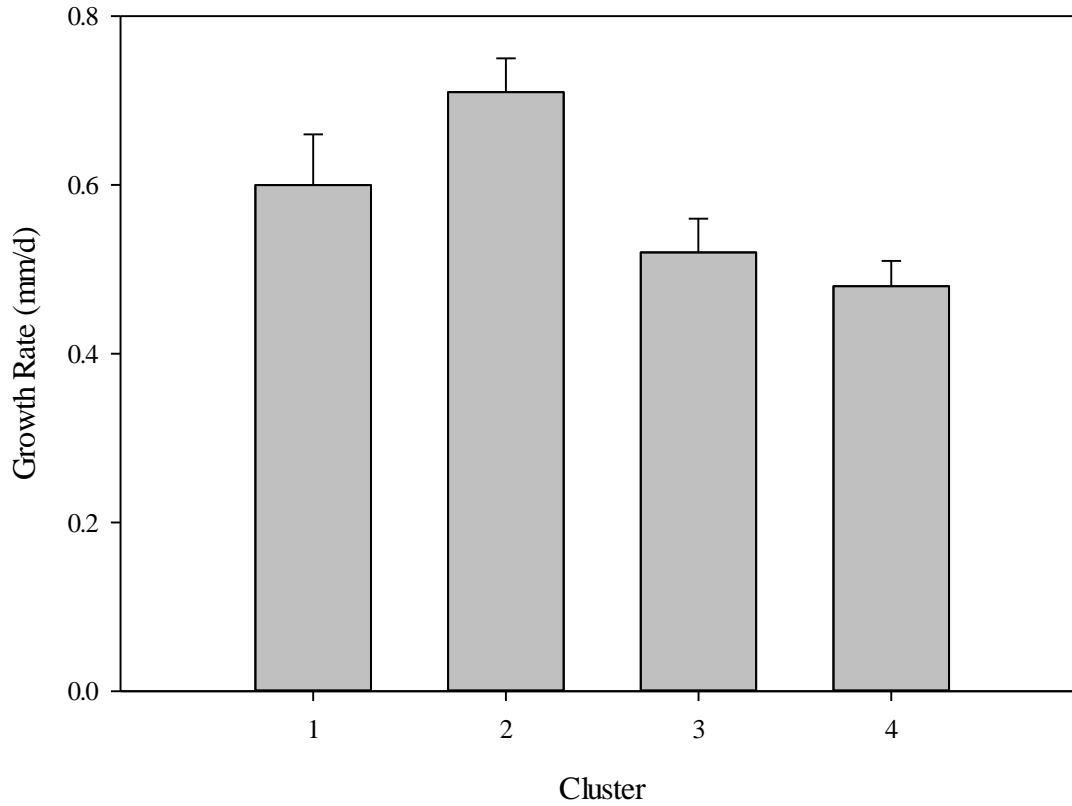


Figure 12. Cluster comparison of growth rates of river herring larvae in four clusters based on water chemistry similarities in the Chowan River, NC, spring 2011. Error bars are 1 SE. Note that Cluster 1 consists of and is represented by site 9 (South Chowan River); Cluster 2 consists of and is represented by site 8 (Rockyhock Creek); Cluster 3 consists of and represented by sites 1 and 7 (North Chowan River and Holiday Island, respectively); Cluster 4 consists of sites 2 – 6 and is represented by sites 2, 4, and 6 (Meherrin River, Wiccacon Creek, and Catherine's Creek, respectively).
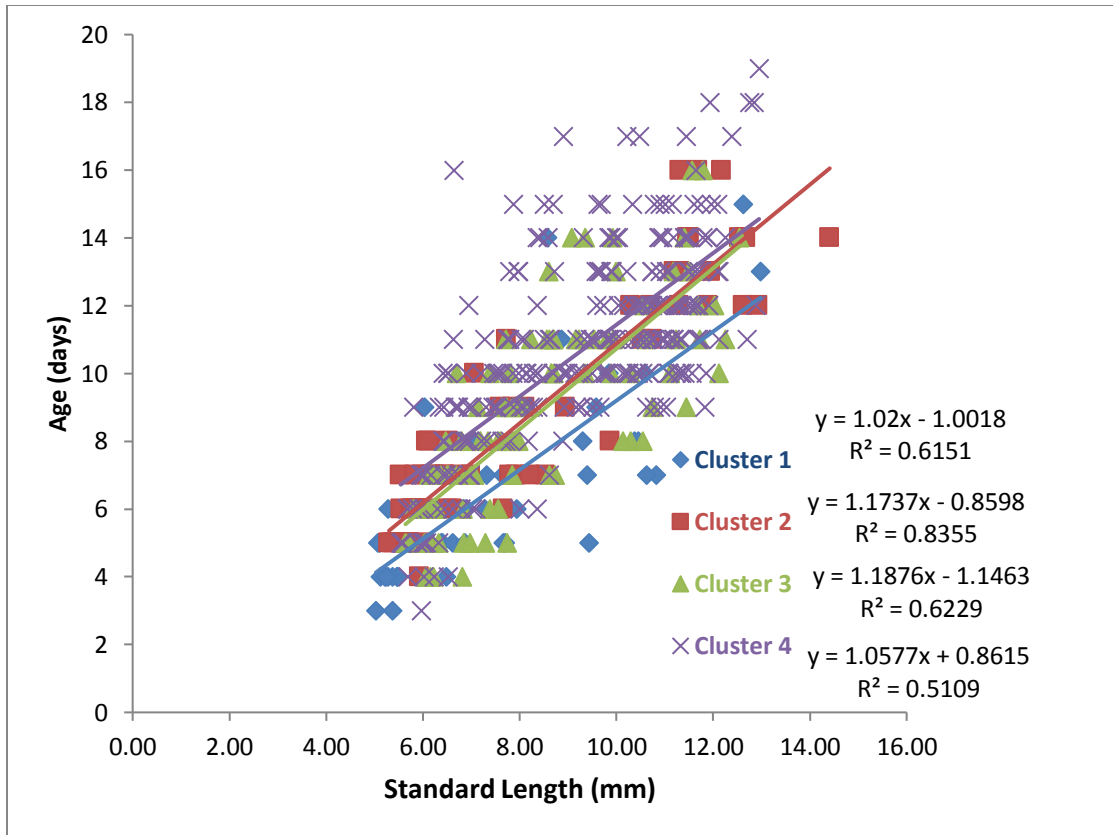
Figure 13. The relationship among larval river herring growth rates from four physicochemically similar clusters sampled in the spring of 2011 on the Chowan River show the positive correlation between standard length (mm) and age (days). Significant growth rate differences were observed between all Clusters except Clusters 2 and 3, note the intersection of the red (Cluster 2) and green (Cluster 3) slopes (ANCOVA; $P< 0.0001$; $F= 239.69$; $n= 489$).
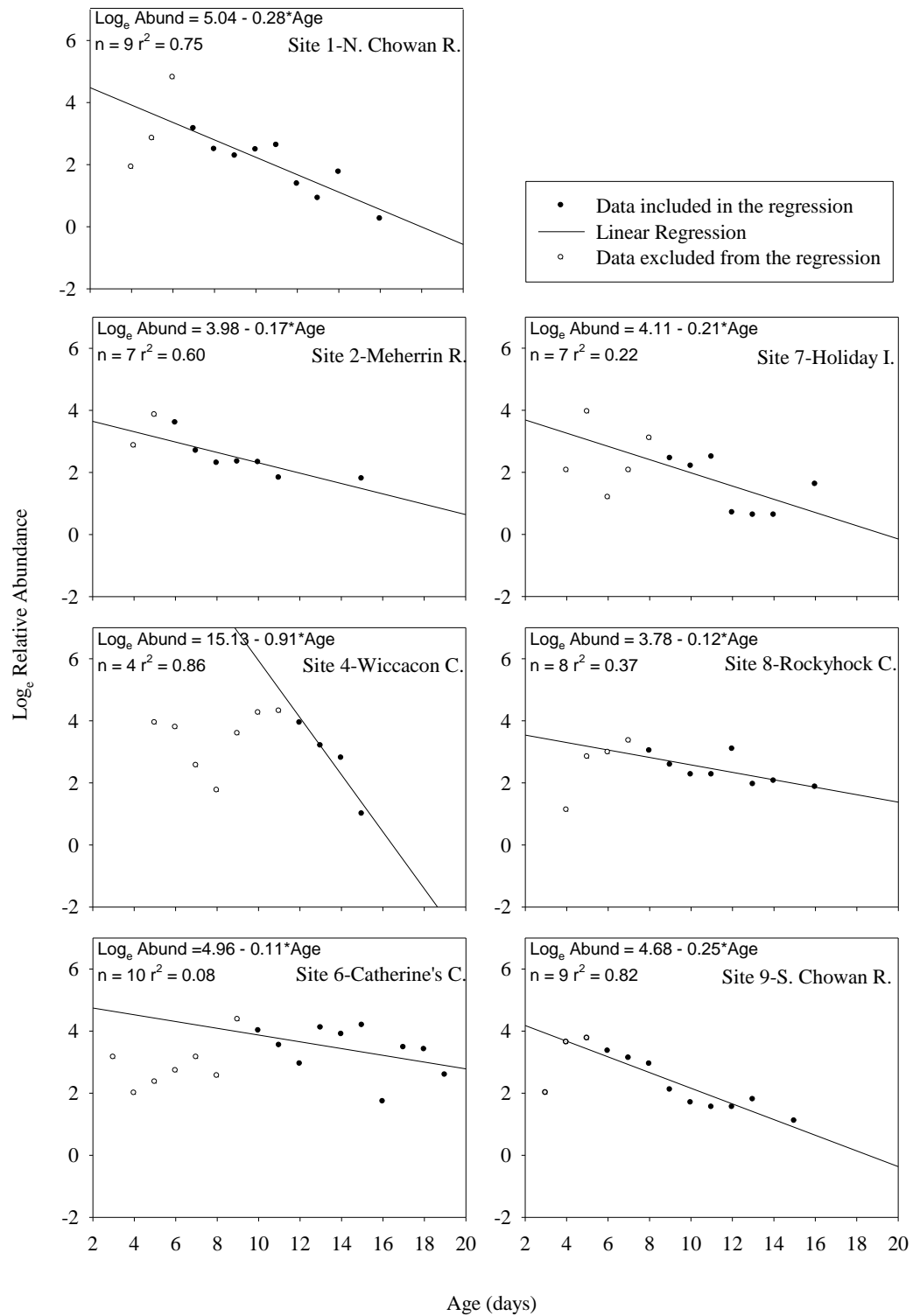
Log$_e$ Abund = 5.04 - 0.28*Age
n = 9 r$^2$ = 0.75          Site 1-N. Chowan R.

Log$_e$ Abund = 3.98 - 0.17*Age
n = 7 r$^2$ = 0.60          Site 2-Meherrin R.

Log$_e$ Abund = 4.11 - 0.21*Age
n = 7 r$^2$ = 0.22          Site 7-Holiday I.

Log$_e$ Abund = 15.13 - 0.91*Age
n = 4 r$^2$ = 0.86          Site 4-Wiccacon C.

Log$_e$ Abund = 3.78 - 0.12*Age
n = 8 r$^2$ = 0.37          Site 8-Rockyhock C.

Log$_e$ Abund =4.96 - 0.11*Age
n = 10 r$^2$ = 0.08          Site 6-Catherine's C.

Log$_e$ Abund = 4.68 - 0.25*Age
n = 9 r$^2$ = 0.82          Site 9-S. Chowan R.

•    Data included in the regression
——  Linear Regression
∘    Data excluded from the regression

Log$_e$ Relative Abundance

Age (days)

Figure 14. Age-specific survival curves of mortality for river herring larvae in the Chowan River, NC, spring 2011. Age in days was estimated from otolith-increment analysis and age-specific abundance was derived from age-length keys.
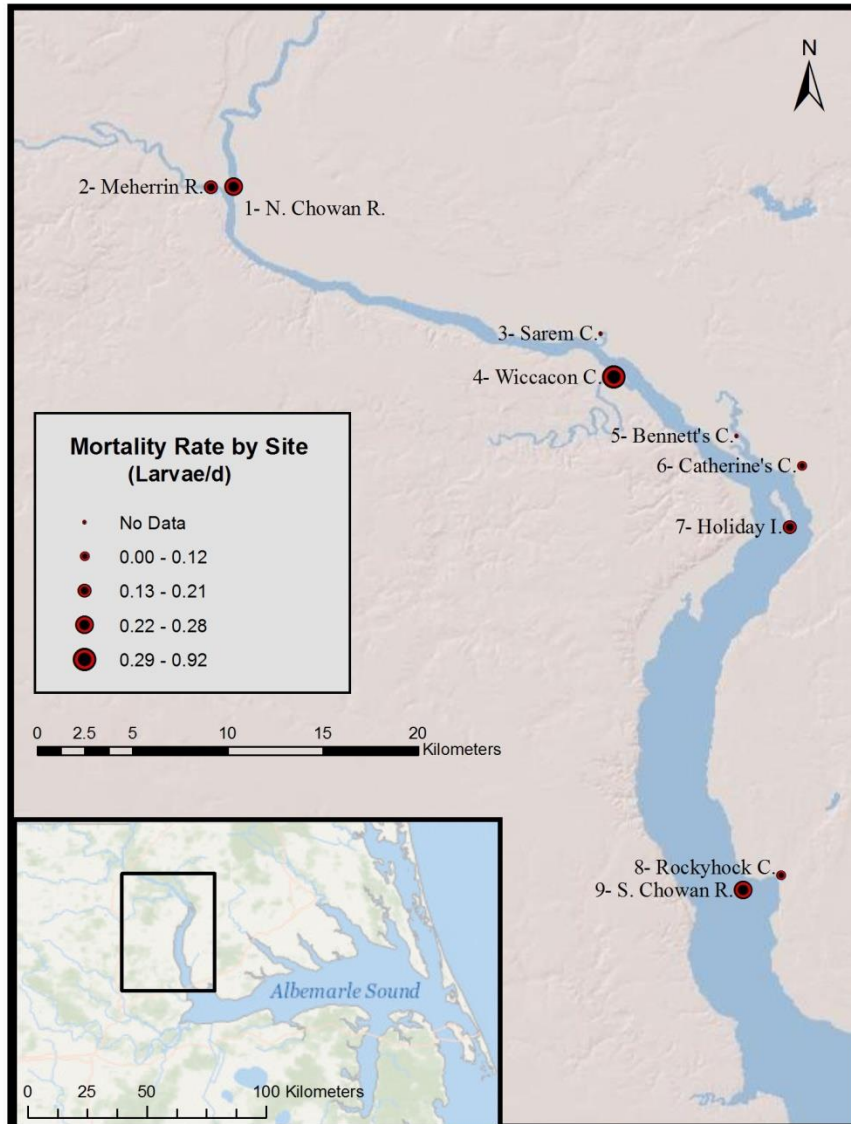


Figure 15. Map of the Chowan River, located in the Northwestern portion of Albemarle Sound, NC showing distribution of larval river herring mortality rates (larvae/day) across sampling sites. Black and red bull's eye circles represent sampling sites including (from North to south): North Chowan River (1), Meherrin River (2), Sarem Creek (3), Wiccacon Creek (4), Bennett's Creek (5), Catherine's Creek (6), Holiday Island (7), Rockyhock Creek (8), South Chowan River (9).

The size of the black and red bull's eye circles is relative to the larval mortality rate (larvae/d) per site, where the largest circles indicate the highest rates and the smallest circles indicate the lowest rates. Age-specific survival curves were used to determine mortality rates at each sampling site. Sites were sampled for larval river herring in spring 2011 (March 18 – May 26).
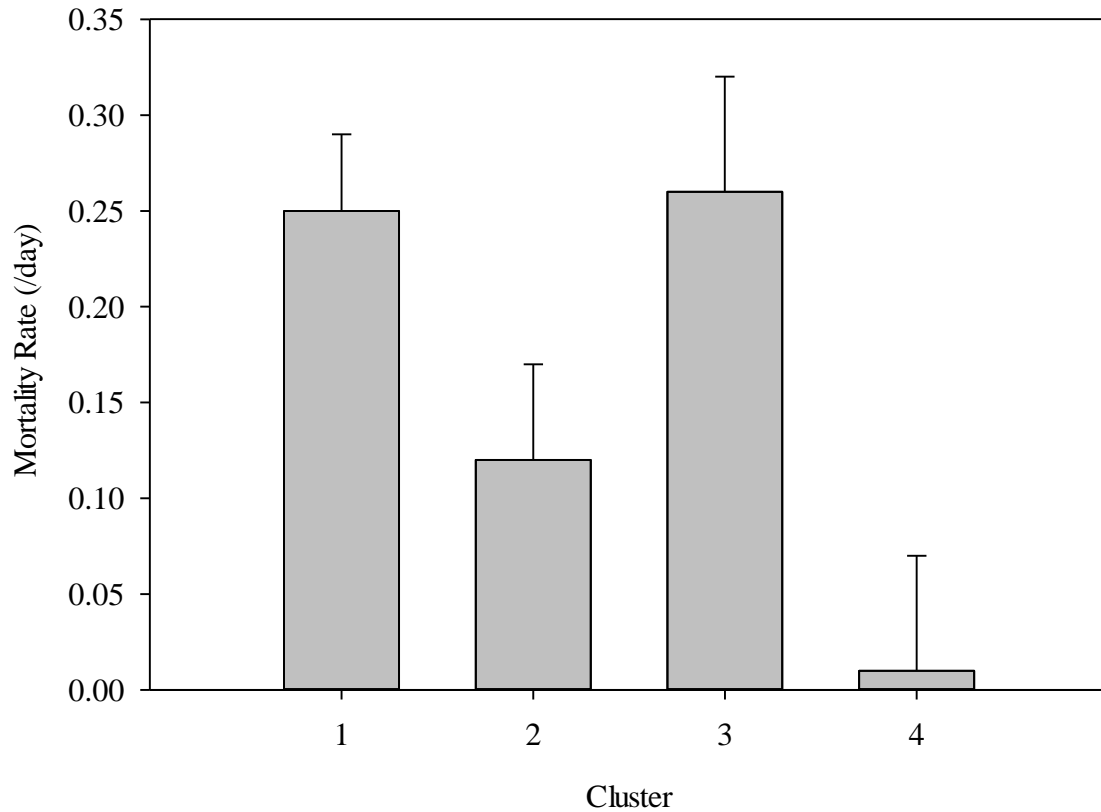


Figure 16. Cluster comparison of mean mortality rates (larvae/day) of river herring larvae in four clusters based on water chemistry similarities in the Chowan River, NC, spring 2011. Error bars are 1 SE. Cluster 1 consists of and is represented by site 9 (South Chowan River); Cluster 2 consists of and is represented by site 8 (Rockyhock Creek); Cluster 3 consists of and is represented by sites 1 and 7 (North Chowan River and Holiday Island, respectively); Cluster 4 consists of sites 2-6 and is represented by sites 2, 4, and 6 (Meherrin River, Wiccacon Creek, and Catherine's Creek, respectively).
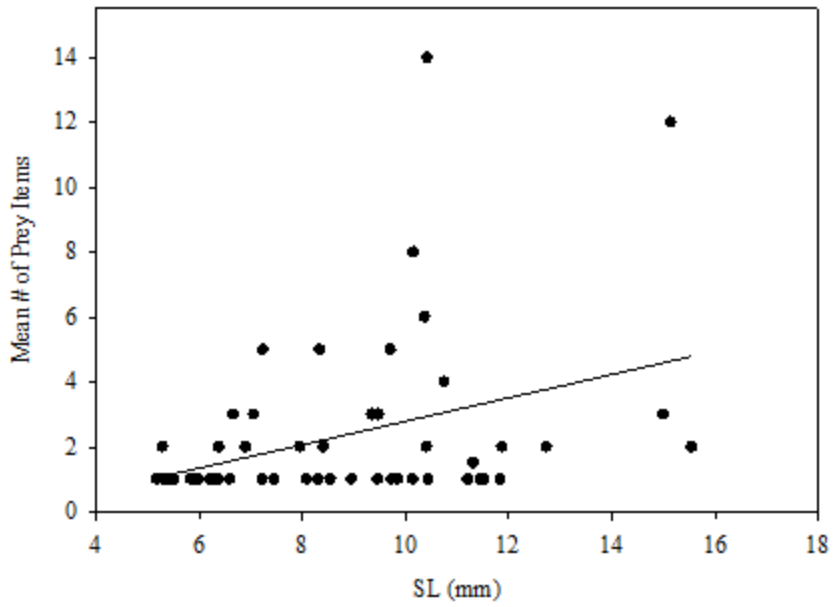
Figure 17. A regression of feeding ratio showing the relationship between larval standard length (mm) and mean number of prey items in gut for river herring larvae collected throughout the Chowan River, NC spring 2011. The mean number of prey items in gut increases with standard length.
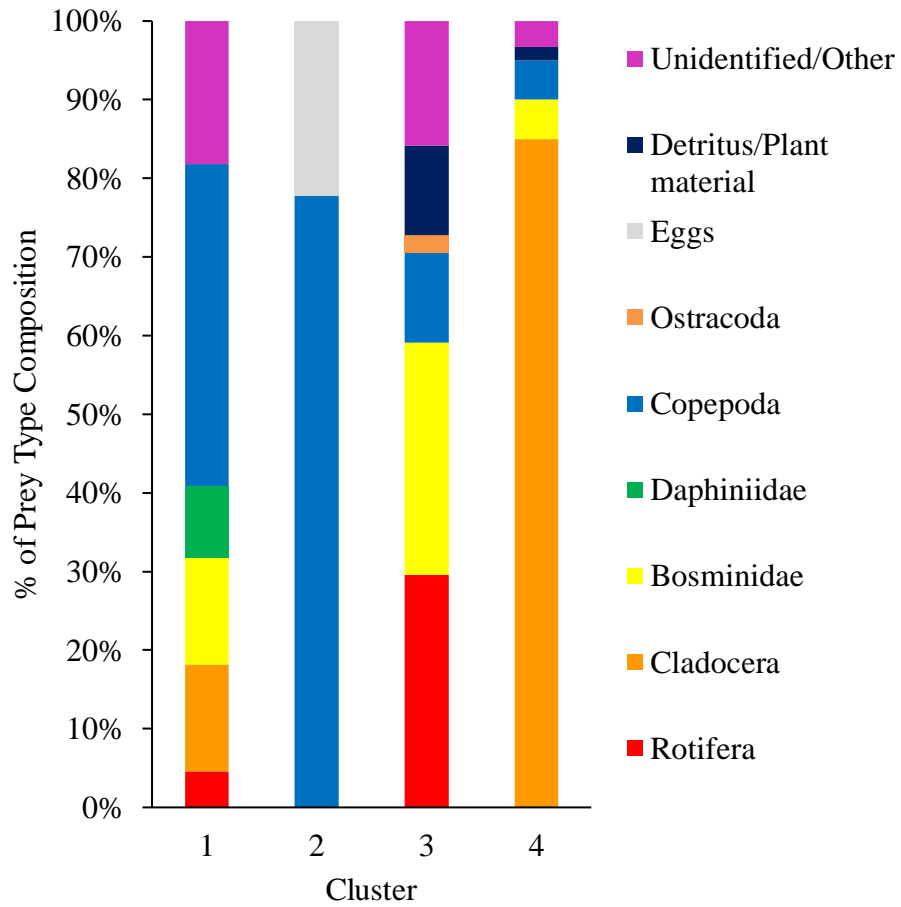
Figure 13. Percent compositions by cluster of larval river herring stomach contents collected in spring 2011 from the Chowan River, NC.
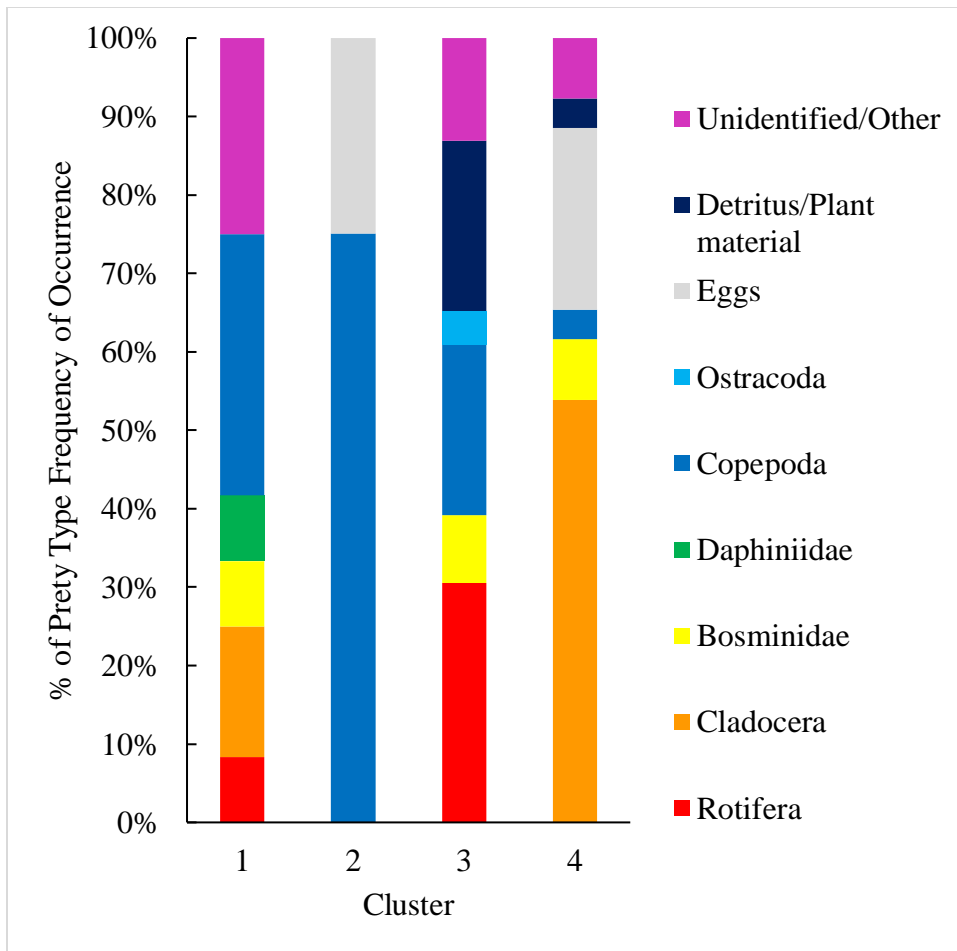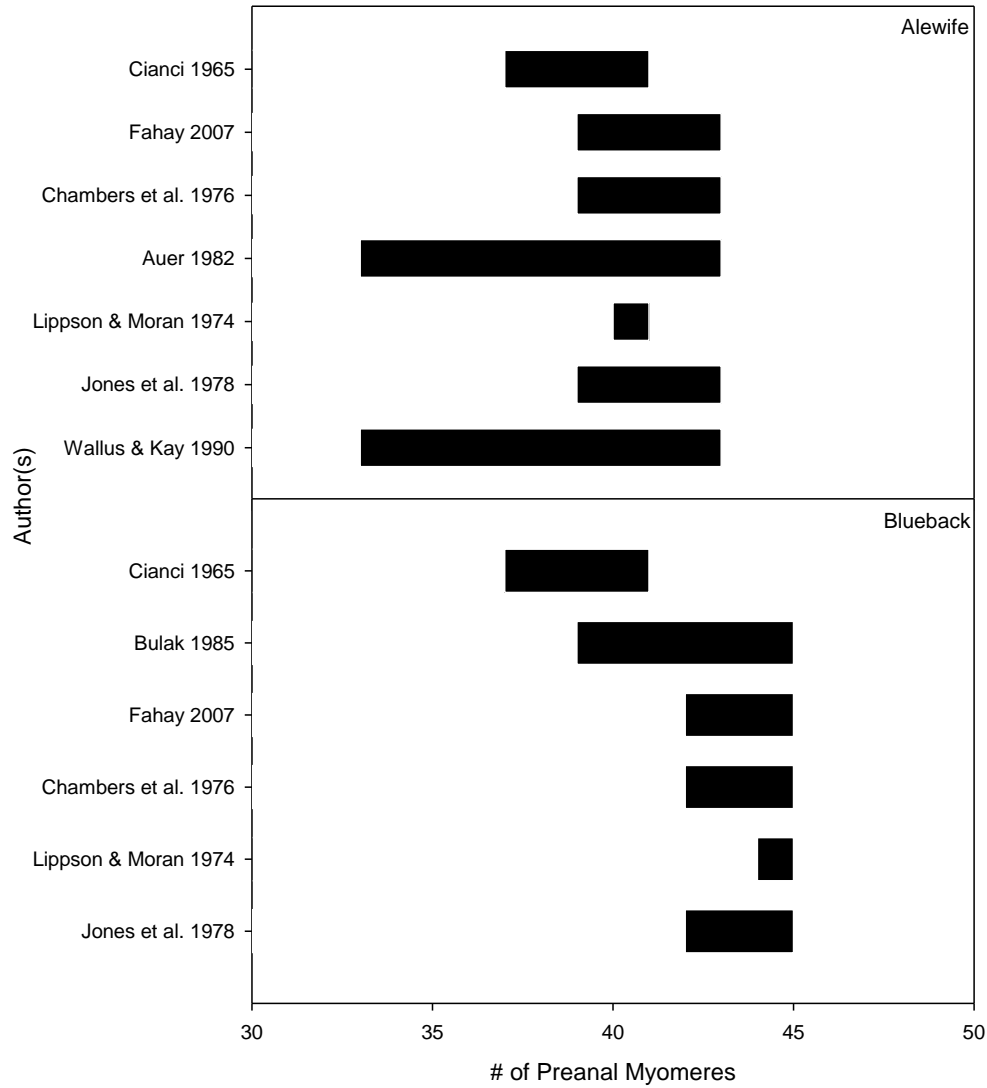
Figure 14. Frequency of occurrence by cluster of larval river herring stomach contents collected in spring 2011 from the Chowan River, NC.

# Appendix A: River Herring Identification Literature Review



A comparison of preanal myomere counts for larval river herring as reported by various references (Cianci 1965; Lippson and Moran 1974; Chambers et al. 1976; Jones et al. 1978; Auer 1982; Bulak 1985; Wallus and Kay 1990; Fahay 2007).
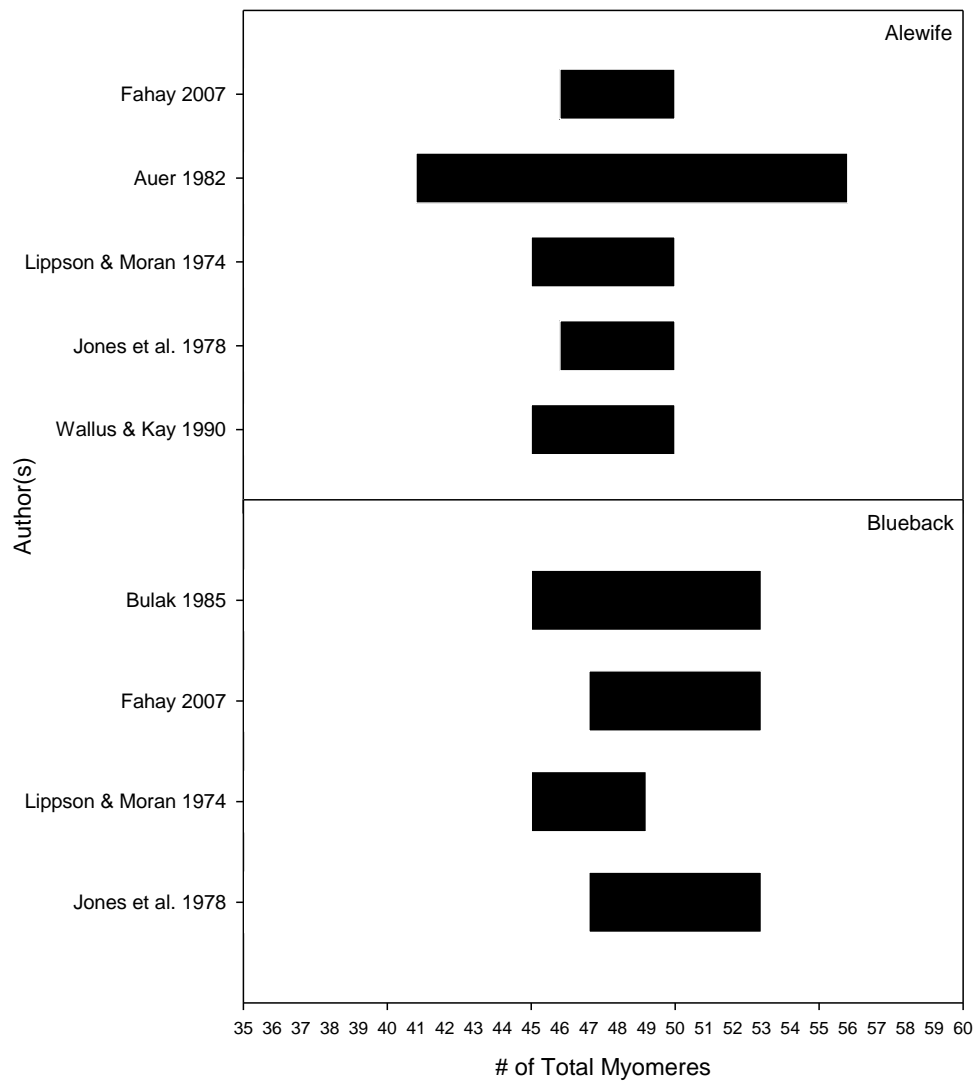
A comparison of total myomere counts for larval river herring as reported by various references (Lippson and Moran 1974; Jones et al. 1978; Bulak 1985; Wallus and Kay 1990; Fahay 2007).
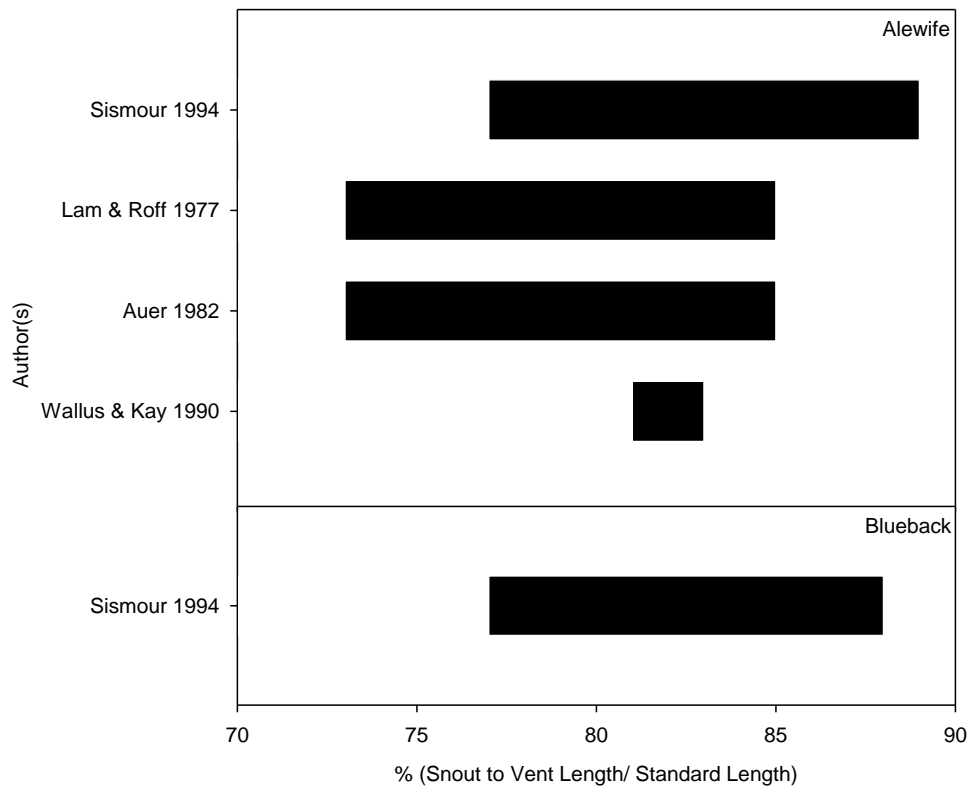
A comparison of snout to vent length (SVL) percent of standard length (SL) values for larval river herring as reported by various references (Lam and Roff 1977; Auer 1982; Wallus and Kay 1990; E. N. Sismour 1994; E. Sismour 1994).

## Ⅲ East Carolina University.
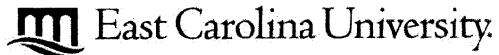
**Animal Care and Use Commitee**

212 Ed Warren Life
Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

March 1, 2011

Anthony Overton, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Overton:

Your Animal Use Protocol entitled, "Can Spawning Habitat be Characterized and Prioritized Based on the Presence of Early Life Stages of River Herring?" (AUP #D254) was reviewed by this institution's Animal Care and Use Committee on 3/1/11. The following action was taken by the Committee:

   "Approved as submitted"

A copy 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.

Sincerely yours,

Scott E. Gordon, Ph.D.
Chairman, Animal Care and Use Committee

SEG/jd

enclosure

**Dauphin Island Sea Lab (DISL)**

**Ichthyoplankton Sorting Protocols - DISL Samples**

Submitted by:     Frank Hernandez, Jr.

Revision Date:     September 2011

**1.0     Procedures for processing DISL plankton samples.**

    1.1     Mininess Samples

        1.1.1   Measure plankton displacement volume for all Mininess samples.

        1.1.2   When excessively large numbers of fish are present, sort an aliquot of the sample that is sufficient to yield 1,000 fish larvae, juveniles and adults. Clearly note on the data sheet the size of the aliquot sorted for fish larvae. **REMEMBER:  ALL FISH LARVAE MUST BE <u>REMOVED</u> FROM THE ALIQUOT.**

        1.1.3   In general, the sample should never be split more than 5 times with the Folsum Plankton Splitter.

        1.1.4   Sort an aliquot of the sample that is sufficient to yield about 200 fish eggs when excessively large numbers of fish eggs are present. Clearly note on the data sheet the size of the aliquot sorted for fish eggs. **REMEMBER: ALL FISH EGGS MUST BE <u>REMOVED</u> FROM THE ALIQUOT.**

        **1.1.5   All fish eggs and larvae removed from any DISL sample must be placed in 95% ethanol.**

        <u>REMEMBER:</u> CHECK THE PERFORMANCE OF THE PLANKTON SPLITTER FREQUENTLY BY ALLOWING THE SAMPLE SPLITS TO SETTLE UNDISTURBED FOR TWENTY MINUTES ON A LEVEL SURFACE THEN COMPARE THE AMOUNT OF SAMPLE IN EACH JAR TO ASCERTAIN IF THE AMOUNT OF SAMPLE IN EACH JAR IS EQUAL. IF THE AMOUNT OF SAMPLE IN EACH JAR IS NOT EQUAL REPOSITION AND/OR ADJUST THE SPLITTER, RECOMBINE THE SPLITS AND ALIQUOT THE SAMPLE AGAIN.

1.2 Neuston Samples

1.2.1 Measure plankton displacement volume for all Neuston samples.

1.2.2 When excessively large numbers of fish larvae are present, sort an aliquot of the sample that is sufficient to yield 1,000 fish larvae, juveniles and adults. Clearly note on the data sheet the size of the aliquot sorted for fish larvae. **REMEMBER: ALL FISH LARVAE MUST BE <u>REMOVED</u> FROM THE ALIQUOT.**

1.2.3 In general, the sample should never be split more than 5 times with the Folsum Plankton Splitter.

1.2.4 Sort an aliquot of the sample that is sufficient to yield about 200 fish eggs when excessively large numbers of fish eggs are present. Aliquots for eggs sorts may be smaller than ½. Clearly note on the data sheet the size of the aliquot sorted for fish eggs. **REMEMBER: ALL FISH EGGS MUST BE <u>REMOVED</u> FROM THE ALIQUOT.**

**1.2.5 All fish eggs and larvae removed from any DISL sample must be placed in 95% ethanol.**

1.3 Ring Net Samples

1.3.1 Measure plankton displacement volume for all Ring Net samples.

1.3.2 When excessively large numbers of fish larvae are present, sort an aliquot of the sample that is sufficient to yield 1,000 fish larvae, juveniles and adults. Clearly note on the data sheet the size of the aliquot sorted for fish larvae. **REMEMBER: ALL FISH LARVAE MUST BE <u>REMOVED</u> FROM THE ALIQUOT.**

1.3.3 In general, the sample should never be split more than 5 times with the Folsum Plankton Splitter.

1.3.4 Fish eggs are to be **<u>counted but not removed</u>** from the samples. Sort an aliquot of the sample that is sufficient to yield about 200 fish eggs when excessively large numbers of fish eggs are present. Aliquots for eggs sorts may be smaller than ½. Clearly note on the data sheet the size of the aliquot sorted for fish eggs.

**REMEMBER: ALL EGGS MUST BE <u>COUNTED</u> FROM THE ALIQUOT.**

**1.3.5  All fish eggs and larvae removed from any DISL sample must be placed in 95% ethanol.**

**2.0  Identification and measurement of ichthyoplankton in DISL samples.**

2.1  Identification

2.1.1  All fishes regardless of size or stage of development are to be removed from samples, identified, counted and placed in labeled specimen vials or jars that match the size of the specimens.  An exception to this would be when only a few specimens of the taxon would require a larger vial. In that case place all the specimens in the larger vial. Please do not break spines off specimens or force large specimens into small vials. Use larger vials for these specimens.

2.1.2  Please follow the classification of scientific (Latin) fish names listed in Table 1 of the SEFSC protocols.

2.1.3  Identify specimens of <u>ONLY</u> the following families to the lowest possible taxon (i.e., genus and species):  CLUPEIDAE\*, SCIAENIDAE, SERRANIDAE,  SCOMBRIDAE,  STROMATEIDAE,  MUGILIDAE, LUTJANIDAE AND CARANGIDAE.

\*It is now permissible to use the combined name "*Sardinella/Harengula*" for larvae that belong to one of those two genera but that cannot be reliably distinguished from each other.  Write this name on the data sheet and enter this name in the computer.

*\*Please note that "Brevoortia tyrannus" is not a species that occurs in the northern Gulf of Mexico and therefore it CANNOT be in DISL samples. Possible identifications for these specimens include "Brevoortia patronus", "Brevoortia smithi", "Brevoortia sp." or "Clupeidae".*

2.1.4  Identify larvae of all other groups to the genus or species level only when such identification can be made easily with on extra time required. Otherwise identify to the family level only.

2.1.5  Use question marks and general comments to denote or bring attention to an uncertain or "best guess" identification of a specimen.  This practice should be used whenever necessary.  Make sure that all question

marks and comments are entered on the electronic copies of the data sheets.

2.2    Specimen counts

2.2.1   COUNT the number of specimens of ALL taxa including Unidentified, Perciformes and Clupeiformes, and record the count on the data sheet and inside vial label. Place the specimens in labeled 3-dram vials.

2.2.2  When samples contain many parts and pieces of larvae of Unidentified, Clupeiformes and Perciformes, count only the 'heads'.

2.2.3   Remove and count all fish eggs from Mininess samples and record the aliquot that was sorted (entire or fraction). Place eggs in labeled 3-dram vials.

2.2.4   Count (do not remove) all fish eggs from Neuston or Ring Net samples and record the aliquot that was sorted (entire or fraction).

2.3    Measurement

2.3.1   Measure up to 20 larvae from a randomly selected subsample for all taxa identified to the species level.

2.3.2   Measure up to 20 larvae from a randomly selected subsample for all taxa identified from the following genera : *Lutjanus*, *Brevoortia*, *Cynoscion,* and *Scomberomorus*.

**This includes *Lutjanus* sp.*, Lutjanus campechanus, Lutjanus griseus, Lutjanus synagris, Lutjanus analis, Brevoortia patronus, Brevoortia smithi, Brevoortia* sp.*, Cynoscion* sp.*, Cynoscion arenarius, Cynoscion nothus, Cynoscion nebulosus, Scomberomorus maculatus, Scomberomorus cavalla,* and *Scomberomorus* sp.**

2.3.3   Measure up to 20 larvae from a randomly selected subsample for all taxa identified from the families Lutjanidae and Clupeidae.

REMEMBER: CHOOSE SPECIMENS TO BE MEASURED RANDOMLY FROM THE DISH.

2.3.4 Measure the smallest and largest specimens only for larvae identified to genus or family (except as noted above). Specimens identified

84

as 'Unidentified' or to the order level (e.g., Clupeiformes, Anguilliformes, and Perciformes) need not be measured (but must *be counted*).

2.3.5   Use the appropriate measurement of body length, i.e., notochord or standard length, depending on the stage of development of the specimen.

## 3.0   Data Sheet and Vial or Jar Labeling

3.1   Data Distribution

3.1.1   Send all original data sheets and the sorting record sheets to Dr. Frank Hernandez, Jr. at the Dauphin Island Sea Lab, 101 Bienville Blvd., Dauphin Island, Alabama 36528, USA.

3.1.2   Send computer-generated identification data sheets along with specimens to Dr. Frank Hernandez, Jr. at the Dauphin Island Sea Lab, 101 Bienville Blvd., Dauphin Island, Alabama 36528, USA.

3.1.3   Microsoft Excel files containing data for each cruise should be sent to Dr. Frank Hernandez, Jr. (fhernandez@disl.org) as mail attachments once they are completed.

3.2   Identification Sheets and Vial Labels

3.2.1   The TOTAL NUMBER of vials in which a taxon has been placed must be recorded in the "vial number" column on the identification data sheet. The label inside a vial containing specimens should specify which vial of the total number of vials it is, for example: 1 of 2, or 2 of 2, etc. This information can be placed on the back of the label if necessary.

3.2.2   Record whether the larvae or eggs were sorted from the entire sample, denoted by 1, or ½ (or smaller for eggs) aliquot on both the identification data sheets and on the inside vial labels.

3.2.3   3-dram vials will be sent to ZSIOP. ZSIOP staff will add inside labels. Label paper (Resistall) will be provided by DISL. Labels must be written with India ink pens on Resistall paper provided by DISL.  DISL will provide pre-printed internal labels.

3.2.4   Record the following information on inside vial labels for MININESS Samples: Cruise, Mininess ID number, sample number, taxon, number of

specimens, bin and station name.  Aliquot size and vial number of total vials used for that taxon (to be placed on back of label).

3.2.5   Record the following information on inside vial labels for NEUSTON Samples: Cruise, Gear, sample number, taxon, number of specimens, and station. For Gear, write in the word  "Neuston" in the space labeled for "Mini #".

3.2.6   Record the following information on inside vial labels for Ring Net Samples: Cruise, Gear, sample number, taxon, number of specimens, and station. For Gear, write in the word "Ring" in the space labeled for "Mini #".

3.2.7   Record the following information on the external (dot) labels for MININESS , NEUSTON and Ring Net Samples: Cruise and sample number.

3.2.8   Please note which samples have been resorted to check for sorting accuracy and record results of these resorts on the identification sheets.

## 4.0   Quality Control Protocol for Computer Data Entry

4.1     All data on fish eggs, fish larvae and zooplankton are recorded on   paper log sheets.  Each taxonomist will error check original paper   log sheets to ensure that tally marks are correctly summed and that  decimal  points  are  included  for each length measurement.

4.2     All  computer  data  entry  must  be  verified  to  ensure  that  the database/spreadsheet data and original log sheet are the same.     This checking procedure will be done by printing out            database/spreadsheet hard copy, and reading data back to the data   entry   individual. After  data  are verified, the electronic        database/spreadsheet data are sent to the appropriate DISL  scientists as email attachments.  Samples and original paper log     sheets are sent separately by surface mail as soon as possible as    required    by    the appropriate protocol.