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Predator-prey interactions among fish-eating birds and selected fishery resources in the Chesapeake Bay: temporal and spatial trends and implications for fishery assessment and management.

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Draft Report: Predator-prey interactions among fish-eating birds and selected fishery resources in the Chesapeake Bay: temporal and spatial trends and implications for fishery assessment and management.

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Executive Summary

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Area of Interest: Ecosystem Based Fisheries Research, Monitoring, and Modeling and Integrated Science Program-cross-cutting multidisciplinary efforts

Project Title: Predator-prey interactions among fish-eating birds and selected fishery resources in the Chesapeake Bay: temporal and spatial trends and implications for fishery assessment and management.

Project duration: Multi-year (48 months); Beginning July 1, 2006 – June 30, 2007 (Year 1) and through 2010 (Year 4).

Objectives:

I.   To understand the relationship between temporal and spatial trends in the distribution and abundance of avian predators and selected fishery resources

II. To estimate the overall metabolic demand/consumption of fish-obligate breeding bird communities in order to parameterize current Chesapeake Bay ecosystem models

III. To develop a novel, fishery independent tool for stock assessment of Atlantic menhaden and American shad by identifying diagnostic isotopic markers which will allow tracking of Atlantic population trends using feathers from sentinel bird species (e.g. Osprey).

Summary of work performed:

SPECIFIC PROJECT OBJECTIVES:

1) Quantify the complex relationships among temporal trends and geospatial patterns, and across multiple scales, using archival, long-term databases of the geographic distribution and abundance of avian predators and selected fishery resources in the Chesapeake Bay region (*Chapter 1*).

2) Complete a two-part retrospective analysis consisting of an analysis of stable isotopes from Bald Eagles and Osprey occupying the Chesapeake Bay circa 1850 – 2002 in order to estimate historical trends in the contribution of anadromous fishes, including American shad, to the diet of Osprey and Bald Eagles over very broad temporal scales. Feathers have been collected from the Smithsonian Institution (historical period) and active nests throughout Chesapeake Bay and its tidal tributaries (*Chapter 2*).

3) Use conventional energetics-based methods to estimate the overall metabolic demand and consumption of fishery resources for selected avian species in order to contribute to the parameterization of existing Chesapeake Bay ecosystem models (*Chapters 3*).

4) For most piscivorous birds comprehensive data on the composition and size distribution of fish prey are lacking. We stratified estimates of avian consumption according to fish species by compiling existing diet information for bird consumers and conducting avian diet studies for those species for which data are lacking (e.g. Osprey, Double-crested Cormorants, and Pelicans). Concurrent with avian diet studies, fishery hydroacoustic surveys were conducted to estimate available fish biomass and quantify the impact of local consumption by fish-eating birds (*Chapter 4*).

5) Develop and test novel, fishery independent stock assessment tool based on diagnostic stable isotope biomarkers (fatty acid signature analysis) for Atlantic menhaden and American shad in sentinel avian predators (*Chapter 5*).

Populations of fish-eating birds within the Chesapeake Bay and its major tributaries have increased dramatically during the past 40 y, resulting in a novel—and potentially significant— source of competition and predation for important Chesapeake Bay finfish stocks. For example, the rapidly increasing abundance of Double-crested Cormorants has impacted commercial aquaculture (Glahn and Brugger 1995) and inland and marine fisheries management (Simmonds *et al.*2000; Crecco and Howell 2006; J. Uphoff, MD DNR, personal observation) in other geographic regions. In spite of significant population growth and geographic expansion by piscivorous birds in the Chesapeake Bay region, the impact of avian predation and competition on marine, estuarine, and riverine fish assemblages in the Chesapeake Bay has not been quantified or incorporated into ecosystem models. Similarly, the potential role of fishery population dynamics in regulating populations of bird species that are of national conservation concern has never been evaluated within the region. In fact, Chesapeake Bay ecosystem models typically ignore avian predators and competitors (e.g. see Baird and Ulanowicz 1989), and fishery stock assessments for the region generally fail to incorporate these potentially important ecological interactions. Several ecologically, culturally, and economically important Chesapeake Bay fishes, including Atlantic menhaden (*Brevoortia tyrannus*) and American shad (*Alosa sapidissima*), contribute substantially to the diets of fish-eating birds (Watts *et al.*2007, McLean and Byrd 1991). Thus, piscivorous birds, which are used widely as a sentinel species for tracking ecosystem health elsewhere (Steidl *et al.*1991a and b, Elliot *et al.*2002, Henny *et al.*2003), may be useful indicator species for fishery population status and trends in the Chesapeake Bay.

The document *Fisheries Ecosystem Planning for the Chesapeake Bay* (Chesapeake Fisheries Ecosystem Plan Technical Advisory Panel 2004) emphasizes the importance of food web dynamics and modeling as essential elements in an effective ecosystem approach to fisheries management. According to the 2004 FEP: "Although conventional single-species management approaches do not typically address predator-prey dynamics, these dynamics form the heart of interactions among species, affecting abundance and production. Such interactions have dramatic and substantial effects on community structure, ultimately affecting fisheries yields in the Bay, and must be

considered when developing or amending ecosystem-based fishery management plans."
In fact, most of the food webs and trophic models illustrated in the 2004 FEP assign to
piscivorous birds the position of apex predator, together with humans, and a recent
NOAA-CBO Chesapeake Bay diet matrix (CFEPTAP 2004) classified piscivorous birds
as the most highly connected predators in the system. Predator-prey interactions are also
important parameters in most Chesapeake Bay ecosystem-based models (e.g. Ecopath
with Ecosim or EWE) and multispecies fish stock assessments (e.g. multispecies virtual
population analysis, MSVPA) requiring estimates of natural mortality. However, a
fundamental problem with current ecosystem and multi-species fisheries models is that
although fisheries data are occasionally precise and fully parameterized, incomplete data–
or no data–exist for closely linked and potentially important ecosystem components such
as piscivorous birds (Silvert and Murta, *in press*).

Effective ecosystem management also requires the ability to document and
forecast system responses to change (NCBO Chesapeake Bay Integrated Science
Program 2006). By tracking changes in the diet, distribution, and reproductive output of
avian predators, fishery biologists and managers will develop a critical understanding of
spatial distributions and system-wide abundances of target fish species, as well as
responses to management-initiated changes and natural disturbances. For example,
historical and well-documented shifts in the spatial distribution, abundance, and
reproductive output of Bald Eagles and Osprey in the Chesapeake Bay region are
associated with concomitant changes in distribution and abundance of important prey
species, including American shad and possibly Atlantic menhaden (McLean and Byrd
1991, Watts *et al.*2007, Viverette *et al.*2007, Glass and Watts 2009). Understanding the
historic role of anadromous species in the diets of avian predators can help identify
significant interactions in Chesapeake Bay food webs over time. Restoration of American
shad and related alosine (*Alosa* spp.) populations is a major focus of the region's
Chesapeake Bay 2000 commitments (Chesapeake Bay Program 2000) and pre- and post-
restoration assessment of Bald Eagle populations could provide valuable data for
evaluating restoration success. Similarly, tracking Atlantic menhaden contributions to the
diets of a sentinel avian predator like Osprey may provide a unique, cost-effective, and
independent tool for consistent, integrated, and long-term monitoring of Atlantic

menhaden stocks in the region. Specifically, the development, testing, and application of new monitoring protocols based on stable isotope analysis of feathers from avian predators to track American shad and Atlantic menhaden population trends would meet the Atlantic States Marine Fisheries Commission's mandate to: "…develop novel methodologies for stock assessment including fishery-independent surveys and variable natural mortality at age or by area."(ASFMC 2001).

Finfish-Waterbird trophic interactions in Tidal Freshwater Tributaries of the Chesapeake Bay

As piscivorous bird populations rebounded in the Chesapeake Bay, *circa* 1970-2010, coastal and riparian habitats were being transformed by activities such as shoreline development, over-harvesting of estuarine and riverine fisheries, and industrial and agricultural pollution. In addition, the relatively recent introduction and establishment of several non-native fishes within Chesapeake Bay tributaries may have significantly altered prey resources for avian predators (Edmonds 2003). The resultant changes in the fish resources available to avian predators over the past 40 years include changing temporal and spatial distribution of fish prey as well as shifts in taxonomic and trophic structure of resident and migratory fish assemblages (Viverette *et al.*2007). Specifically historical ecological changes likely influencing current piscivorous bird distributions and abundance in the Chesapeake Bay include long- and short-term changes in the abundance of anadromous clupeid fishes (Foerster and Reagan 1976), Atlantic menhaden (Uphoff 2003a, b), and the relatively recent introduction and establishment of non-indigenous blue catfish and flathead catfish within coastal rivers (Edmonds 2003; McAvoy *et al.*2000).

For many species of fish-eating birds, there may be considerable spatial variation in the rates of population growth. Tidal freshwater and oligohaline reaches of major Chesapeake Bay tributaries appear to be one area of convergence for this expanding consumer community. Several species, including Bald Eagles and Osprey experienced significantly greater population growth rates in riverine tidal freshwater and oligohaline regions than in higher salinity portions of the bay (Watts *et al.*2004, Watts *et al.*2006).

Shifting fish prey resources may provide an explanation for the observed influence of salinity on distribution of piscivorous bird populations (Watts *et al.*2004, Watts *et al.*2006). The resultant changes in the fish resources available to avian predators over the past 40 years include changing temporal and spatial distribution of fish prey, as well as shifts in taxonomic and trophic structure of resident and migratory fish assemblages (Viverette *et al.*2007).

Access to relatively predictable, annual concentrations of prey, as represented by spawning migrations of anadromous fish, may have profound effects on the distribution and abundance of predators such as Bald Eagles (Willson and Halupka 1995; Restani *et al.*2000). Migratory shads and herrings were once abundant and geographically widespread in the Chesapeake Bay and its tributaries until the construction of dams in the past century largely confined anadromous clupeid spawning activity to the tidal freshwater regions (Jenkins and Burkhead 1994; McIninch and Garman 1999) resulting in a shift from spatially widespread to spatially concentrated fish resources. At the same time a shift from temporally concentrated to temporally widespread (resident year-round) fish resources has taken place in low salinity and tidal freshwater zones. As migratory clupeids declined, there was a concomitant shift from migratory to non-migratory species, i.e., from a seasonally abundant resource to one that is available year-round. For instance, on an annual basis, non-migratory (i.e., resident) *Dorosoma* species, both gizzard shad (*Dorosoma cepedianum*) and the non-native threadfin shad (*D. petenense*), dominate clupeid assemblages in tidal freshwater habitats within the Chesapeake Bay.

In addition to a shift from migratory to resident species, tidal freshwater fish communities experienced a shift in trophic and size structure. Concurrent (*ca*. 1975) with the severe declines in anadromous clupeid populations, the nonindigenous blue catfish (*Ictalurus furcatus)* and flathead catfish (*Pylodictus olivarus*) were introduced to the Atlantic slope of Virginia (Schloesser *et al* In Press). A comparison of total lengths of the ten most abundant fish species in collections in the tidal freshwater James River in 1969 and 1999 suggests a substantial increase in available prey size during that 20-year period (Viverette et al 2007). Increased availability of larger prey may improve foraging efficiency by avian predators. Abundant freshwater prey may provide a nutritional substitute for declining populations of traditionally important forage fishes such as migratory and marine clupeid species.

The availability of alternative prey may account for the fact that recovering Bald Eagle and Osprey populations in tidal freshwater and oligohaline reaches now have the highest density and population growth rates in the Chesapeake Bay (Watts and Paxton 2007. Watts *et al.*2007).

Contribution of marine derived nutrients to Bald Eagles and Osprey nesting in the Chesapeake Bay: A retrospective analysis.

Stable isotopic analysis of tissue samples from consumers record the nutrients assimilated from dietary sources. Bulk $\delta 13C$, $\delta 15N$, and $\delta 34S$ isotope analyses can be a valuable tool in reconstructing diets of historical predator populations. Naturally occurring carbon and sulfur stable isotopes in tissues can distinguish the source of dietary nutrients, i.e. marine versus freshwater, and nitrogen stable isotopes indicate trophic status of consumers (Garman and Macko 1998). A marine signature in tissues of piscivorous birds nesting within tidal freshwaters indicates anadromous (or migratory) fish prey. An additional advantage to stable isotope analysis is that samples from museum collections can be used to study historic diets, allowing for detection of patterns and trends over long periods of time. We conducted an analysis of stable isotopes in feathers collected from Bald Eagles and Osprey occupying the Chesapeake Bay circa 1850 – 2009 in order to estimate historical trends in the contribution of anadromous fishes, including American shad, to the their diets over broad temporal and spatial scales. Specifically we were interested in evaluating the hypothesis that upstream migrations of anadromous clupeid fish represent, at least historically, an ecologically important seasonal subsidy in the form of marine-derived organic matter (MDOM) to piscivorous birds nesting within the Bay's tidal tributaries.

Significant declines in marine stable isotopic signatures in feathers of juvenile Osprey occupying the Chesapeake Bay, particularly in tidal freshwater reaches, over the last 140 years may reflect long-term declines in the abundance of anadromous clupeids (*Alosa* spp., e.g. American shad, Watts *et al.*2006, Viverette *et al.*2007). The results of spatial and temporal analysis of the isotopic signatures of Bald Eagles and Osprey appear to support the hypothesis that Chesapeake Bay piscivorous birds have, over time, shifted

from a diet based on seasonally abundant, native migratory fish to non-indigenous and resident species available year-round within tidal freshwaters and oligohaline reaches. Effective ecosystem management requires the ability to document and forecast system responses to change (NCBO Chesapeake Bay Integrated Science Program 2006). Understanding the current and historic role of critical prey species in the diets and distribution of Bald Eagles and Osprey may help identify significant interactions in Chesapeake Bay food webs over large temporal and spatial scales, and aid in forecasting responses to future change.

For example, a recent study documented shifts in the diet of Bald Eagles occupying the Channel Islands between the late Pleistocene and the mid-20th century using stable isotope analysis (Newsome *et al.*2010). Channel Island Bald Eagles shifted from feeding on native prey species to non-indigenous species introduced and available in high densities starting in the mid-1850's. Both historic prey sources are now depleted or extirpated from the islands. The study highlights the difficult challenges to management of species such as Bald Eagles if historic prey populations, native or introduced, are no longer abundant and an appropriate substitute not available. Similarly, results of the current study highlight the importance of prey distribution to predator abundance and distribution over short and long temporal scales within the Chesapeake Bay. Declines in native anadromous and marine prey, and concentration of these and alternative prey in low salinity habitats may result in a reduction in carrying capacity of the Chesapeake Bay watershed for avian piscivores (Watts *et al.*2007). In addition, substitution of traditional prey species with species occupying higher trophic levels may increase the risk of bioaccumulation of contaminants. By tracking changes in the diet, distribution, and reproductive output of avian piscivores and their prey, biologists and managers will develop a critical understanding of spatial distributions and system-wide abundances of target fish species, as well as community wide responses to management-initiated changes and natural disturbances on predator communities.

Estimates of Energetic Demand by Selected Avian Predators in the Chesapeake Bay

The combined energetic demand of the rapidly expanding avian consumer community—and the implications for effective management of Chesapeake Bay fish stocks—has never been evaluated adequately. Conversely, the potential role of fish population dynamics, distribution, and commercial harvest in regulating bird species that are of national conservation concern is unknown. For most piscivorous birds, comprehensive data on the taxonomic composition and size distribution of fish prey are lacking as inputs for consumption (energetic) models. We used a bioenergetics approach to estimate the amount of fish biomass consumed by breeding piscivorous birds within the tidal reach of the Chesapeake Bay. This approach combined a multi-stage population model with a breeding model and applied allometric relationships between field metabolism and body mass to estimate annual demand across years and daily demand within years. Species-specific models were created for Bald Eagles, Osprey, Great Blue Herons, Double-crested Cormorants, and Brown Pelicans. We also stratified estimates of avian consumption according to fish species by conducting avian diet studies.

Estimated fish consumption by the 5 populations examined increased exponentially from 1,588,084 to 16,014634 kg with an average doubling time of 9.0 years between 1975 and 2005 (Figure 1). This reflects the exponential growth in these populations and the recent colonization of the Bay by Double-crested Cormorants and Brown Pelicans. Due to their large population size, Great Blue Herons consumed the greatest biomass followed by Double-crested Cormorants, Brown
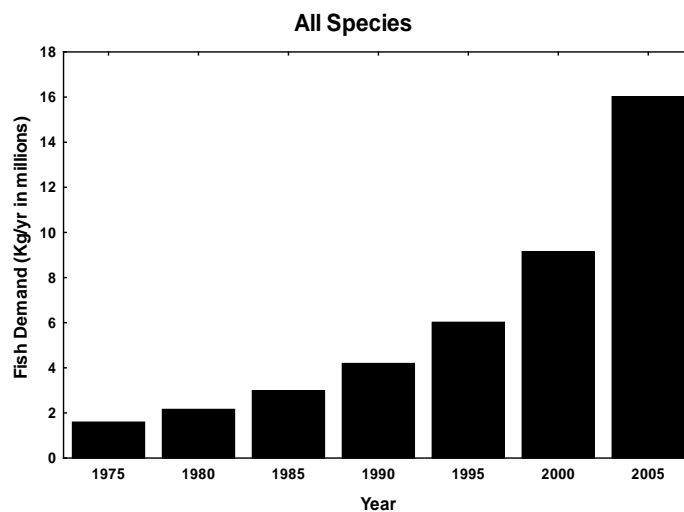

Figure 1. Long-term trend in fish demand for all fish-eating bird populations combined (1975-2005). Projected demand has grown exponentially with an average doubling time of 9 years.

Pelicans, Bald Eagles, and Osprey. Fish demand is governed by both the size of the population and the length of residency in the Bay. Brown Pelicans and Double-crested Cormorants did not occur in the Chesapeake Bay and have become significant fish consumers in a relatively short period of time.

Seasonal Pattern – Estimated seasonal fish consumption reached a peak in July around the time when young are fledging (Figure 2). This is the time when the overall consumer biomass reaches a high before steadily declining due to mortality. The rapid periods of transition in spring and fall reflect the migration periods in and out of the Bay for species that are not resident. Because species vary in phenology and in the details of breeding, seasonal patterns are species-specific. Because populations have grown at different rates over the years and the composition of the community has changed, there has been a slight shift in the pattern of seasonal consumption.
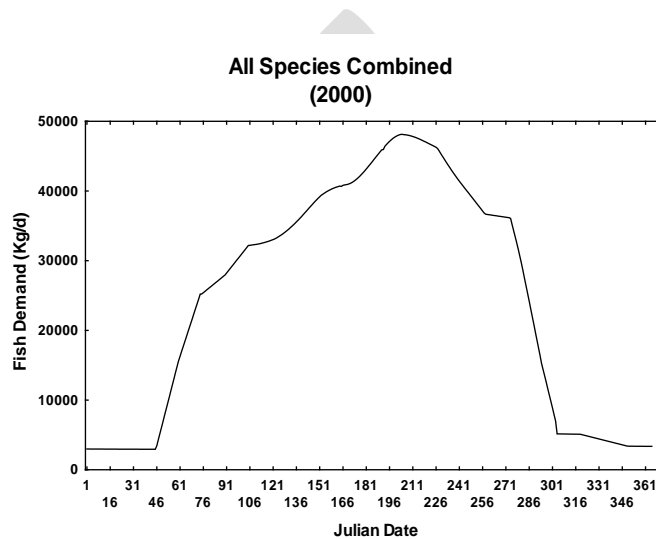
Figure 2. Projected seasonal fish demand for all fish-eating birds in the Chesapeake Bay combined. Demand peaks in July around the time when most young have fledged.

Prey availability studies: Comparison of estimated consumption of menhaden by avian and fish piscivores

For most piscivorous birds (e.g. Brown Pelicans, Osprey, Double-crested Cormorants, Great Blue Herons), comprehensive data on the taxonomic composition and size distribution of fish prey are lacking as inputs for consumption (energetic) models. We stratified estimates of avian consumption according to fish species by conducting avian diet studies. In 2008 we targeted Osprey and Double-crested Cormorants; specifically, the nesting colonies located in tidal freshwater portions of the James River, VA where synoptic avian diet studies and fish community sampling demonstrated that Osprey and

Double-crested Cormorants foraging in tidal freshwater nursery habitats were not targeting YOY menhaden in spite of YOY menhaden being extremely abundant and available.

In 2009, diet studies were expanded to further stratify avian demand and consumption for Double-crested Cormorants and Brown Pelicans in selected locations of the mainstem Chesapeake Bay: 1) Cormorants and Pelicans breeding on Shanks Island located along the southern end of Smith Island, Accomack County, Virginia; and 2) Cormorants breeding on Poplar Island, Talbot County, Maryland. In contrast to results from 2008 that found Double-crested Cormorants and Osprey were not preying on abundant and available YOY menhaden in tidal freshwater, 2009 results of the diet of cormorants and pelican indicate that sub-adult (age 1 and 2) Atlantic menhaden and bay anchovy were the most numerous fish prey consumed during the six week study period. When count data are converted to biomass estimates, Atlantic menhaden dominate species consumed, followed by spot, croaker, and bay anchovy (Fig. 3)
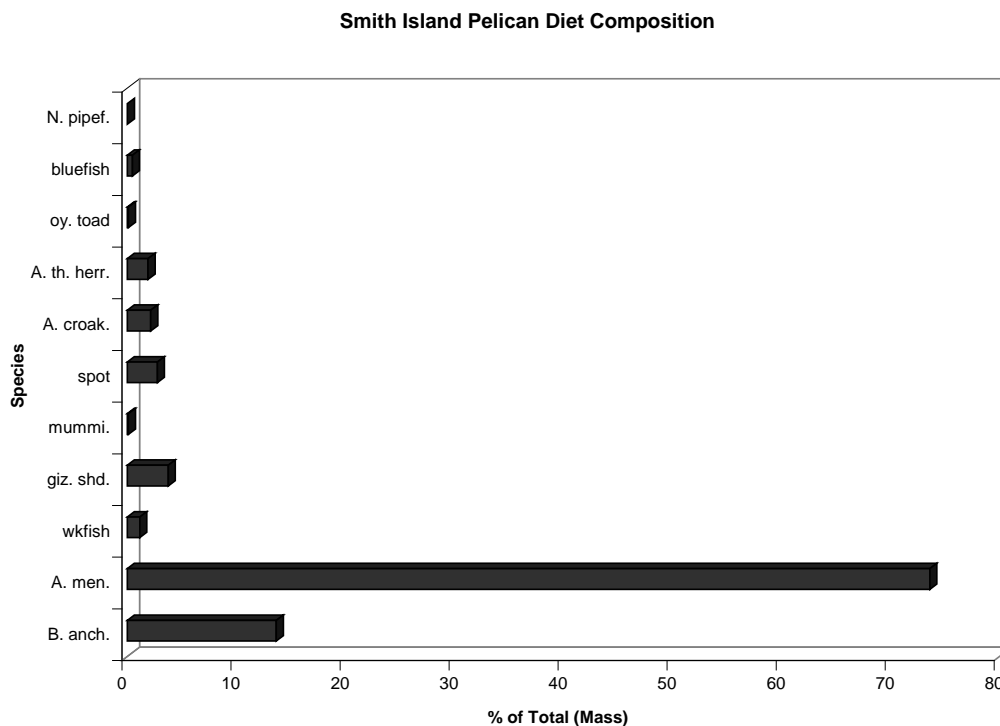
Smith Island Pelican Diet Composition

Figure . 3 Diet composition by percent of total biomass for Brown Pelicans nesting on Smith Island, Maryland.
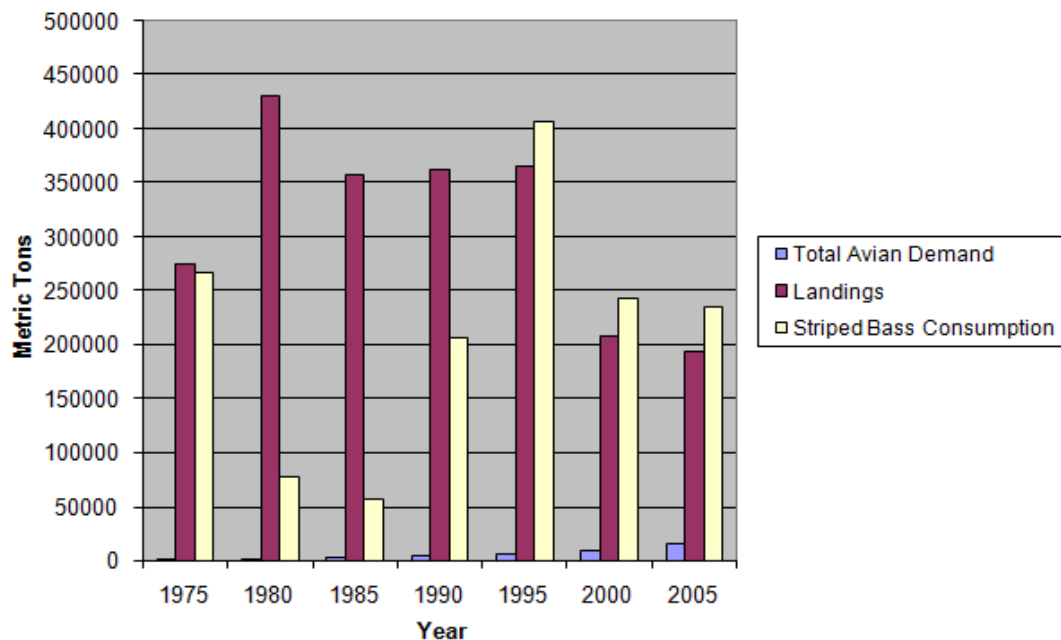
Fig 4. Comparison of estimated total metabolic demand of avian piscivores in the Chesapeake Bay, estimated striped bass consumption of Atlantic menhaden, and Fishery landings of Atlantic menhaden in the Chesapeake Bay.

However, analysis of fishery landings and estimated striped bass consumption indicate that menhaden consumption by striped bass and exploitation by commercial fisheries combined is several orders of magnitude greater than the estimated total metabolic demand of the five largest avian piscivores. The estimated striped bass consumption is based on aggregate biomass models that incorporate both catch and predation functions (biomass dynamic with a type 3 predator-prey function for striped bass and menhaden). So while the relative impact due to continued exponential growth of avian populations in the Bay is likely to increase the relative importance of avian predation on menhaden in the future, conversely, falling menhaden stocks may negatively impact population growth in some avian species. Glass and Watts (2008, Appendix 1) linked dramatically declining condition and reproductive success by populations of Osprey breeding in high salinity regions in the Chesapeake Bay to declines in menhaden stocks over the last several decades (Glass and Watts 2009, Appendix 1). The proportion of menhaden in the diet of populations of Osprey occupying lower estuarine locations (>

18 ppt) locations has declined from 75% in the 1980's to 25% in 2006. Over the same period Osprey population growth, reproductive output, and nestling growth rates in those sites has declined to levels close to those recorded during the period when Osprey reproduction was negatively impacted by organochlorine (e.g. DDT) contamination (Watts and Paxton 2007). In contrast, Bald Eagle and Osprey condition and reproductive output has surged in tidal freshwater habitats, likely due to abundant freshwater fish prey of high nutritional quality. Fish prey consumed by Osprey in the high salinity, lower estuary (> 18 ppt.) were smaller in size and 40% lower in energy content than fish prey consumed by Osprey occupying the upper tidal fresh estuary (see Glass and Watts 2009) .

In addition to populations of breeding birds, on which these analyses were based, the Chesapeake Bay supports much larger numbers of migrant and wintering Double-crested Cormorants and other avian predators. Significant numbers of Double-crested Cormorants winter along tidal tributaries of the Chesapeake Bay in Virginia and Maryland (Wires *et al.*2001) in numbers much larger than breeding populations (B. Watts, pers. comm.). Winter roosts in North Carolina can reach 10,000 birds or more. Estimates for the number of Double-crested Cormorants migrating through Virginia range from 20,000 to 30,000 at Fisherman's Island, VA (Wires *et al.*2001). Estimating the predatory impact of much larger populations of overwintering (cp. breeding, this study) and migratory fish-eating birds was beyond the scope of the current study. However, the impact of winter and migratory waterbirds on fishery stocks is likely greater than the combined impact of nesting waterbirds in the Chesapeake Bay, but no comprehensive survey or analysis of metabolic demand of these predator groups has been undertaken to date (B. Watts, pers. comm.). It appears that Cormorants may concentrate in tidal tributaries during the winter. Upstream reaches of tidal tributaries currently support some of the highest population growth in breeding waterbirds so it would appear that densities of wintering cormorants are not significantly depressing the availability of prey resources in those reaches. The larger concern might be in estuarine and marine habitats (e.g. Eastern Shore) where Atlantic menhaden populations may be impacted.

Species specific biomarkers For Atlantic menhaden and other target fishes:

Piscivorous birds are used widely as a sentinel species for tracking ecosystem health elsewhere (Steidl *et al.*1991a and b, Elliot *et al.*2002, Henny *et al.*2003) and may be useful indicator species for fishery status and trends in the Chesapeake Bay. Grove *et al.* (2009) recently proposed Osprey as a worldwide sentinel species due to their position on the food web, their widespread distribution, and accessibility of nests. Tracking Atlantic menhaden contributions to the diets of a sentinel avian predator like Osprey may provide a unique, cost-effective, and independent tool for consistent, integrated, and long-term monitoring of Atlantic menhaden stocks in the region. To that end we undertook the development, testing, and application of new monitoring protocols based on stable isotope analysis of feathers from avian predators to track Atlantic menhaden population trends using isotopic markers for target fishes extracted from renewable Osprey tissues (e.g. blood, feathers, uropygeal oils).

Bulk isotopic methods, such as those used in the long-term analysis of Bald Eagle and Osprey diets above, have a limited ability to elucidate the specific species composition of consumer diets. One technique that does allow for the determination of specific prey items is fatty acid signature analysis (FASA; Iverson *et al.*1997, Kirsch *et al.*1998, Logan *et al.*2000). This novel approach allows researchers to trace a specific fatty acid from prey to consumer and provide specific information about diet composition. FASA has not yet been applied to the study of eagles or ospreys, but it has been successfully used in dietary studies of other predators (Smith *et al.*1996, Iverson *et al.*1997, Worthy and Abend, 1998). The initial period focused on two questions *re*: lipids in Atlantic menhaden. First, what is the isotopic variability of the lipids in a population of Atlantic menhaden and second, can a relationship be discerned between the chemistry and isotope signatures of the major fatty acids extracted from menhaden prey and those of higher trophic level consumers, including birds? The results of an analysis of menhaden and American shad preliminary compound-specific, isotopic characterization (CSIA, Figure 5), as well as that of other potential prey fish species and predators (see MacAvoy *et al.* 2009, Appendix 2), showed variability in the fatty acid isotope signals suggesting that fatty acid CSIA signatures
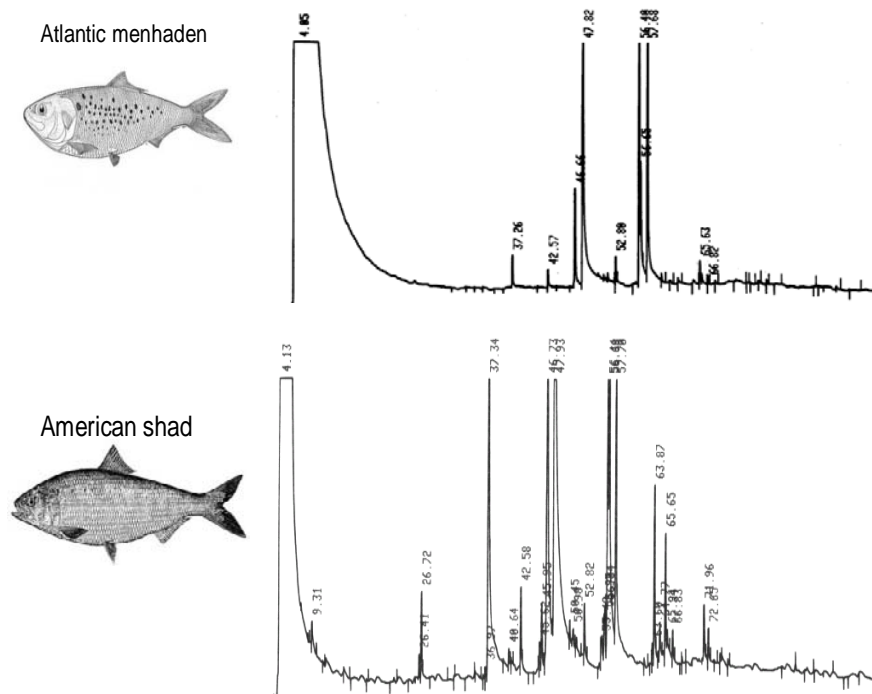
Figure 5 . Atlantic menhaden and American shad Fatty acid characterization (GC/MS) Compound-specific, stable isotope analysis (CSIA)

from Atlantic menhaden can potentially yield a discrete (i.e., diagnostic) chemical and isotope signature that could be identified in the lipid extracts of predatory birds (e.g. Osprey feathers).

During the second phase, in order to test if biomarkers accurately reflected the proportion of Atlantic menhaden and American shad in the diet of local Osprey populations, tissue samples from Osprey consuming a known quantity of target fish species were needed. To this end, Osprey nests in tidal freshwater James River were assigned to experimental (supplemented with menhaden or shad) and control (not supplemented) groups and following cessation of provisioning, we collected tissue samples from Osprey nestlings for isotopic analysis. Together with our earlier laboratory studies, analyses of bulk stable isotopic signatures of Osprey tissues from experimental *versus* control nests in locations where marine-derived isotopic values would be unique suggested that biomarkers in avian tissues reflected the relatively brief and known period (~ 4 weeks) while Osprey nestlings were provisioned with adult menhaden or American shad by researchers. In addition, rapid isotopic turnover for some avian tissues (e.g. blood), as well as short and well-documented foraging distances for nesting adult Osprey, insured relatively discrete geospatial resolution.

However, when FSAs were extracted from Osprey uropygial oils, the signature long chain fatty acids characteristic of Atlantic menhaden and American shad, and which were identified earlier in this project, were not evident. Instead, Osprey uropygial oils were made up of short chain fatty acids only, presumably following metabolism and synthesis of diet-derived lipids by avian predators. As a consequence, initial attempts to identify and use species-specific isotope biomarkers for selected fish prey (e.g. Atlantic menhaden), and based on non-invasive sampling of tissues from avian predators, as a fishery independent tool for fishery stock assessment was not successful. Additional analyses using the same approach but based on analysis of different avian tissues might yield more useful results.

1: Finfish-Waterbird trophic interactions in Tidal Freshwater Tributaries of the Chesapeake Bay

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Finfish-Waterbird Trophic Interactions in Tidal Freshwater Tributaries of the Chesapeake Bay

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Abstract.—With the DDT ban enacted in the early 1970s, piscivorous bird populations have grown exponentially throughout the tidal reach of the Chesapeake Bay. However, avian population growth is not uniform throughout the Chesapeake Bay watershed; several species including Bald Eagles (*Haliaeetus leucocephalus*) and Ospreys (*Pandion haliaetus*) experienced significantly greater population growth rates in riverine tidal freshwater and oligohaline regions than in higher salinity portions of the bay. Shifting fish prey resources may provide an explanation for the observed influence of salinity on distribution of piscivorous bird populations. Changes in the fish resources available to avian predators over the past 40 years include changing temporal and spatial distribution of fish prey, as well as shifts in taxonomic and trophic structure of resident and migratory fish assemblages. Historical ecological changes, including long- and short-term changes in the abundance of anadromous clupeid fishes, Atlantic Menhaden (*Brevoortia tyrannus*), and the relatively recent introduction and establishment of non-indigenous fishes, within tidal freshwater rivers may be influencing piscivorous bird distributions and abundance, particularly for Bald Eagles and Ospreys, in the Chesapeake Bay. Predator-prey interactions among piscivorous birds and fish prey have received little attention from wildlife managers. Collaborative efforts between fishery scientists and avian ecologists will ultimately lead to better ecosystem management of the Bay's living resources.

Key words.—Chesapeake Bay, fresh tidal river, Bald Eagle, Osprey, fish-bird interactions, American Shad, Atlantic Menhaden, catfishes.

After DDT was banned in the early 1970s, many piscivorous bird populations have grown exponentially throughout the tidal reach of the Chesapeake Bay (Watts and Byrd 1998; Watts *et al.* 2004; Watts and Byrd in press; Watts *et al.* in press). Several species experienced dramatic population declines prior to 1970 and have now recovered to near-historic levels. For example, after reaching a low of less than 60 breeding pairs in the early 1970s, the Bald Eagle (*Haliaeetus leucocephalus*) breeding population now likely exceeds 900 breeding pairs (Watts *et al.* in press). An estimated additional 1,500 to 2,000 eagles migrate north to spend the summer months within the Bay from breeding populations throughout the southeast, and during the late fall and early winter the Chesapeake Bay supports migrant Bald Eagles from the northeastern United States and Canada (Watts *et al.* 2007). Other species show similar population recoveries. In less than 30 years, Ospreys (*Pandion haliaetus*)

increased from 1,400 pairs to 3,500 pairs (Watts *et al.* 2004), Great Blue Herons (*Ardea herodias*) increased from approximately 1,000 to more than 18,000 pairs, and Great Egrets (*Ardea alba*) increased from 1,400 to 3,600 pairs (Watts and Byrd 1998; Watts 2004; Watts and Byrd in press; D. Brinker, Maryland Department of Natural Resources, unpubl. data). However, avian population growth is not uniform throughout the Chesapeake Bay watershed; several species including Bald Eagles and Ospreys experienced significantly greater population growth rates in riverine tidal freshwater and oligohaline regions than in higher salinity portions of the bay (Watts *et al.* 2004; Watts *et al.* 2006).

Shifting fish prey resources may provide an explanation for the observed influence of salinity on distribution of piscivorous bird populations (Watts *et al.* 2004; Watts *et al.* 2006). As piscivorous bird populations rebounded in the Chesapeake Bay, *ca.* 1970-2006, coastal and riparian habitats were be-

ing transformed by activities such as shore-line development, over-harvesting of estuarine and riverine fisheries, and industrial and agricultural pollution. In addition, the relatively recent introduction and establishment of several non-native fishes within Chesapeake Bay tributaries may have significantly altered prey resources for avian predators (Edmonds 2003). The resultant changes in the fish resources available to avian predators over the past 40 years include changing temporal and spatial distribution of fish prey (Viverette 2004), as well as shifts in taxonomic and trophic structure of resident and migratory fish assemblages (CBV, unpubl. data). In this paper we will discuss how historical ecological changes, including long- and short-term changes in the abundance of anadromous clupeid fishes (Foerster and Reagan 1976), Atlantic Menhaden (Uphoff 2003a, b), and the relatively recent introduction and establishment of non-indigenous fishes within tidal freshwater rivers (McAvoy *et al.* 2000; Edmonds 2003) may be influencing piscivorous bird distributions and abundance, particularly for the Bald Eagle and Osprey, in the Chesapeake Bay.

FISHERY RESOURCES

Tidal Freshwater Fish Assemblage

Located between non-tidal freshwater and estuarine ecosystems, tidal freshwater habitats support a unique and diverse assemblage of estuarine, marine, and freshwater fish species (Wagner and Austin 1999). The resulting fish community is not only taxonomically diverse compared to adjacent non-tidal and estuarine habitats (Fig. 1), but also more temporally dynamic than adjacent aquatic systems (Viverette 2004) because many of the fish species are transitory and only inhabit tidal freshwaters during specific seasons or life-stages (Setzler-Hamilton 1987; Peterson and Ross 1991; Garman and Macko 1998; Yozzo and Smith 1998). Among the seasonal inhabitants of tidal freshwaters are the anadromous (migratory) clupeids—marine planktivores that migrate into freshwaters every spring to spawn. Anadromous clupeids
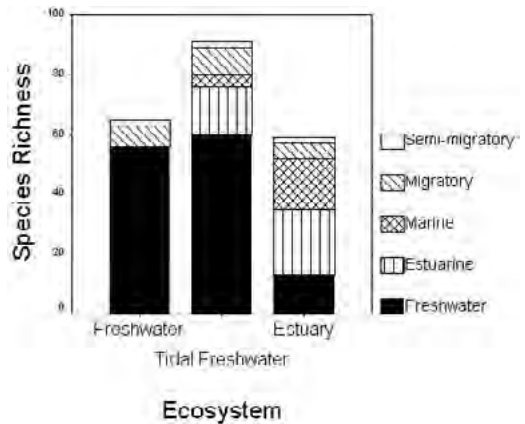
Figure 1. A comparison of fish species richness among non-tidal freshwater, tidal freshwater, and the saline estuary in the Virginia portion of the Chesapeake Bay. Species richness based on Garman and Smock 1999; Wagner 1999; Wagner and Austin 1999; Viverette 2004; and unpubl. data (from GCG).

native to the Chesapeake Bay include the American Shad (*Alosa sapidissima*), Hickory Shad (*A. mediocris*), Alewife (*A. pseudoharengus*), and Blueback Herring (*A. aestivalis*) (Jenkins and Burkhead 1994). The upstream migration of anadromous clupeids provides a substantial subsidy in the form of marine-derived carbon to the nutrient and energy budgets of coastal freshwater habitats each spring (Garman 1992; Garman and Macko 1998; MacAvoy *et al.* 2000). Reproductive fish are particularly nutritious prey due to lipid-rich eggs and sperm (Poole 1989) and represent a potentially important and predictable seasonal nutritional subsidy for piscivorous birds nesting within the Chesapeake Bay.

Shift from Spatially Widespread to Spatially Concentrated Fish Resources

Historically, migratory shads and herrings were abundant and geographically widespread in the Chesapeake Bay and its tributaries. Early European colonists describing the annual spawning runs along Chesapeake Bay tributaries consistently noted the immense quantity of herring and shad moving upstream each spring (Loesch and Atran 1994). This abundant fishery soon became an economically important industry (Foerster and Reagan 1977), with catches increas-

ing dramatically during the 1800s as fishing techniques improved. The fishery peaked in the early 1900s with catches of American Shad in the Chesapeake Bay reaching eight million pounds annually. Archeological evidence indicates American Shad migrated upstream in Virginia tributaries as far as West Virginia (Garman and Nielsen 1992), and records from Thomas Jefferson's estate at Monticello, indicate a herring fishery as far upstream as Charlottesville, Virginia (J. Kauffman, Virginia Department of Game and Inland Fisheries, pers. comm.).

By the early 20th century however, overfishing, combined with dams blocking migration began to impact populations of anadromous fish along the Atlantic coast (Loesch and Atran 1994). Anadromous fish stocks declined steadily throughout the 20th century and in the 1970s, just as Bald Eagle and Osprey populations were beginning to recover, populations of anadromous fish in the Chesapeake Bay basin declined precipitously, experiencing up to a 90% reduction in abundance (Fig. 2; Garman and Nielsen 1992). The causes for the most recent declines include commercial overfishing, barriers to upstream migration, habitat loss, and the introduction of non-native fishes (Foerster and Reagan 1977; Garman and Macko 1998). The construction of dams in particular has restricted the range of anadromous fish by limiting access to inland spawning and nursery grounds (Loesch and Atran 1994). Until recently (e.g., construction of fishway at Boshers Dam, James River ca. 1999, Weaver

Figure 2. Commercial American Shad catch for the Chesapeake Bay from 1880-1972 (from Foerster and Reagan 1976).

et al. 2003), dams at the upstream limit of tidal influence have largely confined anadromous clupeid spawning activity to the tidal freshwater regions of large Chesapeake Bay tributaries (Jenkins and Burkhead 1994; McIninch and Garman 1999).

Shift from Temporally Concentrated to Temporally Widespread (Resident Year-Round) Fish Resources

As migratory clupeids declined, there was a concomitant shift in the tidal freshwater fish community from migratory to non-migratory species, i.e., from a seasonally abundant resource to one that is available year-round. On an annual basis, non-migratory (i.e., resident) *Dorosoma* species, both Gizzard Shad (*Dorosoma cepedianum*) and the non-native Threadfin Shad (*D. petenense*), dominate clupeid assemblages in tidal freshwater habitats within the Chesapeake Bay. In a study of the relative abundance of clupeids in the James and Rappahannock rivers (Garman and Mitchell 1989), Gizzard Shad were the numerically dominant species. By the late 1990s migratory clupeids made up less than 1% of individuals and relative biomass of shads and herrings sampled annually in the tidal freshwater James River (Fig. 3; CBV, unpubl. data). Threadfin shad, introduced into the Chesapeake Bay system in the 1950s, and rare in the James through the 1960s (Jensen 1974), are now well established in western tributaries of the Chesapeake Bay (Jenkins and Burkhead 1994). However, recent data from Virginia and Maryland indicate that Gizzard Shad abundance may be declining from a peak in the late 1990s (J. Uphoff, Maryland Department of Natural Resources, unpubl. data; Maryland Department of Natural Resources 2007). The decline may be attributable to increasing populations of novel apex predators introduced into tidal freshwaters in the last 40 years (R. Greenlee, Virginia Department of Game and Inland Fisheries, pers. comm).

Shift from Migratory Planktivores to Apex Predators

In addition to a shift from migratory to resident species, tidal freshwater fish com-
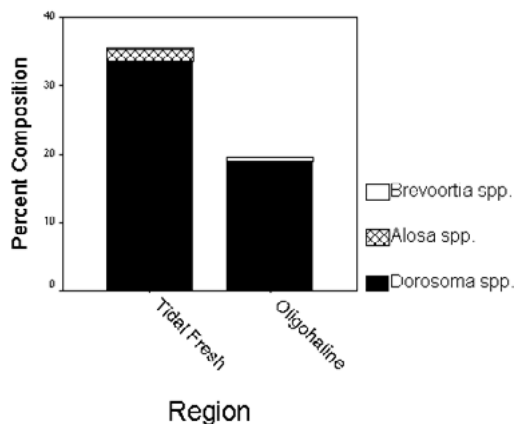
Figure 3. Contribution of anadromous shads (*Alosa* spp.) and resident shads (*Dorosoma* spp.) to fish assemblages of the James River, Virginia (R. Greenlee, Virginia Department of Game and Inland Fisheries unpubl. data; W. Bolin, Dominion Power, unpubl. data).

Figure 4. Frequency of occurrence of native and introduced catfish fish species in the James River, Virginia (Jensen 1974; R. Greenlee, Virginia Department of Game and Inland Species, unpubl. data; W. Bolin, Dominion Power, unpubl. data).

munities experienced a shift in trophic structure. Tidal freshwater fish assemblages along the Atlantic slope have few native piscivorous species, explaining perhaps the evolution of anadromous life-history strategies among migratory clupeids (McAvoy *et al.* 2000). However, concurrent (ca. 1975) with the severe declines in anadromous clupeid populations, the nonindigenous Blue Catfish (*Ictalurus furcatus)* and Flathead Catfish (*Pylodictus olivarus*) were introduced to the Atlantic slope of Virginia. Both catfish species are large and long-lived (up to 50 kg and 30 years) predators; adults prey extensively on fish and are able to ingest most native fishes found in tidal freshwater reaches (Chandler 1998; Graham 1999). Blue Catfish introductions occurred in the James, Rappahannock, Mattaponi, and Potomac drainages between 1974 and 1989, and Flathead Catfish introductions took place in the tidal James and Potomac River drainages (Occoquan Reservoir) between 1965 and the mid-1970s (Jenkins and Burkhead 1994; Edmonds 2003). Both Blue and Flathead catfishes are now well established in Virginia's coastal rivers, particularly tidal freshwater reaches (Fig. 4; Jenkins and Burkhead 1994; Edmonds 2003). More recently, Blue Catfish populations have been expanding in the fresh tidal portion of the Potomac River (SPM, pers.

obs.). Flathead Catfish have recently been documented in the Potomac River (summer 2005, D. Hopler, Virginia Commonwealth University, pers. comm.) and the upper Bay in Maryland (J. Uphoff, Maryland Department Natural Resources, pers. comm.).

The Channel Catfish (*Ictalurus punctatus*) populations also increased substantially in the Chesapeake Bay since the 1970s. Like Blue Catfish and Flathead Catfish, Channel Catfish are not native to the Chesapeake Bay, but were introduced to the mid-Atlantic over 100 years ago (Sauls *et al.* 1998). Channel Catfish were the most common catfish in the James River of Virginia in the late 1990s (Fig. 4) and in 1996, comprised 93% of the commercially harvested catfish in Maryland's portion of the Chesapeake Bay (J. Uphoff, Maryland Department of Natural Resources, unpubl. data). However, since that time, Channel Catfish populations in some Chesapeake Bay tributaries may be declining as Blue Catfish and Flathead Catfish populations continue to expand (Jim Uphoff, Maryland Department of Natural Resources, unpubl. data; Maryland Department of Natural Resources 2007). Over the same 40-year period that the three non-indigenous catfish populations were expanding in the Bay, the smaller, native catfish species including the Brown Bullhead (*Ameiurus nebulosus*) and

White Catfish (*A. catus*), became rare in the mainstem of many of the Chesapeake Bay's tidal tributaries (Fig. 4).

Another introduced piscivore, the Largemouth Bass (*Micropterus salmoides*), also became more common in the last 40 years, along with the native Striped Bass (*Morone saxatilis*), an anadromous piscivore that spawns in tidal rivers (Fig. 5). Striped Bass experienced a population decline in the 1960s and 1970s but by the late 1990s the population was recovered fully (Uphoff 2003a).

Although the effect of introducing apex predators such as the Blue Catfish and Flathead Catfish to these relatively predator-poor coastal rivers is not well documented, introductions of apex predators elsewhere have been linked to declines in native fish populations (Moyle and Light 1996). Flathead Catfish and Blue Catfish may prey heavily on anadromous clupeids during the spring spawning run (Chandler 1998; Garman and Macko 1998) and the impact of these novel predators on on-going recovery efforts for American Shad and other migratory species, as well as their impact on native and naturalized catfish species, is not well understood.

Shift from Smaller to Larger Size Classes of Fish Resources

The increase in abundance and diversity of top predators within tidal freshwater fish communities resulted in a shift in size distribution of available fish prey toward larger size classes. A comparison of total lengths of the ten most abundant fish species in collections in the tidal freshwater James River in 1969 (Fig. 5; Jensen 1974) and 1999 (Fig. 6; Greenlee, VDGIF, unpubl. data; W. Bolin, Dominion Power, unpubl. data) suggests a substantial increase in available prey size during that 20-year period. Increased availability of larger prey may improve foraging efficiency by avian predators.

ECOLOGICAL IMPACTS OF SHIFTING FISHERY RESOURCES ON AVIAN PREDATORS

Bald Eagles

Access to relatively predictable, annual concentrations of prey, as represented by spawning migrations of anadromous fish, may have profound effects on the distribution and abundance of predators such as Bald Eagles (Willson and Halupka 1995; Restani *et al.* 2000). The annual spring spawning run of anadromous clupeids within the Chesapeake Bay coincides with the nesting season of Bald Eagles, which begin nesting in January and are feeding young during the peak of the runs in April and May (Markham 2004; Watts *et al.* 2006; ACM and

Figure 5. Total length of ten most frequently occurring fish species in the tidal freshwater reach of the James River, Virginia *ca.* 1968-1971 (Jensen 1974). Species ranked by frequency of occurrence (left to right).
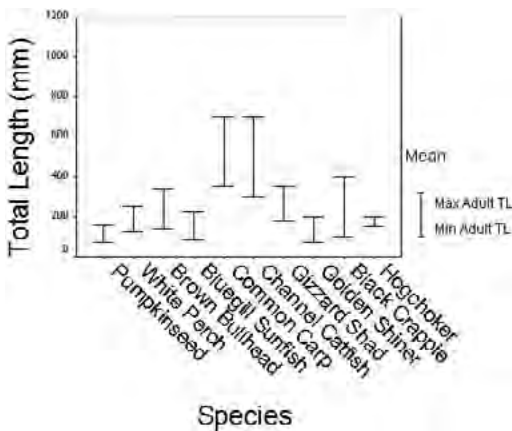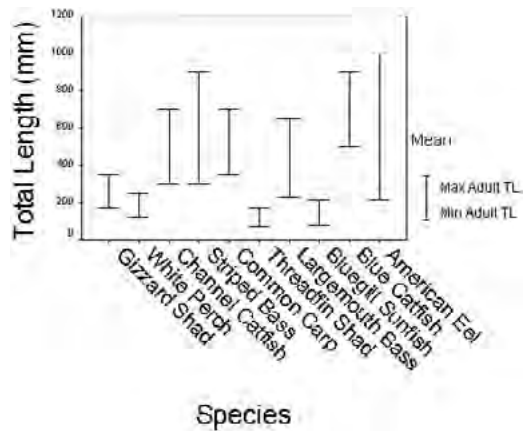

Figure 6. Total length of ten most frequently occurring fish species in the tidal freshwater reach of the James River, Virginia *ca.* 1998-1999 (Greenlee, Virginia Department of Game and Inland Fisheries, unpubl. data; W. Bolin, Dominion Power, unpubl. data). Species ranked by frequency of occurrence (left to right).

BDW, unpubl. data). In addition, Bald Eagles feed on carrion as well as live fish (Skagen *et al.* 1991). Carrion, in the form of post-reproductive carcasses of *Alosa* species, may be relatively plentiful during spring months because a significant percentage (50% to 70% in the mid-Atlantic region) of the adult clupeids dies after spawning (Leggett and Carscadden 1978; Browder 1995).

Anadromous fishes are important components of Bald Eagle diets in other regions of North America, where they congregate near spawning streams in both breeding and non-breeding seasons (Willson and Halupka 1995; Bennetts and McClelland 1997; Restani 2000; Restani *et al.* 2000). In Alaska, anadromous salmonids are an important prey item, and up to 90% of the salmon consumed by Bald Eagles is carrion (Imler and Kalmbach 1955). Studies in Manitoba and Saskatchewan documented a direct, positive relationship between Bald Eagle nest success and proximity to salmon spawning streams (Gerrard *et al.* 1975). In Maine, managers determined that recovery goal success for the Bald Eagle population was linked to restoration of anadromous clupeid populations, specifically Alewife (B. Owens, University of Maine, pers. comm.).

The importance of anadromous clupeids to the diets of Bald Eagles nesting along the mid-Atlantic coast has not been well documented. Only two published studies (Table 1) document Bald Eagle feeding activities in the Chesapeake Bay region during the breeding season. Tyrell (1936) did not report anadromous herrings or shads (*Alosa* spp.) in prey remains, a substantial proportion (55%) of which were unidentified. However, analyses of prey remains are biased in favor of prey with indigestible parts that decompose slowly (Todd *et al.* 1982; Simmons *et al.* 1991). Assessing the percentage of anadromous clupeids in bird diets using traditional methods is difficult because clupeids are relatively soft-bodied and leave scant skeletal remains that are unlikely to persist in the environment. To avoid these potential errors, Markham (2004) used nest cameras to identify fish prey delivered to Bald Eagle nests during the breeding season. Monitored nests were located along the Rappahannock, York, and James Rivers in Virginia. Fish accounted for 90% of prey items delivered and clupeids represented 45% of the identified fish (N = 625). Of the clupeid remains photographed and handled in nests, only anadromous species were observed (BDW, pers. obs.).

Gizzard Shad and Threadfin Shad are year-round (i.e., nonmigratory) residents of tidal freshwater rivers (Jenkins and Burkhead 1994), are increasing in abundance in many Chesapeake Bay habitats, and may, therefore, represent important prey resources for both resident and migrant Bald Eagles. Non-migratory shad are consumed by Bald Eagles in other regions (Southern 1973; Fischer 1982; Thompson *et al.* 2005) during breeding and non-breeding seasons. Gizzard Shad were a numerically important component (13% of prey) of breeding and migrant Bald Eagles diets in the tidal freshwater Hudson River during the mid-summer months (Thompson

Table 1. Bald Eagle (*Haliaeetus leucocephalus*) feeding studies from the Chesapeake Bay region (considering fish only). Note that Markham (2004) and Mersmann (1989) were observational studies and Haines (1998) and Tyrell (1936) were from prey remains.

Fish species	Markham (2004) (%, N = 695) Breeding	Mersmann (1989) (%, N = 253) Non-breeding	Haines (1988) (%, N = 45) Non-breeding	Tyrell (1936) (%, N = 44) Breeding
Shads and Herrings	40.86[a]	15.01[b]	0.00	0.00
Catfish	33.67	3.56	95.45[c]	44.44
Other (unknowns)	25.47	81.42 (68.38)	4.55	55.55

[a]Migratory and resident.
[b]Gizzard shad.
[c]Native brown bullhead (75%).

et al. 2005). In a study of wintering Bald Eagles in Illinois, Gizzard Shad was the primary prey item (Southern 1973; Fischer 1982) and Mersmann (1989) reported that Bald Eagles on northern Chesapeake Bay foraged heavily on winter-killed Gizzard Shad. In addition, introduced Threadfin Shad experience high mortality at water temperatures below 7°C (Jenkins and Burkhead 1994) and may provide an important food resource for resident and migrant Bald Eagles occupying the Chesapeake Bay during severe winters.

Catfish species (Ictaluridae) comprise a substantial proportion of Bald Eagle diets in North America, particularly inland populations (Haywood and Ohmart 1986; Grubb 1995; Mabie *et al.* 1995). Catfish prey remains persist in the environment due to the large pectoral girdle and spines, and may result in overestimation of catfish dietary importance. However, in a study of prey preference conducted on the upper Chesapeake Bay, Bald Eagles chose catfish species over other fish species (e.g., Gizzard Shad), and other prey types (e.g., mammals and waterfowl, DeLong 1990). Catfish were a numerically dominant item in the diets of breeding and migrant eagles on the tidal freshwater Hudson River between April and September (Thompson *et al.* 2005). Bullhead catfish comprised 35% of prey remains in Bald Eagle nests in Minnesota (Dunstan and Harper 1975) and 25% of prey identified in a diet study of both wintering and nesting Bald Eagles at inland sites in Maine (Todd *et al.* 1982).

In Markham's (2004) diet study of Chesapeake Bay Bald Eagles, catfish species comprised 34% of fish delivered to the nest and 31% of all nest deliveries. The catfish in Markham's study were not identified to species; however, based on anecdotal evidence (BDW, pers. obs.), non-native Blue Catfish, Channel Catfish, and in the James River, Flathead Catfish, likely provided the bulk of the catfish consumed. Native catfishes were a food resource for Chesapeake Bay Bald Eagles prior to the widespread introduction of non-indigenous Blue and Flathead Catfishes, *ca.* 1975. Tyrell's (1936) breeding season study of nest remains included catfish in nearly 45% of collections. Similarly, in a study of

prey remains at a summer roosting site on the Potomac River, approximately 95% of prey remains consisted of catfish species, primarily native Brown Bullhead (Haines 1988). Catfish remains were observed during 232 (37%) of 630 visits to nests distributed throughout the Chesapeake Bay during the breeding seasons between 1978 and 1986 (K. Cline, Virginia Dept. of Game & Inland Fisheries, unpubl. data). Of 106 nest visits where catfish species were identified, White Catfish were present on 55 (52%), Channel Catfish were present on 52 (49%), bullhead species were present on 18 (17%), and Blue Catfish were present on only one (<1%).

Osprey

Since the early 1970s, the Osprey population in the Chesapeake Bay has more than doubled. Since recovery from pesticide-related declines, Osprey populations were initially concentrated in the Chesapeake Bay mainstem and the mouths of the major tributaries (Watts *et al.* 2004). Ospreys occurred rarely in tidal fresh and brackish portions the Chesapeake Bay tributaries in the 1970s, and were extirpated from some areas such as the tidal freshwater James River (Kennedy 1972; Watts and Paxton 2007). However, by the mid-1980s, Osprey populations in higher salinity regions had reached pre-pesticide levels, appeared to be approaching carrying capacity (Watts *et al.* 2004), and localized populations were beginning to exhibit signs of food stress such as brood reduction and sibling aggression (McLean and Byrd 1991a). In contrast, since the 1980s, Osprey populations within tidal freshwaters have experienced the highest colonization and growth rates in the Chesapeake Bay, and exhibit no signs of approaching carrying capacity (Watts *et al.* 2004).

The only published diet study of Ospreys within the Chesapeake Bay, conducted in the higher salinity reaches of the lower bay during the mid-1980s, showed that Atlantic Menhaden (*Brevoortia tyrannus*) comprised 75% of nest deliveries to Osprey nests (McClean and Byrd 1991b). Atlantic Menhaden are a major component of the diet of coastal

Osprey populations in New England (Poole 1989), coastal New Jersey (Steidl *et al.* 1991a) and the Delaware Bay (Steidl *et al.* 1991b). Unlike the anadromous clupeids, Atlantic Menhaden, a marine clupeid, spawn over the continental shelf. Larval Atlantic Menhaden move into the Chesapeake Bay as far upstream as tidal freshwater, but the larger, forage-size juveniles are most common in the middle to lower tributaries and mainstem areas of the Chesapeake Bay, where they remain throughout the spring, summer and fall (Murdy *et al.* 1997). Atlantic Menhaden are important forage for a variety of fish predators in the Chesapeake Bay such as Striped Bass, Weakfish (*Cynosion regalis*), and Bluefish (*Pomatomus saltatrix*), as well as supporting one of the most important commercial fisheries in the United States (Murdy *et al.* 1997; Uphoff 2003a).

During the early to mid-1980s Atlantic Menhaden stocks began to decline in the Chesapeake Bay (Fig. 7; Uphoff 2003b), coinciding with the first evidence of brood reduction and sibling rivalry recorded in lower Chesapeake Bay Osprey populations (McClean and Byrd 1991). Similar evidence of food stress was not apparent a decade earlier

(Stinson 1977) when Atlantic Menhaden stocks were comparatively larger (Uphoff 2003b). By the early 1990s symptoms related to food stress were also being reported for fish piscivores, including Striped Bass and Weakfish, that are dependent on Atlantic Menhaden (Uphoff 2003b, 2006). Declining abundance of Atlantic Menhaden in higher salinity regions (e.g., Bay mainstem) may be negatively affecting Osprey population stability in high salinity areas of the Chesapeake Bay at the same time that comparatively abundant fish prey resources in oligohaline and tidal freshwater river habitats may be supporting expansion and local population growth in Ospreys.

The Osprey's arrival on Atlantic slope breeding grounds coincides with the beginning of the spring anadromous clupeid spawning run and later, during the height of the spawning season, Ospreys are laying and incubating eggs (Poole 1989; M. Byrd, College of William and Mary, unpubl. data). Anadromous clupeids are an important dietary component for Osprey nesting along the Atlantic coast, particularly riverine populations (Jamieson *et al.* 1982). Along the southern coast of New England, newly arrived adult Ospreys fed on anadromous her-
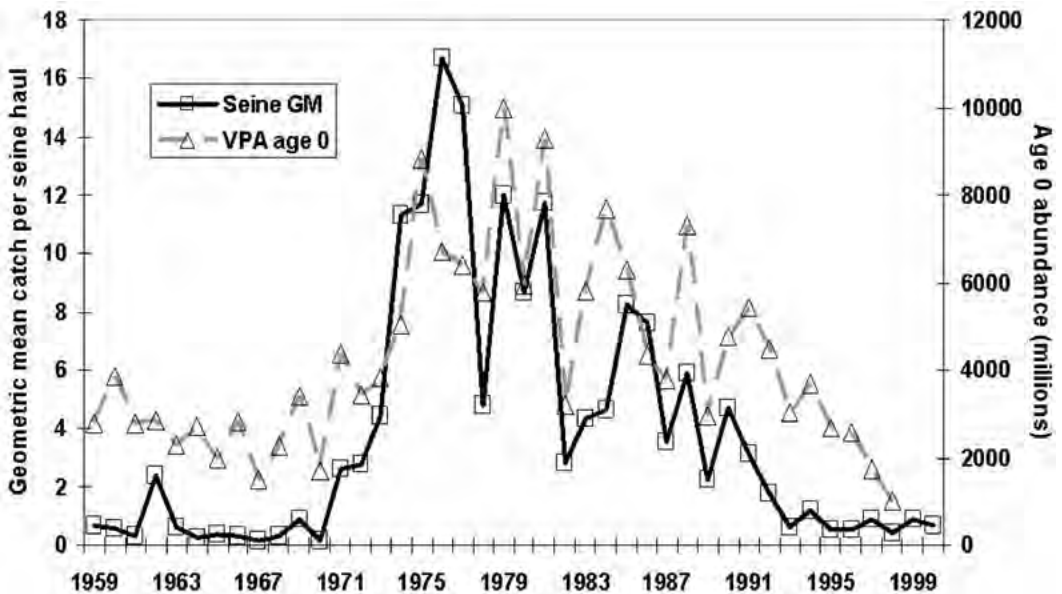

Figure 7. Geometric mean catches per standard seine haul for Atlantic Menhaden collected from Maryland's portion of Chesapeake Bay, and VPA (virtual population analysis) estimates of age zero Atlantic Menhaden abundance, 1959-2000 (from Uphoff 2003).

ring (*Alosa* spp.) almost exclusively, but switched to other locally abundant species after herring availability declined (Poole 1985, 1989). Ospreys from mid-Atlantic and New England regions may continue feeding nestlings into late July and August (Poole 1989), well after the anadromous clupeids have left spawning grounds, requiring a switch to alternative prey. For instance, in Nova Scotia, inland Osprey populations nesting on rivers and lakes fed heavily on Alewife and Blueback Herring early in the breeding season when spawning fish were abundant, but switched to foraging for alternative prey in the estuary, an average distance of 23 km from nest sites, later in the breeding cycle (Jamieson *et al.* 1982).

Unlike Ospreys in Nova Scotia that travel to the lower estuary to feed when the spawning migration ends (Jamieson *et al.* 1982), Ospreys inhabiting tidal freshwaters in the Chesapeake Bay may exploit a variety of locally abundant fish prey during the latter portion of the breeding season. For instance, catfish prey contributed to Osprey diets in Delaware Bay (Steidl *et al.* 1991b) and made up a small but significant proportion of prey deliveries in the lower tributaries and mainstem regions of the Chesapeake Bay (McClean and Byrd 1991). Catfish made up the bulk of prey taken by Ospreys nesting in Idaho, but consumption varied significantly with the availability of spawning salmonids, the second most numerous prey item observed (Van Daele and Van Daele 1982). In addition to anadromous clupeids and catfish, inland populations of Ospreys in other regions of North America feed on Gizzard Shad and Threadfin Shad (Swenson 1979; Edwards 1988), centrarchids (Dunstan 1974; Swenson 1979; Edwards 1988), and a variety of benthic species (Swenson 1979; Van Daele and Van Daele 1982; Grover 1984), all of which are abundant in tidal freshwaters of the Chesapeake Bay.

Other Waterbird Species

Great Blue Heron distribution within the Chesapeake Bay is also heavily skewed toward oligohaline and tidal freshwater habitats (BDW, unpubl. data). The first breeding

record for Double-crested Cormorants (*Phalacrocorax auritus*) occurred in 1978 within the tidal freshwater James River (Blem *et al.* 1980). By 1995, the cormorant breeding population in the tidal freshwater James River grew to over 200 pairs (Watts and Bradshaw 1996). Heron and cormorant feeding studies are lacking for the Chesapeake Bay, and most such studies conducted elsewhere are in response to perceived depredation of commercial fisheries or aquaculture facilities. These studies indicate that both waterbird species feed on a variety of fish species in tidal freshwaters (Hoy 1994; Trapp 1998; Simmonds *et al.* 2000; Glahn *et al.* 2002; Steinmetz 2003; Fenech *et al.* 2004) including migratory and non-migratory shads and herrings, yellow perch, catfishes, and centrarchid species.

MANAGEMENT IMPLICATIONS

The predator-prey interactions among piscivorous birds and fish prey has received little attention from wildlife managers (Steinmetz *et al.* 2003). The potential role of fish population dynamics and commercial harvest in affecting avian distribution, including those that are of national conservation concern such as the Bald Eagle, is largely undescribed for the Chesapeake Bay. In fact, most Chesapeake Bay ecosystem and management models (e.g., Baird and Ulanowicz 1989) ignore avian predators and competitors, and fishery stock assessments for the region generally fail to incorporate these potentially important ecological interactions (Chesapeake Fisheries Ecosystem Plan, Technical Advisory Panel 2004). Fisheries management decisions may, however, directly impact piscivorous bird populations in the Chesapeake Bay. For example, considerable resources have been invested in American Shad recovery efforts in the Chesapeake Bay watershed (Weaver *et al.* 2003) and successful restoration of anadromous fishes into historical habitats could have an impact on distribution of avian predators. The effect on American Shad recovery efforts of recently introduced piscivorous fishes, which feed on anadromous clupeids (McAvoy *et al.* 2000), is unclear. Thus, catfish manage-

ment could influence both prey (shads) and predator (piscivorous birds) distribution in the region. Additionally, Maryland has conducted a Chesapeake Bay-specific stock assessment of Atlantic Menhaden (Uphoff 2003a) and the NOAA Chesapeake Bay Program is currently supporting a similar assessment (J. Uphoff, pers. comm.), the results of which could influence future management decisions within the Bay.

Further conservation implications may result from documented shifts in historic trophic relationships among the fish and piscivorous bird communities within tidal freshwaters. Bald Eagles, Osprey, and other piscivorous birds are feeding at a higher trophic level in Chesapeake Bay tributaries where large, long-lived, and nonidigenous fish predators are now established. Such shifts may lead to greater risks from bioaccumulation of toxic compounds. Garman *et al.* (1998) documented critical levels of PCB's in James River Blue Catfish populations in an area also inhabited by the east coast's largest population of both breeding and non-breeding Bald Eagles (Watts and Whalen 1997). High methyl mercury levels have led to fish consumption advisories within tidal freshwater tributaries of the York and Piankatank Rivers in Virginia, as well as impoundments along the James and Chickahominy (Virginia Department of Environmental Quality 2007). Because the Chesapeake Bay Bald Eagle populations represents a nexus of three distinct breeding populations (Buehler *et al.* 1991; Watts *et al.* 2007), the conservation implications may, in fact, reach well beyond the borders of the Chesapeake Bay basin.

Finally, in order to understand and address research and conservation issues surrounding fish-bird interactions in the Chesapeake Bay, better communication and collaboration among fisheries and avian researchers should be encouraged. Development of, and access to, accurate and relevant data regarding the status, distribution, and abundance trends for fish communities in estuarine and tidal freshwater habitats of the Chesapeake Bay is integral to understanding patterns of distribution and abundance of waterbird populations. However, the challenges in obtaining, analyzing, and interpreting existing fisheries data are considerable, and include: 1) few published studies; 2) published studies that do exist are primarily driven by concerns about perceived depredation of fish stocks by avian predators (e.g., see Cowx 2003); 3) limited access to fisheries data; and 4) fish stock assessment techniques are unfamiliar to avian ecologists, include inherent biases, and may compromise accurate data interpretation. Collaborative efforts between fishery scientists and avian ecologists, along with the use of new technologies, including nest video cameras (Watts *et al.* 2004), stable isotope analyses (MacAvoy *et al.* 1998; Knoff *et al.* 2001), and hydroacoustics (Speckman 2005) may overcome these challenges, eliminate data gaps, and ultimately lead to better ecosystem management of the Bay's living resources.

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Chapter 2: Contribution of marine derived nutrients to Bald Eagles and Osprey nesting in the Chesapeake Bay: A retrospective analysis.

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INTRODUCTION:

Bald Eagle and Osprey Populations

Since banning of organochlorine pesticides in the early 1970s, Chesapeake Bay Bald Eagle and Osprey populations have recovered to historic levels. For example, after reaching a low of less than 50 breeding pairs in the early 1970s, the Bald Eagle breeding population has increased to more than 900 breeding pairs (Watts *et al.* 2007.). In addition, up to 2,000 non-breeding eagles migrate annually from throughout the southeast to spend summer months within the Bay (Watts 2005). Declining Osprey populations that had contracted to higher salinity locations prior to the 1970's, having been extirpated from low salinity tributaries, increased to over 1500 breeding pairs, approximately 20% of the total U.S. Osprey population, by the early 1980's (Henny 1983) and now number in excess of 3500 breeding pairs, possibly the largest breeding population in the world (Watts 2004).

The spatial distribution, abundance, and reproductive output of Bald Eagles and Osprey since recovering from DDT related declines have been associated with concomitant changes in distribution and abundance of important fish prey, including American shad (*Alosa sapidissima*) and Atlantic menhaden (*Brevoortia tyrannus*; Watts *et al.* 2006, Viverette *et al.* 2007, Markham and Watts 2008, Glass and Watts 2009). Shifts have occurred in an upstream direction with tidal freshwater and oligohaline reaches of major Chesapeake Bay tributaries becoming areas of greatest population growth for both species. Bald Eagle colonization rate, nesting density, and reproductive rate are significantly negatively correlated with salinity and average population doubling time for tidal fresh reaches is less than 6 y compared to more than 16 y for polyhaline areas. (Watts *et al.* 2007). For Osprey populations, average doubling times as low as 4 years have been documented for tidal fresh areas compared to greater than 40 years in polyhaline areas (Paxton and Watts, 2007).

Shifting fish resources, including long- and short-term declines in the abundance of anadromous clupeids (*Alosa* spp.), Atlantic menhaden, and the relatively recent introduction and expansion of non-indigenous fishes (e.g. blue catfish) within tidal fresh and oligohaline reaches are the most likely explanations for the observed salinity effects (Glass and Watts, 2009, Markham and Watts, 2008, Viverette *et al.* 2007). Spawning fish, such as American shad and related migratory clupeids native to the East Coast, are particularly nutritious prey due to their loads of fat-rich eggs and sperm (Poole 1989). Anadromous fish stocks have declined somewhat steadily throughout the 20th century. Most recently however, beginning in the 1970's just as Bald Eagles and Osprey populations were beginning to recover, populations of anadromous fish in the Chesapeake Bay basin began to decline precipitously; experiencing as much as a 90% reduction in abundance (Garman and Macko 1998). The causes for the most recent declines are not fully understood but probably involve a combination of factors including commercial over-fishing, barriers to upstream migration, habitat alteration, as well as the introduction of non-native aquatic species (Foerster and Reagan 1977, Garman and Macko 1998, Chandler 1998).

Similarly, annual concentrations of lipid rich, pelagic marine fish species in nearshore and estuarine habitats can be critical to maintaining local piscivore communities (Murdy *et al.* 1997; Uphoff 2003, Mullers *et al.* 2009). Forage-size juvenile Atlantic menhaden, a marine clupeid, are most common in the higher salinity middle to lower tributaries and mainstem areas of the Chesapeake Bay seasonally (Murdy *et al.* 1997). By the mid-1980's, Osprey populations in these core high salinity areas had largely recovered from DDT related declines, and population growth rates were rising. At that time Atlantic menhaden stocks were high and menhaden comprised 75% of nest deliveries to Osprey nests (McClean and Byrd 1991a). However, Atlantic menhaden stocks in the Bay subsequently began a steep decline (Uphoff 2003) and by 2006, the proportion in the diet of Osprey occupying high salinity locations (> 18 ppt) had declined to 25% (Glass and Watts 2009) along with local Osprey population growth rates (Watts and Paxton 2007).

Concurrent with recent declines in anadromous shads and menhaden, the blue catfish (*Ictaluris furcatus*) and the flat-head catfish (*Pylodictus olivarus*) were introduced and became established in tidal freshwater and brackish portions of Chesapeake Bay tributaries (Jenkins and Burkehead 1994). Both introduced catfish are highly piscivorous (Chandler 1998). Tidal freshwater systems along the Atlantic slope have very few native piscivores and stable isotope analysis suggest that the introduction of these two non-indigenous catfish species has effectively added a new "top-tier' to the community structure of the tidal freshwater reach; essentially introducing a new trophic level that has not historically existed (Garman and Macko 1998). Recent diet analysis suggest blue catfish and flathead catfish make up a significant proportion of the diet of Bald Eagles and Osprey nesting in low salinity habitats of the Bay (Markham *et al* 2008, Glass and Watts 2009), perhaps providing alternative sources of energy as traditional native prey species decline.

Stable isotopic analysis of tissue samples from consumers record the nutrients assimilated from dietary sources. Bulk $\delta13C$, $\delta15N$, and $\delta34S$ isotope analyses can be a valuable tool in reconstructing diets of historical predator populations. Naturally occurring carbon and sulfur stable isotopes in tissues can distinguish the source of dietary nutrients, i.e. marine versus freshwater, and nitrogen stable isotopes indicate trophic status of consumers (Garman and Macko 1998). A marine signature in tissues of piscivorous birds nesting within tidal freshwaters indicates anadromous (or migratory) fish prey. An additional advantage to stable isotope analysis is that samples from museum collections can be used to study historic diets, allowing for detection of patterns and trends over long periods of time. The objective of this study is to conduct an analysis of stable isotopes in feathers collected from Bald Eagles and Osprey occupying the Chesapeake Bay circa 1850 – 2009 (Figure 1) in order to estimate historical trends in the contribution of anadromous fishes, including American shad, to the their diets over broad temporal and spatial scales. Specifically we were interested in evaluating the hypothesis that upstream migrations of anadromous clupeid fish represent, at least historically, an ecologically important seasonal subsidy in the form of marine-derived organic matter (MDOM) to piscivorous birds nesting within the Bay's tidal tributaries.

METHODS

Stable Isotope Analysis:

Feathers from birds occupying the Chesapeake Bay prior to 1997 were provided by the Smithsonian Institution's Natural History Museum Bird Collection. Feathers collected from 1999 – 2009 were taken from active Bald Eagle and Osprey nests in the Chesapeake Bay mainstem and tidal tributaries (Figure 1). Nest locations represent a range of salinities from tidal freshwater to polyhaline. When multiple birds were sampled from one nest, mean stable isotope values for all the nestlings were taken and the nest treated as one sample. Bulk stable isotope analysis of the feathers was conducted at UVA's Stable Isotope Laboratory at the Center for Environmental Studies. For a detailed methodology see MacAvoy *et al.* 1998.

GIS Analysis:

Specific sampling locations including latitude and longitude were only available for birds collected after 2000. Birds collected in 1999 were recorded on paper USGS maps and hand digitized. Museum specimens had only very general location data, usually a city or county name. USGS Quad layer files were used to find a central point within each city or county and then a point associated with the closest appropriate water body assigned to the individual bird. Only those birds whose locations fell within the Chesapeake Bay tidal region and were collected during the breeding season of March through August were included in the analysis.

Bird point locations were buffered to reflect an average foraging distance for each species based on published data. Ospreys were assigned a foraging area of 3.0 km (Poole 1989) and Bald Eagles a foraging area of 5 km (Watson 2002). Foraging areas were overlaid on a salinity coverage that included a salinity model developed for the Chesapeake Bay (Chesapeake Bay Program) merged with selected polygons taken from the National Wetlands Inventory (NWI) that included any freshwater areas (including ponds, lakes, and riverine habitats) not included in the Chesapeake Bay Program salinity model. All freshwater features from the NWI were assigned a salinity of 0. A mean salinity was calculated within each bird's or nest's foraging area (Figures 2 and 6). Once a salinity value was attributed to each bird or nest location, regression analyses were performed using species, age, date sampled, and salinity in order to analyze temporal and spatial trends in the contribution of marine derived nutrients to Bald Eagles and
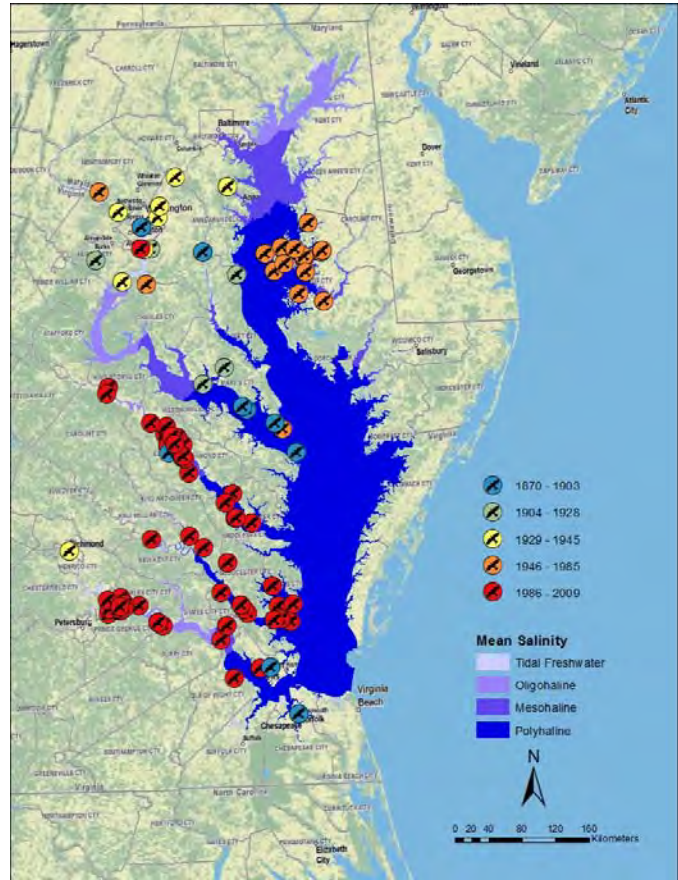

Figure 1: Bald Eagle and Osprey Sampling Sites 1870 - 2009 (1870 - 1997 Smithsonian Museum of Natural History Collections) (1999 - 2009 Field Collected).

Osprey occupying the Chesapeake Bay over the last 150 years. All statistical analyses were performed with software package SPSS v18. with an alpha value for statistical significance of 0.05.

RESULTS:
Osprey:

Historical collections of Osprey were sufficient to allow an analysis of isotopic values over both temporal and spatial scales. One-way Anova showed a significant difference in Osprey δ^{13}C (F= 27.824, p = 0.00), δ^{15}N (F = 96.38, p = 0.00), δ^{34}S (F= 30.336, p = 0.00) between adult and juvenile Osprey so the two groups were analyzed separately. For adult Osprey, a stepwise linear regression with

Fig. 2. Osprey locations and foraging areas. Salinity values of polygons within each foraging area were used to calculate mean salinity for each bird or nest location.

δ^{13}C (N=36), δ^{15}N (N=32), δ^{34}S (N=36) as dependent variables, and year sampled and mean salinity as independent variables, yielded no significant relationships (p< 0.05). The lack of trends for adult Osprey likely reflect the fact they may molt and re-grow their feathers outside of the breeding season, possibly on migration or southern wintering grounds. Thus isotopic values of feathers from adults may not reflect conditions within the Chesapeake Bay.

However, feathers from juvenile Osprey (hatch year) should have isotopic values reflective of the diet within the Chesapeake Bay during the period they are growing feathers while nestlings. Isotopic analysis (Fig. 3) shows that modern Osprey (collected since 1970) have more depleted carbon and sulfur values than historic specimens (collected prior to 1970) consistent with a more terrestrial, freshwater diet, particularly in low salinity reaches. However, historic specimens from low salinity reaches (> 5 ppt) have more enriched carbon and sulfur isotopic values similar to modern and historic Osprey specimens inhabiting high salinity reaches (> 5 ppt), indicating a more estuarine or marine contribution. For nitrogen, the isotopic signatures of modern populations are highly variable, ranging from low values similar to planktivorous clupeids up to piscivorous prey such as blue catfish.

Regression analysis of isotopic values since 1883 (Fig 4 and 5) indicate a significant (p < .05) decrease in carbon and sulfur stable isotopic values. When time (year collected) and mean salinity are included in a regression model, 64% (N = 45, F=36.879, p = 0.0, r² = 0.637) of the variation in δ^{34}S and 48% (N = 45, F = 19.675, p = 0.0, r² = 0.477) of the variation in δ^{13}C is explained. The results are consistent with a decline in the contribution of marine nutrients to freshwater habitats over time. When only samples from low salinity zones are included in the analysis, the temporal component accounts for an even greater proportion of the variation in isotopic values.
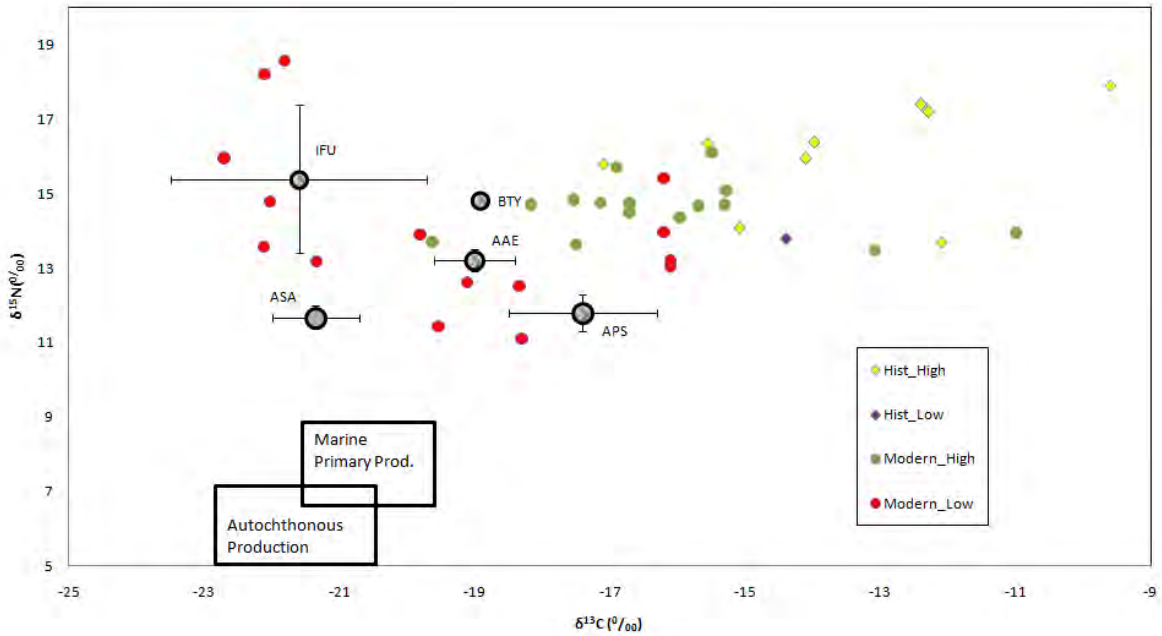
a.
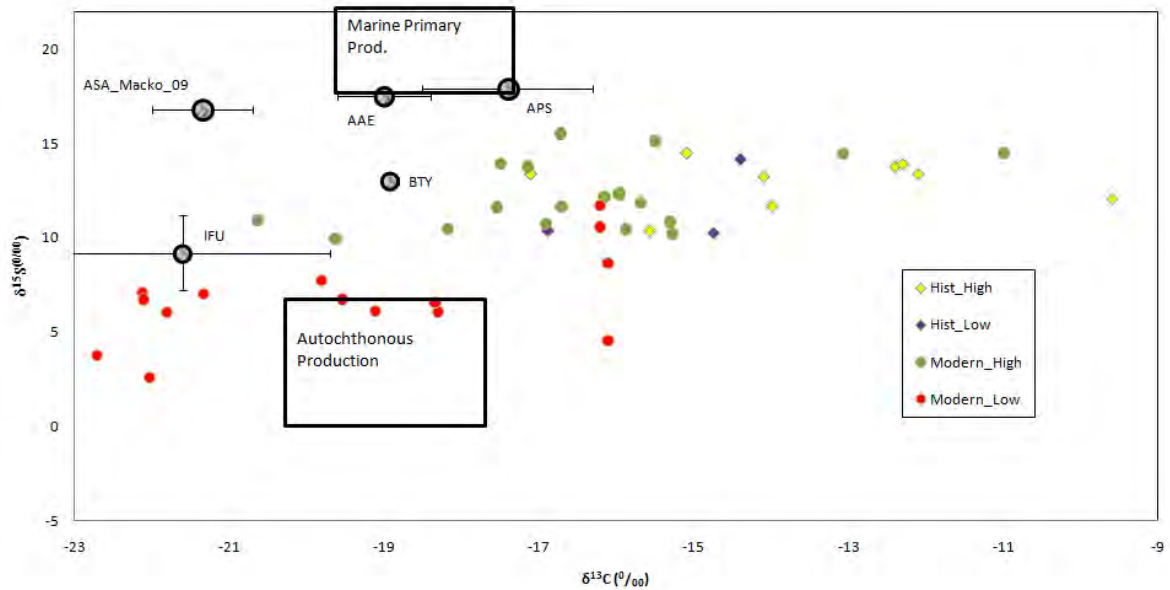
b.

Fig. 3. Bivariate plots of a) $\delta^{13}C$ and $\delta^{15}N$, and b) $\delta^{13}C$ and $\delta^{34}S$ values for juvenile Osprey feathers collected from the Chesapeake Bay between 1883 and 2009. Also included are means (+/_ SD) for fish prey including American shad (ASA), blueback herring (AAE), alewife (APS), Atlantic menhaden (BTY), and blue catfish (IFU). Values for ASA and BTY are from fish collected during the current study, values from all others are from MacAvoy *et al.* (2009).
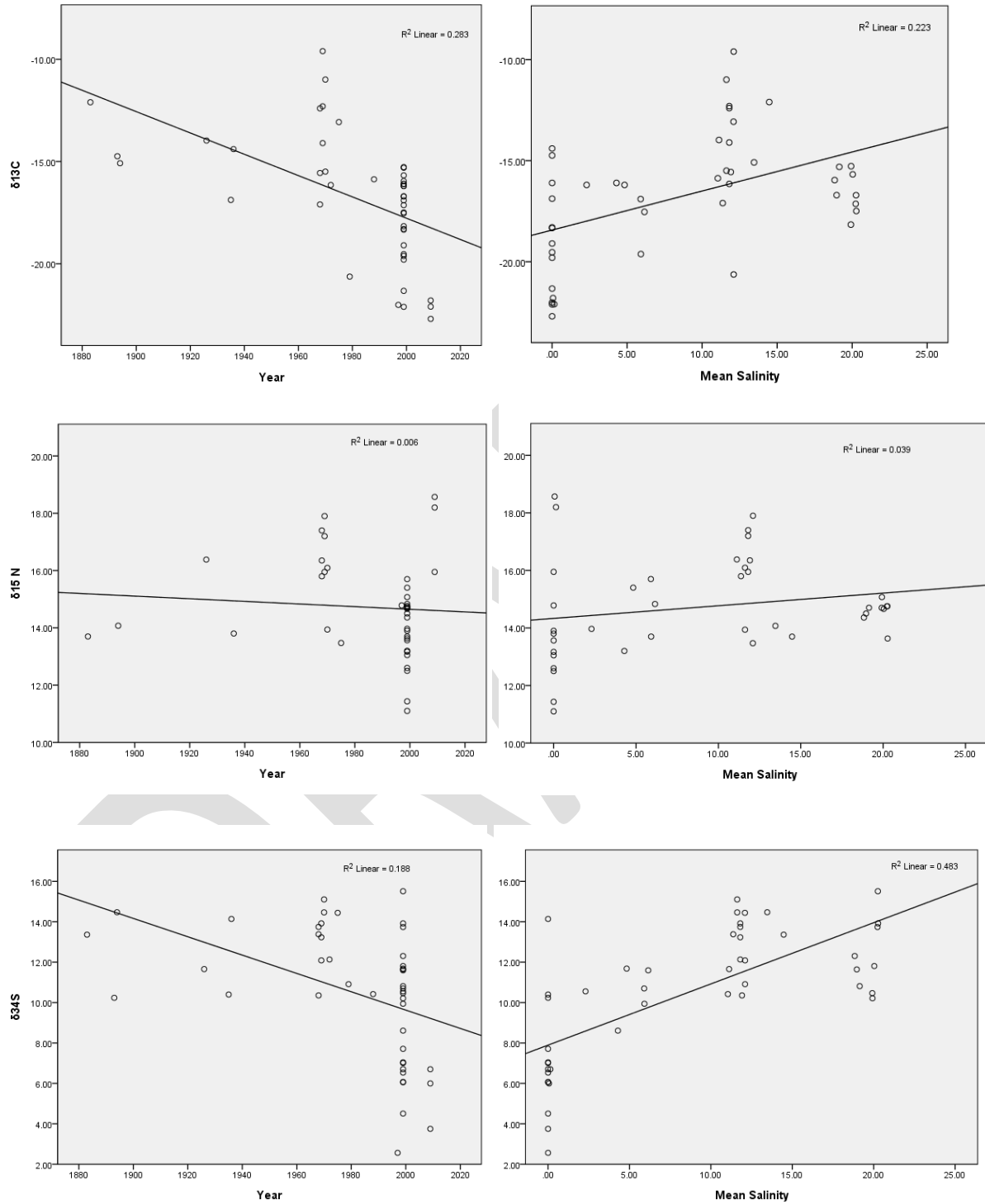
Fig. 4. Results of regression analysis of Carbon, Nitrogen, and Sulfur stable isotope ratio analyses of juvenile Osprey feathers from museum and field collections dated 1840 – 2009 from sites across the Chesapeake Bay.
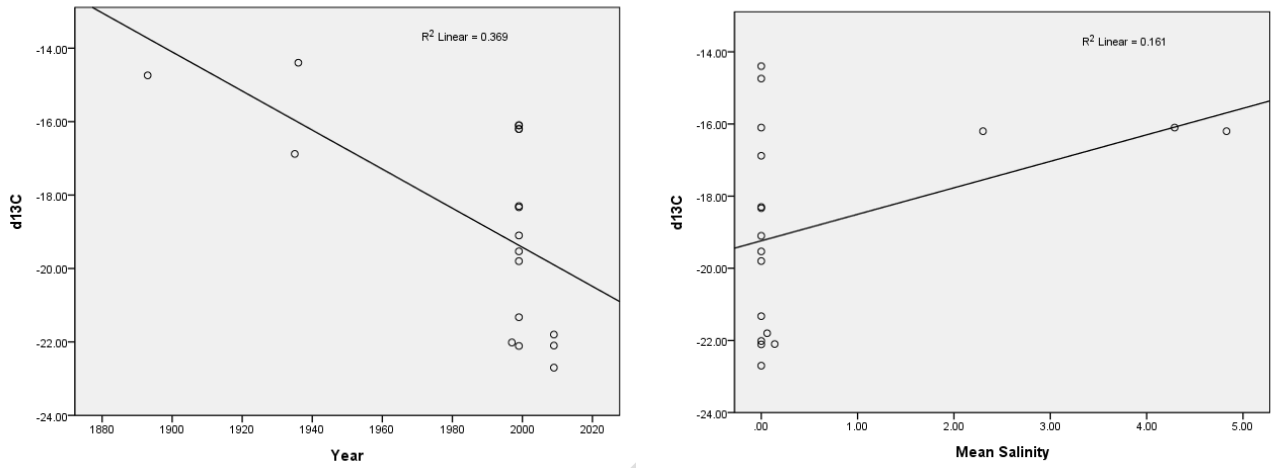
a.
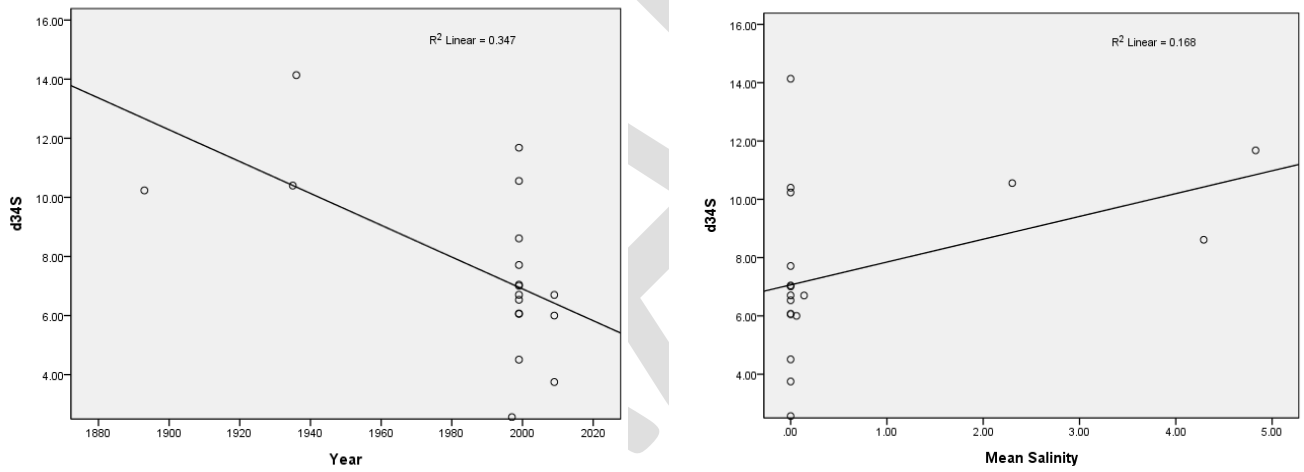
b.

Figure 5: Results of regression analysis of Carbon and Sulfur stable isotope ratio analyses of juvenile Osprey feathers from museum and field collections dated 1840 – 2009 for low salinity (< 0.5 ppt) zone only of tidal tributaries of the Chesapeake Bay.

Bald Eagles:

The Chesapeake Bay supports two large migratory populations of Bald Eagles (from south-eastern and north-eastern US and Canada) in addition to the resident population (Watts *et al.* 2007), so feathers from adults and juvenile (1 year – 3 year) museum specimens were removed from the analysis because it is not possible to distinguish resident from migrant individuals, the latter of which may not reflect diet within the Chesapeake Bay. Because nestlings located within the Bay
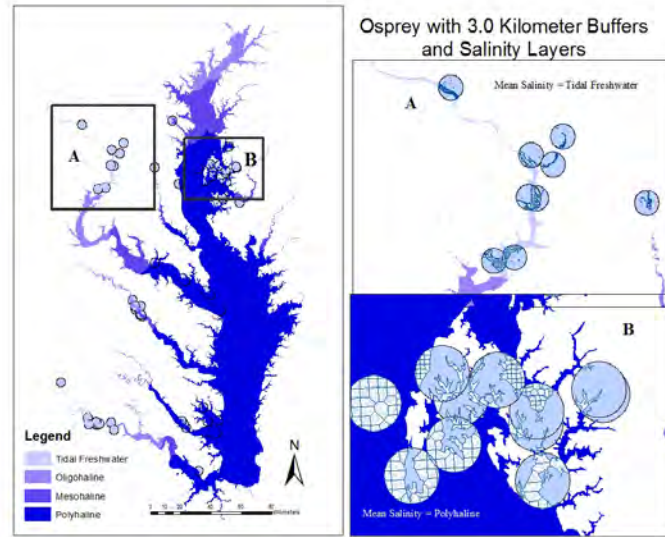

Fig. 6. Bald Eagle locations and foraging areas. Salinity values of polygons within each foraging area were used to calculate mean salinity for each bird or nest location.

are actively growing feathers during the breeding season, they are most likely to reflect the fish prey available in the Bay during that period, however only two nestling specimens from one nest sampled in 1870 were included in the Smithsonian collections, so we lacked adequate samples for a temporal analysis of Bald Eagles.

However, feathers from adult Bald Eagles collected from under nests in the Chesapeake Bay from 1999 – 2006 were included in the spatial analysis along with feathers from nestlings collected from 2004 - 2006. Spatial trends related to salinity are evident in the stable isotopic values of Bald Eagle feathers collected from both adults (n = 10) and nestlings (n = 29). There were significant differences in $\delta^{13}C$ (F = 19.29, p = 0.00), $\delta^{34}S$ (F =23.869, p = 0.00), and $\delta^{15}N$ (F = 8.469, p = 0.006) values between Bald Eagles sampled in low salinity (0.0 – 5.0 ppt) and high salinity (5.0 – 18.0 ppt) regimes. There were no significant differences between adults and nestling Bald Eagles for $\delta^{13}C$ (F = 1.61, p = 0.213) and $\delta^{34}S$ (F = 0.155, p = 0.696) values so they were analyzed together. There were significant differences in $\delta^{15}N$ values (F = 5.119, p = 0.03) between nestlings and adults so the two groups were analyzed separately for nitrogen stable isotope values.

Bivariate plots (Fig. 7) of carbon and sulfur isotopic values show a pattern consistent with a marine to freshwater gradient with most values clustering mid-way between marine and terrestrial inputs. Bald Eagles occupying mesohaline reaches (0.5-18.0 ppt) have a more enriched isotopic signal indicating estuarine prey such as Atlantic menhaden and/or a mix of freshwater and marine prey. Bald Eagles occupying low salinity habitats (0 – 0.5 ppt) have less enriched carbon and sulfur isotopic values than those occupying mesohaline reaches, with values more similar to freshwater prey such as blue catfish. Bivariate plots of carbon and nitrogen (Fig 8) indicate adult Bald Eagles in both low salinity and high salinity zones are feeding at a relatively high trophic level, more similar to the piscivorous blue catfish than planktivores such as anadromous shad and herring species. For juvenile Bald Eagles, nestlings from mesohaline
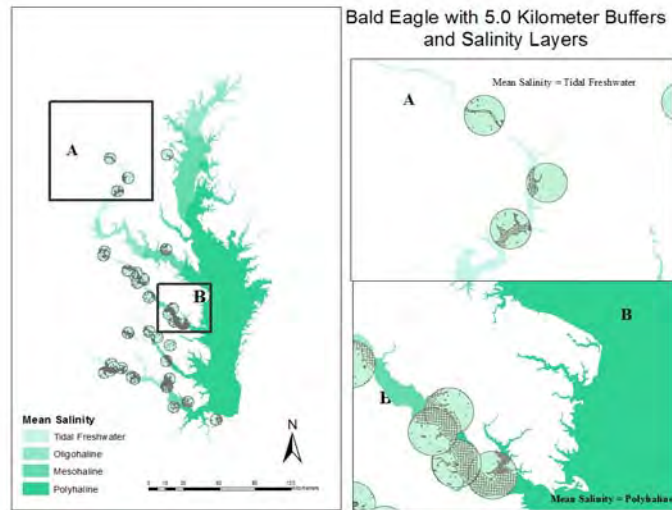
reaches have more consistently high nitrogen values, while those from low salinity reaches show a greater range from very low values to very high consistent with the range of available prey.

Results of regression analysis (Fig. 9) of stable isotopic values of carbon (n = 39, p = 0.00, r^2 = 0.358) and sulfur (n = 39, p = 0.00, r^2 = 0.466) showed a significant positive relationship with salinity, reflecting a decrease in marine nutrients in the diet of Bald Eagles in an upstream direction. However, eagles occupying tidal freshwater reaches display a greater range of values reflecting the fact that tidal freshwater fish communities are more diverse, including forage fish species ranging from freshwater to marine.

Isotopic values of nitrogen in feathers from both nestling (hatch year, n = 29, p = 0.011) and adult Bald Eagles (n = 10, p = .001) had significant positive relationship with salinity, but adults have a higher mean nitrogen value (adult mean = 15.76 +/- 1.65, mean nestling 14.38 +/- 1.53). In addition, a greater portion of the variation in nitrogen stable isotope values is explained by salinity (r^2 = 0.787) than for nestlings (r^2 = 0.215).
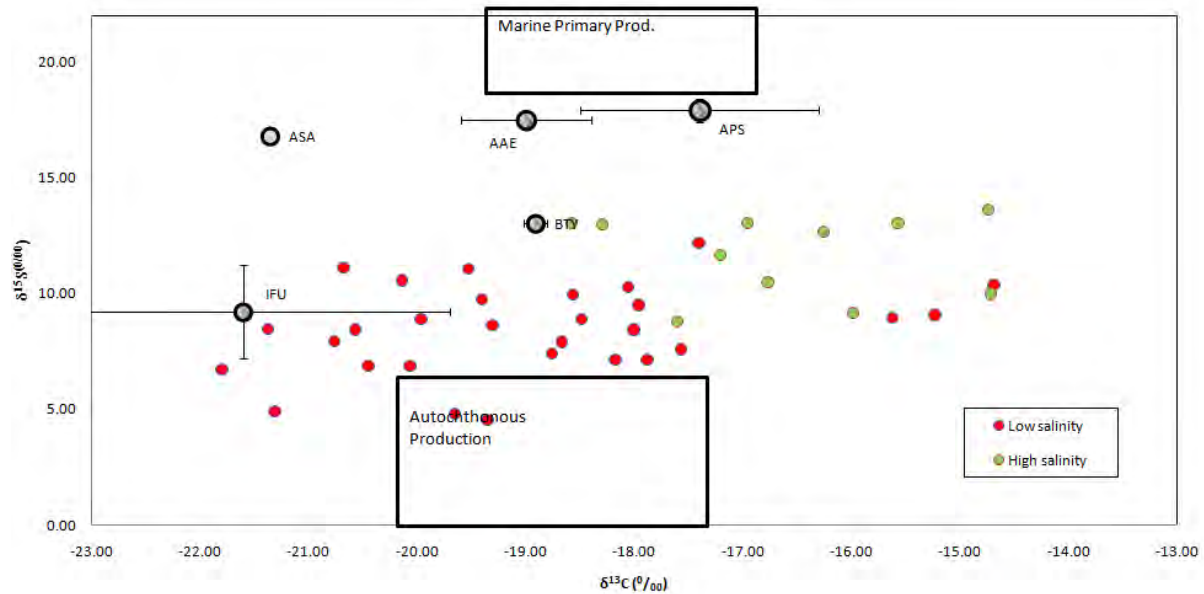
Fig. 7. Bivariate plot of $\delta^{13}C$ and $\delta34S$ values for Bald Eagle feathers collected from the Chesapeake Bay from 1999 through 2006. Also included are means (+/_ SD) for fish prey including American shad (ASA), blueback herring (AAE), alewife (APS), Atlantic menhaden (BTY), and blue catfish (IFU). Values for ASA and BTY are from fish collected during the current study, values from all others are from MacAvoy *et al.* (2009).
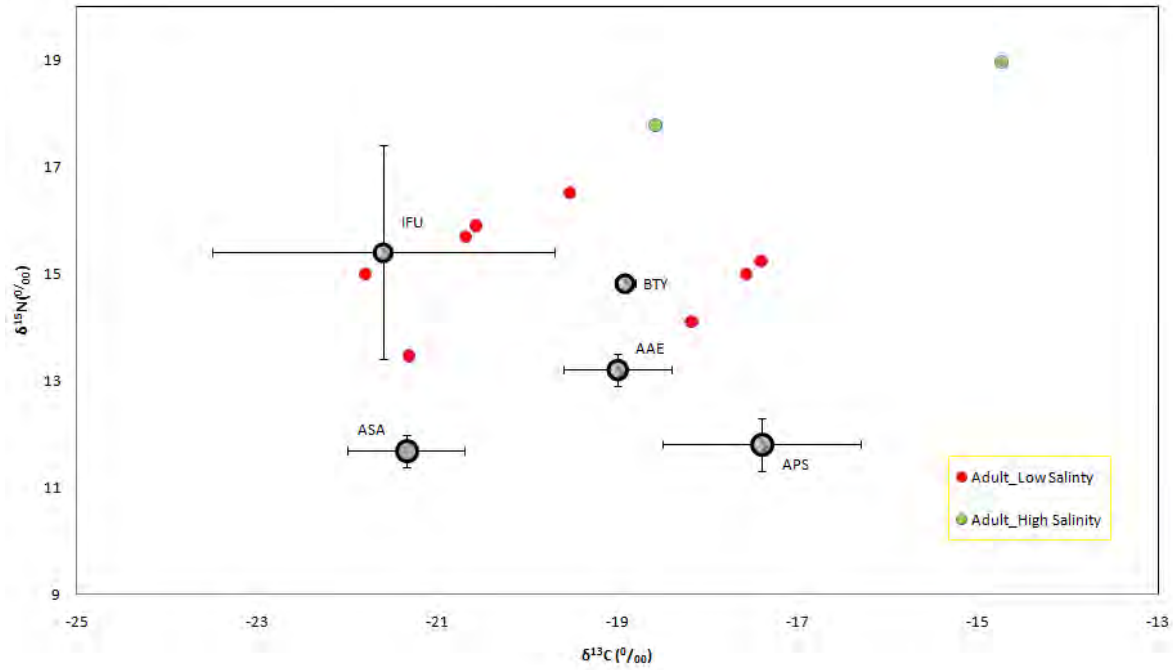
a.
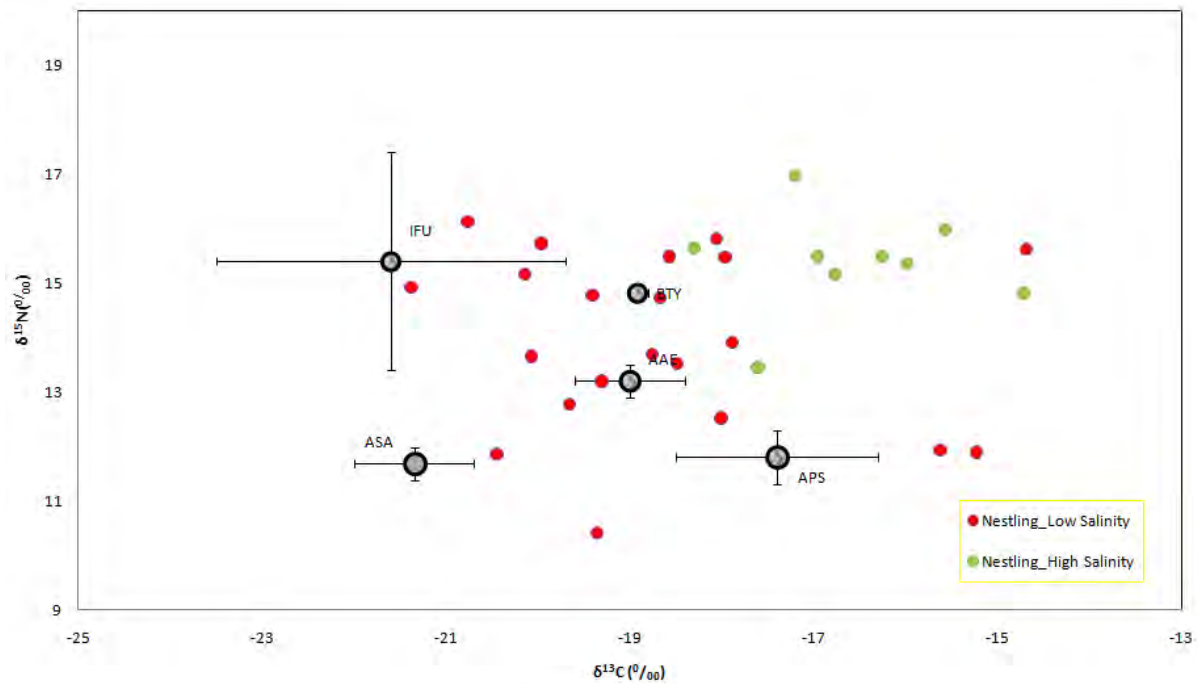
b.

Figure 8. Bivariate plots of a) adult and b) nestling $\delta^{13}C$ and $\delta^{15}N$ values for Bald Eagle feathers collected from the Chesapeake Bay between1999 and 2006. Also included are means (+/_ SD) for fish prey including American shad (ASA), blueback herring (AAE), alewife (APS), Atlantic menhaden (BTY), and blue catfish (IFU). Values for ASA and BTY are from fish collected during the current study, values from all others are from MacAvoy *et al.* (2009).

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a.

b.

c.

d.
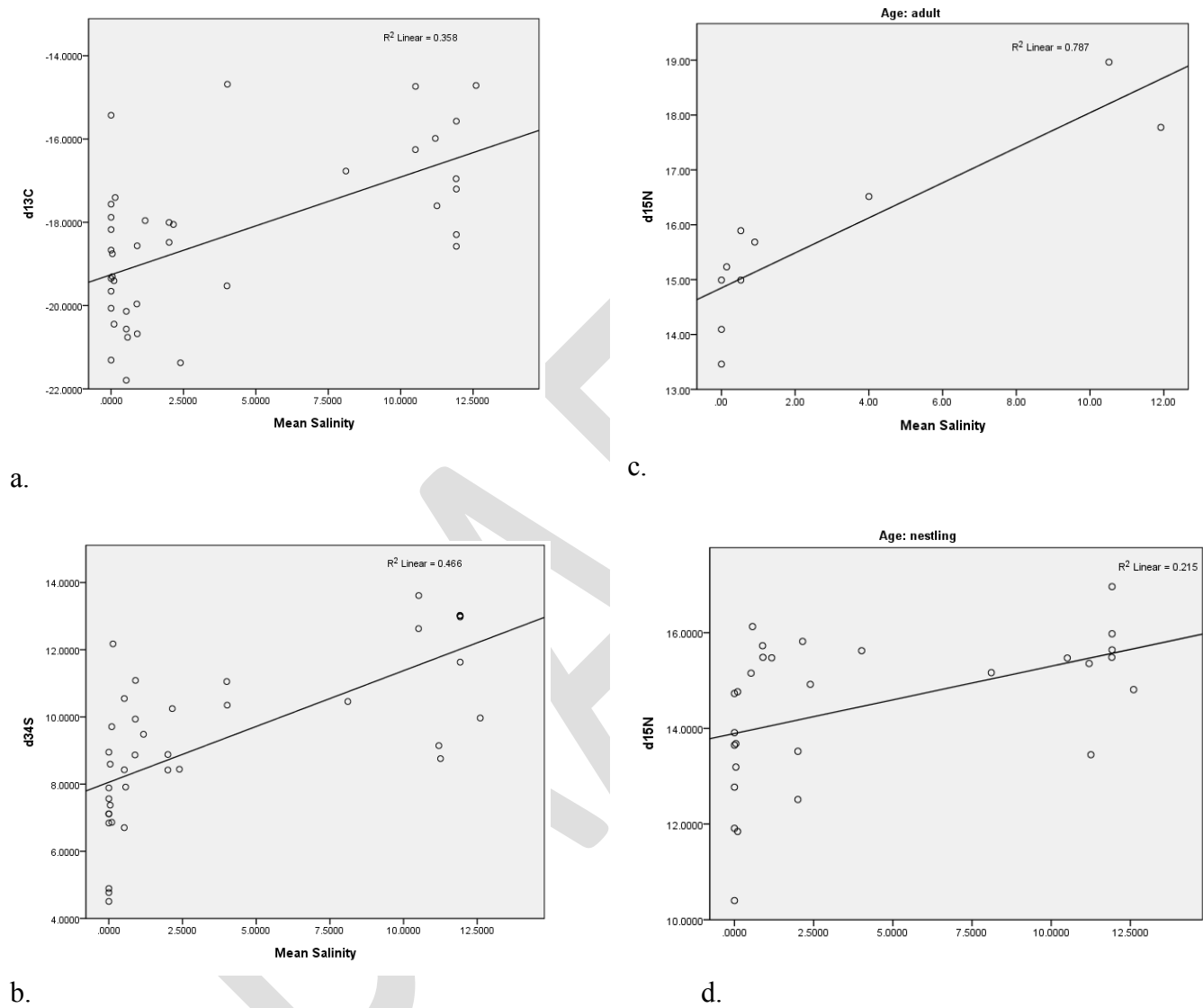
Fig. 9. Results of regression analysis of Carbon, Nitrogen, and Sulfur stable isotope ratio analyses of adult and juvenile Bald Eagles collected from sites along a salinity gradient from mesohaline to tidal freshwater within tidal tributaries of the Chesapeake Bay.

DISCUSSION:

Osprey

Stable isotopic signatures of anadromous fish reflect the marine environment in which they feed, and diagnostic isotopic values should be evident in tissues of avian predators (MacAvoy *et al.* 1998, Anderson *et al.* 2009, Jones *et al.* 2010). Results of the temporal analysis of carbon and sulfur stable isotopes indicate a statistically significant decrease in marine derived organic carbon and sulfur in the diets of Osprey nesting in tidal freshwater reaches of the Chesapeake Bay over the last 150 years, likely reflecting long-term declines in the abundance of anadromous clupeid prey (*Alosa* spp, e.g. American shad, Watts *et al.* 2006, Viverette *et al.* 2007). These results support the hypothesis that Chesapeake Bay piscivorous birds have, over time, shifted from a diet based on seasonally abundant, native migratory fish to resident and non-indigenous freshwater species available year-round and are consistent with recent diet studies of Osprey nesting in tidal freshwater habitats (Glass and Watts 2009) where gizzard shad and Ictalurid species dominated nest deliveries.

Abundant resident freshwater prey may provide a nutritional substitute for declining populations of traditionally important forage fishes such as migratory and marine clupeid species, driving up densities and reproductive rates of piscivorous birds in brackish and freshwater habitats. The availability of alternative prey likely account for the fact that recovering Bald Eagle and Osprey populations in tidal freshwater and oligohaline reaches now have the highest colonization rates, density and population growth rates in the Chesapeake Bay (Watts *et al.* 2006). In contrast, Osprey populations in higher salinity regions of the Chesapeake Bay are experiencing declining population growth and reproductive success likely due to the decline in Atlantic menhaden in those regions. Unlike tidal freshwater zones, high value alternative prey may not be available, driving the population to shift to brackish and freshwater zones.

Bald Eagles

Although we were unable to conduct a temporal analysis of Bald Eagle isotopic values due to a lack of sufficient historical samples, a spatial analysis indicates a significant decline in marine isotopic signature from mesohaline through tidal freshwater zones. In contrast to the isotopic results, Markham and Watts (2008) found no significant difference in species composition of fish delivered to Bald Eagle nests in mesohaline and tidal freshwater zones. The feathers from nestling Bald Eagles analyzed in the isotopic analysis were collected from the same nests observed in Markham and Watt's feeding study. In the feeding study, Clupeid and Ictalurid species dominate all prey deliveries, and overall diet composition does not vary spatially along the salinity gradient. The lack of significant differences in diet between the two salinity zones is unexpected because Bald Eagles are opportunistic, generalist piscivores, preying mainly on abundant, schooling fish in shallow waters. As generalist feeders, the diet is expected to reflect the composition of the available fish community, and the fish community does vary along the salinity gradient (Jenkins and Burkhead 1993, Murdy *et al.* 1997, Viverette 2004).

Within freshwater and brackish zones, resident Clupeid and Ictalurid species are some of the most abundant species both as a percent composition and percent biomass. For instance, in a 1999 analysis, 2 species of non-migratory clupeids (Dorosoma sp.), made up approximately 35% of the tidal freshwater fish community and 20% of the biomass in the James River (Viverette *et al.* 2007). Some current estimates of total biomass of blue catfish in the tidal freshwater James River are as high as 70% (R. Greenlee, VDGIF, pers.com). So the diet composition reported in Markham and Watts (2008) in freshwater zones generally reflects the local fish community (Viverette *et al.* 2007). However, although blue catfish are known to tolerate salinities up to 12 ppt. (Murdy *et al.* 1997), resident freshwater clupeids and catfish likely make up a relatively small percentage of available prey in mesohaline zones of Chesapeake Bay tributaries (Murdy *et al.* 1999).

One explanation for the similarity in diet composition in both freshwater and mesohaline zones may be that Bald Eagles nesting in mesohaline reaches are traveling much greater distances from their nests in order to forage in freshwater reaches. However, our analysis indicates a significant difference in isotopic values among Bald Eagles nesting in mesohaline and freshwater/brackish zones, and a positive relationship between isotopic values and salinity, suggesting the two groups are not foraging in the same areas. Bald Eagles occupying the higher salinity zones have more enriched isotopic signatures indicative of greater marine and estuarine contribution to the diet than Bald Eagles occupying freshwater zones.

An alternative explanation is that a greater percentage of clupeid species consumed in mesohaline zones may be anadromous and estuarine species (Alosa and Brevoortia), the former preyed on as they move upstream through mesohaline zones on their way to spawn in freshwater zones. In contrast, Bald Eagles occupying freshwater and brackish zones may consume more resident, freshwater Dorosoma species which make up greater than 90% of available clupeids in low salinity reaches (Viverette *et al.* 2007), far outnumbering migratory individuals, even during spawning. In addition, catfish occupying mesohaline reaches are likely consuming more estuarine and marine prey which could lead to more enriched isotopic value, in turn reflected in isotopic values of avian predators. However turnover in fish tissues can be as long from several months to a year and it is unknown if individual catfish remain in mesohaline conditions for extended period of time (MacAvoy et al. 2009).

Although adult Bald Eagles nesting in the Chesapeake Bay are resident year round (unlike Osprey) the period of growth of feathers collected under nests is likely not concurrent with the period of growth of feathers collected from nestlings. Differences in δ^{15}N values between adult and nestlings may reflect differences in diet composition seasonally or differences in prey size between adults and nestlings. Some piscivorous fish species eaten by eagles, including blue catfish, become more pisivorous, thus feed at a higher trophic level, as they age. Feeding studies of Bald Eagles have shown that fish delivered to nests in mesohaline zones are larger on average than fish delivered to nests in tidal freshwater zones (Markham and Watts 2008b) which may also account for the stronger relationship between nitrogen stable isotope values and salinity in adult Bald Eagles. Catfish may be consuming different prey in mesohaline zones. In tidal freshwater zones for instance blue catfish are known to feed heavily on the abundant gizzard shad (R. Greenlee, VDGIF, pers. Com), which feeds at a relatively low trophic level. Catfish

diets in higher salinity areas may be more variable and include fishes from a range of trophic guilds.

Bald Eagles nesting in mesohaline zones have lower densities but higher provisioning rates, reproductive rates, and nestling growth rates than Bald Eagles nesting in tidal freshwater zones (Markham and Watts 2008b). If Bald Eagles nesting and foraging in mesohaline zones are in fact preying on larger numbers of anadromous and estuarine species of clupeids, it may contribute to the higher reproductive and provisioning rates. Although freshwater reaches may provide large concentrations of alternative prey, including resident freshwater clupeids and introduced catfish species, these substitutes may not provide the nutritional 'bang for the buck' of traditional anadromous and estuarine prey such as American shad and Atlantic menhaden.

CONCLUSION:

Effective ecosystem management requires the ability to document and forecast system responses to change (NCBO Chesapeake Bay Integrated Science Program 2006). Understanding the current and historic role of critical prey species in the diets and distribution of Bald Eagles and Osprey may help identify significant interactions in Chesapeake Bay food webs over large temporal and spatial scales, and aid in forecasting responses to future change. Recently, a study documented shifts in the diet of Bald Eagles occupying the Channel Islands between the late Pleistocene and the mid-20th century using stable isotope analysis (Newsome *et al.* 2010). Over time, Channel Island Bald Eagles shifted from feeding on native prey species to non-indigenous species introduced and available in high densities starting in the mid-1850's. The study highlights the difficult challenges in current Channel Island Bald Eagle re-introduction efforts since historic prey populations, both native and introduced, are no longer abundant and an appropriate substitute may not exist.

Likewise, the current study highlights the importance of prey distribution to predator abundance and distribution over both small and large temporal and spatial scales, and the application of this knowledge to conservation strategies for the future. Declines in native anadromous and marine prey, and concentration of these and alternative prey in low salinity habitats may result in a reduction in carrying capacity of the Chesapeake Bay watershed for avian piscivores (Watts *et al.* 2007). By tracking changes in the diet, distribution, and reproductive output of avian piscivoress and their prey, biologists and managers will develop a critical understanding of spatial distributions and system-wide abundances of target fish species, as well as community wide responses to management-initiated changes and natural disturbances on predator communities.

Chapter 3: Estimates of Energetic Demand by Selected Avian Predators in the Chesapeake Bay

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Background:

Breeding Populations -- Since the end of the DDT era in the early 1970s, piscivorous bird populations have increased exponentially throughout the tidal (freshwater and polyhaline) reaches of the Chesapeake Bay. Several species that experienced dramatic population declines prior to 1970 have recovered to historic levels. For example, after reaching a low of less than 50 breeding pairs in the early 1970s, the Bald Eagle breeding population has increased dramatically with an average doubling time of 8.2 years and more than 900 breeding pairs (Watts *et al. In press a*.). Average reproductive rates (chicks/breeding attempt) increased from 0.2 during the early 1960s (Abbott 1963) to more than 1.5 in recent years (Watts *et al. In press a*). In addition, up to 2,000 non-breeding eagles migrate annually from throughout the southeast to spend summer months within the Bay (Watts 2005). Other avian species have shown similar population trends. In less than 30 years, Osprey increased from 1,400 pairs to 3,500 pairs (Watts *et al.* 2004) and the Chesapeake Bay now supports the largest breeding population in the world. In the same time period, Great Blue Herons increased from approximately 1,000 to more than 18,000 pairs, and Great Egrets have increased from 1,400 to 3,600 pairs (Watts 2004, Brinker, unpub. data).

In addition to the increased abundance of avian species that bred historically within Bay waters, other piscivorous species have recently expanded their range into the Bay and have exhibited dramatic rates of population growth. Double-crested Cormorants colonized the Chesapeake Bay in 1978 and have grown to more than 2,000 breeding pairs (Watts and Bradshaw 1996). Brown Pelicans colonized the Bay in 1987 and have

increased to 2,500 breeding pairs (Watts 2004). The combined energetic demand of this rapidly expanding consumer community—and the implications for effective management of Chesapeake Bay fish stocks—has never been evaluated adequately. Conversely, the potential role of fish population dynamics, distribution, and commercial harvest in regulating bird species that are of national conservation concern is unknown for the region.

The objective of this study is to use conventional energetics-based methods to estimate the overall metabolic demand and consumption of fishery resources for selected avian species during the breeding season, in order to contribute to the parameterization of existing Chesapeake Bay ecosystem models. The breeding season was chosen because data for avian populations is most complete for breeding populations within the Bay and the reproduction season is an energetically demanding period. In addition, it is during the breeding season that avian piscivores may rely most heavily on seasonally abundant fish such as Atlantic menhaden and American shad (Markham and Watts 2008a, Watts and Glass 2009, Jones et al., 2010)

Populations outside the breeding season - In addition to populations of breeding birds, on which these analyses were based, the Chesapeake Bay supports much larger numbers of migrant and wintering Double-crested Cormorants and other avian predators. For instance, substantial numbers of Double-crested Cormorants winter along tidal tributaries of the Chesapeake Bay in Virginia and Maryland (Wires *et al.*2001) in numbers much larger than breeding populations (B. Watts, pers. comm.). Winter roosts in North Carolina can reach 10,000 birds or more. Estimates for the number of Double-crested

Cormorants migrating through Virginia range from 20,000 to 30,000 at Fisherman's

Island, VA (Wires *et al.*2001). Estimating the predatory impact of much larger

populations of overwintering (cp. breeding, this study) and migratory fish-eating birds

was beyond the scope of the current study. However, the impact of winter and migratory

waterbirds on fishery stocks is likely greater than the combined impact of nesting

waterbirds in the Chesapeake Bay, but no comprehensive survey or analysis of metabolic

demand of these predator groups has been undertaken to date (B. Watts, pers. comm.). It

appears that Cormorants may concentrate in tidal tributaries during the winter. Upstream

reaches of tidal tributaries currently support some of the highest population growth in

breeding waterbirds so it would appear that densities of Cormorants are not

significantly depressing the availability of prey resources in those reaches. The larger

concern might be in estuarine and marine habitats (e.g. Eastern Shore) where Atlantic

menhaden populations may be impacted.

Methods:

We used a bioenergetics approach to estimate the amount of fish biomass consumed by

breeding piscivorous birds within the tidal reach of the Chesapeake Bay. This approach

combined a multi-stage population model with a breeding model (Figure 1) and applied

allometric relationships between field metabolism and body mass to estimate annual

demand across years and daily demand within years. Species-specific models were

created for Bald Eagles, Osprey, Great Blue Herons, Double-crested Cormorants, and

Brown Pelicans. Bay-wide survey data was used to parameterize the population model and 28 general, nesting, feeding, and demographic parameters were used to develop the breeding models. Metabolic demand was measured in Kj/d. Demand was converted into fish biomass using an energy density of 5.5 Kj/g of a generic fish. Population and community-wide projections were made in 5-y intervals over a 30-year period between 1975 and 2005.

Spatially-explicit maps of fish demand were generated for nesting Bald Eagles and Osprey. Maps for Bald Eagles were produced in 5 year intervals (1975-2005) along the James River. A map for Osprey was produced for the comprehensive survey conducted in 1995 for the tidal reach of the Bay. Fish demand per pair was used along with foraging ranges to produce maps.

We estimated fish consumption by waterbird populations within the tidal reach of the Chesapeake Bay. For this analysis we considered the tidal reach of the Bay to include all waters from Cape Henry and Fisherman Island up the main stem and tributaries to their conclusion or to the fall line. Breeding populations of Bald Eagles, Osprey, Great Blue Heron, Brown Pelican, and Double-crested Cormorants were modeled. These include all of the fish-obligate breeding species within the Bay with a body mass greater than 1.5 kg.

We used a bioenergetics approach similar to that employed in previous studies (e.g. Wiens and Scott 1975, Furness 1978, Wiens 1984, Cairns *et al.* 1991, Madenjian and Gabrey 1995) to estimate the amount of fish biomass consumed by each population on

daily and annual time scales. Our approach combined a multi-stage population model with a breeding model (Figure 1) and applied allometric relationships between field metabolism and body mass to estimate annual demand across years and daily demand within years. This approach allowed for the estimation of daily energy expenditure (DEE) for all individuals within each population.

Post-Fledged Birds - DEE of a fledged bird (DEE-F) was calculated using the allometric relationship presented by Birt-Friesen *et al.* (1989).

$$DEE\text{-}F = 1737.8W^{0.727}$$

Where DEE-F = the daily energy expenditure, in kilojoules, of a fledged bird, and W = mass in kilograms. This allometric equation was based on measurement of metabolic rates of free-flying seabirds so was appropriate for this application. DEE was converted to kilocaloric units using the equation:

$$1\ kilojoule = 0.23892\ kilocalories$$

DEE divided by consumption and assimilation efficiency equaled the total daily energy consumption for an individual. Total daily energy consumption divided by the average energy density of the diet yielded the daily food mass per individual. The product of the daily food mass and the proportion of fish in the diet represented the daily fish consumption per individual.

Pre-Fledged Birds – DEE of a pre-fledged bird (DEE-P) was estimated using the allometric relationship presented by Kendeigh *et al.* (1977).

$$DEE\text{-}P = 1.230W0.7749$$

Estimates of Fish Consumption

Long-term Pattern - Estimated fish consumption by the 5 populations examined increased exponentially from 1,588,084 to 16,014634 kg with an average doubling time of 9.0 years between 1975 and 2005 (Figure 2). This reflects the exponential growth in these populations and the recent colonization of the Bay by Double-crested Cormorants and Brown Pelicans (Table 2). Due to their large population size, Great Blue Herons consumed the greatest biomass followed by Double-crested Cormorants, Brown Pelicans, Bald Eagles, and Osprey. Fish demand is governed by both the size of the population and the length of residency in the Bay. Brown Pelicans and Double-crested Cormorants did not occur in the Chesapeake Bay and have become significant fish consumers in a relatively short period of time.

Seasonal Pattern – Estimated seasonal fish consumption reached a peak in July around the time when young are fledging (Figure 3). This is the time when the overall consumer biomass reaches a high before steadily declining due to mortality. The rapid periods of transition in spring and fall reflect the migration periods in and out of the Bay for species

that are not resident. Because species vary in phenology and in the details of breeding, seasonal patterns are species-specific (Figure 4a-4e). Because populations have grown at different rates over the years and the composition of the community has changed, there has been a slight shift in the pattern of seasonal consumption (Figure 5).

Distribution of Fish Consumption

Osprey Bay-wide – The distribution of fish consumption by Osprey throughout the tidal reach of the Chesapeake Bay is restricted to areas within the littoral zone (Figure 6). However, within this zone there is considerable spatial variation in breeding density and related fish consumption. Projected consumption is highest within small tributaries where breeding density is particularly high. The factors contributing to variation between tributaries are not clear but may relate to fish availability or to differences in nesting substrate availability.

Bald Eagles-James River – The distribution of fish consumption by Bald Eagles within the James River has increased dramatically over the 25-year sequence of projections (Figure 7). Projected consumption is confined to the littoral zone and has increased the most within the tidal fresh reaches where breeding density is high.

Table 1. Matrix of parameters used to develop annual metabolic demand and population models. Values are from best available sources for Chesapeake Bay populations. Where no information is available for Bay populations, published values for other populations were substituted. GBHE, OSPR, BAEA, DCCO, and BRPE refer to Great Blue Heron, Osprey, Bald Eagle, Double-crested Cormorant, and Brown Pelican respectively.

Parameter	GBHE	OSPR	BAEA	DCCO	BRPE
General					
Adult Male Wt (g)	2576	1437	3522	1808	3702
Adult Female Wt (g)	2204	1798	4630	1540	3174
Arrival Date (breeders)	2/15-3/15	3/15-4/15	resident	2/15-3/15	4/1-5/1
Arrival Date (non-breeders)	2/15-3/15	5/1-6/1	resident	2/15-3/15	4/1-5/1
Departure Date	10/1-10/31	8/15-9/15	resident	9/20-10/20	11/15-12/15
Nesting					
Early Laying Date	3/15	4/25	1/24	3/15	3/1
Late Laying Date	4/15	5/31	3/7	7/15	7/15
Laying interval (d)	2	1	2	1	2
Incubation Time (d)	27	37	35	27	32
Egg Wt (g)	72	66	114	46.5	103
Hatching Wt (g)	53.4	50.3	85	31	73.5
Asymptotic Wt (g)	2322	1647	4046	1760	4000
Fledging Wt (g)	2390	1647	4076	1760	3440
Logistic K	0.173	0.173	0.0942	0.191	0.071
Time to Asymptote (d)	60	49	55	45	50
Time to Fledging (d)	70	55	80	49	76

Feeding					
Fish Diet (%)	72	100	94	100	100
Consumption (%)	100	100	90	100	100
Assimilation Efficiency (%)	87	80	75	75	80
Demographic					
Clutch Size	3	3	2	4	3
Hatching Success (%)	92	82	85	75	60
Reproductive Rate (yng/pr)	1.57	1.2	1.5	2.2	0.9
Age to breeding	3 yr	4 y	5 yr	3 yr	4
First Year Survival (%)	31	45	77	48	30
Second Year Survival (%)	63.7	82	90	75	75
Third Year Survival (%)	78.10	82	90	85	75
Adult Survival (%)	78.10	82	90	85	75
r-value	0.069	0.038	0.084	0.2445	0.354

Table 2. Breeding populations (in breeding pairs) of fish-eating birds and estimated fish demand (in kg of fish) in the Chesapeake Bay (1975-2005)

	GBHE	**OSPR**	**BAEA**	**DCCO**	**BRPE**
Population					
1975	2,163	1,564	70	0	0
2005	16,950	4,888	854	4,417	3,528
Fish Demand					
1975	1,042,441	389,286	156,357	0	0
2005	8,168,920	1,216,646	1,907,562	2,504,407	2,217,099

Figure 1. Flow diagram of population and metabolic demand models.

All Species
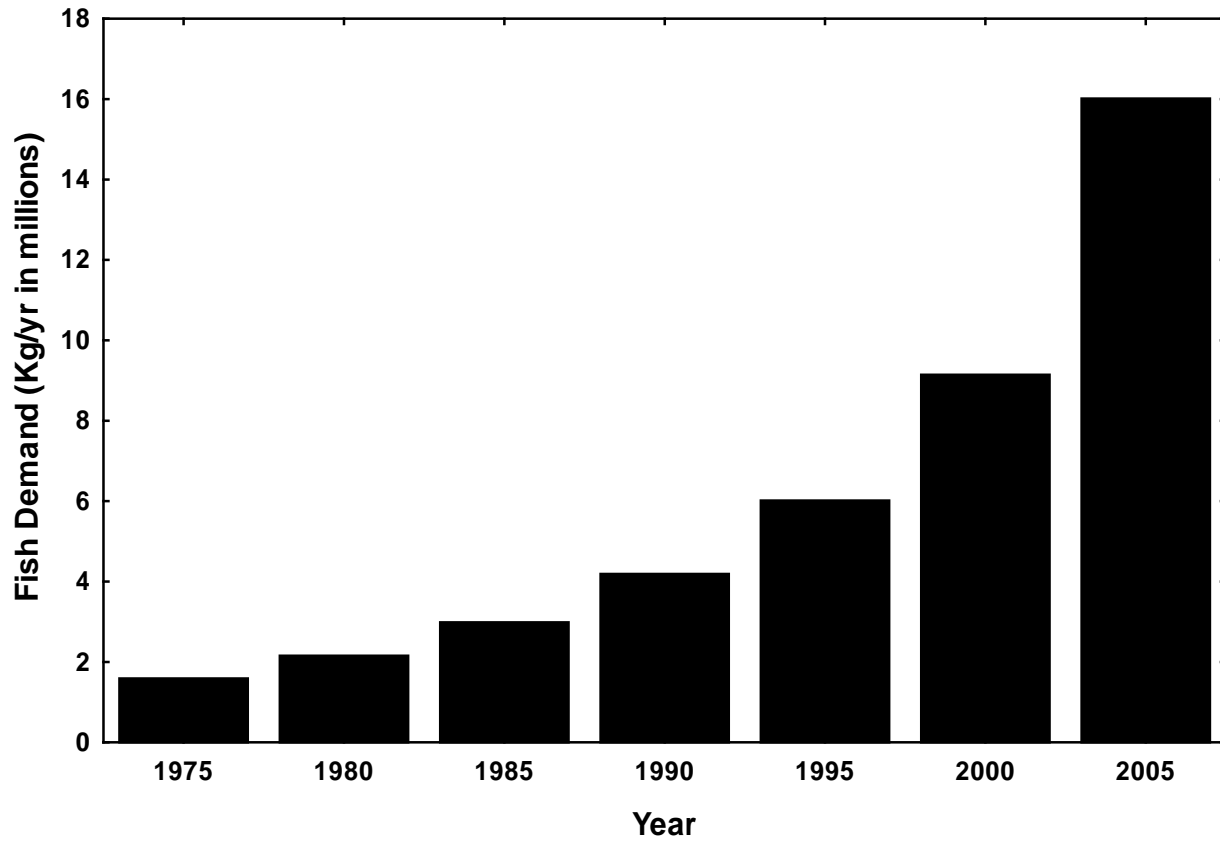
Figure 2. Long-term trend in fish demand for all fish-eating bird populations combined (1975-2005). Projected demand has grown exponentially with an average doubling time of 9 years.

All Species Combined
(2000)
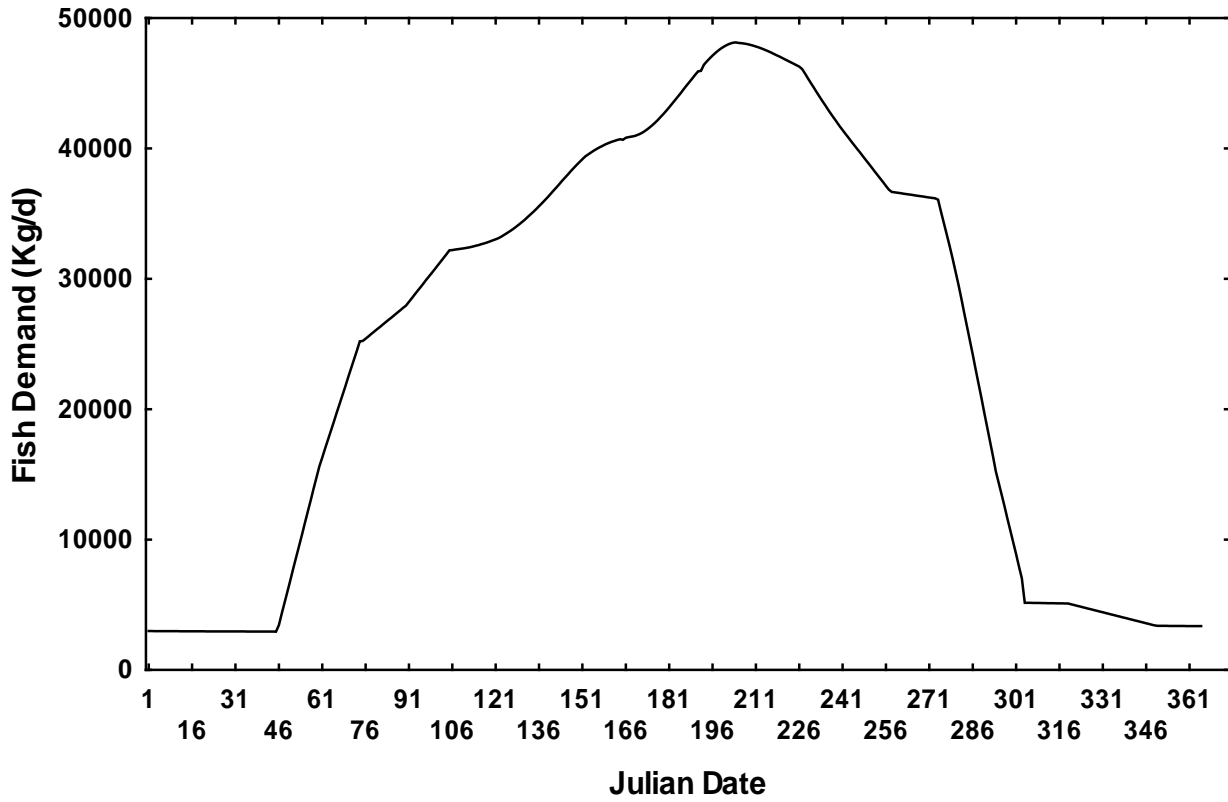
Figure 3. Projected seasonal fish demand for all fish-eating birds in the Chesapeake Bay combined. Demand peaks in July around the time when most young have fledged.
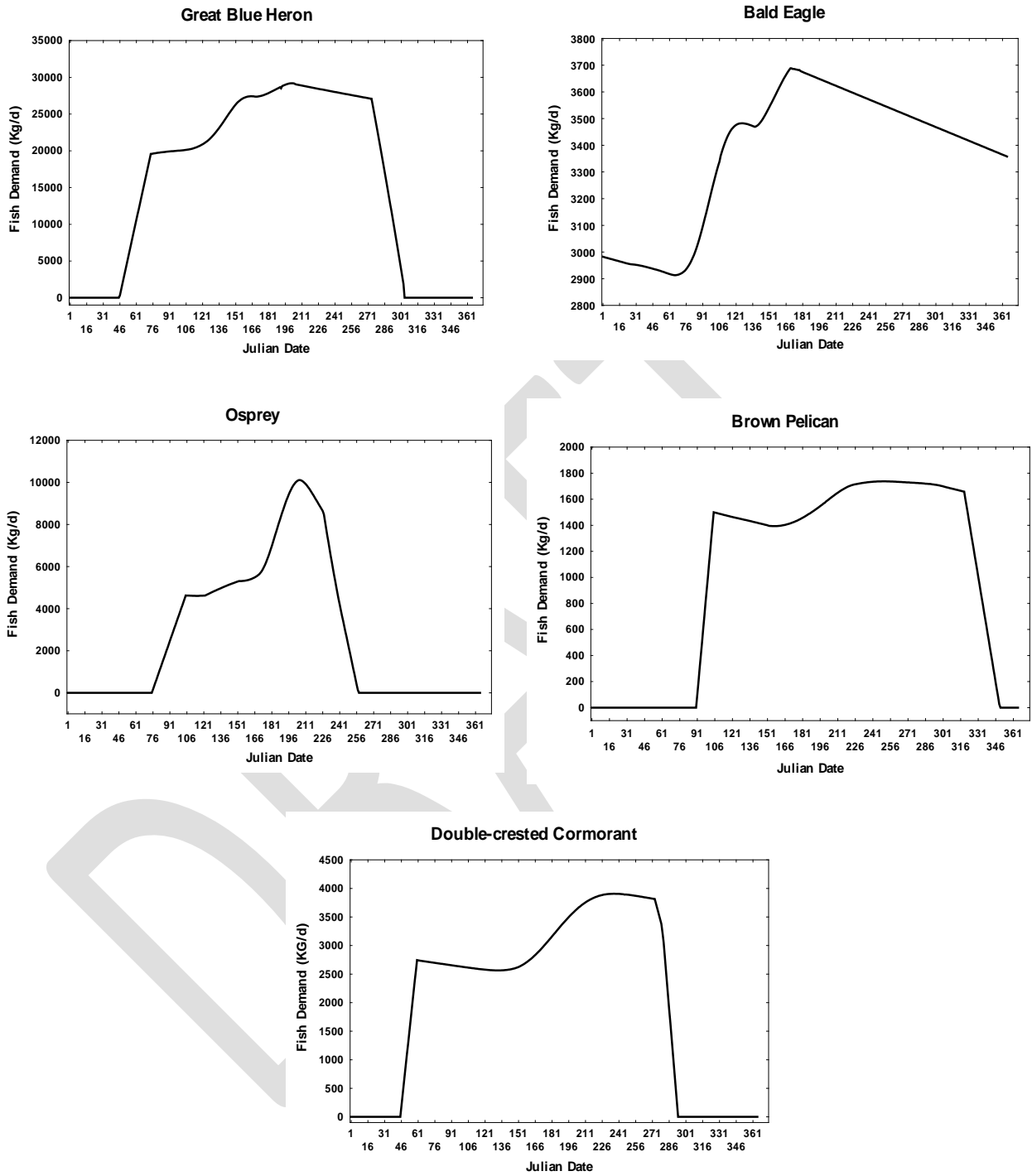
Figure 4. Projected seasonal fish demand patterns for individual species included in this study. Patterns vary between species according to residency and details of breeding ecology.

Seasonal Fish Demand Comparison
(1975 vs 2005)
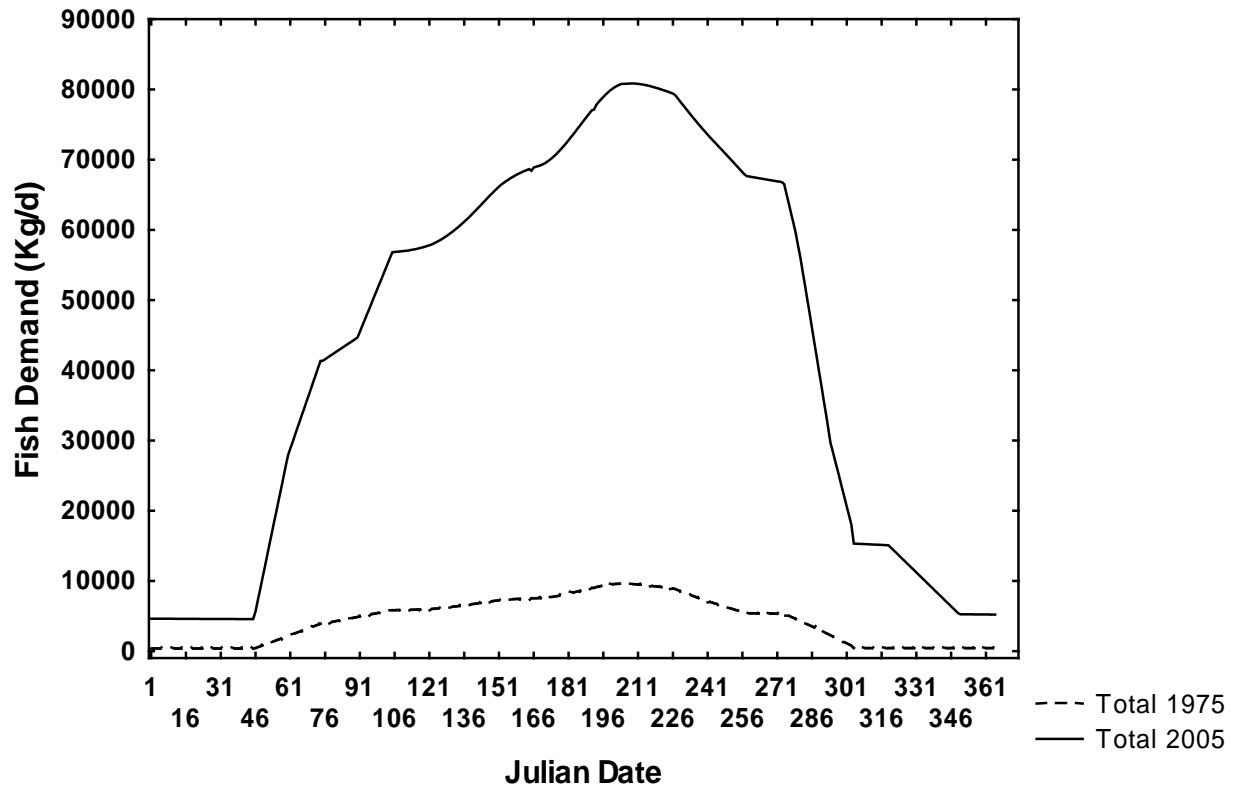
Figure 5. Comparison of projected seasonal fish demand between 1975 and 2005.
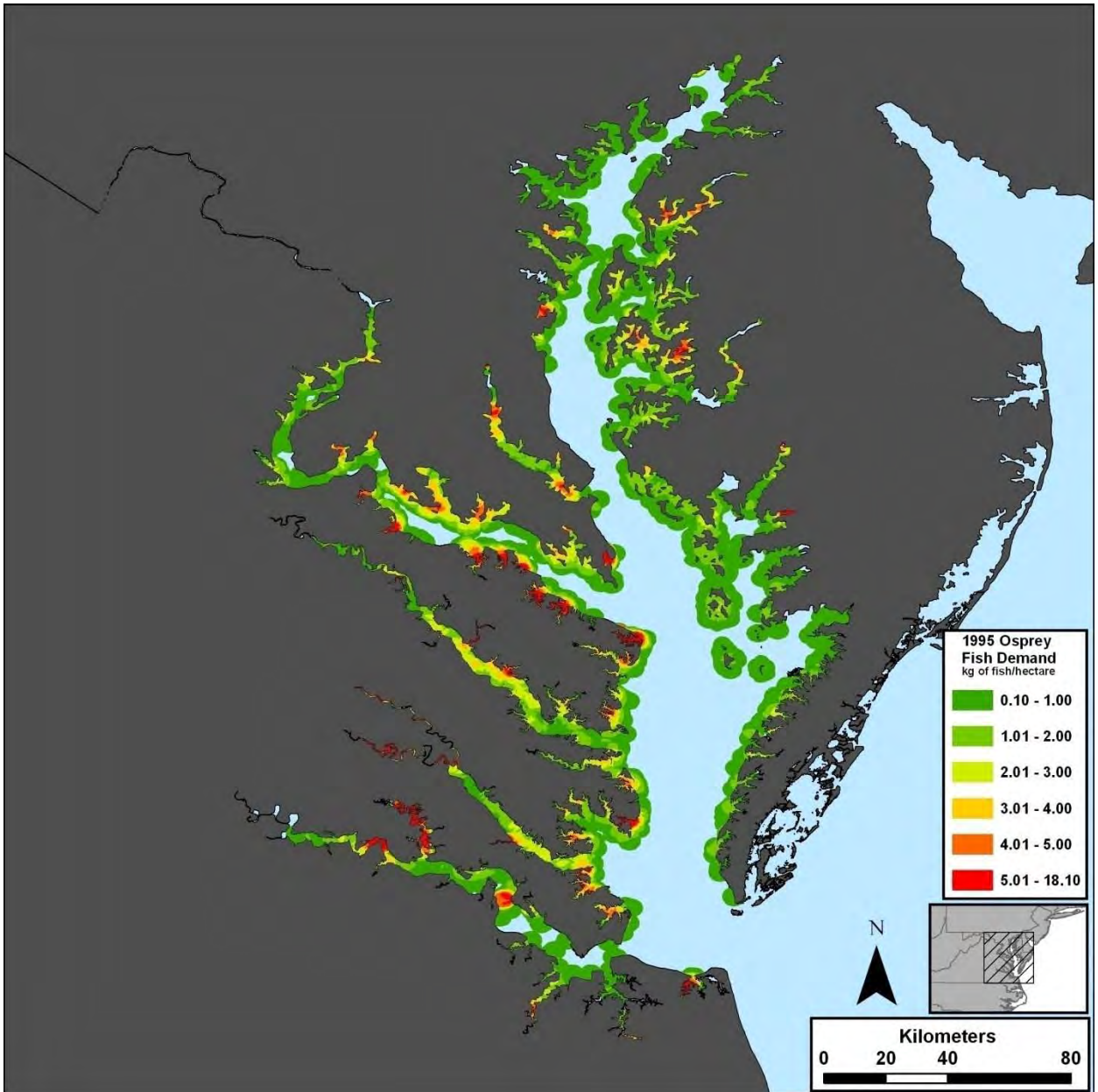
Figure 6. Distribution of projected fish consumption by Osprey throughout the Chesapeake Bay (1995).
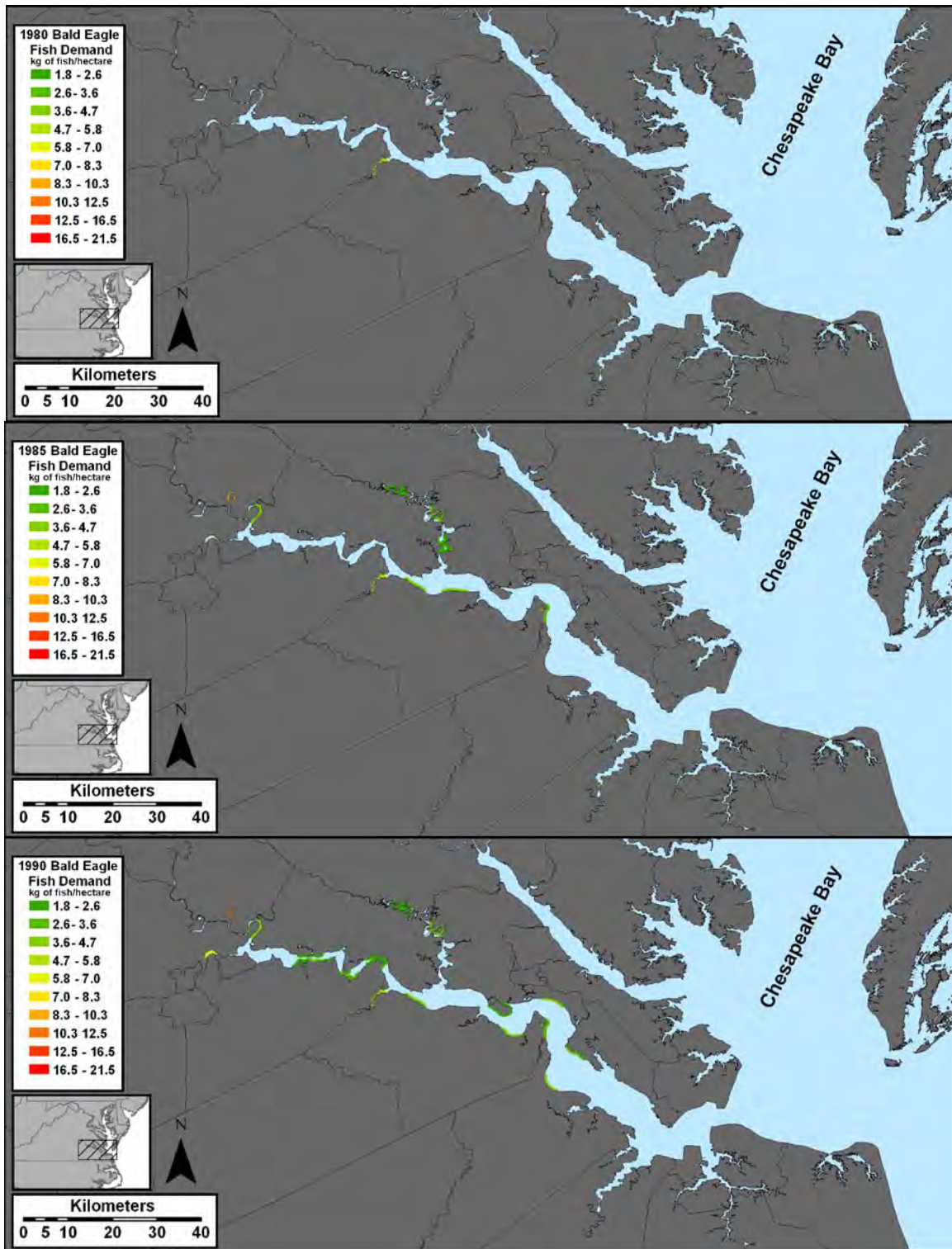
Figure 7. Time series of fish demand projections for Bald Eagles within the James River. Consumption has increased dramatically over this 25-year period, particularly within the tidal fresh reaches.
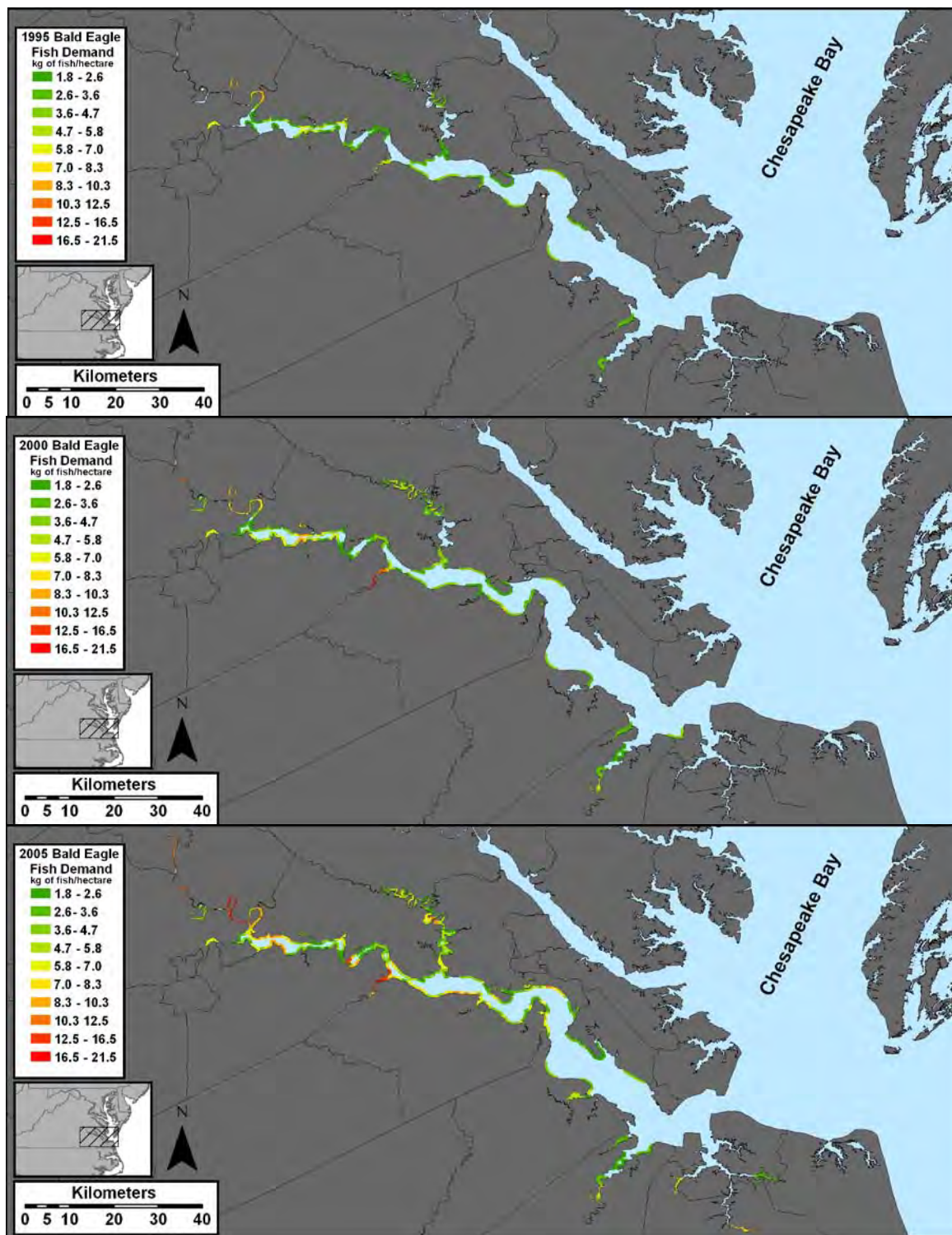
Figure 7 cont. Time series of fish demand projections for Bald Eagles within the James River. Consumption has increased dramatically over this 25-year period, particularly within the tidal fresh reaches.

Chapter 4: Stratification of Avian Consumption by Prey Species

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2008 Avian Diet Studies

For most piscivorous birds (e.g. Brown Pelicans, Osprey, Double-crested Cormorants, Great Blue Herons), comprehensive data on the taxonomic composition and size distribution of fish prey are lacking as inputs for consumption (energetic) models. We stratified estimates of avian consumption according to fish species by conducting avian diet studies.

In the spring and summer of 2008 (Project Years 2&3), we stratified diets of Osprey and Double-crested Cormorants nesting in tidal freshwater by species consumed. For Cormorants, we visited the colony on a weekly basis during the breeding season to collect pellets and partially digested prey remains that Cormorants regurgitated. We collected a total of 266 prey remains and 694 pellets. The prey remains were identified to the lowest taxonomic level possible. Preliminary analysis indicates that Cormorants consumed mostly hogchoker (*Trinectes maculatus*, 29% by number) and gizzard shad (*Dorosoma cepedianum,* 22% by number); however, analysis by biomass will likely show that Gizzard shad is a much more important prey item given the larger relative size of this species in the diet (Tables 1 and 2).

For Osprey, we attempted to identify species of fish that birds captured while we were making behavioral observations. Because of the distance between observers and foraging Osprey, we were not able to identify prey to species; however, data that we collected provides for a comparison to previous work completed on Osprey diet in the Chesapeake Bay (Table 2). In our sample of 138 observations, 4 were catfish (Ictaluridae), 51 were shad (Clupeidae), 1 perch (Percidae), and 82 were not identified. Of prey items identified, 7% were catfish and 91% were clupeids. When studying

Osprey diet in lower-saline tributaries of the Chesapeake Bay in 2006-2007, Glass and Watts (2009) used digital video cameras to record food items that Osprey brought to their nests. They found diets were composed of 52% catfish and 32% shad by number. When examining their data for just the James River near Hopewell, VA, Osprey diets included 50% catfish and 46% clupeids (Glass and Watts, 2009). The difference between Osprey diets in 2008 and 2006-2007 reflects either temporal shifts in diets of Osprey in the James River or differences attributable to our study methods.

In addition to describing Cormorant and Osprey diets, we also recorded locations where Cormorants and Osprey foraged, so we could describe the foraging distribution for each species. By determining distances that each species travel to forage, we can assess relationships between their prey and the bird populations. For example, the farthest that Cormorants moved to foraging locations during the breeding season was about 10km, which is 25-50% of the maximum distance that cormorants breeding on Lake Champlain travel from their colonies to forage (Duerr 2007). This difference is likely due to very high densities of blue catfish and gizzard shad present in that section of the James River (Table 1).

Table 1. Preliminary data on diet of Double-crested Cormorants from partially digested prey remains collected at the colony on the James River during the breeding season of 2008.

Species	Number Identified	Percent of diet by number	Number measured for backbone length	Average backbone length (mm)
American Eel	16	6.0	2	208.6
Blue Catfish	10	3.8	4	115.5
Blue Crab	4	1.5	0	
Bluegill	15	5.6	7	72.8
Catfish sp.1	8	3.0	1	171.3
Catfish sp.2	6	2.3	0	
Gizzard shad	59	22.2	24	124.8
Herring sp.	3	1.1	0	
Hogchoker	77	28.9	34	37.2
Largemouth Bass	1	0.4	0	
Atlantic menhaden	1	0.4	1	82.6
Threadfin shad	37	13.9	18	73.8
Unknown	9	3.4	0	
White Perch	20	7.5	13	53.4
Total	266		104	

Table 2. Relative contribution of prey taxa identified in Osprey diets within lower- and upper-estuarine sites in lower Chesapeake Bay during the 2006 and 2007 breeding seasons and along the James River in 2008 . Chi-square tests were conducted to detect significant differences in frequencies of occurrence between habitats samples in 2006-2007. Data from 2006-2007 are from Glass and Watts (In Press).

	2006-2007 LOWER		2006-2007 UPPER		2006-2007 OBSERVED VS. EXPECTED FREQUENCY		2008 JAMES RIVER	
SPECIES	N	% TOTAL	N	% TOTAL	χ^2	P	N	% TOTAL
Alewife	0	0.0	1	0.3	1.0	0.330		
Atlantic croaker	27	12.3	26	6.6	0.1	0.745		
Atlantic menhaden	53	24.2	6	1.5	39.9	<0.001		
Atlantic thread herring	5	2.3	0	0.0	5.3	0.022		
Bluefish	1	0.5	0	0.0	1.0	0.330		
Clupeidae	0	0.0	15	3.8	14.3	<0.001	4	2.9
Gizzard shad	9	4.1	110	28.0	80.7	<0.001		
Hickory shad	0	0.0	3	0.8	2.9	0.091		
Hogchoker	1	0.5	0	0.0	1.0	0.330		
Ictaluridae	0	0.0	203	51.7	192.8	<0.001	51	37.0
Largemouth bass	0	0.0	1	0.3	1.0	0.330		
Herring sp.	4	1.8	0	0.0	4.2	0.040		
Spot	19	8.7	0	0.0	20.0	<0.001		
Spotted seatrout	63	28.8	0	0.0	66.3	<0.001		
Striped bass	10	4.6	5	1.3	1.9	0.164		
Summer flounder	12	5.5	0	0.0	12.6	<0.001		
Threadfin shad	1	0.5	4	1.0	1.7	0.199		
White perch	2	0.9	8	2.0	3.3	0.069	1	0.7
Unindentified	12	5.5	11	2.8			82	59.4
TOTAL	**219**		**393**				**138**	

2008 Tidal James River Fish Community Assessment:

During the 2008 field season, concurrent with avian diet studies, the oligohaline and tidal fresh James River fish community was examined for structure and density of potential food items for feeding Double-Crested Cormorants. Fishes were examined using boat electrofishing techniques for accurate species identification, enumeration, and size ranges, as well as synoptic acoustic assessment of the community (Table 3). Where and when possible, these assessments were made immediately following observations of feeding birds. The general procedure was to move the boat-mounted echosounder over the bird-populated area, collecting data on fish density in the vicinity of avian predators. The echosounding boat was followed immediately by an electrofishing boat that sampled the same transect. After the initial run, an extended electroshocking transect was also sampled using low frequency settings to assess ictalurid populations more efficiently.

Hydroacoustic data were collected using a boat-mounted Biosonics DT-X Echosounder operating at a frequency of 430 kHz and maximum ping rate of 10 per second. A pulse duration of 0.4 ms and bandwidth of 5 kHz were used . Maximum target strength threshold was set at -70 dB, with single echo targets filtered within 0.8-1.2 of the echo pulse length. We used a split-beam transducer with a 10.3 degree beam width. Data from all water column targets were processed in real time by echocounting using BioSonics Visual Acquisition ver. 5.0 software. Fish densities and number of accepted targets were calculated by BioSonics Visual Analyzer 4 software.

The fish community was examined further for composition, density and size structure by synoptic, quantitative electrofishing immediately following the acoustic surveys. Fish were sampled using an 18-foot Smith-Root electrofishing boat. Pulsed direct current was employed using various frequencies and voltage output so as to maximize catch and as a function of water temperature, depth and conductivity. All fish stunned by electrofishing were netted and placed into a live well for recovery and processing. Fishes were identified, enumerated, measured for size class, examined for anomalies, and then released unharmed following Virginia Commonwealth University IACUC protocol AD20042. Additional electrofishing using low-frequency settings were used to capture ictalurid fishes that respond better to those specific techniques. In cases where stunned catfishes were too numerous to capture, floating fishes near the boat were identified visually and enumerated.

After initial calibration and test runs in the James River, transects were sampled weekly from mid-May through late June, 2008. The total numbers of targets identified in the area sampled are listed in Table 4. Figures 4 and 5 illustrate sampling area corresponding to recent Cormorant presence. Figure 6 is an example of a data file representation produced by the BioSonics Visual Analyzer software. Note various targets (singular) in the deeper regions of the channel as well as schools of smaller fishes (perhaps Atlantic menhaden) near the surface (clouded appearance). Fourteen fish species representing seven families were collected from the sample area (Table 4). The most abundant species were blue catfish, gizzard shad, Atlantic menhaden, and threadfin shad, respectively.

After adjustment for size (inclusive of potential fish prey <30 cm TL), the order of abundance from greatest to least is blue catfish, Atlantic menhaden, gizzard shad, and threadfin shad.

Comparison of electrofishing and hydroacoustic equipment suggests that in water of moderate depths (<6 m on average) the two gear types were comparable in sampling the same community. Electrofishing is inefficient in deeper water and therefore the number of targets (putative fish) detected by the hydroacoustic gear is generally greater in synoptic comparisons. Similarly, shallow water fish prey communities are sampled more effectively with electrofishing gear due to the inefficiency of hydroacoustic methods in shallow water (smaller transducer 'cone' samples less water and therefore reduced likelihood of target recognition). Further analysis of the hydroacoustic data will assess target strength as it relates to fish size and placement in water column to better refine species identification from the data and support comparison to avian diet data.

Table 3. Results from hydroacoustic and combined electrofishing sampling in James River, 2008. Number of targets acquired and number of fish captured from same transect are listed along with the three most abundant species under 30 cm total length. Numbers is parentheses are results of low frequency electrofishing.

Date	Area	Number of Targets	Number of Captures	Dominant species <30 cm
16 May 2008	Buoy channel	94	0	n/a
23 May 2008	Kimages	262	249	BTY,DCE,IFU
23 May 2008	Powell's	506	75	DCE,DPE,BTY
23 May 2008	Tar Channel	669	72	IFU, DCE
23 May 2008	Berkeley	23	269	BTY,DCE,DPE
27 May 2008	Triangle	162	159	DPE,DCE,MBE
27 May 2008	Marina	49	25	IFU, BTY
27 May 2008	Tar West	98	72	DCE,BTY, IFU
3 June 2008	Rice upstream	35	131	DPE,DCE,IFU
3 June 2008	Marina	155	64	BTY,DPE,DCE
3 June 2008	Colony	238	523 (500)	IFU, DPE,DCE
9 June 2008	Bridge channel	983	63	DCE,DPE, IFU
9 June 2008	Triangle	59	52	DCE,BTY, IFU
9 June 2008	Colony	382	244 (200)	DCE,DPE
17 June 2008	Kimages	25	0	n/a
17 June 2008	Main channel	1068	0	n/a
17 June 2008	Marina flats	130	0	n/a
26 June 2008	Colony	516	217 (152)	IFU,DCE, BTY
26 June 2008	Allied up	822	340 (295)	IFU,BTY,DCE,
26 June 2008	Dredge Island	178	91 (3)	DCE,BTY, IFU

BTY - *Brevoortia tyrannus;* DCE - *Dorosoma cepedianum;* IFU - *Ictalurus furcatus*
DPE - *Dorosoma petenense;* MBE - *Menidia beryllina*

Table 4. Fishes collected by boat electrofishing (normal and low-frequency) from the tidal James River between Powell's Creek and Hopewell, Virginia. Numbers represent catch at all sites and all collections combined.

Species	Common Name	Total Catch	Total Catch <30cm
Ictalurus furcatus	blue catfish	944	512
Dorosoma cepedianum	gizzard shad	553	330
Brevoortia tyrannus	Atlantic menhaden	454	454
Dorosoma petenense	threadfin shad	170	170
Menidia beryllina	inland silverside	21	21
Morone saxatilis	striped bass	12	8
M. americana	white perch	4	4
Notropis hudsonius	spottail shiner	2	2
Alosa sapidissima	American shad	2	2
A. aestivalis	blueback herring	1	1
Lepisosteus osseus	longnose gar	29	0
Cyprinus carpio	common carp	5	0
Anguilla rostrata	American eel	1	0
Pylodictis olivaris	flathead catfish	1	0

Figure 4. Area of sampling (hydroacoustic run and electrofishing) above the Benjamin Harris bridge, James River, Virginia.
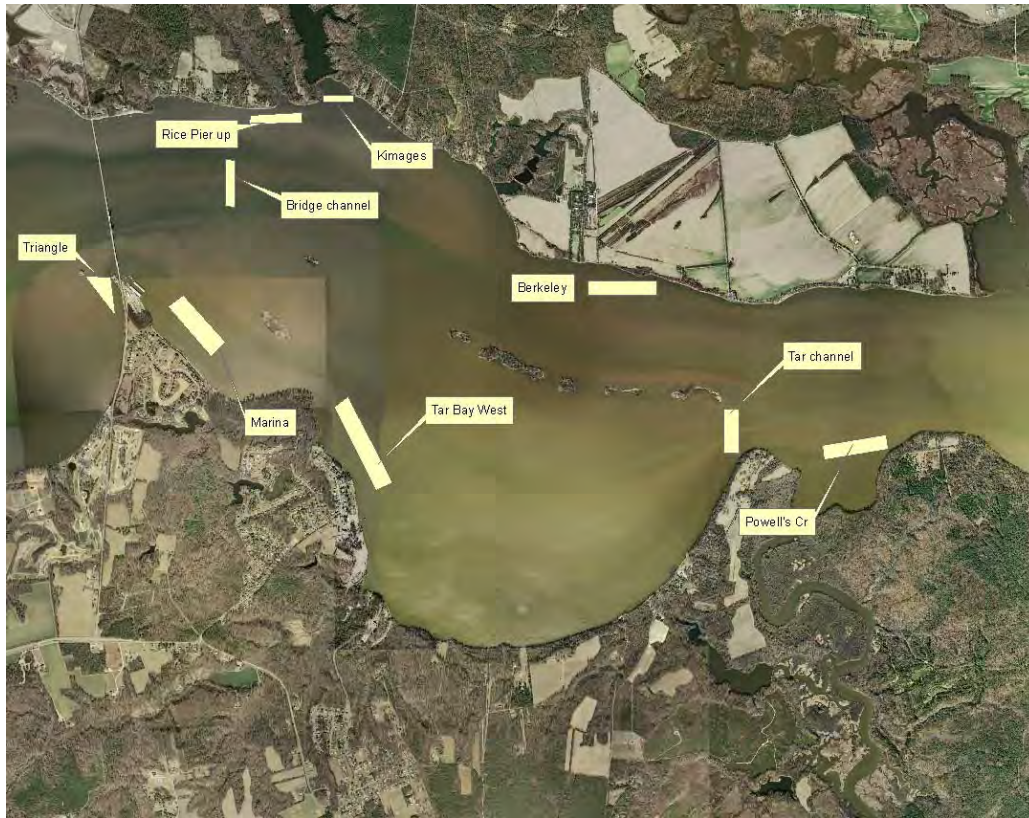
Figure 5. Area of sampling (hydroacoustic run and electrofishing) below the Benjamin Harris bridge, James River, Virginia.
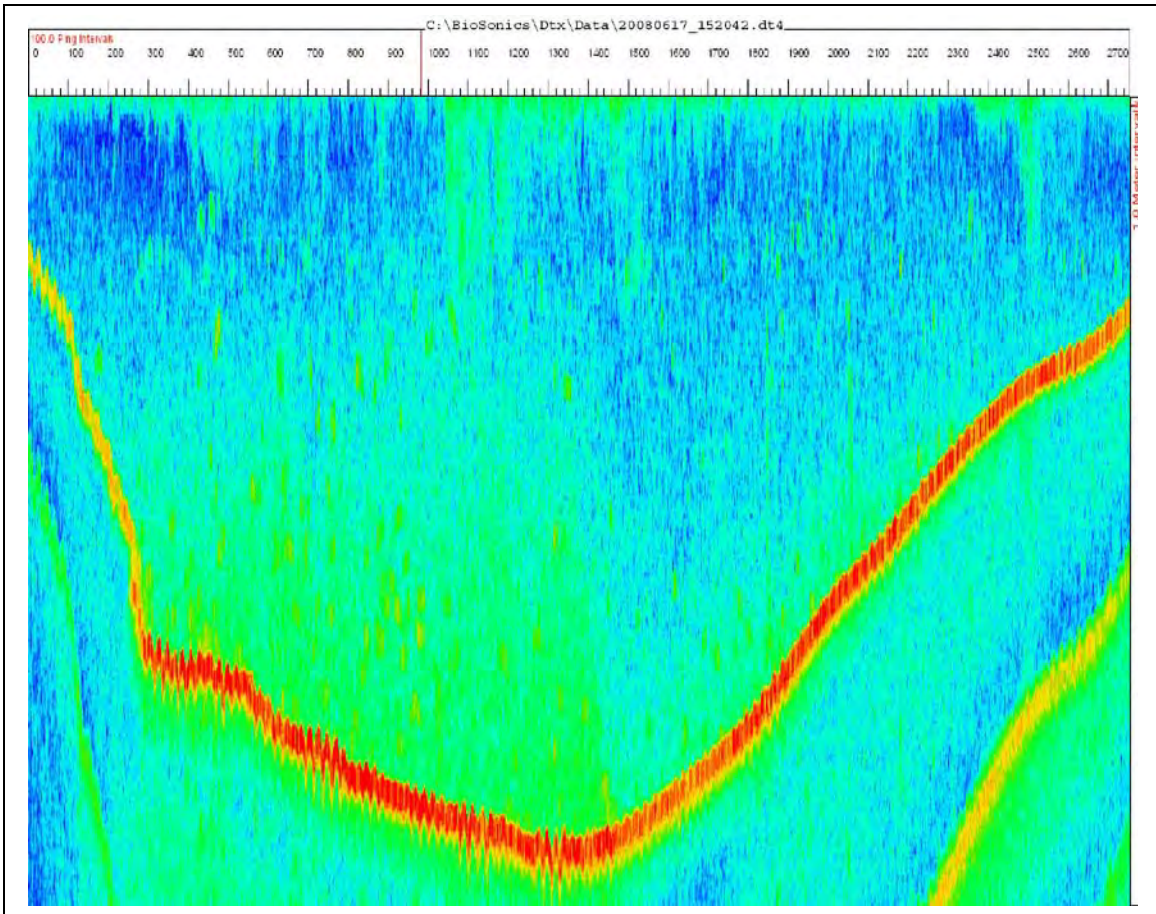
Figure 6. Example Echogram display representing the main James River channel. Red line represents the bottom. Visual Analyzer software identified a total of 1,068 targets (i.e., fish) from the associated data file.

2009 Avian Diet Studies

Diet sampling at nesting colonies.

In 2008 we targeted Osprey and Double-crested Cormorants; specifically, the nesting colonies located in tidal freshwater portions of the James River, VA where synoptic avian diet studies and fish community sampling demonstrated that Osprey and Double-crested Cormorants foraging in tidal freshwater nursery habitats were not targeting YOY menhaden in spite of YOY menhaden being extremely abundant and available. In 2009, diet studies were expanded to further stratify avian demand and consumption for Double-crested Cormorants and Brown Pelicans in selected locations of the mainstem Chesapeake Bay: 1) Cormorants and Pelicans breeding on Shanks Island located along the southern end of the Smith Island, Accomac County, Virginia; and 2) Cormorants breeding on Poplar Island, Talbot County, Maryland.

We assessed diets by systematically walking through colonies and recording fish regurgitated by Cormorants and Pelicans (Fig's. 7 – 12). Visits were scheduled to coincide with the time period that young nestlings were present in the breeding colonies. During this time, Cormorant and Pelican nestlings remain in or very close to their nests and regurgitate food when disturbed. For fish that could be attributed to the bird species that regurgitated it, we recorded the species, number and size (recorded in 5cm intervals). We revisited colonies about every 10 days until cormorants and pelicans were 4-5 weeks old. After this age, cormorant and pelican nestlings become mobile in nesting colonies, limiting our ability to attribute regurgitant samples to individual birds, and increasing aggressive interactions between these species. When nestlings reached 4-5 weeks old, we

captured them by hand and collected tissue samples (see Species Specific Biomarkers section below).

In contrast to results from 2008 that found Double-crested Cormorants and Osprey were not preying on abundant and available YOY menhaden in tidal freshwater, preliminary 2009 results (Figure 11) of the diet of cormorants and pelican indicate that sub-adult (age 1 and 2) Atlantic menhaden and bay anchovy were the most numerous fish prey consumed during the six week study period. However when count data are converted to biomass estimates, Atlantic menhaden dominate species consumed, followed by spot, croaker, and bay anchovy (Figure 12).

Figure 7. Project personnel conducting prey sampling in pelican and cormorant colony, Smith Island, Maryland.

Figure 8. Pelican hatchlings and anchovy prey, Smith Island, Maryland.

Figure 9. Menhaden on cormorant nest, Smith Island, Maryland.

Figure 10. Cormorant chicks in colony, Smith Island, Maryland.

Cormorant Diet (Poplar Island)
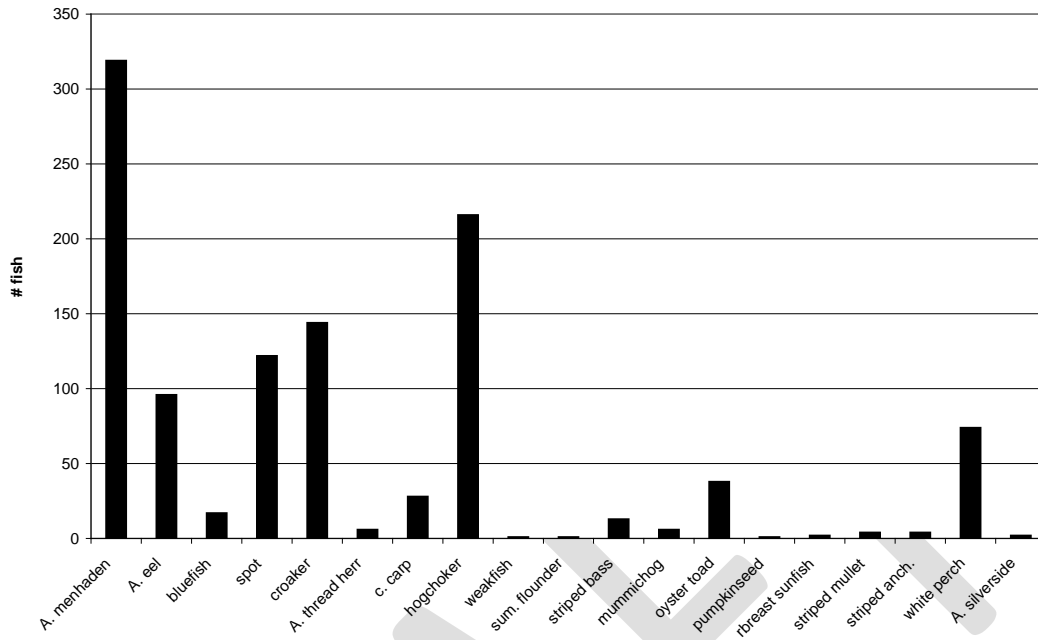
A.

Brown Pelican Diet (All Smith Colonies Combined)
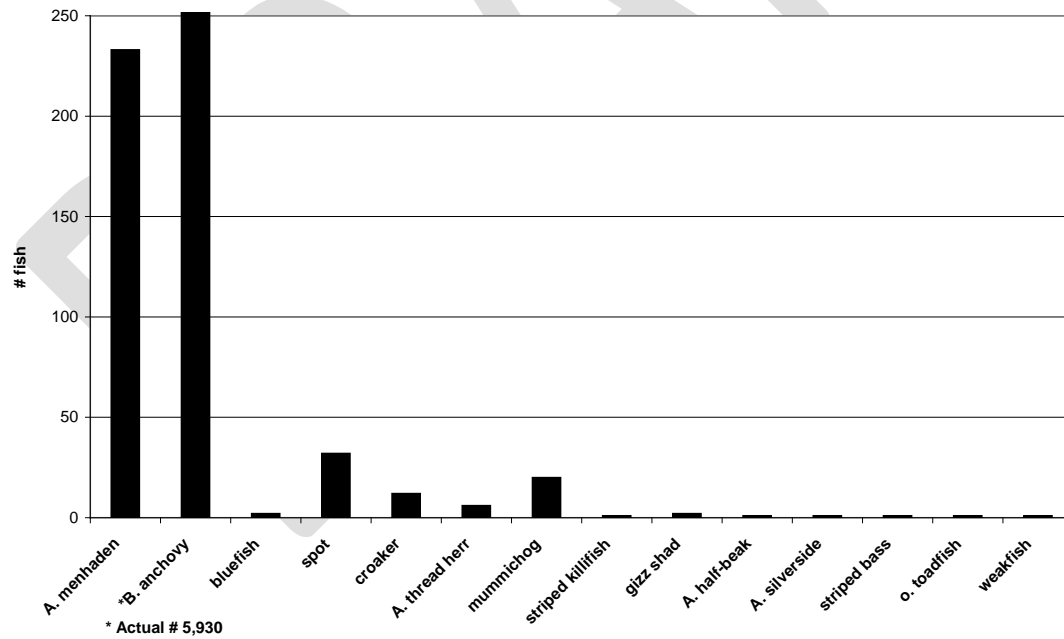
B.

Figure 11. Diet composition by total number of specimens for A) Double-crested Cormorants and B) Brown Pelicans nesting on Smith and Poplar Islands, Maryland.

Cormorant Diet Composition
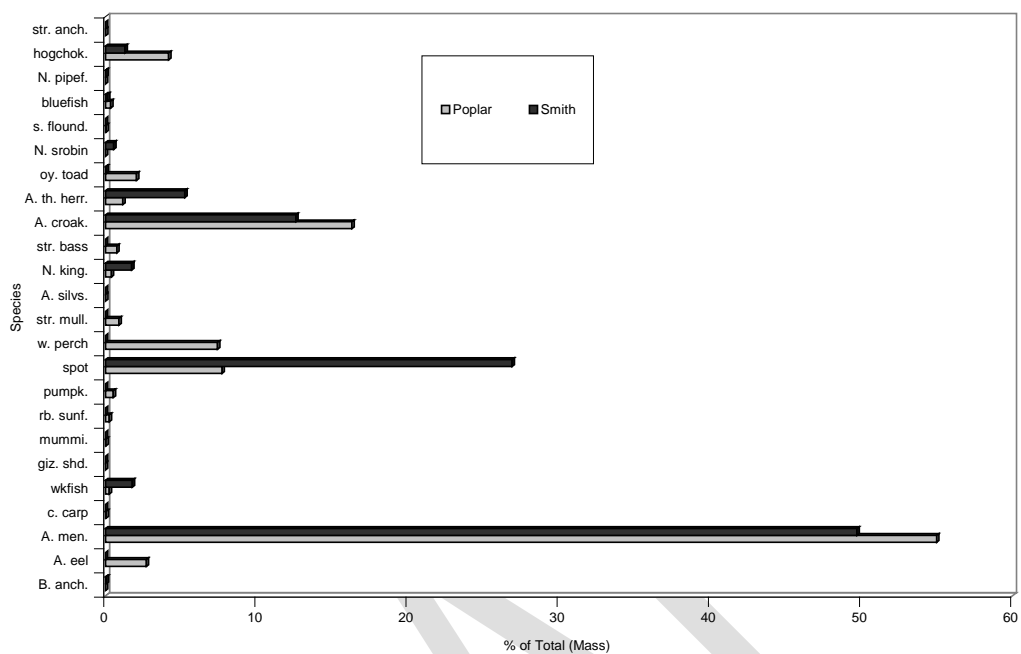
Smith Island Pelican Diet Composition
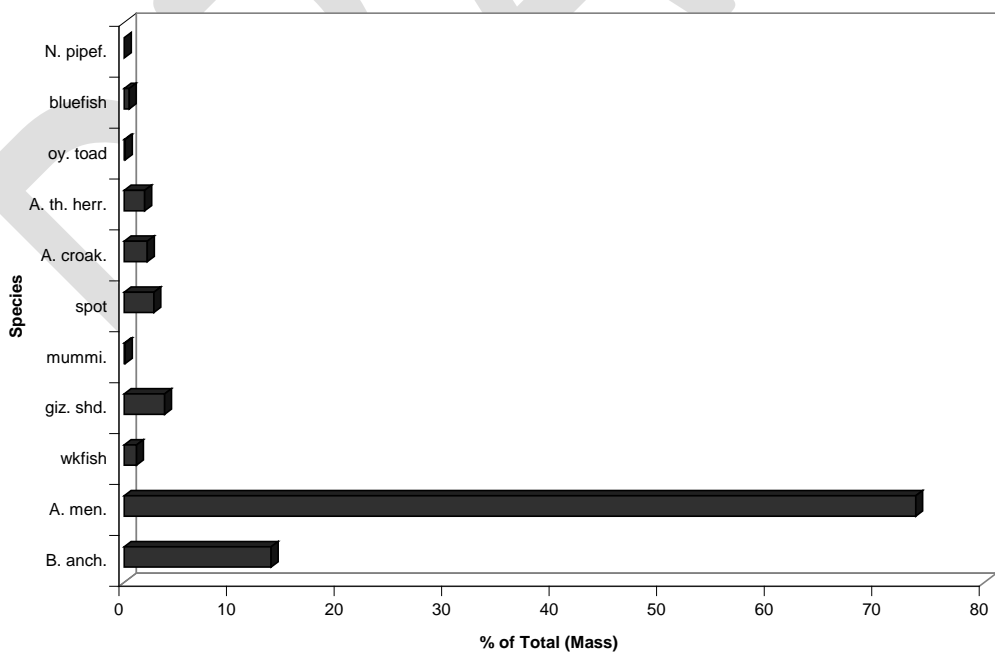
Figure 12. Diet composition by percent of total biomass for A) Double-crested Cormorants and B) Brown Pelicans nesting on Smith and Poplar Islands, Maryland.

Prey availability studies: Comparison of estimated consumption of menhaden by avian and fish piscivores

Although the project team had hoped to analyze fish availability using synoptic avian diet and fish hydroacoustic surveys, foraging observations of pelicans and cormorants in the upper Bay indicated that foraging ranges around targeted colonies were too large to adequately sample using boat mounted hydoacoustic equipment. In fact, pelicans forage in both the Bay and the Atlantic.

However, recent analyses by J. Uphoff of fishery landings and estimated striped bass consumption indicate that menhaden consumption by striped bass and exploitation by commercial fisheries combined is several orders of magnitude *greater* than the estimated *total metabolic demand* of the five largest avian piscivores (Bald Eagle, Osprey, Great Blue Heron, Brown Pelican, Double-crested Cormorant, Figs. 13 and 14, Table 5). The estimated striped bass consumption is based on aggregate biomass models that incorporate both catch and predation functions (biomass dynamic with a type 3 predator-prey function for striped bass and menhaden). The estimated fish demand by avian piscivores was based on a bioenergetics approach combining a multi-stage population model with a breeding model and applied allometric relationships between field metabolism and body mass to estimate annual demand across years and daily demand within years (see Chapter 3). Bay-wide survey data was used to parameterize the population model and 28 general, nesting, feeding, and demographic parameters were used to develop the breeding models (for more bioenergetics model see Garman *et al.* 2007).

So while the relative impact due to continued exponential growth of avian populations in the Bay is likely to increase the relative importance of avian predation on menhaden in the future (Fig. 13), conversely, falling menhaden stocks may negatively impact population growth in some avian species. Dr. Bryan Watts has linked dramatically declining condition and reproductive success by populations of Osprey breeding in high salinity regions in the Chesapeake Bay to declines in menhaden stocks over the last several decades. The proportion of menhaden in the diet of populations of Osprey occupying lower estuarine locations (> 18 ppt) locations has declined from 75% in the 1980's to 25% in 2006 (Glass and Watts 2009). Over the same period Osprey population growth, reproductive output, and nestling growth rates in those sites has declined to levels close to those recorded during the period when Osprey reproduction was negatively impacted by organochlorine (DDt) contamination (Watts and Paxton 2007). In contrast, eagle and osprey condition and reproductive success has surged in tidal freshwater habitats which is also linked to fish prey availability. Fish prey consumed by Osprey in the high salinity, lower estuary (> 18 ppt.) were smaller in size and 40% lower in energy content than fish prey consumed by Osprey occupying the upper tidal fresh estuary (> 5 ppt, see Watts and Paxton 2006 and Glass and Watts 2009, Appendices 3 and 4)).

All Species
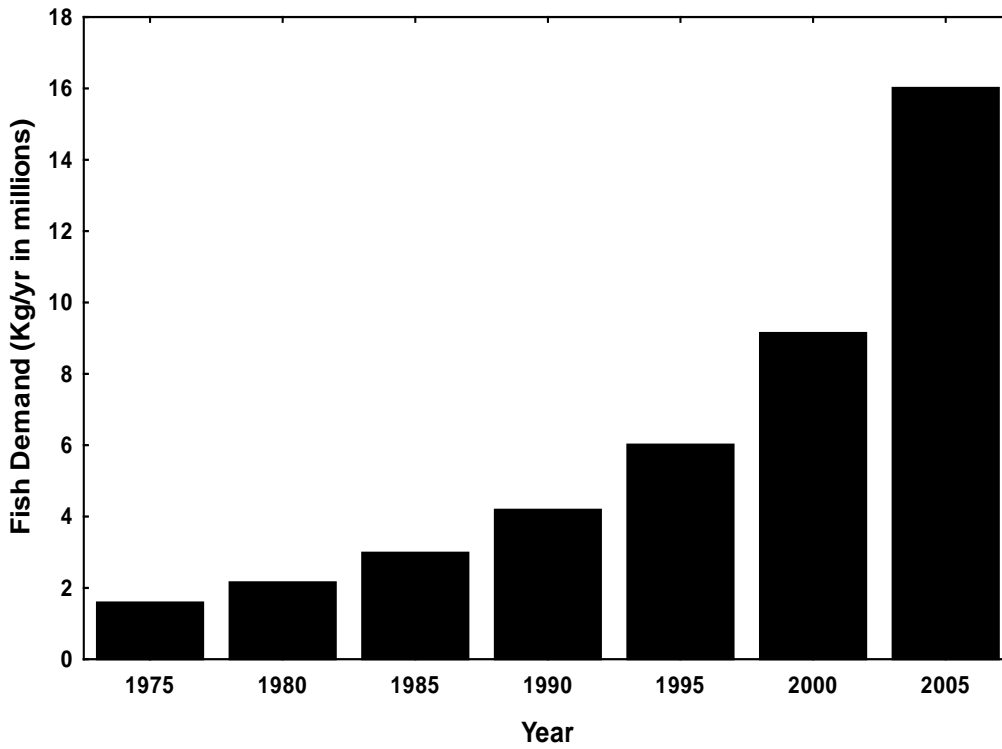
Figure 13. Long-term trend in fish demand for all fish-eating bird populations combined (1975-2005). Projected demand has grown exponentially with an average doubling time of 9 years.

Table 5. Breeding populations (in breeding pairs) of fish-eating birds and estimated fish demand (kg of fish) in the Chesapeake Bay (1975-2005)

	GBHE	OSPR	BAEA	DCCO	BRPE
Population					
1975	2,163	1,564	70	0	0
2005	16,950	4,888	854	4,417	3,528
Fish Demand					
1975	1,042,441	389,286	156,357	0	0
2005	8,168,920	1,216,646	1,907,562	2,504,407	2,217,099

Fig. 14. Comparison of estimated total metabolic demand of avian piscivores in the Chesapeake Bay, estimated striped bass consumption of Atlantic menhaden, and Fishery landings of Atlantic menhaden in the Chesapeake Bay.

Chapter 5: **SPECIES SPECIFIC BIOMARKERS FOR ATLANTIC MENHADEN AND OTHER TARGET FISHES**

Atlantic menhaden are a critical food resource for the temporally and spatially dynamic guild of fish, bird, and mammalian predators along the Atlantic Coast (Rogers and Van Den Ayvle 1989; Munroe and Smith 2000) and represent an important link in the coastal marine food chain, affecting the conversion and exchange of energy and organic matter within coastal marine systems (CFEPTAP 2004). Atlantic menhaden stocks also support one of the most valuable commercial fisheries in the region, representing nearly 40% of total Atlantic coast landings by weight since 1980 (Munroe and Smith 2000). Abundance (biomass) of Atlantic menhaden stocks was low in the 1960s, increased rapidly in the early 1970s and remained relatively high through 1980s. Abundance thereafter declined and reached an asymptotic low in the mid-1990s and has remained at these levels through 2006. Low available biomass of Atlantic menhaden in Chesapeake Bay has been linked recently to declines in growth and reproductive success of economically and ecologically important piscivores that are dependent on menhaden for forage. These putative impacts include decreased feeding success and condition of striped bass in Chesapeake Bay (Uphoff, 2003b) and decreased reproductive success of Osprey (Viverette *et al.* 2007, Glass and Watts 2009).

Atlantic menhaden contribute substantially to the diets of fish-eating birds (Watts *et al.* 2006, McLean and Byrd 1991) such as Osprey, Brown Pelicans, Bald Eagles, and Cormorants. Since the end of the DDT era in the early 1970s, piscivorous bird populations have increased exponentially throughout the tidal (freshwater and polyhaline) reaches of the Chesapeake Bay. In less than 30 years, Osprey increased from 1,400 pairs to 3,500 pairs (Watts *et al.* 2004) and the Chesapeake Bay now supports the largest

breeding population in the world. Diet studies of Osprey within the Chesapeake Bay, conducted in higher salinity regions during the mid-1980s showed that Atlantic menhaden comprised 75% of nest deliveries to Osprey nests on the mainstem Bay and the mouths of tidal tributaries (McClean and Byrd 1991). Coastal Osprey populations in New England (Poole 1989), coastal New Jersey (Steidl *et al.* 1991), and the Delaware Bay (Steidl *et al.* 1991b) also relied heavily on Atlantic menhaden during the 1980s. However by 2006, Atlantic menhaden comprised only 25% of Osprey diets in the higher salinity (>18 ppt) portion of the Chesapeake Bay (Glass and Watts 2009). The decline in menhaden as a portion of Osprey diet may reflect Atlantic menhaden stocks declining in the Chesapeake Bay, a trend that started in the early to mid-1980's (Uphoff 2003). The decline coincides with the first evidence of brood reduction and sibling rivalry recorded in Chesapeake Bay Osprey populations (McClean and Byrd 1991). Similar evidence of food stress was not apparent a decade earlier (Stinson 1977) when Atlantic menhaden stocks comparatively larger (Uphoff 2003).

Piscivorous birds are used widely as a sentinel species for tracking ecosystem health elsewhere (Steidl *et al.* 1991a and b, Elliot *et al.* 2002, Henny *et al.* 2003) and may be useful indicator species for fishery status and trends in the Chesapeake Bay. Grove *et al.* (2009) recently proposed Osprey as a worldwide sentinel species due to their position on the food web, their widespread distribution, and accessibility of nests. Tracking Atlantic menhaden contributions to the diets of a sentinel avian predator like Osprey may provide a unique, cost-effective, and independent tool for consistent, integrated, and long-term monitoring of Atlantic menhaden stocks in the region. Specifically, the development,

testing, and application of new monitoring protocols based on stable isotope analysis of feathers from avian predators to track Atlantic menhaden population trends would meet the Atlantic States Marine Fisheries Commission's 5 mandate to "…develop novel methodologies for stock assessment including fishery-independent surveys and variable natural mortality at age or by area" (ASFMC 2001). Sentinel species are typically used to empirically assess bioavailability and concentration of contaminants; however avian predators may also represent a fishery-independent source of distribution and relative abundance data for forage fishes such as menhaden, using isotopic markers for target fishes extracted from renewable Osprey tissues (e.g. blood, feathers, uropygeal oils).

3) Isotope Biogeochemistry of Atlantic Menhaden Lipids:

Stable isotope analysis as an analytical tool:

A large number of laboratory and field studies have demonstrated that the stable isotopic ratio of an organism's diet is consistently and reliably reflected in the isotopic ratio of consumer tissues (Macko *et al.* 1982, Tieszen *et al.* 1983, Macko *et al.* 1987). Consistent isotopic differences among marine, freshwater, estuarine, and pelagic environments are reflected throughout the food web and can be utilized to indicate a consumer's relative reliance on different prey resources (Michener and Schell, 1994). The isotopic composition of a consumer integrates that organism's assimilated diet over time (Ostrom and Fry 1993, Michener and Schell, 1994), in contrast to traditional methods of diet determination such as stomach content analysis and visual observations, which reflect recently ingested foods only. However, although bulk δ^{13}C, δ^{15}N, and δ^{34}S isotope analyses may be valuable tools (e.g. see Chapter 2), their application may be limited. In particular, bulk isotopic methods have a limited ability to elucidate the specific composition of consumer diets. One technique that does allow for the determination of specific prey items is fatty acid signature analysis (FASA; Iverson *et al.* 1997, Kirsch *et al.* 1998, Logan *et al.* 2000). This novel approach allows researchers to trace a specific fatty acid from prey to consumer and provide specific information about diet composition. FASA has not yet been applied to the study of eagles or ospreys, but it has been successfully used in dietary studies of other predators (Smith *et al.* 1996, Iverson *et al.* 1997, Worthy and Abend, 1998).

Compound specific isotope analysis (CSIA):

Compound specific isotope analysis (CSIA) of fatty acids has never been applied to the study of diets of eagles or ospreys; similarly, this technique has not been applied to the assessment of Atlantic menhaden and American shad stocks. This study used a novel CSIA

technique— fatty acid signature analysis (FASA)—in an attempt to establish relationships between observed molecular distributions and isotopic compositions of tissues from target predator and prey species. By comparing the isotopic compositions of fatty acids obtained through CSIA, consistent differences in the types and concentrations of various fatty acids, as well as their respective isotopic values, will potentially provide a valuable means for identifying unique, species-specific compounds that could be used to elucidate ecosystem-based foodwebs.

Although FASA can provide information about specific diet composition that is often unclear based on bulk isotopic measurements, the assessment of diet with this technique may still provide ambiguous results. The technique employed in this study, compound specific isotope analysis (CSIA) expands the capabilities of this technique by coupling analysis of the fatty acid signatures in prey and consumers with consequent analysis of the isotopic composition of these fatty acids (Macko, 1994). Knowledge of the isotopic value of the fatty acids greatly improves resolution of the specific diet composition. Previous studies have utilized CSIA to examine diet and nutrition in ducks (Hammer *et al.* 1998), mollusks (Pond *et al.* 1998), and shrimp (Pond *et al.* 2000). No published study has examined Bald Eagle or Osprey feeding ecology using CSIA and the technique has the potential to track over time the energetic contribution of specific prey species (e.g. menhaden) using non-invasive sampling (e.g. feathers).

Methods

Methodologically, this technique involves extraction and saponification of the fatty acids from the tissues, followed by esterification of the fatty acids into methyl esters (FAMEs). The FAMEs are chemically analyzed (GC/MS) as well as characterized for their stable carbon isotope compositions using a gas chromatograph interfaced through a combustion furnace with isotope ratio mass spectrometer (GC/C/IRMS; Ballentine *et al.* 1996). The FAMEs are chemically

characterized with a Hewlett Packard 5890 Series II gas chromatograph equipped with an HP-1 (30m x 0.2 mm i.d.) column interfaced to a Hewlett Packard 5971A mass selective detector (GC/MSD). Compound-specific carbon isotope data is obtained using a GV Isoprime isotope ratio mass spectrometer (IRMS) and a Hewlett Packard 5890 Series II gas chromatograph equipped with the same column as above and coupled through a combustion furnace and water trap to the Isoprime. Samples will be injected five times for reproducibility and precision. A compound of known isotopic composition (naphthalene, $\delta^{13}C$ -26.3‰ ± 0.1) is coinjected with the samples for calibration and evaluation of the combustion furnace. Fractionation resulting from the methylation of the fatty acids and the kinetic isotope effect associated with esterification is determined using pure fatty acid standards (Ballentine *et al.* 1996). The amount of isotopic alteration depends on chain length, and can be corrected accordingly. Carbon added during the esterification is isotopically identical to the parent methanol (Abrajano *et al.* 1994. Therefore, the $\delta^{13}C$ values for the fatty acid compounds are calculated by a simple mass balance equation:

$$\delta^{13}C_{FAME} = (x)\delta^{13}C_{FA} + (1-x)\ \delta^{13}C_{Methanol}$$

where $\delta^{13}C_{FAME}$ is the carbon isotopic value for the fatty acid methyl ester, $\delta^{13}C_{Methanol}$ is the isotopic value for the methanol, $\delta^{13}C_{FA}$ is the corrected value for the fatty acid, and x is the fraction of carbon contributed by the fatty acid.

Results:

Isotopic variation in Atlantic menhaden and American shad

The initial period focused on two questions *re*: lipids in Atlantic menhaden. First, what is the isotopic variability of the lipids in a population of Atlantic menhaden and second, can a relationship be discerned between the chemistry and isotope signatures of the major fatty acids extracted from menhaden prey and those of higher trophic level

consumers, including birds? To address the first question, a random sample of Atlantic menhaden (samples provided through the cooperation of Omega Protein, Reedville, Virginia) from the lower Bay, and representing a variety of sizes (ages), were measured, lyophilized, and Soxhlet extracted with a methanol:dichorobenzene azeotropic mixture to remove lipids. The residues were dried and isotopically characterized for ^{13}C and ^{15}N. The solvents from the extraction were distilled off, and the lipid residue was also isotopically assessed for ^{13}C. Portions of lipid extracts from other prey fish species and probable predators were saponified, and derivitzed to methyl esters, which allow for the chemical (using gas hromatography/mass spectrometry; GC/MS) and gas chromatography/ combustion/ isotope ratio mass spectrometry (GC/C/IRMS) for compound specific isotope analysis of major fatty acid compounds. Isotope compositions were corrected for the methyl ester addition to the fatty acids.

The bulk isotope ^{15}N compositions of Atlantic menhaden samples changed with increasing size and (putative) age. However, no trend was observed with tissue ^{13}C, and, in fact, the lipid extracts (Fig 1) show remarkable consistency across the size (age) ranges of the Atlantic menhaden samples. This result suggests that the Atlantic menhaden, while varying diet to some degree, (reflected in the ^{15}N abundances) obtain their lipid chemical and isotope signature from a more uniform source, and this signature can be resolved from other potential lipid sources because of its relative proportion of the total lipid pool. Furthermore, a preliminary compound-specific, isotopic characterization (CSIA) of other potential prey fish species and predators showed variability in the fatty acid isotope signals (Fig. 2). The potential predators of the marine fish had isotope signatures of both saturated and unsaturated fatty acids (especially 16:0, 16:1, 18:0, 18:1) that are nearly

identical to the marine prey fish, while more depleted fatty acid signatures were seen in the prey fish more influenced by terrigenous sources. The results suggested that fatty acid CSIA signatures from Atlantic menhaden can potentially yield a discrete (i.e., diagnostic) chemical and isotope signature that could be identified in the lipid extracts of predatory birds (e.g. Osprey feathers).
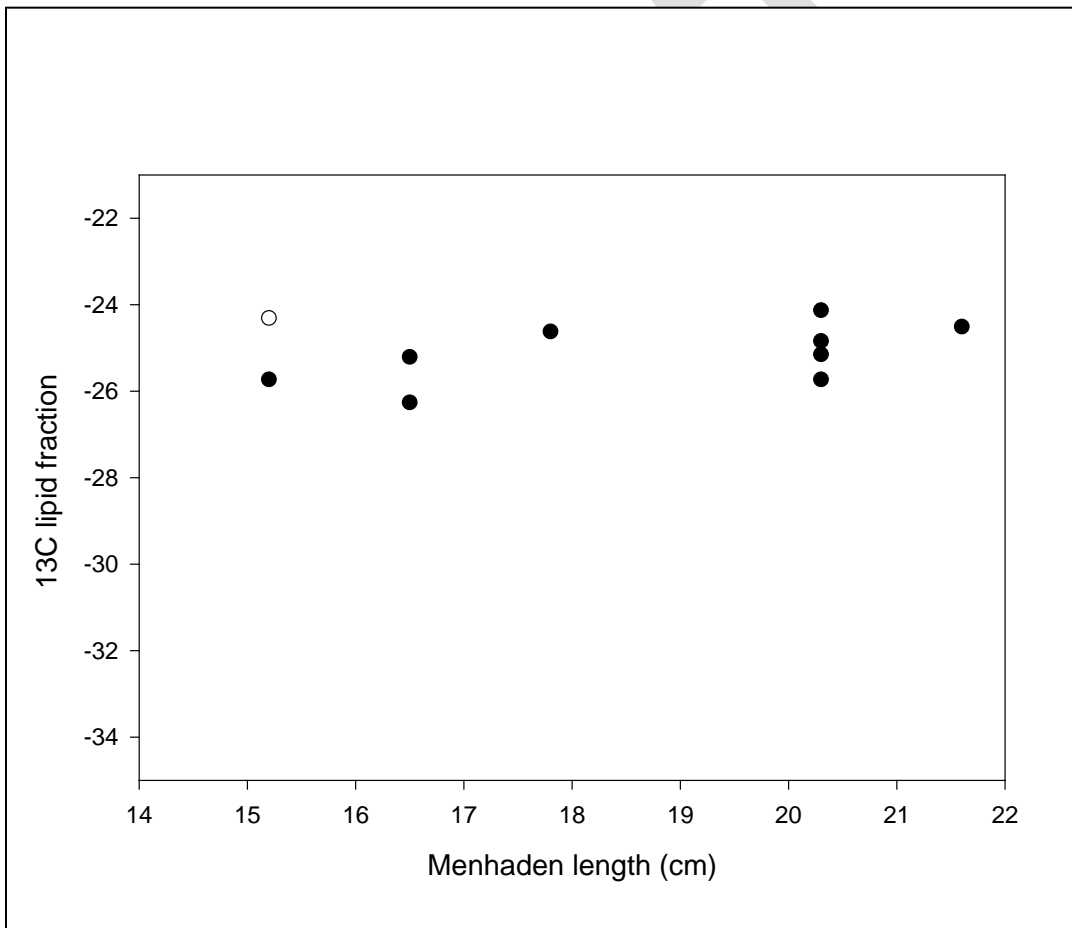
Figure 1. Carbon Stable Isotope results from Atlantic menhaden of different lengths. Fish were collected in the Chesapeake Bay and its tributaries in October 2006

Menhaden 1

C12:0   C14:0   C16:1; C16:0   C:18:3; C18:2; C18:1; C18:0

Atlantic menhaden
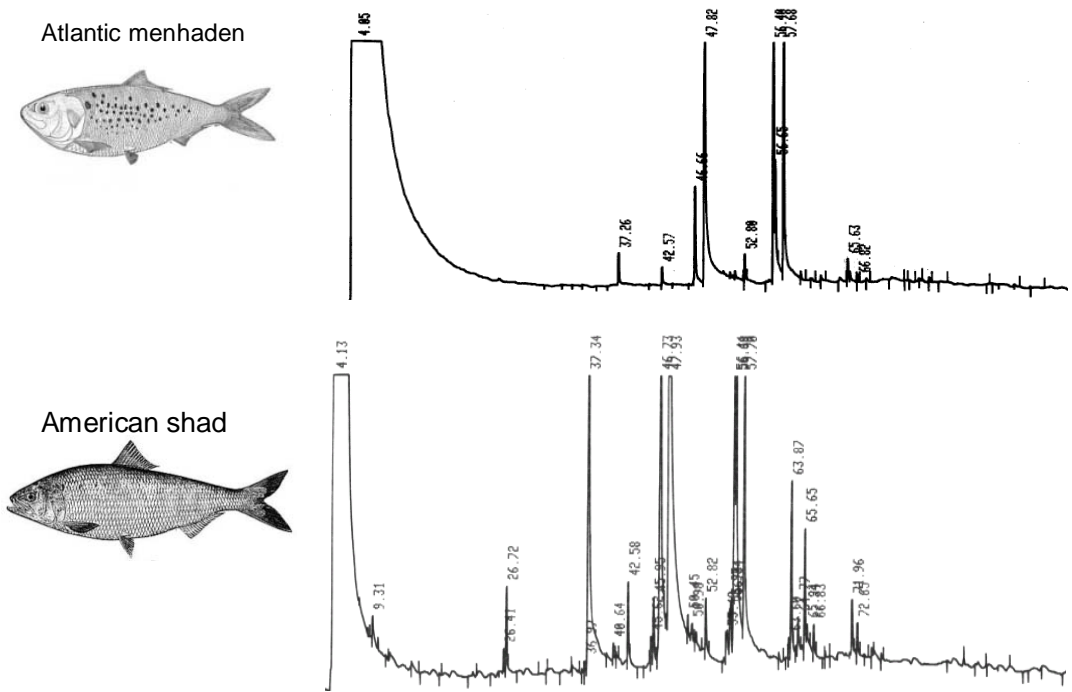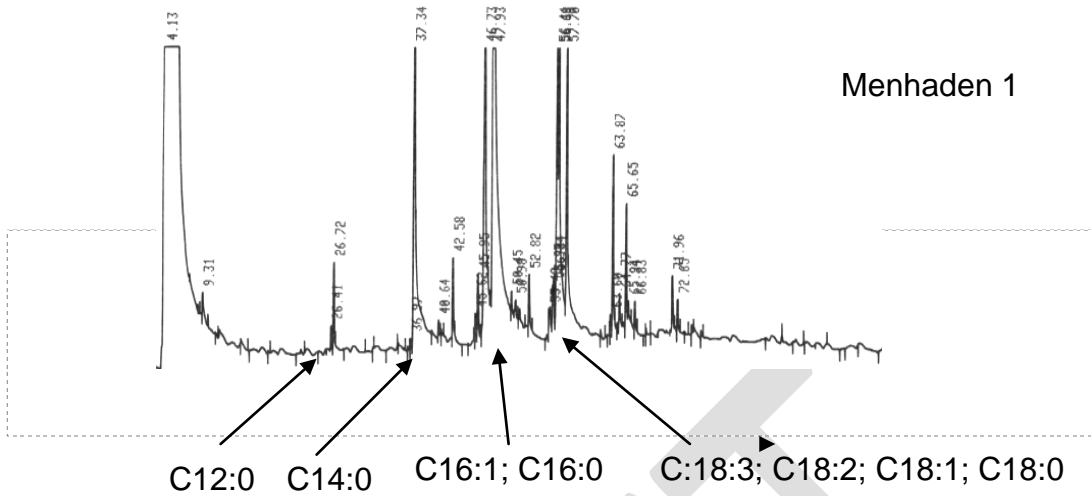
American shad

Figure 2. Atlantic menhaden and American shad Fatty acid characterization (GC/MS)
Compound-specific, stable isotope analysis (CSIA)

Feeding experiments:

In 2009, in order to test if biomarkers accurately reflected the proportion of Atlantic menhaden and American shad in the diet of local Osprey populations, tissue samples from Osprey consuming a known quantity of target fish species were needed. To this end, Osprey nests in tidal freshwater James River were assigned to experimental (supplemented with menhaden or shad) and control (not supplemented) groups (Figure 3). A total of 42 Osprey nests along a transect from Turkey Island Cut downstream to Sturgeon Point were monitored from May 15 through August 3 2009. In order to have sufficient tissue samples for isotopic analysis only nests with at least two nestlings were chosen for supplemental feeding so many nests were dropped from the study due to nestling loss prior to provisioning.

It is known from earlier fish consumption surveys in this region (Glass and Watts 2009) that neither Atlantic menhaden nor American shad are regular components of Osprey diets during June and July, the period when nestlings are actively growing feathers. Glass (2008) studied Osprey provisioning rates and food habits when nestlings were >2 weeks old. This is the age that growth of Osprey nestlings and provisioning rates by adults has peaked. Glass found that adults provision an average of 46g of fish per hour in the upper reaches of the Chesapeake Bay, which equates to an energy budget of 4410 kJ/day/nest. For our supplemental feeding, we used one of Glass' sites, the James River near Hopewell, Virginia. Based upon the energy budget for a nest and the energy density of menhaden (6.7 – 7.9 kJ/g; Frimodt 1995, Thayer *et al.* 1973) and American shad (8.0 –

8.2 kJ/g; USDA Agricultural Research Service Nutrient Data Laboratory 2009, Watt and Merrill 1975) we determined that approximately 250g of fish would be needed on a daily basis to provide about 40% of the food at each nest.

We provisioned Atlantic menhaden at 6 Osprey nests and American shad at 4 nests that contained 2-3 nestlings (Fig's. 4-6). We began provisioning when nestlings were 2-3 weeks old and continued for an additional 2 to 3 weeks (Table 1). Consumption of supplemented fish was confirmed by observation of adults feeding young within a short time after fish were placed in nests and by skeletal remains of provisioned fish. To limit disturbance at nests, we provisioned approximately 500 - 700g of fish every other day. Within 1-2 days following cessation of provisioning, we collected tissue samples from Osprey nestlings (Table 2). We also collected tissue samples from 6 nests from the study area that were not provisioned to act as controls for comparison of biomarker levels. In addition, we collected samples from 1 nest that we used to determine if Osprey in our study area would consume fish placed at nests. A trace level of menhaden biomarker may be detected from samples collected from these nestlings because 2 menhaden were provisioned at the nest when nestlings were about 2 weeks old.
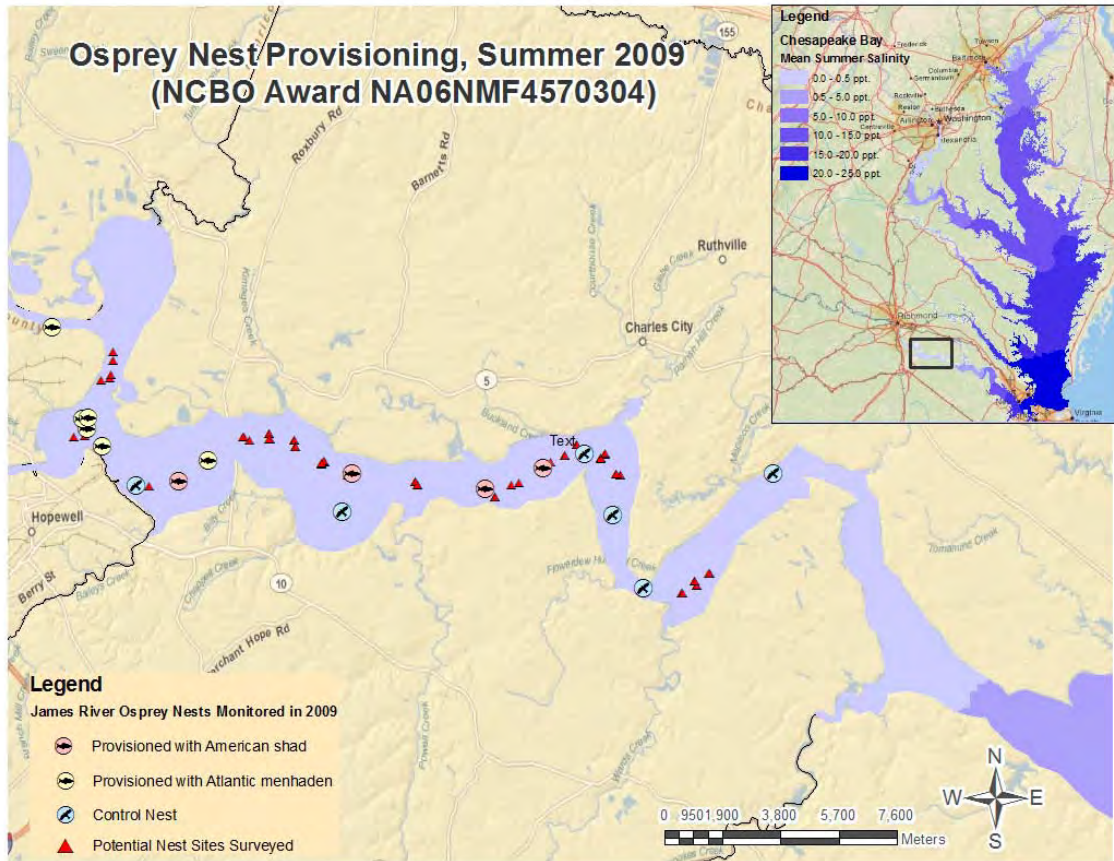
Figure 3. Locations of Osprey nests sampled for isotopic analysis during 2009 breeding season. Osprey nests in tidal freshwater James River were assigned to experimental (supplemented with menhaden or shad) and control (not supplemented) groups. A total of 42 Osprey nests along a transect from Turkey Island Cut downstream to Sturgeon Point were monitored from May 15 through August 3, 2009. Only nests with at least two nestlings surviving to fledging age were included in the study.

Fig 4. Project personnel check nests for nestlings.

Fig. 5. Mechanical fish feeder.

Fig 6. Osprey nestlings with supplemented menhaden.

Table 1. Experimental and control nests**.**

Nest	Treatment	No. Nestlings	Provisioning Period	Total Wt. (g) suppl. Fish	Tissue Samples collected
G135	BTY	2	6/8 - 6/28	5740	30-Jun
R132	TRACE BTY	2			30-Jun
G121	BTY	2	6/08-6/28	5860	30-Jun
R122	BTY	2	6/8-6/28	5650	30-Jun
R120	BTY	2	6/8-6/28	5910	30-Jun
R118	BTY	2	6/8-6/38	5910	30-Jun
PR1	Control	2			30-Jun
G107	BTY	3	6/8-6/28	5910	30-Jun
G85	Control	3			1-Jul
G79	Control	2			1-Jul
R76A	Control	3			1-Jul
R74C	Control	2			1-Jul
Stump	Control	2			14-Jul
R98	ASA	2	6/30-7/14	4675	14-Jul
R92	ASA	2	6/30-7/14	5555	14-Jul
G97	ASA	3	7/8-8/3	6930	3-Aug
R86	ASA	2	7/10-8/3	5940	3-Aug

Tissue sampling of birds.

We collected 3 sets of tissue samples from Osprey, Cormorants and Pelicans (Tables 2
and 3). Blood was collected from the brachial vein of each bird using sterile butterfly
needles and vaccutainers. We also plucked 8 – 10 contour feathers from the belly, breast,
and back of each bird. Oil from the uropygial gland was gently expressed onto feathers
of the gland. We then clipped the terminal ends of these feathers to collect the oil
sample. We followed protocols approved by Institutional Animal Care and Use
Committee when handling birds and collecting tissue samples.

Table 2. Tissue samples from Osprey nests collected in the tidal fresh James River

Date collected	Nest	Treatment	No. feathers	No. blood	No. oil
30-Jun	G135	BTY	2	2	2
30-Jun	R132	TRACE BTY	2	2	2
30-Jun	G121	BTY	2	2	2
30-Jun	R122	BTY	2	2	2
30-Jun	R120	BTY	2	2	2
30-Jun	R118	BTY	2	2	2
30-Jun	PR1	Control	2	2	2
30-Jun	G107	BTY	3	2	3
1-Jul	G85	Control	3	3	3
1-Jul	G79	Control	2	2	2
1-Jul	R76A	Control	3	3	3
1-Jul	R74C	Control	2	1	2
14-Jul	Stump	Control	2	2	2
14-Jul	R98	ASA	2	2	2
14-Jul	R92	ASA	2	2	2
3-Aug	G97	ASA	3	3	3
3-Aug	R86	ASA	2	2	2

Table 3. Brown Pelican and Double-crested Cormorant tissue samples collected from Poplar and Smith Islands, Maryland analyzed for Atlantic menhaden and American shad specific biomarkers.

Sample No.	Date	Location	Species	Age (weeks)	Blood?	Feathers?	Oil?
DCCO 1	24-Jun	Poplar Island	DCCO	3-4	y	n	n
DCCO 2	24-Jun	Poplar Island	DCCO	3-4	y	n	n
DCCO 3	24-Jun	Poplar Island	DCCO	3-4	y	n	n
DCCO 4	9-Jul	Poplar Island	DCCO	3-4	y	n	n
DCCO 5	9-Jul	Poplar Island	DCCO	4	y	y	y
DCCO 6	9-Jul	Poplar Island	DCCO	6	y	y	y
DCCO 7	9-Jul	Poplar Island	DCCO	5-6	y	y	y
DCCO 8	9-Jul	Poplar Island	DCCO	5	y	y	y
DCCO 9	9-Jul	Poplar Island	DCCO	5	y	y	y
DCCO 10	9-Jul	Poplar Island	DCCO	5	y	y	y
DCCO 11	9-Jul	Poplar Island	DCCO	4	y	y	y
DCCO 12	9-Jul	Poplar Island	DCCO	dead	n	y	n
DCCO 13	9-Jul	Poplar Island	DCCO	dead	n	y	n
DCCO 14	9-Jul	Poplar Island	DCCO	dead	n	y	n
DCCO 15	9-Jul	Poplar Island	DCCO	dead	n	y	n
DCCO 16	9-Jul	Poplar Island	DCCO	dead	n	y	n
DCCO 17	9-Jul	Poplar Island	DCCO	dead	n	y	n
DCCO18	10-Jul	Smith Island South Point Marsh	DCCO	5	y	y	y
DCCO 19	10-Jul	Smith Island South Point Marsh	DCCO	4	y	y	y
DCCO 20	10-Jul	Smith Island South Point Marsh	DCCO	5	y	y	y
DCCO 21	10-Jul	Smith Island South Point Marsh	DCCO	3.5	y	y	y
BRPE 1	10-Jul	Smith Island South Point Marsh	BRPE	4	y	y	y
BRPE 2	10-Jul	Smith Island South Point Marsh	BRPE	5	y	y	y
DCCO 22	10-Jul	Smith Island South Point Marsh	DCCO	3-4 dead	n	y	n
DCCO 23	10-Jul	Smith Island South Point Marsh	DCCO	3-4 dead	n	y	n
BRPE 3	10-Jul	Smith Island South Point Marsh Cut	BRPE	5	y	y	y
BRPE 4	10-Jul	Smith Island South Point Marsh Cut	BRPE	4	y	y	y
BRPE 5	10-Jul	Smith Island South Point Marsh Cut	BRPE	4-5	y	y	y
BRPE 6	10-Jul	Smith Island South Point Marsh Cut	BRPE	5	y	y	y
BRPE 7	10-Jul	Smith Island South Point Marsh Cut	BRPE	3-4 dead	n	y	n

Results:

Together with our earlier laboratory studies, analyses of bulk stable isotopic signatures of Osprey tissues from experimental *versus* control nests in locations where marine-derived isotopic values would be unique (Figure 7) suggested that the menhaden-specific biomarkers in avian tissues reflected the relatively brief and known period (~ 4 weeks) while Osprey nestlings were provisioned with adult menhaden or American shad by researchers. In addition, rapid isotopic turnover for some avian tissues (e.g. blood), as well as short and well-documented foraging distances for nesting adult Osprey, insured relatively discrete geospatial resolution.

Figure 7. Sulfur stable isotope values (δ34S, ‰) for blood and feathers from experimental Osprey nests provisioned with American shad (ASA), Atlantic menhaden (BTY), no provisioning (Control). Data for tem colonies of other avian predators are included for comparison: Poplar Island Cormorants (PopDCCO), Smith Island Brown Pelicans (smthBRPE), and Smith Island Cormorants (SmthDCCO) during Summer, 2009.

However, when FSAs were extracted from Osprey uropygial oils, the signature long chain fatty acids characteristic of Atlantic menhaden and American shad, and which identified earlier in this project, were not evident. Instead, Osprey uropygial oils were made up of short chain fatty acids only,

presumably following metabolism and synthesis of diet-derived lipids by avian predators. As a consequence, initial attempts to identify and use species-specific isotope biomarkers for selected fish prey (e.g. Atlantic menhaden), and based on non-invasive sampling of tissues from avian predators, as a fishery independent tool for fishery stock assessment was not successful. Additional analyses using the same approach but based on analysis of different avian tissues might yield more useful results.

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Appendix 1: Glass and Watts 2009

OSPREY DIET COMPOSITION AND QUALITY IN HIGH- AND LOW-SALINITY AREAS OF LOWER CHESAPEAKE BAY

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Abstract.—Chesapeake Bay, in the northeastern United States, is believed to support the largest concentration of breeding Ospreys (*Pandion haliaetus*) in the world. Following the banning of DDT, this population exhibited significant spatial variation in growth rates, with the fastest and slowest rates occurring in the lowest and highest salinity areas, respectively. Because salinity can influence fish distributions, we quantitatively analyzed Osprey diet composition along the gradient in the Chesapeake Bay to determine if variation in foraging ecology contributed to this pattern of population recovery. We recorded >1800 hr of food-provisioning behavior for 25 pairs within nine study areas that were classified as either upper estuarine (<5 parts per thousand [ppt] salinity) or lower estuarine (>18 ppt). Atlantic menhaden (*Brevoortia tyrannus*) and seatrouts (*Cynoscion* spp.) were dominant dietary components for pairs within lower-estuarine reaches, whereas gizzard shad (*Dorosoma cepedianum*) and catfish (Ictaluridae) dominated upper-estuarine diets. Lower-estuarine prey fish averaged 6% shorter (Kolmogorov-Smirnov test: $D = 0.203$, $P = 0.004$), 34% lighter ($D = 0.305$, $P < 0.001$), and 40% lower in energy content ($D = 0.247$, $P < 0.001$) than their upper-estuarine counterparts. We conclude that diet quality may be contributing to spatial variation in the growth rate of the Chesapeake Bay Osprey population.

Key Words: *Osprey*; Pandion haliaetus; *Chesapeake Bay*; *diet*; *foraging ecology*; *population regulation*; *salinity*.

COMPOSICIÓN Y CALIDAD DE LA DIETA DE *PANDION HALIAETUS* EN ÁREAS DE SALINIDAD ALTA Y BAJA EN LA PARTE BAJA DE LA BAHÍA DE CHESAPEAKE

Resumen.—Se cree que la bahía de Chesapeake, ubicada en el este de los Estados Unidos, sostiene la concentración más grande de individuos reproductivos de la especie *Pandion haliaetus* del mundo. Tras la prohibición del DDT, existió variación espacial sustancial en la tasa de crecimiento de esta población. Las tasas más altas y más bajas se presentaron en las áreas de salinidad máxima y mínima, respectivamente. Debido a que la salinidad puede influenciar las distribuciones de los peces, analizamos cuantitativamente la composición de la dieta de *P. haliaetus* a lo largo del gradiente en la bahía de Chesapeake para determinar si variaciones en la ecología de forrajeo habrían contribuido a este patrón de recuperación poblacional. Registramos más de 1800 horas de comportamiento de provisión de alimento para 25 parejas en nueve áreas de estudio que habían sido clasificadas ya sea, como estuarinas altas (menos de 5 partes por mil de salinidad) o estuarinas bajas (más de 18 partes por mil). Los peces *Brevoortia tyrannus* y *Cynoscion* spp. fueron componentes dominantes de la dieta de las parejas de las áreas estuarinas bajas, mientras que *Dorosoma cepedianum* y los de la familia Ictaluridae dominaron las dietas de las áreas estuarinas altas. Los peces depredados en las áreas estuarinas bajas fueron, en promedio, 6% más cortos (prueba de Kolmogorov-Smirnov: $D = 0.203$, $P = 0.004$), 34% más livianos ($D = 0.305$, $P < 0.001$) y presentaron un contenido de energía 40% menor ($D = 0.247$, $P < 0.001$) que sus contrapartes de las áreas estuarinas altas. Concluimos que la calidad de la dieta podría estar contribuyendo a la variación espacial en la tasa de crecimiento de la población de *P. haliaetus* de la bahía de Chesapeake.

[Traducción del equipo editorial]

Although restricted to a diet composed almost entirely of live fish, Ospreys (*Pandion haliaetus*) consume a wide array of species and occur in a diversity of habitats (Poole et al. 2002). Fish populations of many coastlines, estuaries, marshes, lagoons, rivers, lakes, and reservoirs support Osprey populations. This dietary plasticity is one of the primary factors

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contributing to their worldwide distribution (Poole 1989). Ospreys are found on every continent except Antarctica (Poole et al. 2002).

Chesapeake Bay is the largest estuary in North America and one of the most productive aquatic ecosystems in the world (Murdy et al. 1997). The bay's high productivity and 13 000-km shoreline make it an ideal body of water for breeding Ospreys. Historically, Chesapeake Bay has supported the largest concentration of breeding Ospreys in the world (Henny et al. 1974, Spitzer and Poole 1980). Although this population suffered from the effects of DDT (Stinson and Byrd 1976), reproductive rates showed signs of recovery through the 1970s and 1980s (Watts and Paxton 2007). By the mid-1990s, the tidal reach of the bay supported an estimated 3473 breeding pairs (Watts et al. 2004). Not all areas of the bay have recovered at the same rate, however. The only bay-wide breeding survey conducted since 1973 revealed that mean doubling times of the within geographic subregions ranged from 4.3 yr to more than 40 yr. The slowest rates generally occurred in higher-salinity areas of the bay proper and the fastest rates along the lower-salinity reaches of upper tributaries (Watts et al. 2004).

Saturation of nesting substrate along the bay proper does not appear to be a primary factor contributing to the slower population growth rate there, because potential nesting sites are plentiful and some historic nest sites are no longer being occupied (M. Byrd pers. comm.). Neither are environmental contaminants likely responsible for the differential population growth rate, because studies have shown that recent contaminant levels have not affected Osprey reproductive success (Rattner et al. 2004). The potential effect of foraging ecology on population growth has not been assessed, however.

Salinity tolerance is an important factor contributing to the distribution of fish species within estuaries (Boesch 1977, Murdy et al. 1997, Jung 2002). Thus, prey availability, and ultimately Osprey foraging behavior, may differ markedly between higher- and lower-salinity areas in Chesapeake Bay. In 1985, McLean and Byrd (1991) documented provisioning behavior at seven nests located in high-saline waters of the bay. Here we compare the diet of Osprey pairs provisioning broods within defined higher- and lower-salinity subregions of Chesapeake Bay and its upper tributaries. We describe for the first time the diet of Ospreys nesting in lower-salinity reaches and discuss how differences across the salinity gradient may relate to the spatial differences

in population growth noted by Watts et al. (2004). Such information is important to Osprey conservation, as well as ecosystem-scale considerations such as fisheries management and contaminant monitoring.

METHODS

We investigated the influence of salinity on diet by observing nesting Ospreys during the 2006 and 2007 breeding seasons within the extremes of salinity found within Chesapeake Bay. For the purpose of this study, we considered ''upper-estuarine'' areas those ranging in salinity from 0 to 5 parts per thousand (ppt) and ''lower-estuarine'' areas those exceeding 18 ppt. We chose salinity replicates to study from a pool of areas delineated by the Chesapeake Bay Program analytical segmentation scheme (Data Analysis Work Group 1997). We chose five upper-estuarine and four lower-estuarine sites (Fig. 1), each of which contained an average of three nests on channel markers or duck blinds over open water that were accessible by boat. We attempted to randomize site locations over as broad an area as was feasible, but we were restricted by the availability of boat ramps. We sampled a total of 29 nests, three of which were sampled during both 2006 and 2007.

We used micro-video monitoring to record provisioning data. The camera unit consisted of a portable digital video recorder (Secumate Mini, Yoko Technology Corp., Taiwan) connected to a 10-cm bullet camera (CM25SH CCD Color Sunshield, MicroAmerica, U.S.A.), both of which were powered by a 12-V deep-cycle marine battery. To obtain the highest resolution image of provisioning behavior, we secured the bullet camera approximately 1 m from the nest. We attached the camera directly to either a channel marker railing or duck blind beam, and we stored the recording unit and battery inside a weatherproof container placed nearby. We mounted cameras after nestlings reached at least 2 wk old, and generally filmed during all daylight hours for 1–2 d/wk, until nestlings approached fledging age. Logistical difficulties, however, precluded us from collecting video footage equally at all nests and sites.

The provisioning parameters we assessed included prey taxonomy, length, mass, and energy content. We identified prey items to the lowest taxonomic level possible and estimated prey size by comparing against Osprey morphological characters visible on images. We identified most prey to species; however, due to the lack of strong morpho-
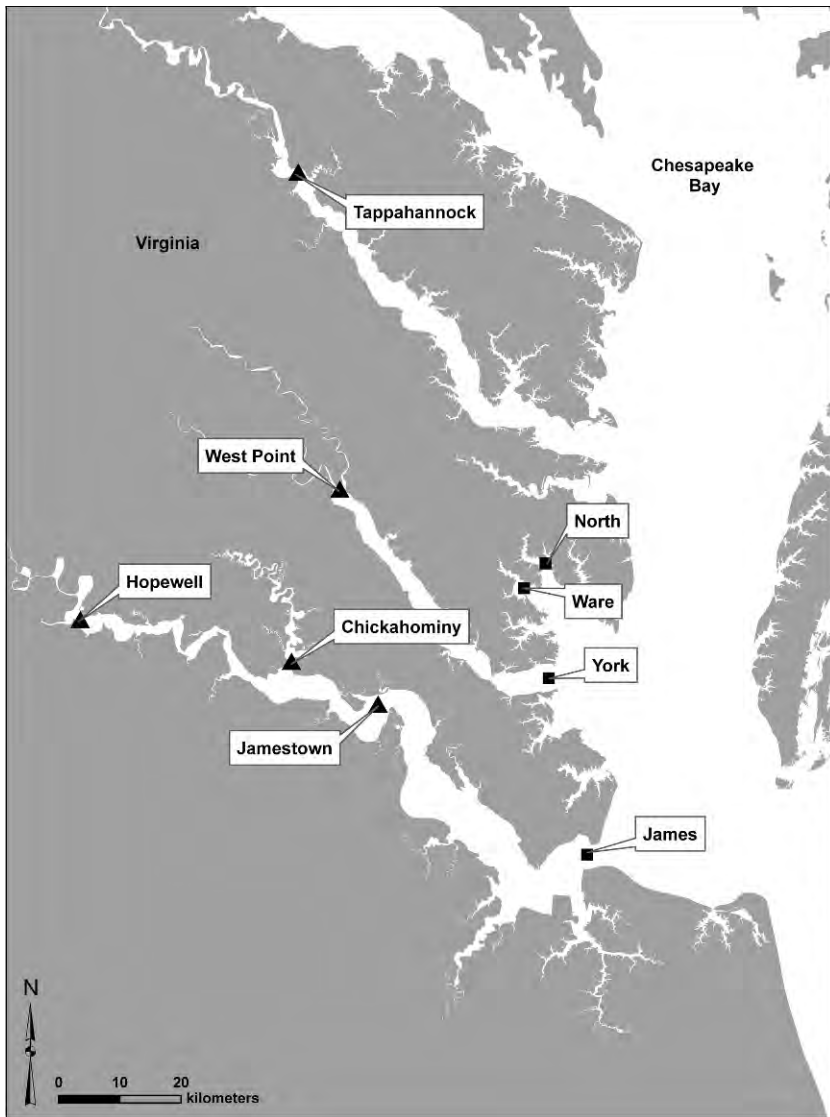
Figure 1. Osprey study sites within southwestern Chesapeake Bay during the 2006 and 2007 field seasons. Triangles indicate upper-estuarine, low-salinity sites and squares indicate lower-estuarine, high-salinity sites.

logical distinctions between some species (principally catfish [Ictaluridae] and shad [*Dorosoma* spp.]), we were able to identify some fish only to genus or family. We estimated fish length to the nearest cm using multiples of a typical adult Osprey's bill or talon length (values obtained from Poole et al. 2002). We minimized potential biases associated with these estimations by having a single individual conduct all video reviews. We used published morphometric data to extrapolate total fish length in cases where prey were only partially visible, and ultimately estimated fish mass based on published length-mass conversion equations (Appendix 1). Finally, because energy content per unit mass varies among species, we calculated the total kilocalories delivered per prey item by using published energy-density data (Appendix 2). In the few cases where length-mass conversion equations or energy-density data were unavailable for identified taxa, we calculated values using data for closely related taxa. As in

previous Osprey diet studies, we considered most fish to be entirely edible and therefore wholly consumed (e.g., Stinson 1977, Poole 1982, Van Daele and Van Daele 1982, McLean and Byrd 1991, Steeger et al. 1992). Catfish >31 cm in total length were an exception; we assumed them to be only 90% consumable (Dykstra 1995, Markham 2004).

We summarized identified taxa by number of individuals, biomass, and energy content for upper- and lower-estuarine sites. We used chi-square tests to detect differences between habitats in the frequency of occurrence of each taxon. We calculated expected values by averaging the frequencies observed in the two salinity habitats and incorporating a correction factor that accounted for incidental unequal sampling effort. For example, because only 48% of the total sampling effort occurred in the lower-estuarine habitat, we calculated the expected frequency of a given taxon for this habitat by multiplying its cumulative observed frequency for both habitats by 0.48 rather than the usual 0.50.

We evaluated diet breadth and prey characteristics using a subset of nests where prey diversity reached an asymptote. We projected the asymptotic number of species consumed at each nest by fitting each distribution to the following negative exponential function: accumulated number of species $= b0 * (1 - \exp(-b1 * \text{accumulated number of observations}))$, where $b0 = $ asymptote (Miller and Wiegert 1989). Based on this subset of nests, we compared the frequency distributions of prey lengths, masses, and energy contents in the two salinity habitats using nonparametric Kolmogorov-Smirnov tests. We estimated diet breadth using Simpson's (1949) 1-D species-diversity index and evaluated differences in diet breadth between the habitats using a t-test.

We used chi-square analyses to assess the spatial and temporal uniformity of delivery rates (g/hr) for major fish taxa within each habitat. We used average site values for each habitat as the expected values for spatial comparisons and average annual values for each habitat as the expected values for temporal comparisons.

RESULTS

We recorded 667 hr and 748 hr of video footage in the lower- and upper-estuarine sites, respectively. On average, we recorded 177 hr of footage per site (range 50–308 hr, SD of 120 hr) and 59 hr of footage per nest (range 19–161 hr, SD of 38 hr). We pooled the prey data from the five upper-estuarine sites, and similarly pooled prey data from the four lower-estuarine sites. We positively identified 589 prey items: 15 taxa to species, one taxon to genus, and two taxa to family.

The frequency of occurrence of species dominating the Osprey diet differed between the two salinity habitats for all species except the Atlantic croaker (*Micropogonias undulatus*). Catfish and gizzard shad (*Dorosoma cepedianum*) represented the greatest percentage (80%) of total prey items provisioned in the upper-estuarine sites, whereas seatrouts (*Cynoscion* spp.), Atlantic menhaden (*Brevoortia tyrannus*), spot (*Leiostomus xanthurus*), and Atlantic croaker composed the major percentage (74%) of fish provisioned in the lower-estuarine sites (Table 1). Occurrences of less common species, including Atlantic thread herring (*Opisthonema oglinum*), unidentified Clupeidae, round herring (*Etrumeus teres*), and summer flounder (*Paralichthys dentatus*), also differed between salinity habitats (Table 1).

Prey species that dominated the Osprey diet by frequency of occurrence were similarly represented as percentages of total energy delivered to nests (Table 1). Catfish and gizzard shad made up 77% of the total energy provisioned to nestlings in upper-estuarine sites, whereas *Cynoscion* spp., Atlantic menhaden, and gizzard shad composed 76% of the total energy delivered to nestlings in lower-estuarine sites.

Fish length averaged 7% longer in upper-estuarine sites (range 10.2–42.9 cm, mean 23.7 ± SD of 7.0 cm) than in lower-estuarine sites (range 12.7–42.0 cm, mean 22.2 ± 5.0 cm; Kolmogorov-Smirnov test: $D = 0.203$, $P = 0.004$; Fig. 2). Fish biomass averaged 52% greater in upper-estuarine sites (range 10.2–850.0 g, mean 239.8 ± 194.9 g) than in lower-estuarine sites (range 18.1–850.0 g, mean 157.8 ± 112.8 g; $D = 0.305$, $P < 0.001$). Whole-fish energy content of fish averaged 66% higher in upper-estuarine sites (range 69.5–5904.5 kJ, mean 1491.6 ± 1475.7 kJ) than in lower-estuarine sites (range 83.3–5899.4 kJ, mean 899.6 ± 807.1 kJ; $D = 0.247$, $P < 0.001$). Taxonomic diet breadth, as measured by Simpson's 1-D diversity index, did not differ between the two habitats (upper-estuarine: range 0.236–0.823, mean 0.526 ± 0.163; lower-estuarine: range 0.549–0.844, mean 0.696 ± 0.119; $t = -0.981$, $P = 0.253$).

Significant spatial variation in prey delivery rates (g/hr) occurred among sites within each habitat for all major fish taxa (Table 2). Significant temporal (among year) differences in prey delivery rates occurred only for gizzard shad in the upper-estuarine sites (Table 2).

Table 1. Relative contributions of all prey taxa identified in the Osprey diet within lower- and upper-estuarine sites in lower Chesapeake Bay during the 2006 and 2007 breeding seasons. Chi-square tests were conducted to detect significant differences in frequencies of occurrence between habitats. Scientific names of species are in Appendix 1.

	LOWER		UPPER		OBSERVED VS. EXPECTED FREQUENCY		LOWER		UPPER	
SPECIES	N	% TOTAL	N	% TOTAL	χ^2	P	kJ	% TOTAL	kJ	% TOTAL
Alewife	0	0.0	1	0.3	1.0	0.330	0.0	0.0	3211.6	0.4
Atlantic croaker	27	12.3	26	6.6	0.1	0.745	15238.1	5.5	28875.5	3.9
Atlantic menhaden	53	24.2	6	1.5	39.9	<0.001	123901.2	44.7	33051.1	4.5
Atlantic thread herring	5	2.3	0	0.0	5.3	0.022	2630.1	1.0	0.0	0.0
Bluefish	1	0.5	0	0.0	1.0	0.330	560.2	0.2	0.0	0.0
Clupeidae	0	0.0	15	3.8	14.3	<0.001	0.0	0.0	29870.8	4.0
Gizzard shad	9	4.1	110	28.0	80.7	<0.001	36868.2	13.3	341197.7	46.0
Hickory shad	0	0.0	3	0.8	2.9	0.091	0.0	0.0	21381.5	2.9
Hogchoker	1	0.5	0	0.0	1.0	0.330	394.1	0.1	0.0	0.0
Ictaluridae	0	0.0	203	51.7	192.8	<0.001	0.0	0.0	245045.6	33.0
Largemouth bass	0	0.0	1	0.3	1.0	0.330	0.0	0.0	1595.8	0.2
Round herring	4	1.8	0	0.0	4.2	0.040	5516.6	2.0	0.0	0.0
Spot	19	8.7	0	0.0	20.0	<0.001	10132.8	3.7	0.0	0.0
Spotted seatrout	63	28.8	0	0.0	66.3	<0.001	50187.5	18.1	0.0	0.0
Striped bass	10	4.6	5	1.3	1.9	0.164	12156.2	4.4	13399.7	1.8
Summer flounder	12	5.5	0	0.0	12.6	<0.001	5403.2	2.0	0.0	0.0
Threadfin shad	1	0.5	4	1.0	1.7	0.199	151.0	0.1	2669.8	0.4
White perch	2	0.9	8	2.0	3.3	0.069	2294.9	0.9	4842.6	0.7
Unknown	12	5.5	11	2.8			11913.1	4.3	16586.2	2.2
TOTAL	**219**		**393**				**277347.2**		**741727.1**	

DISCUSSION

Our characterization of Osprey diet during the 2006 and 2007 breeding seasons elucidated marked differences between upper- and lower-estuarine
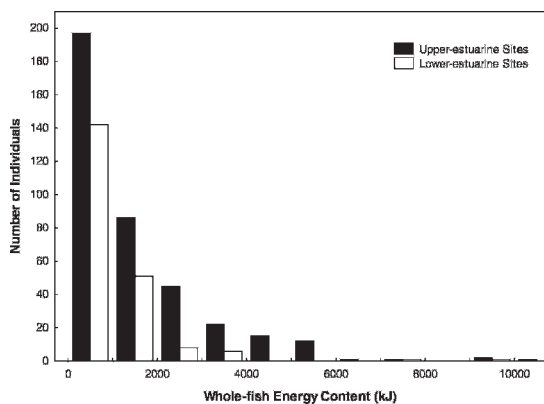
Figure 2. Comparisons of the frequency of occurrence and energy content of individual fish identified in Osprey diets within upper- and lower-estuarine sites during the 2006 and 2007 breeding seasons in lower Chesapeake Bay.

habitats. Fish taxa targeted by Ospreys varied significantly along the salinity gradient in both frequency of occurrence and percentage of total energy content delivered to broods. In the lower-estuarine sites, Atlantic menhaden and *Cynoscion* spp. were the dominant prey items provisioned. Although constituting only 24% of the diet by frequency of occurrence, Atlantic menhaden provided 44% of the total energy provided to broods in the lower-estuarine sites. Due in large part to its high lipid content relative to other species, Atlantic menhaden historically has been shown to be an important prey item for Ospreys breeding throughout the coastal waters of the mid-Atlantic and northeastern United States (Spitzer and Poole 1980, Poole 1989, McLean and Byrd 1991, Steidl et al. 1991). Atlantic menhaden also form large compact schools very near the water surface, making them relatively easy for Ospreys to locate and capture (Munroe and Smith 2000).

Although we were not able to identify to species all individuals in the important group *Cynoscion* spp., it appeared that this group was composed pri-

Table 2.   Spatial and temporal comparisons of provisioning rates (g/hr) for major taxa identified in the Osprey diet during the 2006 and 2007 breeding seasons in lower Chesapeake Bay. Site means were calculated by averaging all site values for both years. Annual means were calculated by averaging all site values within each year. These means were used as expected values in chi-square analyses.

	Site		Observed vs. Expected Frequency		Annual		Observed vs. Expected Frequency	
Zone and Species	Mean	SD	χ^2	P	Mean	SD	χ^2	P
Upper-estuarine zone								
Atlantic croaker (*Micropogonias undulatus*)	12.1	14	64.5	<0.001	1.4	1.5	1.6	0.201
Gizzard shad (*Dorosoma cepedianum*)	78.5	41.8	89.0	<0.001	93.9	57.1	34.7	<0.001
Ictaluridae	55.6	26.1	48.8	<0.001	66.2	7.0	0.7	0.389
Lower-estuarine zone								
Atlantic croaker (*Micropogonias undulatus*)	7.9	5.6	13.1	0.001	4.1	1.8	0.8	0.381
Atlantic menhaden (*Brevoortia tyrannus*)	25.1	30.5	63.0	<0.001	9.1	4.5	2.2	0.138
Spotted seatrout/weakfish (*Cynoscion* spp.)	11	10.6	20.3	<0.001	23.0	5.7	1.4	0.236

marily of spotted seatrout (*Cynoscion nebulosus*). This concurs with McLean and Byrd's (1991) study as well as with the opinions of local recreational anglers (K. Glass unpubl. data) who routinely fished for this species throughout the lower-estuarine sites. By biomass, spotted seatrout are the second largest catch annually landed by the saltwater fishing industry in the southeast United States, and the recreational catch is believed to be greater than the commercial catch (Murdy et al. 1997). Although found throughout the Chesapeake Bay in a wide range of salinities, spotted seatrout occur predominantly in higher-salinity waters and frequent shallow waters with sandy bottoms, making them accessible to Ospreys (Murdy et al. 1997).

In the upper-estuarine sites, gizzard shad and catfish dominated the diet. Although gizzard shad occurred only half as frequently as catfish, which comprised 52% of the diet by frequency of occurrence, gizzard shad constituted 46% of the total energy delivered to broods, whereas catfish constituted only 33%. The dominance of these taxa in the upper-estuarine diet is not surprising because they are abundant in these waters (Murdy et al. 1997). Gizzard shad can occur in salinities as high as 22 ppt within Chesapeake Bay, but they are not anadromous and primarily occur in the tidal fresh and oligohaline waters where they spawn from March to August (Murdy et al. 1997, Munroe and Smith 2000). This species is therefore an ideal prey item because it is available throughout the Osprey breeding season (April–August). Its availability to Ospreys

is further increased by both a rapid growth rate, which quickly precludes consumption by most piscivorous fish, and the schooling behavior it typically exhibits between 0.3–1.6 m below the surface (Jenkins and Burkhead 1994). Furthermore, a large size associated with a very high energy density guarantees that gizzard shad provide a substantial energy return for foraging Ospreys. Previously, gizzard shad had been documented in the Osprey diet only within the resident population of southern Florida (Collopy 1984, Edwards 1988).

Like gizzard shad, catfish also can be found in a wide range of salinities, but occur most frequently in fresher water (Murdy et al. 1997, Virginia Institute of Marine Science unpubl. data). Several species of catfish are well established throughout the lower-saline reaches of Chesapeake Bay (Murdy et al. 1997) and localized spawning ensures their presence throughout the Osprey breeding season (Jenkins and Burkhead 1994). The foraging ecology of catfish likely also contributes to their large presence in the Osprey diet. Catfish primarily feed on benthic organisms (Murdy et al. 1997) and bottom-feeders are more vulnerable to Osprey attacks than limnetic-feeders; presumably because they have their eyes focused predominantly on the underlying substrate (Swenson 1979). Benthic fish are also often drawn to shallower waters to forage (Haywood and Ohmart 1986), thereby further increasing their vulnerability to depredation because they have no downward escape route. We believe that Ictaluridae brought to nests were primarily channel catfish (*Ictalurus punc-*

tatus), blue catfish (*Ictalurus furcatus*), and white catfish (*Ameiurus catus*), as suggested by regular observation of deeply forked caudal fins. Previously, only bullhead catfish (*Ameiurus* spp.) had been documented in the Osprey diet (Van Daele and Van Daele 1982, Collopy 1984, Vana-Miller 1987, Poole 1989, Steeger et al. 1992).

Breeding Bald Eagles (*Haliaeetus leucocephalus*) also have been shown to rely predominantly on catfish and shad species in the upper-estuarine areas of Chesapeake Bay (Markham 2004). As Osprey and Bald Eagle populations both continue to expand in this region, competition for these prey resources will likely escalate. Exploitive or interference competition may subsequently affect population dynamics. Although Bald Eagles may displace Ospreys when territories overlap to a large extent, some researchers have suggested that the dominance may be reversed if Ospreys greatly outnumber Bald Eagles (Ogden 1975).

In other populations, Ospreys have been shown to target fish within a narrow size range (Swenson 1978, Van Daele and Van Daele 1982, Poole 1989). We found that the average lengths, biomasses, and energy contents of consumed fish all differed between upper- and lower-estuarine sites. Differing by 1.5 cm, 82 g, and 592.4 kJ per fish on average, the provisioned lower-estuarine fish were 6% shorter, 34% lighter, and 40% less energy-rich than their upper-estuarine counterparts. The differences in fish biomass and energy content appeared to be primarily due to a variation in diet composition rather than fish length, because each species has unique length-mass and mass-energy conversion factors.

Although spatial differences in diet composition within habitats existed, our results indicate that Ospreys breeding in the upper-estuarine sites enjoy a higher quality diet than those in the lower-estuarine sites. Given the broad spatial scale of our study, extrapolation of our findings to the broader region seems valid. Because diet quality directly influences the reproductive success of breeding Ospreys, spatial differences in diet quality may be influencing the dynamics of the Chesapeake Bay Osprey population. Given that Ospreys rarely breed farther than 50 km from their natal sites and exhibit extreme site fidelity in annual breeding, Osprey population growth and decline are predominantly influenced by local survival and reproductive rates (Poole et al. 2002). Consequently, if Ospreys produce fewer young per breeding attempt in the lower-estuarine sites than in the upper-estuarine sites due to lower diet quality, overall population growth would likely reflect this. Spatial variation in growth rates of the Chesapeake Bay population may therefore ultimately be due to the spatial differences in diet quality elucidated in our study. This has important implications for the long-term stability of this population, as well as for fisheries management and overall ecosystem health. We encourage further studies that characterize both parental provisioning rates and reproductive success to more conclusively assess the influence diet quality may be having on the growth trend of the Chesapeake Bay Osprey population.

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Appendix 1. Length-mass conversions used for fish identified in the diet of Ospreys nesting in lower Chesapeake Bay during the 2006 and 2007 breeding seasons. In conversion equations, mass (M) is in grams and length (L) is in centimeters.

SPECIES	BIOMASS CONVERSION	REFERENCE
Alewife (*Alosa pseudoharengus*)	$M = 0.0085*L^{3.000}$	Madenjian et al. 2003
American shad (*Alosa sapidissima*)	$M = 0.0065*L^{2.959}$	Muncy 1960
Atlantic croaker (*Micropogonias undulatus*)	$M = 0.0052*L^{3.148}$	Wilk et al. 1978
Atlantic herring (*Clupea harengus*)	$M = 0.0075*L^{3.030}$	Muncy 1960
Atlantic menhaden (*Brevoortia tyrannus*)	$M = 0.0161*L^{3.000}$	June and Nicholson 1964
Atlantic thread herring (*Opisthonema oglinum*)	$M = 0.0186*L^{2.920}$	Claro and García-Arteaga 1994
Banded rudderfish (*Seriola zonata*)	$M = 0.0259*L^{2.908}$	Bohnsack and Harper 1988
Black crappie (*Pomoxis nigromaculatus*)	$M = 0.0096*L^{3.075}$	Vanderpuye and Carlander 1971
Blue catfish (*Ictalurus furcatus*)	$M = 0.0185*L^{3.000}$	Crawford 1993
Channel catfish (*Ictalurus punctatus*)	$M = 0.0041*L^{3.407}$	Muncy 1959
Clearnose skate (*Raja eglanteria*)	$M = 0.0022*L^{3.295}$	Sulikowski et al. 2003
Gizzard shad (*Dorosoma cepedianum*)	$M = 0.0182*L^{2.890}$	Lagler and Van Meter 1951
Hickory shad (*Alosa mediocris*)	used American shad	
Hogchoker (*Trinectes maculatus*)	$M = 0.0199*L^{3.001}$	Dawson 1965
Largemouth bass (*Micropterus salmoides*)	$M = 0.0158*L^{2.960}$	Swingle 1965
Round herring (*Etrumeus teres*)	$M = 0.0059*L^{3.158}$	Dawson 1965
Spot (*Leiostomus xanthurus*)	$M = 0.0092*L^{3.072}$	Dawson 1965
Spotted seatrout (*Cynoscion nebulosus*)	$M = 0.0131*L^{3.000}$	Crawford 1993
Striped bass (*Morone saxatilis*)	$M = 0.0061*L^{3.153}$	Mansueti 1961
Summer flounder (*Paralichthys dentatus*)	$M = 0.0102*L^{2.994}$	Smith and Daiber 1977
Threadfin shad (*Dorosoma petenense*)	$M = 0.0035*L^{3.774}$	Carlander 1969
Weakfish (*Cynoscion regalis*)	$M = 0.0088*L^{3.000}$	Crozier and Hecht 1913
White perch (*Morone americana*)	$M = 0.0125*L^{3.020}$	St. Pierre and Davis 1972

Appendix 2.   Mass-energy conversion equations used for fish identified in the diet of Ospreys nesting in lower Chesapeake Bay during the 2006 and 2007 breeding seasons. In conversion equations, energy (E) is in kJ and mass (M) is in grams.

SPECIES	ENERGY CONVERSION	REFERENCE
Alewife (*Alosa pseudoharengus*)	$E = 185*(M/100)$	Frimodt 1995
American shad (*Alosa sapidissima*)	$E = 192*(M/100)$	Watt and Merrill 1975
Atlantic croaker (*Micropogonias undulatus*)	$E = 100*(M/100)$	Frimodt 1995
Atlantic herring (*Clupea harengus*)	$E = 190*(M/190)$	Frimodt 1995
Atlantic menhaden (*Brevoortia tyrannus*)	$E = 189*(M/100)$	Frimodt 1995
Atlantic thread herring (*Opisthonema oglinum*)	used Atlantic herring	
Banded rudderfish (*Seriola zonata*)	used white perch	
Black crappie (*Pomoxis nigromaculatus*)	used white perch	
Blue catfish (*Ictalurus furcatus*)	$E = 103*(M/100)$	Frimodt 1995
Channel catfish (*Ictalurus punctatus*)	$E = 112*(M/100)$	Frimodt 1995
Clearnose skate (*Raja eglanteria*)	used summer flounder	
Gizzard shad (*Dorosoma cepedianum*)	$E = 200*(M/100)$	Watt and Merrill 1975
Hickory shad (*Alosa mediocris*)	used American shad	
Hogchoker (*Trinectes maculatus*)	used summer flounder	
Largemouth bass (*Micropterus salmoides*)	used white perch	
Round herring (*Etrumeus teres*)	used Atlantic herring	
Spot (*Leiostomus xanthurus*)	used Atlantic croaker	
Spotted seatrout (*Cynoscion nebulosus*)	$E = 99*(M/100)$	Frimodt 1995
Striped bass (*Morone saxatilis*)	$E = 92*(M/100)$	Frimodt 1995
Summer flounder (*Paralichthys dentatus*)	$E = 84*(M/100)$	Frimodt 1995
Threadfin shad (*Dorosoma petenense*)	used gizzard shad	
Weakfish (*Cynoscion regalis*)	$E = 99*(M/100)$	Frimodt 1995
White perch (*Morone americana*)	$E = 118*(M/100)$	Watt and Merrill 1975

Appendix 2: *Anadromous fish as marine nutrient vectors*, Fishery Bulletin

Anadromous fish as marine nutrient vectors

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Abstract—The tidal freshwater of Virginia supports anadromous herring (*Alosa* spp.) spawning runs in the spring; however, their importance as nutrient delivery vectors to the freshwater fish food web remains unknown. The stable isotope signatures of fishes from 21 species and four different guilds (predators, carnivores, generalists, and planktivores) were examined in this study to test the hypothesis that marine derived nutrients (MDNs) brought by anadromous fish would be traced into the guilds that incorporated them. Spawning anadromous fish were ^{13}C and ^{34}S-enriched (δ^{13}C and δ^{34}S of approximately 18‰ and 17.7‰, respectively) relative to resident freshwater fish. Of the guilds examined, only predators showed ^{13}C and ^{34}S-enrichment similar to the anadromous fish; however, some generalist catfish also showed enriched signatures. Specific fatty acid δ^{13}C signatures for gizzard shad (*Dorosoma cepedianum*), blue catfish (*Ictalurus furcatus*), and alewife (*Alosa pseudoharengus*), show a 10‰ range among fishes, clearly reflecting isotopically distinct dietary sources. The δ^{13}C and δ^{34}S distribution and range among the freshwater fishes suggest that both autochthonous and allochthonous (terrestrial C3 photosynthetic production and MDN) nutrient sources are important to the tidal freshwater fish community.

Streams in which anadromous fish spawn are often nutrient poor and the spawning anadromous fish may be an important source of nutrients to them (Kline et al., 1993; Wipfli et al., 2003). Sometimes spawning anadromous fish even fertilize near-stream terrestrial environments (Ben-David et al., 1998; Koyama et al., 2005). The spawning fish are frequently semelparous and deliver marine derived nutrients (MDN) to the freshwater as moribund biomass, excreted ammonium ion (NH_4^+), or through gamete release (Cederholm et al., 1989; Browder and Garman, 1994; Wipfli et al., 2003). Several studies in Alaska and the Pacific Northwest of North America have demonstrated the importance of marine nutrients brought to freshwater streams by anadromous salmonids (Bilby et al., 2003; Kline et al., 1993; Francis et al., 2006). In the Gulf of Mexico, migrating Gulf menhaden (*Brevoortia patronus*) transported estuarine nutrients into inshore environments (Deegan, 1993), and returning salmon contributed to the productivity of Lake Ontario tributaries (Rand et al., 2002). However, less work has been done on the East Coast of the United States where coastal development has been much more intense and the dominant anadromous species (*Alosa* spp.; herring (*A. aestivalis*), American shad (*A. sapidissima*), and alewife (*A. pseudoharengus*)) are often not highly abundant (Deegan, 1993; Garman and Macko, 1998). Although the *Alosa* spp. on the east coast tend towards an iteroparous life cycle rather than a semelparous one, they do experience heavy postspawning mortality (alewife postspawning mortality has been measured as 41% (Havey, 1961) and between 39% and 57% (Durbin et al., 1979)). Because tidal freshwater streams receive nutrients from marine and freshwater primary productivity at different times, the incorporation of these nutrients by consumers may be different depending on feeding guilds. Fish found in the same area in a stream may derive nutrition from local or translocated productivity. In nutrient poor systems, such as East Coast United States tidal freshwater areas, it is important to understand nutrient sources to different feeding guilds (e.g., predators, carnivores, generalists, and planktivores).

For more than 20 years now, carbon and nitrogen stable isotopes (re-

ported as a ratio of heavy to light isotopes and given δ notation with units of ‰, see *Materials and methods* section for more detail) have been used to determine the importance of MDN in freshwater systems, and to characterize the trophic structure within those systems (Kline, et al., 1993; Vander-Zanden et al., 1999). For example, carbon and nitrogen isotopes have shown that anadromous Pacific salmon (*Oncorhynchus* spp.) were a significant source of allochthonous nitrogen to coastal streams where spawning occurs (Kline et al., 1993).

Hesslein et al. (1991) used sulfur isotopes to differentiate freshwater migratory and non-migratory fishes in the Mackenzie River Basin, Canada. On the East Coast of the United States, anadromous river herring (*Alosa* spp.) retain their marine isotope signal after spending part of the spring spawning in freshwater, and that some freshwater piscivores are ^{34}S and ^{13}C-enriched after preferentially consuming migrating *Alosa* spp. during the spawning run (Garman and Macko, 1998; MacAvoy et al., 2000).

An additional tool for determining origins and transformations of organic material from different sources is the stable isotope ratio of specific compounds. Isolating a specific compound, or class of compounds, then measuring the isotope ratio on those compounds, may offer a more robust technique to trace biologically significant compounds (such as fatty or amino acids) than would be possible from bulk isotope analysis alone. For example, examining the carbon isotopic composition of fatty acids from an animal, particularly essential fatty acids, allows the direct determination of dietary sources that contribute to the fatty acid pool of that animal (Stott et al., 1997). Although bulk isotope analysis can be an effective nutrient tracer in systems with isotopically distinct nutrient sources (Peterson et al., 1985), the isotopes of specific fatty acids may provide more confidence in identifying sources (Canuel et al., 1997).

Carnivorous heterotrophs are unable to synthesize fatty acids longer than 18-carbons, nor can they desaturate carbon-carbon bonds between the ninth and terminal methyl carbon, therefore, these essential fatty acids must be obtained from diet (Olsen 1999). Because essential fatty acids are not influenced by subsequent metabolism within a eukaryotic heterotroph, they retain their original isotope composition (Stott et al., 1997). Fatty acids synthesized by marine plankton and incorporated into marine fish would be highly enriched in ^{13}C relative to those produced by freshwater primary producers or C3 photosynthesis. Additionally, short chain fatty acids, used as precursors in the biosynthesis of unsaturated or longer chain saturated fatty acids, should be ^{13}C enriched in relation to biosynthesized fatty acid products (Murphy and Abrajano, 1994). In this study, the fatty acid nomenclature used is carbon number:number of double bonds. For example, 18:2 is an 18-carbon fatty acid with two points of unsaturation. The desaturation of 16:0 to 16:1 and 18:0 to 18:1–18:2 occurs by a systematic fractionation of roughly 2‰ per desaturation (DeNiro and Epstein,

1977; Monson and Hayes, 1982). Also, studies have shown that the elongation of fatty acids by *de novo* synthesis results in a 2‰ per 2-carbon acetyl group addition. These fractionations allowed the identification of fatty acids that were directly incorporated from symbiotic bacterial sources in mussels as opposed to those obtained through *de novo* synthesis (Murphy and Abrajano, 1994).

In this study we compared the $δ^{15}N$, $δ^{13}C$, $δ^{34}S$ of bulk tissues, plus the $δ^{13}C$ of specific fatty acids among four guilds of fish plus anadromous *Alosa* spp. in a tidal freshwater stream on the East Coast of the United States. Our objective was to determine if anadromous fish, captured more than 40 km from the salt-wedge, were isotopically distinct from freshwater residents, and to determine if freshwater guilds showed the incorporation of marine allochthonous organic material.

Materials and methods

Field collections by boat electrofisher were made in the tributaries and main-stem of the Rappahannock River, VA (within a 40-mile area between Fredericksburg and Tappahannock, VA) during March and May 1997 and 1998 (Fig. 1). The Rappahannock River is tidal in this region (tidal range: 0.1 to 1 meter) and shares many physicochemical characteristics with other tidal freshwater rivers in the region (Garman and Nielsen, 1992). Fishes were collected and placed on ice in the field, transported back to the laboratory, and muscle tissue samples were taken, which were then dried for later analysis. Analysis of the sulfur and compound specific fatty acid samples took several years and were completed by 2002.

The fishes were placed into four different guilds based on feeding strategies taken from Jenkins and Burkhead's (1993) seminal work on Virginia freshwater fishes, plus an anadromous life cycle group (Table 1).

Bulk isotope tissue analysis, elemental analyzer, and isotope ratio mass spectrometry

Samples of dorsal muscle tissue were dried at 60°C for three days and homogenized in preparation for analysis. The tissues were then lipid extracted by refluxing them in dichloromethane for 35 minutes (Knoff et al., 2002), except for those samples selected for compound specific analysis, which were soxlet extracted (see below; gas chromatography-mass spectrometry (GC-MS) and compound specific stable isotope analysis (CSIA)). One milligram (mg) of dried, lipid-extracted muscle was used for $δ^{13}C$ and $δ^{15}N$ analysis. Six mg was used for $δ^{34}S$ analysis. A Carlo Erba elemental analyzer (EA) (Fisons/VG/Micromass, Manchester, UK) coupled to a Micromass Optima isotope ratio mass spectrometer (IRMS) (Fisons/VG/Micromass, Manchester, UK) was used to obtain $δ^{13}C$, $δ^{15}N$ and $δ^{34}S$ values. The $δ^{13}C$ and $δ^{15}N$ were obtained concurrently, and $δ^{34}S$ was determined during separate analytical runs.
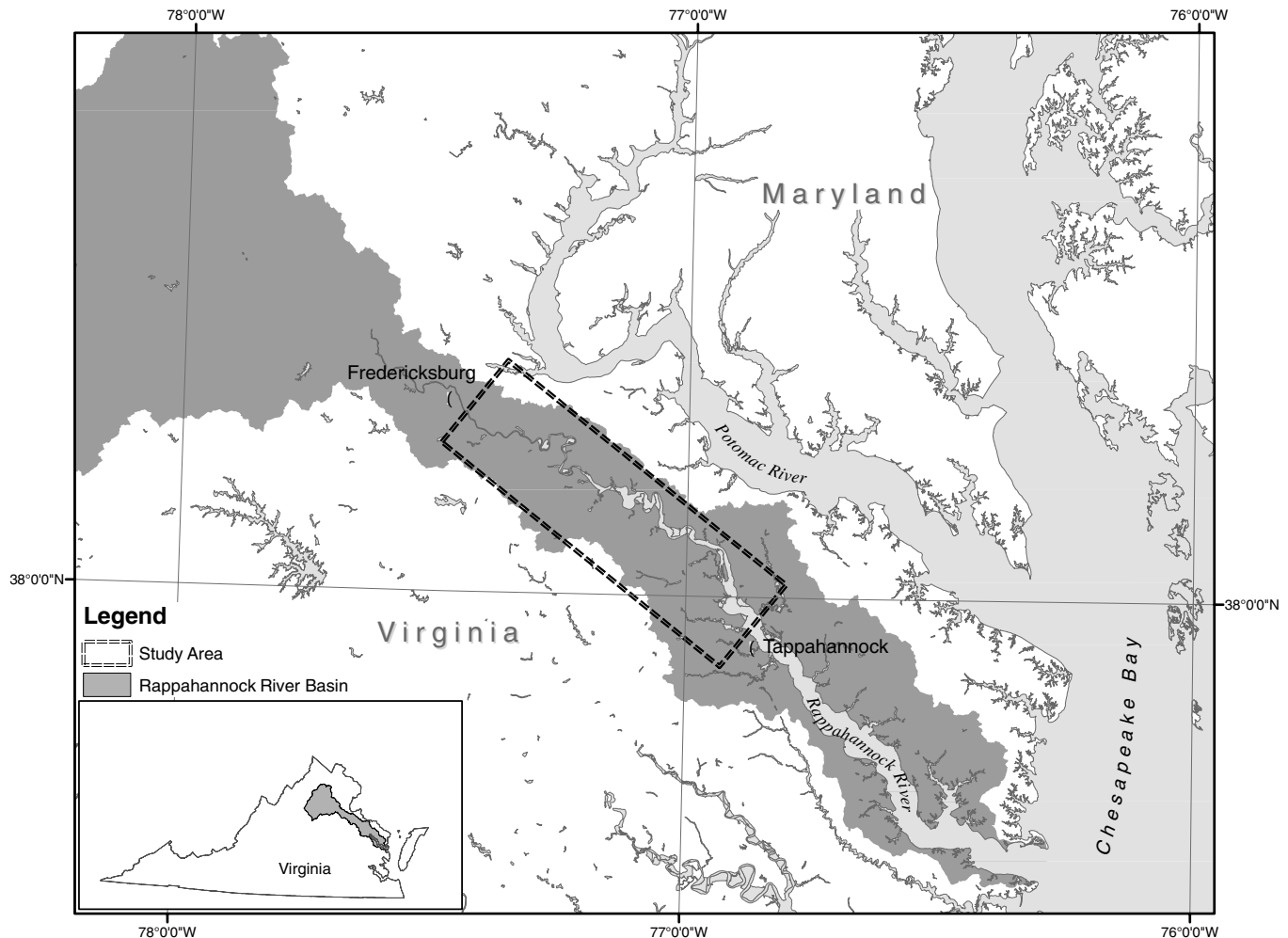
Figure 1

The boxed area indicates the section of the Rappahannock River, Virginia, between the towns of Fredericksburg and Tappahannock, where all fish were captured to determine the role of anadromous fish as marine nutrient vectors to the freshwater environment. Boat electrofishing was conducted between February and May 1997 and 1999. Sampling was conducted so that fish were captured before, during and after the spring spawning run of the anadromous *Alosa* spp.

The isotope compositions are reported in relation to standard material and follow the same procedure for all stable isotopic measurements, as follows:

$$\delta^x E = [(^x E /^y E)\text{sample}/(^x E /^y E)\text{standard}] - 1) \times 1000, \quad (1)$$

where E = the element analyzed (C, N, or S);
$\quad\quad x$ = the molecular weight of the heavier isotope; and
$\quad\quad y$ = lighter isotope (x=13, 15, 34, and y=12, 14, 32 for C, N, and S, respectively).

The standard materials to which the samples are compared are Pee Dee Belemnite for carbon, air N_2 for nitrogen, and Canyon Diablo Triolite for sulfur. Reproducibility of all measurements was typically 0.2‰ or better. Between every 12 samples, a laboratory standard was analyzed. In a typical run of 60 samples (+5 stan-

dards, 65 measurements total) the standard deviations for $\delta^{15}N$ and $\delta^{13}C$ were <0.2‰. For $\delta^{34}S$, standard deviations were <0.3‰.

Gas chromatograph-mass spectrometer (GC-MS)

Once dried, muscle samples selected for compound specific isotope analysis (CSIA) were lipid extracted (Soxhlet method from Ballentine et al., 1996) and the fatty acids had a methyl group added to the carboxyl end (derivitized) so they could be characterized by gas chromatography (GC). This was done by heating with BF_3CH_3OH for eight minutes (Ballentine et al., 1996). The fatty acid methyl esters (FAME) were analyzed by GC-MS using a Hewlett Packard 5890 Series II gas chromatograph (Palo Alto, CA) interfaced to a Hewlett Packard 5971A mass sensitive detector (Palo Alto, CA), with helium gas as the carrier. A 60-meter J&W DB-5

column (J&W Scientific, Folsom, CA) was used for FAME separation. The GC oven temperature program used was as follows: 100°C for 2 minutes, ramp at 3°C/min. to 210°C, hold for 20 min, ramp 1°C/min. to 220°C, hold for 10 min.

Compound specific stable isotope analysis (CSIA)

The FAMEs were analyzed for their stable carbon isotope compositions using a Hewlett Packard 5890 Series II gas chromatograph interfaced through a combustion furnace with a VG Isoprime IRMS (Fisons/VG/Micromass, Manchester, UK). The GC was equipped with the same column that was used for the GC-MS analysis and helium was the carrier gas. The GC oven temperature program was identical to that used for the GC-MS FAME identification. Time elution was used to identify peaks. The CO_2 combustion products of the fatty acids eluting from the column were introduced into the mass spectrometer after passing through a water trap.

All FAME $\delta^{13}C$ values were corrected for the addition of the methyl group to the original fatty acid. The derivatization of the fatty acids to their methyl esters results in a predictable and reproducible isotope effect (Ballentine et al., 1996; Uhle et al., 1997). Adding a methyl group to the fatty acid alters its isotope signature. However, if the isotopic ratio of the methanol (in this case $\delta^{13}C = -46‰$, measured by injecting the methanol into the mass spectrometer through the GC) and

the fatty acid methyl ester are known, then the isotopic signature of the original fatty acid can be determined using a mass balance Equation 2.

$$\delta^{13}C_{\text{FAME}} = f_{\text{FA}-}\delta^{13}C_{\text{FA}} + f_{\text{Methanol}}\,\delta^{13}C_{\text{Methanol}} \qquad (2)$$

where $\delta^{13}C_{\text{FAME}}$, $\delta^{13}C_{\text{FA}}$,
and $\delta^{13}C_{\text{Methanol}}$ = the carbon isotope signatures of the FAME, the underivatized fatty acid, and the methanol, respectively; and

f_{FA} and $f_{Methanol}$ = the fractions of carbon in the FAME due to the underivatized fatty acid and methanol, respectively (Ballentine et al., 1996; Uhle et al., 1997).

Each sample was injected four to eight times (depending on the reproducibility of the analysis). Only $\delta^{13}C$ values that were within 1.5‰ of each other were considered to reflect the $\delta^{13}C$ of the FAME (MacAvoy et al., 2002). Therefore, the $\delta^{13}C$ reported for each FAME identified is represented by an average value and a standard deviation. Every sixth sample injected was an internal, laboratory standard (naphthalene-d, $\delta^{13}C - 25.7‰$) to insure consistent performance of the GC, oxidation furnace, and mass spectrometer.

Statistical analysis

Kruskal-Wallis nonparametric procedures were used to test for differences in isotopic values among anadromous fish and the different guilds (predators, carnivores, generalists, and planktivores, ($\alpha = 0.05$)). The Dunn procedure was used to examine differences between groups (Rosner, 1990). Statview SE + Graphics (Abacus Concepts, Inc., Cary, NC), JMP In (SAS, Cary, NC) and Microsoft Excel version 5.0 (Microsoft, Inc., Redmond, WA) were used for statistical tests. The Dunn procedure reduces the risk of type-1 error inherent in multiple comparison techniques. It does so by increasing the Z-score needed to reject the null hypothesis as the number of individual groups being compared increases. In the present study, a Z-score of ± 3.02 (0.9975 confidence) was needed for a difference to be significant.

Results

The first objective of this study was to establish that the spawning anadromous fish retained the marine isotope signal more than 40 km upstream from saline waters. This was the case for all three isotopes examined.

Table 1

Fish species examined by guild (including an anadromous group) from the Rappahannock River to assess the role of marine fish as nutrient vectors. Guild assignments are based on diet as reported in Jenkins and Burkhead (1993).

Guild	Species name	Common name
Predator	*Ictalurus furcatus*	blue catfish
	Lepisosteus osseus	longnose gar
Carnivore	*Micropterus salmoides*	largemouth bass
	Lepomis gibbosus	pumpkinseed
	Hybognathus regius	eastern silvery minnow
	Notemigonus crysoleucas	golden shiner
	Lepomis macrochirus	bluegill
	Perca flavescens	yellow perch
Generalist	*Anguilla rostrata*	American eel
	Ameiurus catus	white catfish
	Ameiurus nebulosus	brown bullhead
	Ictalurus punctatus	channel catfish
Planktivore	*Menidia beryllina*	inland silverside
	Dorosoma cepedianum	gizzard shad
	Erimyzon oblongus	creek chubsucker
Anadromous	*Alosa aestivalis*	blueback herring
	Alosa pseudoharengus	alewife
	Alosa sapidissima	American shad
	Morone saxatilis	striped bass
	Morone americana	white perch

Table 2

Isotope values for all fish used in this study seperated by Family. "A" indicates anadromous, * indicates euryhaline range. Guild assingments are based on diet as reported in Jenkins and Burkhead (1993). "C" indicates a group with some isotope data derived from MacAvoy et al. (2000). White perch (*Morone americana*) shows elevated ^{13}C content is probably not marine protein given the low $\delta^{34}S$ ratio; *M. americana* is a secondary carnivore and the high $\delta^{13}C$ reflect this. Standard deviation is given after the ± and N is in parentheses.

Family and Species	Common name	Guild: food types	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Anguillidae					
Anguilla rostrata	American eel	generalist: insects, snails, fish, clams	−24.7±0.7 (3)	11.2±0.8 (3)	0.9±2.4 (3)
Atherinidae					
Menidia beryllina	inland sliverside	planktivore	−23.8±0.9 (3)	15.5±0.2 (3)	10.0±0.9 (3)
Catostomidae					
Erimyzon oblongus[C]	creek chubsucker	planktivore: planktonic crustaceans	−28.1 (1)	10.9 (1)	5.1 (1)
Centrarchidae					
Micropterus salmoides	smallmouth bass	carnivore	−23.0±1.9 (5)	14.5±1.3 (5)	7.6±3.2 (5)
Lepomis gibbosus	pumpkinseed	carnivore: insects, worms	−25.4±1.1 (8)	13.1±1.3 (8)	6.5±2.3 (9)
Lepomis macrochirus	bluegill	carnivore: insects, worms	−23.7±2.2 (5)	14.7±1.8 (5)	4.7±2.0 (5)
Clupeidea					
Alosa pseudoharengus[A, C]	alewife spawning	anadromous: copepods, diatoms, ostracods, shrimp, fish	−17.4±1.1 (7)	12.8±0.8 (7)	17.9±0.8 (6)
Alosa aestivalis[A, C]	blueback herring spawning	anadromous: copepods, cladocerans	−19.0±0.6 (7)	13.2±0.3 (7)	17.5±0.4 (7)
Alosa sapidissima[A, C]	juvenile American shadspawning	anadromous: copepods, small invertebrates	−20.2±0.6 (4)	12.6±0.4 (4)	8.0±2.2 (4)
Dorosoma cepedianum	gizzard shad	planktivore: filter feeder	−20.2±2.1 (7)	14.0±0.9 (7)	7.8±2.5 (7)
Cyprinidae					
Hybognathus regius	eastern silvery	minnowcarnivore: diatoms, algae, ooze detritus	−23.0±2.1 (6)	12.4±3.4 (6)	6.5±2.5 (6)
Notemigonus crysoleucas	golden shiner	carnivore: microcrustaceans insects	−24.8±1.1 (5)	13.1±1.6 (5)	2.5±1.7 (5)
Ictaluridae					
Ictalurus furcatus[C]	blue catfish	carnivore/piscivore	−21.6±1.9 (43)	15.4±2.0 (43)	9.2±3.0 (43)
Ictalurus punctatus	channel catfish	opportunistic generalist	−20.5±2.0 (3)	13.4±1.2 (3)	8.5±3.2 (3)
Ameirus nebulosus	brown bullhead	generalist/omnivorous	−24.0±0.8 (3)	13.2±0.5 (5)	5.3±1.6 (5)
Ameirus catus	white catfish	generalist/omnivorous	−21.2±2.7 (10)	15.8±2.3 (10)	8.7±4.7 (10)
Lepisosteidae					
Lepisosteus osseus	longnose gar	predator, piscivore	−23.1	16.8	8.34
Moronidae					
Morone saxatilis[A]	striped bass	generalist, piscivorous	−25.0±2.3 (2)	13.3±2.4 (2)	3.4±4.3 (2)
Morone americana[A*]	white perch	carnivorous: worms, shrimp, fish	−20.7±1.2 (5)	16.7 ±1.4 (5)	7.5±3.9 (5)
Percidae					
Perca flavescens[C]	yellow perch	carnivore: insects small fish	−25.1±2.1 (6)	14.3±2.2 (6)	6.9±1.6 (6)

The second objective was to test whether the different guilds of fish showed the incorporation of the marine isotope signal brought to the tidal freshwater by the anadromous fishes. This was observed, but largely limited to the predator guild.

Of the groups examined, the anadromous fish were the most ^{13}C-enriched, with mean values of approximately −19‰, followed by predators and planktivores (means −21.8‰ and −22.0‰, respectively), which were not significantly different from each other. This suggests that, of the remaining two guilds, carnivores were significantly ^{13}C-depleted relative to generalists (mean −24.1‰ and −23.5‰, respectively; Table 2). There was approximately a 10‰ range in $\delta^{13}C$ among the exclusively freshwater guilds (Table 2, Fig. 2).

Anadromous fish have elevated $\delta^{15}N$ values relative to freshwater fish with similar feeding strategies. However, the trophic enrichment and diet-tissue discrimination associated with $\delta^{15}N$ signatures make using nitrogen a less effective tracer for source than carbon or sulfur. In this study there was less variability within the guilds $\delta^{15}N$ signatures, relative to $\delta^{13}C$, although the range (‰)

Figure 2

$\delta^{15}N$ vs. $\delta^{13}C$ values for the four guilds and anadromous *Alosa* species, showing that most resident freshwater fishes are approximately two trophic levels above primary producers (C3 or autochthonous production), in contrast to the *Alosa* spp., whose $\delta^{15}N$ reveals that they are one trophic level above marine primary production. Boxes indicate the isotope signature of C3 terrestrial plant primary production, freshwater autochthonous production, and marine primary production. *Alosa* spp. are ^{13}C-enriched relative to most freshwater residents, reflecting marine primary production.

Figure 3

$\delta^{34}S$ vs. $\delta^{13}C$ values for the four guilds and anadromous *Alosa* species, with boxes to indicate the isotope signature of C3 terrestrial plant primary production, freshwater autochthonous production, and marine primary production. *Alosa* spp. are highly ^{34}S-enriched relative to most freshwater residents, reflecting marine sulfate (which becomes incorporated into primary producers and *Alosa* spp. while they grow in the Atlantic Ocean). Predators are the only guild showing elevated $\delta^{34}S$, indicating the incorporation of marine protein derived from *Alosa* spp.

of $\delta^{15}N$ values among all fishes was similar to that observed for $\delta^{13}C$ (10‰). The anadromous fish had the lowest $\delta^{15}N$ values and generally grouped between 12‰ and 13‰; however, their values were not lower than generalists or carnivores. The predators were the most ^{15}N-enriched of any group (Table 2). There were no significant differences among the $\delta^{15}N$ values for carnivores, generalists, and planktivores (Table 2).

Sulfur isotopes were hypothesized to be the most useful for tracing marine protein into freshwater, owing to extreme differences between the $\delta^{34}S$ of marine plankton and various sulfur sources in freshwater. Predator fishes and anadromous *Alosa* spp. showed elevated ^{34}S signals relative to other resident freshwater fishes, indicating that the predators incorporated *Alosa* spp. sulfur (protein). The range of $\delta^{34}S$ values among all the fish captured was from approximately 0‰ to 20‰, a considerably larger range than observed for the other two isotopes (Table 2, Fig. 3). Significant differences were observed in $\delta^{34}S$ among several of the separate groups. Anadromous species were highly ^{34}S-enriched relative to all resident freshwater fish (Table 2, Fig. 2), although the striped bass (40 cm total length (TL)) had values between 0.3‰ and 6.4‰, the lowest of the anadromous $\delta^{34}S$ values. Predators were the most ^{34}S-enriched of the resident fish, followed by planktivores (a trend also observed for $\delta^{13}C$). Carnivores and generalists were the most ^{34}S-depleted of the guilds and were not significantly different from each other (Table 2). Sulfur was the only stable isotope that completely separated the anadromous *Alosa* spp. from the full time freshwater residents. All of the *Alosa* spp. individual values were ^{34}S-enriched and outside the ranges observed in the other groups (Table 2).

Fatty acid analysis

Fatty acid (FA) isotope values show that some predators derive fats from anadromous fish and that there is a large variation among FA isotope values. FA $\delta^{13}C$ values were determined for one alewife (anadromous), one gizzard shad (*Dorosoma cepedianum,* a native freshwater planktivore), and two blue catfish (*Ictalurus furcatus*, an introduced piscivorous predator). For the blue catfish bulk $\delta^{13}C$ and $\delta^{34}S$ values from muscle tissue showed that one individual (A in Table 3) was significantly ^{13}C and ^{34}S-depleted relative to the other. This was also the case for the respective $\delta^{13}C$ values of their individual FAs. The anadromous alewife and the more ^{13}C-enriched blue

Table 3

Fatty acid (FA) $\delta^{13}C$ values for Rappahannock River fish. Means ± 1 Standard Deviation. (n=3). Values are corrected for CH4OH derevitization. FAs show that carbon from anadromous fish has been incorporated by *Ictalarus furcatus* but not by other resident fishes. Bulk isotope values show trends similar to the FAs and are as follows: alewife *A. pseudoharengus*, $\delta^{13}C$ –19.3‰, $\delta^{15}N$ 11.9‰, $\delta^{34}S$ 17.1‰; blue catfish *Ictalarus furcatus* (A) $\delta^{13}C$ –26.0‰, $\delta^{15}N$ 13.3‰, $\delta^{34}S$ 6.1‰; *I. furcatus* (B) $\delta^{13}C$ –19.3‰, $\delta^{15}N$ 16.6‰, $\delta^{34}S$ 10.8‰; gizzard shad *Dorosoma cepedianum* $\delta^{13}C$ –21.5‰, $\delta^{15}N$ 14.5‰, $\delta^{34}S$ 10.2‰.

Fatty acid	*Alosa pseudoharengus* alewife (‰)	*Ictalurus furcatus* blue catfish (‰)	A *Ictalurus furcatus* blue catfish (‰)	B *Dorosoma cepedianum* gizzard shad (‰)
12:0	–22.4 (0.4)	–28.5 (0.5)	–22.5 (0.9)	–27.4 (1.0)
14:0	–27.4 (1.8)	–33.6 (0.9)	–26.9 (0.6)	–25.5 (1.4)
16:1	–26.8 (0.8)	–35.4 (0.6)	–25.6 (0.7)	–27.4 (0.6)
16:0	–22.1 (0.1)	–30.3 (0.2)	–23.3 (0.3)	–25.7 (0.6)
18:1	–23.3 (0.6)	–30.5 (0.6)	–24.5 (0.7)	–28.7 (0.4)
18:0	–19.9 (1.8)	–28.8 (0.7)	–20.4 (1.1)	–23.5

catfish (B) had $\delta^{13}C$ FA values that, for the most part, overlapped with each other. Their 16 and 18 carbon length FAs were generally ^{13}C-enriched relative to the gizzard shad and the second blue catfish (A) (Table 3). For all fish, except gizzard shad, the saturated 12:0, 16:0, and 18:0 FAs were more enriched (2‰ to 6‰) than the 14:0, 16:1 and 18:1 FAs. 14:0 FAs are not elongated to 16 or 18 carbons in animals, which is why they are ^{13}C-depleted relative to saturated 16:0 and 18:0 (see Discussion). For the gizzard shad, the 12:0 FAs were 2‰ depleted relative to the 14:0 FAs. The blue catfish (B) with low $\delta^{13}C$ and $\delta^{34}S$ bulk values, generally had more ^{13}C-depleted FAs than other fishes. There was up to a 10‰ range among the FAs within an individual fish, with unsaturated FAs ^{13}C-depleted relative to saturated, and longer saturated chains being generally ^{13}C-depleted relative to shorter chain FAs (Table 3).

Discussion

The fact that the anadromous *Alosa* spp. were the most ^{13}C-enriched of the groups examined was expected because they retain the ^{13}C-enriched (relative to freshwater) signal of marine carbon fixation (Garman and Macko, 1998, MacAvoy et al., 2000, Hoffman et al., 2007). High $\delta^{13}C$ in freshwater systems with anadromous fish does not necessarily indicate trophic status (Garman and Macko, 1998; MacAvoy et al., 2000; Gregory-Eaves et al., 2007). The ^{13}C-enriched predators (mostly piscivorous catfish) show a wide range in $\delta^{13}C$, from –16 to –27‰ (white perch also show elevated $\delta^{13}C$ relative to most resident freshwater fish, but they also are ^{34}S-depleted, indicating that their carbon signature reflects their status as a secondary carnivore, not marine carbon). The most ^{13}C-enriched of the predators reflect the consumption of marine material, probably spawning adult *Alosa* spp., which had the most ^{13}C-enriched values of any prey item found. A number of predators, however, clearly derive very little carbon from marine

migrants; they are strictly freshwater feeders, as shown by their ^{13}C-depleted carbon isotope values. Among the remaining three guilds, the planktivores (within which the anadromous *Alosa* spp., mainly filter feeders, were not included) were the most ^{13}C-enriched, driven largely by the migratory and filter-feeing gizzard shad (Jenkins and Burkhead, 1993). Gizzard shad ^{13}C enrichment probably reflects consumption of autochthonous production and not marine derived nutrients, because the gizzard shad $\delta^{34}S$ are too low to reflect substantial marine material (Table 2 and see below). The $\delta^{13}C$ range among the resident freshwater fishes suggest, not surprisingly, that both autochthonous and allochthonous production contribute to carbon fixation in this tidal freshwater stream. Indeed, in the York River estuary, a few kilometers south of the Rappahannock River, Raymond and Bauer (2001) estimate that between 38% and 56% of dissolved organic carbon was derived from internal (autochthonous) sources.

Only a small percent of the residents show an exclusive allochthonous signal in the region of the Rappahannock River examined, and most of the resident freshwater fish show an autochthonous $\delta^{13}C$ signature, which is characteristic of small tributaries close to the main stem of a large piedmont river. The $\delta^{13}C$ range of allochthonous productivity in Virginia tidal freshwater streams is between –25‰ and –28‰ (Garman and Macko, 1998; Hoffman et al., 2007). Because CO_2 solubility is limited in water, systems dominated by autochthonous production tend to be ^{13}C-enriched relative to C3 plants that appear in small streams dominated by C3 allochthonous production (Michener and Schell, 1994). Garman and Neilson (1992) note that the presence of gizzard shad and detritivores in Virginia tidal freshwater suggest that autochthonous production is important in these systems relative to non-tidal areas upstream, where fishes primarily consume terrestrial arthropods (Garman, 1991). Most of the guilds examined in this study reflected the predominance of autochthonous production and have $\delta^{13}C$ values that are lower

than would be expected for a C3 dominated system. The anadromous *Alosa* spp. were also ^{13}C-enriched relative to other guilds. All of their δ^{13}C values cluster between –22‰ and –16‰, whereas all other guilds range to approximately –28‰ range (the most ^{13}C-depleted values reflecting allochthonous production). This ^{13}C enrichment in *Alosa* spp. is not due to incorporating autochthonous freshwater production. The ^{13}C-enrichment is a signal from the marine environment from which the *Alosa* spp. biomass was derived. This interpretation is supported by the markedly ^{34}S-enriched values of the *Alosa* spp., which are in most cases 7‰ greater than any other fish in this study (δ^{34}S value of sulfur fixed from marine SO_4 in the ocean at present is highly enriched relative to freshwater [Kaplan et al., 1963]). Therefore, the ^{13}C enrichment of the *Alosa* spp. biomass (and other anadromous fishes) is due to a marine influence, not an autochthonous influence.

Of the guilds examined, predators show the highest δ^{34}S value after the *Alosa* spp., but are not significantly enriched in ^{13}C relative to other guilds. The elevated ^{34}S in predators (many of whom are piscivores) shows that more marine sulfur is incorporated by this guild relative to others. The predator's elevated δ^{15}N values place them at the top of the fish food web, although some smaller individuals (blue catfish), feed at lower trophic levels while young (Jenkins and Burkhead, 1993).

The link between anadromous *Alosa* spp. and the predators is also supported by the fatty acid carbon isotope signatures. *Alosa* spp. 16 and 18 carbon FAs were generally the most ^{13}C-enriched of the fish examined (Table 3). The two large (53cm TL) blue catfish show two very different FA isotope profiles. One blue catfish (B in Table 3) had a series of highly ^{13}C-enriched FAs (bulk muscle tissue δ^{13}C and δ^{34}S are also enriched in this individual) and the other had FAs with isotope signatures similar to allochthonus primary production (also consistent with bulk muscle tissue δ^{13}C and δ^{34}S). Shorter chain (12 carbon) and more saturated FAs reveal the original δ^{13}C of the fats in the diet. Longer chain and unsaturated FAs can be subject to *de novo* transformations, which result in well established fractionations as chain length is systematically increased or as a double bond between carbons is created (making a point of unsaturation in a saturated FA). Generally, there is a 2‰ depletion in δ^{13}C arising from each unsaturation and another 2‰ depletion for each two carbon acetyl group addition (Deniro and Epstein, 1977). The most conservative tracer of dietary FAs, are the enriched precursors to long chain and unsaturated FAs. Among the FAs analyzed, the 12:0, 16:0, and 18:0 yield the best δ^{13}C estimate for dietary FAs, which clearly show distinct isotope signals depending on the carbon sources listed below: 1) ^{13}C-enriched marine isotope signals (represented by alewife and blue catfish B), 2) allochthonus production (represented by blue catfish A), or 3) a mix of autochthonous and allochthonus production, with the possibility of marine influences (represented by gizzard shad, although their δ^{34}S values do not reflect the typical marine signal).

The δ^{13}C and δ^{34}S distribution and range among the freshwater fishes suggest, not surprisingly, that both autochthonous and allochthonous nutrient sources, with the allochthonous sources being terrestrial C3 vegetation and marine primary production inwelling to this tidal freshwater stream, more than 40 km from the Chesapeake Bay. Unlike streams on the West Coast of the United States, where marine derived nitrogen and carbon can be an important nutrient source to inland ecosystems (Kline et al., 1993; Bilby et al., 2003; Chaloner et al., 2002), for all fish guilds in the study reported here, except the predators, there was not significant marine nutrient uptake. Several West Coast studies have shown that marine derived nitrogen, and some marine derived carbon, contributed to invertebrates (Francis et al., 2006; Hicks et al., 2005), primary producers, and juvenile fish within or near the sites receiving the spawning anadromous fish (Bilby et al., 2003; Koyama et al., 2005). For example, Bilby et al. (1996) found that 17% and 30% of the nitrogen in collector-gathers and juvenile coho salmon *(Oncorhynchus kisutch)* in Washington, were derived from spawning salmon. Ben-David et al. (1998) found that salmon carcasses may have contributed to the nitrogen incorporated by some terrestrial plants, as well as deer mice, squirrels, and voles; and Wipfli et al. (2003) found that salmon carcasses fueled increased growth rates among young salmonids. However, those studies show that only some material from decaying salmon makes its way into invertebrates and riparian vegetation (Bilby et al., 1996, 1998; Francis et al., 2006). There is strong evidence however, that the nutrients deposited as a result of the postspawning death of anadromous adults did significantly sustain fry the following year (Bilby et al., 1996, 1998).

In the East Coast stream examined here, carnivores and generalists, which consume benthic invertebrates as part of their diet, did not show a marine signal. Compared with anadromous salmonids on the West Coast, East Coast herring have a lower postspawning mortality and their runs have less biomass. Both of these facts indicate that a limited amount of marine protein and nitrogen maybe be delivered to spawning streams unless it is consumed directly by predatory fish. This is consistent with findings suggesting benthic insects in *Alosa* spp. spawning streams do not accumulate large amounts of marine derived material, even if they are living closely with post-spawning anadromous fish carcasses (Francis et al., 2006; Garman, 1992). It should be noted that in West Coast streams associated with spawning salmon, invertebrate uptake can be substantial (Hicks et al., 2005; Chaloner et al., 2002). Unlike most West Coast streams however, some tidal streams in Virginia have large piscivorous fish (introduced from Texas, Louisiana, or Mississippi in the 1970s) and these fish clearly incorporate marine material. So, while salmon (and presumably herring) on the West Coast import nutrients to the base of the food web (terrestrial autotrophs, young-of-the-year fish, and some invertebrates), in the steams examined here the marine material enters the top of the aquatic food web

where spawning adult anadromous fish are consumed by piscivorous fish. In order to fully understand the importance of a migratory or transitory nutrient source to consumers, the time required for that nutrient to be incorporated must be understood, thereby allowing a temporal evaluation of ecosystem structure. While the results of this study suggest that marine material does not form a substantial nutrient source to most of the fish community, more work needs to be done to investigate marine inputs derived from spawning anadromous fish, to other, lower order components of East Coast United States tidal freshwater systems.

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Appendix 3: Bay Journal Article

Yes, there is something fishy about scientists' spying on Bay's fish-eating birds

Increased bird numbers take a bite out of fish populations, though some osprey don't get enough

By Karl Blankenship

Scientists have lately taken to snatching feathers from osprey nests around the Bay. And, in some cases, they've set up cameras to spy on everything going on in eagle nests.

And when it comes to cormorants, they wade straight into their colonies.

"The adults flush, but the young stay and regurgitate whatever is in their stomach," said Adam Duerr, a biologist with the Center for Conservation Biology, a research center operated by the College of William and Mary and Virginia Commonwealth University.

The object, of course, is not to wade through bird vomit but to learn what's been on the menu of fish-eating birds around the Bay.

Unlike fisheries scientists, who simply cut fish open to see what's inside, those studying birds need to be more creative. "You can't go out and kill hundreds of osprey to see what they've been eating," said Greg Garman, director of the Virginia Commonwealth University's Center for Environmental Studies.

Instead, they can get some diet information by analyzing osprey feathers, or watching what species birds bring back to nests to feed their young. In the case of cormorants, Duerr said, biologists "can go around the colony and see whatever they have spit up, then count, measure and identify the fish they were consuming."

Knowing what birds eat is important because populations of birds that eat fish-eagles, osprey, cormorants, brown pelicans and great blue herons-have soared in recent decades.

After DDT nearly eliminated them in the 1970s, thousands of eagles and ospreys now nest along the Bay and its tidal tributaries. Brown pelicans and double-crested cormorants, which were not previously present, have moved into the Bay, and their numbers have increased dramatically.

That, in turn, has resulted in a huge potential demand for fish.

Using crude estimates, the scientists say birds may have consumed about 4.5 million pounds of fish when populations bottomed out in 1975. By 2005, avian predators around the Bay needed about 38 million pounds of fish-and scientists expect that number to increase for at least another decade.

In some lakes, cormorants have been shown to have significant impacts on fish populations.

In the Bay, birds appear to consume a relatively small, but still significant, number of fish

compared with other predators-mainly other fish and humans.

Nonetheless, the increase could affect management. Fishery managers set catch limits based on models that estimate how many fish are in the population. Those models assume that "natural mortality"-all sources of death except fishing-remains constant over time.

Those models don't account for the fact that birds are eating eight times as many fish as 30 years ago, and that number could increase over the next decade.

"In the world of single-species assessments, these things are all constants," said Jim Uphoff, a fisheries biologist with the Maryland Department of Natural Resources, who is cooperating with the study. "We don't typically do assessments with that thought in mind."

The research is being funded by the National Oceanic and Atmospheric Administration's Chesapeake Bay Office in the hope that it will provide information for future models that will better reflect predator-prey relationships, and therefore better inform management decisions.

To that end, the scientists-who are midway through a four-year project-are trying to learn what types and amounts of fish the birds are eating to better refine their estimates.

The greatest impacts, though, may not be how birds are affecting fish, but rather how changes in fish populations may be affecting birds.

For instance, many believe the Bay's menhaden population is in decline, possibly depriving striped bass and other predators of food. The research suggests effects could reach into bird nests as well.

Several studies in the last three decades have examined osprey diets in Mobjack Bay, located between the mouths of the Rappahannock and York rivers in Virginia.

In the mid-1980s, those studies found that 75 percent of the diet of nesting osprey was menhaden. By 2005, only a quarter of their diet was menhaden. The switch from menhaden, an oily, energy-rich food, to other species appears to have dramatically affected osprey.

Production of young osprey in Mobjack Bay today is as poor as it was during the DDT era. The difference, said Bryan Watts, director of the Center for Conservation Biology, is that during the DDT era, when the pesticide caused thinning of egg shells, only 30 percent of the eggs even hatched. Today, more than 90 percent of the eggs hatch, but the young birds die.

"The chicks just are not being fed enough and die at a young age," Watts said. "I believe that because menhaden are so energy-rich, they are not a replaceable component in the diet."

As a result, the osprey population in Mobjack Bay has stagnated.

A similar pattern is emerging around the Chesapeake. With somewhere between 6,000 and 8,000 active nests, the Bay region holds the largest osprey population in the world, but the number of nesting osprey in high-salinity areas has leveled off.

"If menhaden are a critical component of osprey diet and the rug is essentially pulled out from under them, it is possible we could see a population collapse," Watts said. "But if menhaden came back, they would likely recover."

Meanwhile, osprey populations in tidal-fresh areas-those near the upper limit of the Bay's tidal influence-continue to grow exponentially.

Tidal freshwater areas contain a unique mix of marine and freshwater species. Osprey can still find other clupeoids-a fish group that includes menhaden, herrings and shads-to eat as migratory hickory shad and nonmigratory gizzard shad abound. Thriving populations of exotic predators, such as blue catfish, flathead catfish and others, are present in huge numbers.

The booming populations of fish in tidal-fresh areas may help bald eagles, as well. Their nesting was once timed to coincide with the spring migration of shad and herring into Bay tributaries. Nesting would occur in winter so the hatching of hungry young birds occurred as vast numbers of fish, filled with energy-rich eggs and sperm, began migrating up the rivers.

Populations of herring and shad have been decimated by loss of habitat from dam construction, pollution and overfishing. But like the osprey in tidal fresh areas, bald eagles have found substitutes, including blue catfish, an introduced species and voracious predator that seems to make up a good portion of the eagles' diet.

"The only good thing that I can say about blue catfish is they've probably been pretty important in the recovery of bald eagle and osprey, and it explains why the greatest concentration of both of these bird species has been in the tidal fresh waters," Garman said.

The change in diet does have a downside. Some areas, such as the tidal fresh portion of the James, have fish consumption advisories because of elevated levels of PCBs and other toxins in blue catfish.

"In a few years, are we going to see some sort of toxic effects from the contaminants in the blue catfish?" Garman asked. "It is a reasonable expectation that these birds might be affected."

Meanwhile, cormorants and pelicans, which are generalists in what they eat, appear to be doing well around the Bay as their populations continue to expand. Work is continuing this year to analyze their diets.

Understanding the blue heron diet is the most problematic. They are widely dispersed around the Bay and its tributaries, so their diets may vary considerably from place to place. But with more than 18,000 pairs estimated to be around the Bay, they are also the most numerous avian predator.

"The great blues are the real gorilla of those species here, the population is huge now, and they are here most of the year," Watts said.

Scientists say getting fisheries biologists and ornithologists to work together to understand a significant part of the Bay food web has been as important as their findings.

"It's a great opportunity for fisheries people to work with the bird people, and for the first time begin to see what some of the relationships are," Watts said. "I think as we begin to look at some of the fisheries regulations, considering some of the other consumers in the equation would be a great thing."

That has begun to happen in some places, as fishery regulations along the mid-Atlantic coast work to conserve horseshoe crabs because their eggs are essential food for migrating red knots, which stop each spring to eat.

"If you're going to start looking at things on an ecosystem basis, these things are important," Uphoff said. "If you don't consider it, you're living in a fool's paradise.

Feathers offer glimpse into ospreys' diet

Ospreys-or at least their feathers-may soon become a key tool to monitor menhaden populations around the Bay.

By examining stable isotopes in their feathers, scientists already can determine how much of an osprey's diet in the previous few weeks or months came from freshwater fish or marine species.

The scientists, who are studying fish predation by birds with a grant from the National Oceanic and Atmospheric Administration's Chesapeake Office, have used the technique to study historical changes in bald eagle diets.

They obtained a feather from every eagle in the Smithsonian collected around the Bay since the mid-1800s. The feather analysis showed their diet overwhelmingly originated from marine environments, as they ate shad and herring returning from the ocean to spawn, until the 1970s, when shad stocks collapsed around the Bay. After that, freshwater fish became the mainstay of their diet.

Now, Stephen Macko, at the University of Virginia, is trying to use isotope analysis to identify an individual species-menhaden. This spring, scientists are replacing some of the food in several osprey nests with menhaden, and comparing isotopes in their feathers with those from nests without menhaden.

If they can determine a specific "marker" for menhaden, they will eventually be able to determine the relative size of the menhaden stock by collecting feathers from osprey around the Bay and seeing what portion of their diet consisted of menhaden in the previous weeks.

The osprey are ideal for such monitoring because they seem to prefer menhaden, unlike other birds which are more general in their feeding, and they are widely distributed around the Bay.

"You could get a snapshot of different places around the Bay at the same time," said Greg Garman, director of the Virginia Commonwealth University's Center for Environmental Studies. "Over time, you could track the stock more effectively than you can just from landings alone."

Cap on menhaden catch may be extended until research is complete

The catch limit for menhaden in the Chesapeake may be capped for an additional three years as research aimed at determining the health their population in the Bay continues.

The existing annual cap of 109,020 metric tons of menhaden from the Chesapeake Bay is set to expire after next year.

In 2006, the Atlantic States Marine Fisheries Commission adopted a five-year cap based on the average landings from 2000 through 2005. The intent was to allow for research to determine whether the Bay is suffering from "localized depletion" of menhaden.

While ASMFC stock assessments show the coastwide menhaden stock is healthy, sportsmen and some conservation groups have charged that the Bay has too few of the small, oily fish to support striped bass and other predators because of fishing pressure in the Bay. The menhaden fishing

fleet is based in Reedville, VA.

The three-year extension was proposed because much of the research will not be completed and analyzed before the cap expires.

Unlike other fisheries, Virginia's menhaden catch is regulated by the General Assembly, rather than the Virginia Marine Fisheries Commission. To extend the cap past 2010, the General Assembly would have to approve the change when it meets next winter.

To keep that timetable, the ASMFC may need to approve a draft amendment to its management plan when it meets in August, and then submit the plan for public comment. Final action could happen when the commission meets this fall.

But an extension may face opposition unless the cap is changed.

Ken Hinman, president of the National Coalition for Marine Conservation, said he was disappointed that no other other extension options were proposed, such as resetting the cap to reflect the most recent five years of catch data.

That would certainly result in a lower limit as almost all recent catches in the Bay have been less than 100,000 metric tons.

He also said that recent studies raise "red flags" about menhaden. The studies show increased striped bass mortality in the Bay, while menhaden reproduction remains low. Overall menhaden landings along the East Coast continue to decline.

"There are a lot of reasons to want to take more precautionary actions as opposed to a cap that is not really constraining the fishery at all," he said. "We probably need to do more than we've been doing, so we're not in favor of just extending the current cap."

Karl is the Editor of the Bay Journal.

Appendix 4: Comparison of weekly boat electrofishing and hydroacousitic surveys.

Kimages Cove 23 May 2008 N 4133040.88 E305176.27						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	178				178	672	
DCE		27		23	50		
LOS				4	4		
CCA				1	1		
DPE		15			15		
IFU		1			1		
Depth	1-6.2m				249	672	262

Powells Cr mouth 23 May 2008 N 4140351.22 E 309324.18						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	5				5		
DCE		22	2	13	37		
ARO				1	1		
LOS				3	3		
MBE	1				1		
DPE		23			23		
IFU		2	3				
Depth	0.2-6.9 m				75	1056	506

Tar Bay channel 23 May 2008 N 4130831.95 E 308128.62						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
DCE		7		1	8		
MSX				1	1		
IFU		24		39	63		
Depth	.49-11m				72	480	669

Berkely 23 May 2008 N 4131370.32 E 308028.13						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	182				182		
DCE		85		4	89		
DPE	1	13			14		
MSX	1				1		
MBE	1				1		
IFU				2	2		
Depth	.25-2.2m				289	600	50

Jordan Pt Triangle 27 May 2008							
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	4				4		
DCE		13	1	44	58		
DPE	1	65			66		
CCA				2	2		
MBE	11				11		
MSX				1	1		
IFU		1	1	15	17		
Depth	0.5-5.5				159	660	341

Jordan Pt Marina 27 May 2008							
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	3				3		
DCE				8	8		
IFU	1	6	4	3	14		
Depth	0.5-4.8m				25	560	49

Tar Bay West End 27 May 2008							
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
LOS				1	1		
BTY	8				8		
DCE		15		16	31		
DPE		2			2		
IFU		3		27	30		
Depth	0.5-6.6m						
AvgD	3.965m	MaxD	6.6m		72	720	98

Rice to mouth of Harris Cr 3 June2008						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	6				6		
ASA	1				1		
DCE		9	1	9	19		
DPE		39			39		
IFU			10	52	62		
CCA				2	2		
					129	501	37

Colony near Allied Chemical 3 June2008						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	1				1		
DCE		4	1	4	9		
DPE		13			13		
IFU			40	500	540		
MSX				2	2		
					565	505	467
Avg D	4.78m	Ma xDepth	6.3 m				

Tar Bay 3 June2008						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	11				11		
ASA	1				1		
DCE		2		10	12		
DPE		5			5		
IFU				35	35		
					64	485	66
Avg D	3.27m	Ma xDepth	4.4 m				

Cross-Channel below Bridge 9 June 2008 N 4132608.79 E 303469.84 start						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	1	1			2		
DCE		21		12	33		
DPE		5			5		
MAM		1			1		
IFU		2		20	22		
					63	720	983

Jordan Pt Triangle 9 June 2008						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	5	3			8		
DCE		29		8	38		
DPE		1			1		
MBE	2				2		
MAM		1			1		
IFU		2		1	3		
					53	690	59

Colony Run 9 June 2008						
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
LOS				20	20		
DCE		2		21	23		
IFU				200			
					243	580	382

Colony area across from Allied 26 June 2008							
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	8	12			20		
DCE	1	32		6	39		
DPE		1			1		
IFU			137	16	153		
MSX	1				1		
					214	942	443
AvgD	6.94m	MaxD	8.4m				

Shoreline upstream from Allied 26 June 2008							
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	9	12			21		
DCE			9	1	10		
AAE	1				1		
NHU	2				2		
IFU			275	20	295		
MSX	5				5		
					334	650	821
AvgD	8.78m	MaxD	10.1m				

Dredge Island across from Allied 26 June 2008							
Species	0-10	10-20	20-30	>30	Total	Seconds	Hyrdo-count
BTY	113				113		
DCE			38	43	81		
IFU			3		3		
MAM	1				1		
MSX	1				1		
					199	628	138
AvgD	1.54m	MaxD	2.4m				84top 54 bot

Site	Date	Fish/sec	Ping/sec	
Kimages Cove	24 April 08	0.25		
Tar Bay	24 April 08	0.12		
TarBay shoreline	24 April 08	0.15		
Turkey Isl cut	29 April 08	0.12		
Jordan triangle	14 May 08	0.07		
Tar Bay	14 May 08	0.09		
Kimages	23 May 08	0.37	0.54	
Powells Creek	23 May 08	0.07	0.32	
Tar Bay Channel	23 May 08	0.1	1.3	
Berkeley	23 May 08	0.45	0.06	
Jordan Triangle	27 May 08	0.18	0.25	
Jordan Marina	27 May 08	0.03	0.36	
Tar Bay West	27 May 08	0.12	0.33	
Rice Pier Channel	3 June 08	0.26	0.17	
Tar Bay	3 June 08	0.13	0.23	
Allied channel	3 June 08	1.1	0.55	
Bridge Channel	9 June 08	0.09	2.6	
Jordan Triangle	9 June 08	0.08	0.13	
Colony	9 June 08	0.4	1.2	
Kimages Cr	17 June 08		0.11	
Main channel	17 June 08		1.9	
Marina flats	17 June 08		0.48	
Colony	26 June 08	0.24	1.2	
Allied up	26 June 08	0.52	2.5	
Dredge Island	26 June 08	0.15	0.42	