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NCU-T-04-001 C2

**Development and validation of juvenile abundance indices (JAIs) for
selected North Carolina finfish**

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Completion report for Study IV
NOAA award NA16FG1220

November, 2004

Introduction

It is the perpetual objective of fisheries scientists to accurately describe the state of fish stocks and to understand population fluctuations. Through stock assessments, this information is incorporated into management plans that are intended to safeguard the viability of fish stocks and fisheries. A key component of a stock assessment is knowledge of early life history dynamics and variability in recruitment (Houde, 1987).

In North Carolina, fishery management plans must be developed for exploited marine and estuarine species as mandated by the Fisheries Reform Act of 1997. The North Carolina Division of Marine Fisheries (NCDMF) is responsible for the development of those fishery management plans and for conducting the prerequisite stock assessments of the species. Through multiple sampling programs, the NCDMF has conducted fisheries-independent monitoring of young fishes in the sounds and rivers of the state since the early 1970s. Environmental and catch data, among other types of data, are archived in a mainframe biological database and are available by query. The long time series of these data presents a valuable opportunity to examine fluctuations in recruitment through the years. However, indices of young-of-the-year (YOY) abundance must be developed in an unbiased manner. The data must first be examined for consistency, and collection methodologies must be appropriate to the species of interest.

The NCDMF is scheduled to complete stock assessments and management plans for white perch (*Morone americana*) in 2009-2011, yellow perch (*Perca flavescens*) in 2010-2012, spotted seatrout (*Cynoscion nebulosus*) in 2007-2009, and southern kingfish (*Menticirrhus americanus*), northern kingfish (*Menticirrhus saxatilis*), and Gulf kingfish (*Menticirrhus littoralis*) in 2005-2007. Time series of juvenile abundance indices (JAIs) for each species will be an important part of the assessments. None of these species were the targets of any of the fishery-independent sampling programs, but they were captured in those programs. The purpose of this study was to evaluate the usefulness of existing data for description of recruitment in these species and to make recommendations that may improve future monitoring. Specifically, the procedural jobs were:

- Determine the most appropriate gear(s) to capture YOY and age-1 white perch, yellow perch, kingfishes, and spotted seatrout.
- Examine trends in catch and effort by appropriate gear for each of these species.

- Determine the validity of JAI through correlation analyses between annual values of age-1 abundance to YOY abundance that is lagged by one year.

The species under consideration exhibit disparate environmental preferences and life history strategies. It is important to be cognizant of those differences in evaluating the usefulness of data and in determining what additional data may be of value. The timing and duration of spawning and early growth rates are particularly relevant pieces of information when age is to be determined primarily through length-based methods.

The species of interest can be divided into two general categories: perches and sciaenids. The white perch is semi-anadromous, inhabiting estuarine waters until migrating upstream to fresh water for spawning from late March to May (in the Patuxent Estuary, Maryland) (Mansueti, 1961). The yellow perch is primarily a freshwater fish, but it does occur in brackish and tidal fresh waters. Spawning occurs in freshwater over a month's time (March in the Chesapeake Bay) (Muncy, 1962). Growth of young perch varies greatly between cohorts (Noble, 1975). In contrast, the kingfishes and spotted seatrout, all members of the family Sciaenidae, spawn over a protracted season (late spring through early fall) and exhibit rapid, sexually dimorphic growth (Nieland et al., 2002; Roumillat and Brouwer, 2004; Smith and Wenner, 1985). Differences between the two groups of fishes influenced the strategies applied for the identification of YOY.

Methods

Age determination and selection of sampling programs

In order to calculate juvenile abundance indices (JAIs) and indices of age-1 abundance for validation of those JAIs, it was necessary to identify YOY and age-1 fishes and to determine the sampling programs, gear types, and geographic locations in which these age classes of each species were most prevalent. To that end, the NCDMF biological database was queried for data pertaining to each of the species of interest. The resulting datasets were checked for obvious errors, such as unreasonable values and frame shifts in data entry. These were corrected in the downloaded datasets and all extraneous data records (i.e., non-target species and duplicate data records) were deleted

to yield species-specific datasets encompassing all DMF sampling programs. Unless otherwise noted, all data manipulations were performed with SAS version 8.02 software.

Within a sample, here defined as a single net tow or seine haul, total number of fish per status (i.e., broad size class) per species was recorded. Only a subset of those fish had been measured for length (fork length for perches and total length for spotted seatrout and the kingfishes), so the total number of a species status at length per sample was calculated by multiplying the sample total by the proportion of the measured subset at that length. Resulting values were not rounded to the nearest integer. Fish lengths were converted to 10 mm length classes, with the name of the class as the starting length (e.g., fish 60 - 69 mm composed length class 60).

In each species-specific dataset, fish numbers were summed over length class by month and year, excluding samples in which fish length was recorded as either a mode or maximum and minimum values, as opposed to lengths of individuals. Monthly length frequency distributions were created for each species for each year and evaluated for potential for identification of YOY and age-1 age classes by modal progression analysis.

Modal progression analysis is better suited to species with relatively restricted spawning seasons, such as the white perch and yellow perch, and as opposed to the sciaenids (Maceina et al., 1987). Therefore, additional sources of data to aid in age determination of the latter group were sought in the literature and among NCDMF personnel. A North Carolina-specific otolith-derived age length key (length classes divided into percentages at each age) was used to assign age groups to spotted seatrout age-1 and older (NCDMF Annual progress report, Grant F-42, 2003). The youngest fish included in that key were age-1, so YOY spotted seatrout were identified by length frequency modes. Southern kingfish, northern kingfish, and Gulf kingfish ages were determined entirely by age length key (Chip Collier, NCDMF, unpublished data). Age length keys for these species were initially divided by sex, but this information was not available in the NCDMF biological database, so the keys were combined for both sexes. Though the age length keys used to assign ages to these species were developed using fish collected within a small time period relative to temporal coverage of the database, lack of other age data made it necessary to apply proportions of age at length across all time periods.

In both perch species, growth slows greatly after the first year of life, causing overlap in length frequency distributions of ages one and older. Tools within the software package FISAT II (FAO-ICLARM Stock Assessment Tools) were utilized to estimate the shapes of age-specific distributions when there was perceived overlap in lengths of age-1 and older fish. An age length key was developed using these distributions such that each length class was assigned proportions of the overlapping age classes. Length frequency distributions of white perch and yellow perch were examined separately by month and year for assignment of ages because of reported high variability of growth in these species (Noble, 1975).

In the species-specific datasets, numbers of fish caught in each sample were summed by length class. Numbers in each length class in each sample were divided into numbers at age according to either the modal progression analysis or applicable age length key. Numbers at age were summed across each sample, so that for every sample, the numbers of YOY and age-1 fish caught were known.

Numbers of YOY and age-1 fish caught in all samples in each species-specific dataset were summed over sampling program to determine which programs resulted in the capture of these age classes and that were most appropriate in developing abundance indices. Fishery-dependent and non-quantitative sampling programs were excluded from these analyses.

Development of abundance indices

Those programs deemed to contain sufficient information on one or more of the species of interest, based on analysis of species-specific datasets, were downloaded from the NCDMF database. Within the chosen sampling programs, the geographic location in which YOY and age-1 fishes occurred and the sampling gears most effective in their capture were determined. Program-specific datasets were error checked and samples were chosen for the development of a time series of abundance indices based on consistency of sampling methods, areas, and times. Broader sets of samples were also analyzed in an attempt to detect if changes in sampling methodology affected the ability of the program to index the species and age class of interest.

Arithmetic means of catch per unit effort (CPUE) were calculated by each survey year, and by each month within each year. Geometric means were also calculated by month and year, but because calculation involved logarithms and zeros were prevalent, $\ln(\text{CPUE}+1)$ was used to transform data. Monthly indices were used in the evaluation of changes in the sampling program; yearly indices were more illustrative of population fluctuations. The geometric mean is favored as an abundance index because occasional extremely large catches do not unduly influence the mean value. Pearson correlation coefficients were calculated (PROC CORR, SAS Institute, 1999) for JAIs and the next year's index of age-1 abundance when both indices were available for the same species.

Results

Age determination

The spotted seatrout age length key did not contain data from YOY fish, but in most cases, this age class was reasonably separated from older fish in length frequency distributions. Length frequency distributions were not helpful in classifying any of the kingfish species.

Modal progression analysis of length frequency distributions adequately defined month- and year-specific length ranges for YOY white perch and yellow perch. Low sample sizes and high degree of length overlap of the next few age classes made identification of age-1 perches extremely difficult. Use of the FISAT II software made this task more objective, but did not completely eliminate subjectivity of interpretation of frequency distributions. Particularly with low sample sizes, it was difficult to evaluate the appropriateness of this method of identifying age-1 fish.

Selection of sampling programs

Program 100 (Juvenile Anadromous Survey- seine and trawl, primarily in Albemarle Sound) was clearly the best source of data on YOY and age-1 white perch (Fig. 1). It was also chosen as the data source for yellow perch, though catches of this species were an order of magnitude lower than those of white perch. The second large peak of YOY yellow perch abundance (Fig. 2; Program 160- Anadromous Egg and

Larval Sampling) resulted from two very large collections of larval yellow perch and was disregarded.

Fig. 1. White perch YOY and age-1 abundance in the NCDMF biological database by program. All areas and gears encompassed by a sampling program included.

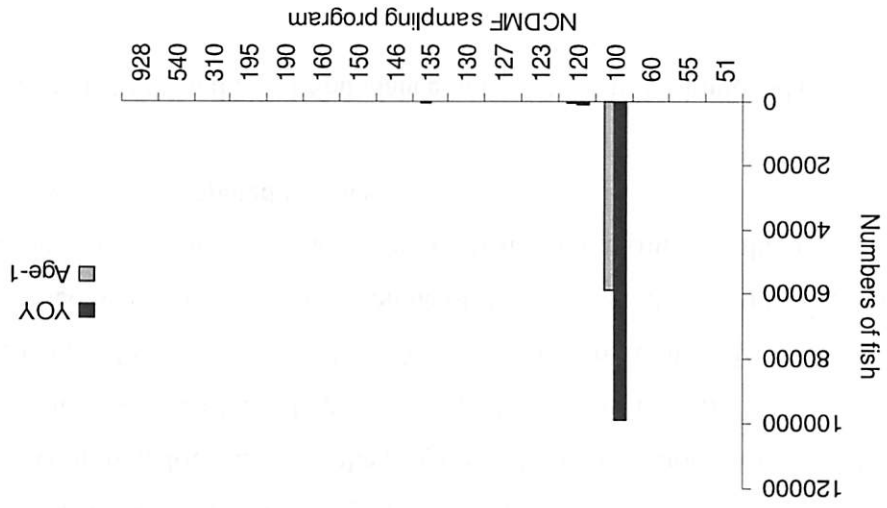
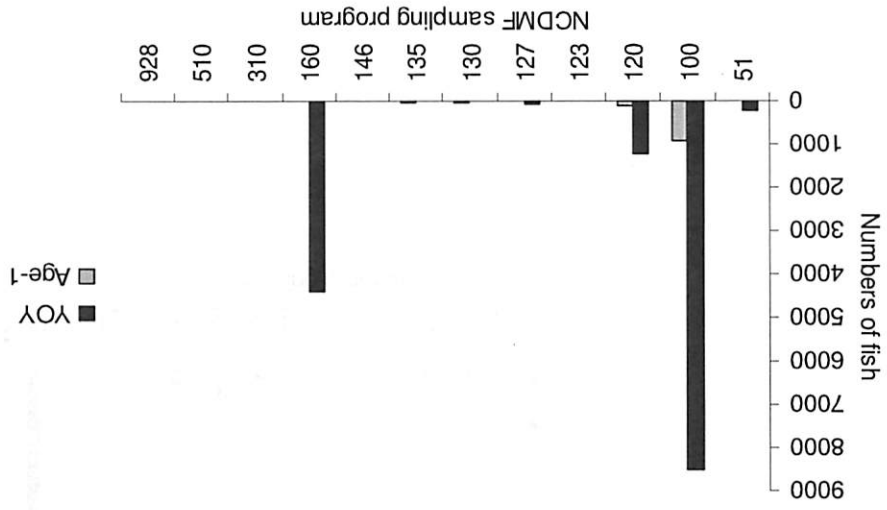


Fig. 2. Yellow perch YOY and age-1 abundance in the NCDMF biological database by program.



Few records of spotted seatrout, southern kingfish, Gulf kingfish, and northern kingfish were found in fishery-independent sampling programs. Program 120 (Estuarine Trawl Survey- primarily in Pamlico Sound) offered the largest collection of catch data for spotted seatrout YOY (Fig. 3). Program 135 (Striped Bass Independent Gill Net Survey- primarily in Albemarle Sound) was explored for its utility in devising an index of spotted seatrout age-1 abundance, but was eventually discarded due to low sample size (Fig. 3) and difference in geographic location from the program taking the majority of spotted seatrout YOY. No good source of age-1 spotted seatrout data was identified.

Highest catches of southern kingfish YOY and age-1 were found (Fig. 4) in Program 195 (Pamlico Sound Trawl Survey). None of the fishery-independent sampling programs caught significant numbers of northern kingfish or Gulf kingfish; therefore, abundance indices were not computed for these species.

Fig. 3. Spotted seatrout YOY and age-1 abundance in the NCDMF biological database by program.

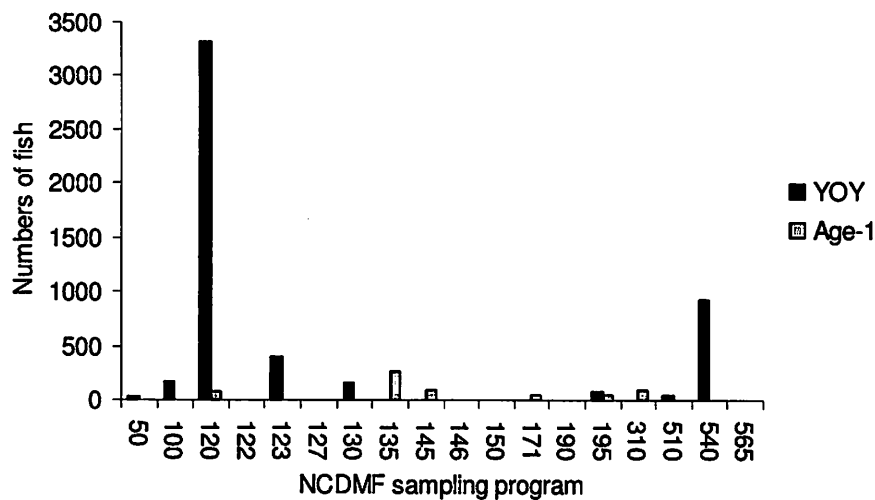
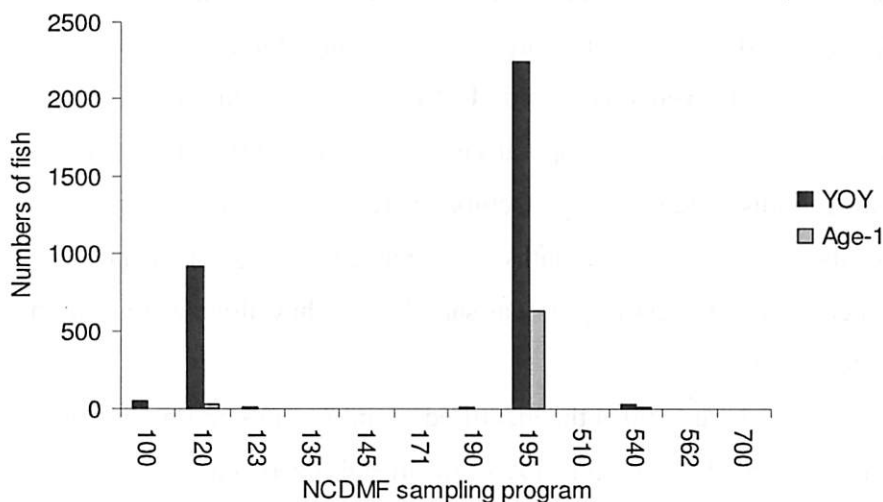


Fig. 4. Southern kingfish YOY and age-1 abundance in the NCDMF biological database by program.



Development of abundance indices

Program 100, white perch and yellow perch

White perch and yellow perch were taken in Program 100, which began in 1972, by seine and trawl. Throughout the time series, the seine was a 60 ft knitted bag seine with 0.25 in mesh (NCDMF biological database variable *gear*, code 311). The unit of effort was one seine haul. The predominant trawling gear used in the early years of the program was a Carolina wing trawl with 26 ft head rope, 4 in body mesh and 0.125 in tailbag mesh (*gear* 531). In later years of the program, continuing to present, the trawling gear was the North Carolina State University semi-balloon trawl (developed by Dr. William Hassler) with 18 ft head rope, 0.75 in body mesh and 0.126 in tailbag mesh (*gear* 535). There was a period of overlap between these two gears from 1982 to 1984, but a study of the comparative efficiency of these two gears was not conducted. The duration of trawl tows varied among and within sampling days, and in many cases (particularly samples in 1994), durations of tows were not recorded. Because of the mid-series gear change and the compromised ability to standardize catch to duration of trawl tows, only seine data were used in the development of abundance indices for time series examination.

Sampling in Program 100 took place predominantly in Albemarle Sound and its tributaries; this area yielded the majority of white perch and yellow perch. Fixed seining stations were dropped and added through the years, but 20 stations within Albemarle Sound were sampled consistently. For the purposes of creating abundance indices, only samples taken at these stations were considered. Until 1976, seine sampling was conducted nearly year-round. Though temporal sampling coverage fluctuated somewhat starting in 1977, the months of June through October were regularly sampled in the remaining years of the program. These months encompassed the highest catches of white perch and yellow perch when the entire year was sampled, so they alone were used in calculation of abundance indices.

Seining effort in Program 100 at the identified sampling sites and in the identified months was high but variable from 1973 to 1978. Effort stabilized at a lower level through the 1980s and into the early 1990s. The years 1993 and 1994 were characterized by a decline in sampling effort, but 1995 brought a slight increase above the effort of the 1980s. That level of effort continued through the remainder of the time series. Comparison of the arithmetic mean and geometric mean yearly catch per unit effort (CPUE) for YOY white perch (Fig. 5) indicates that the large fluctuations in population size evident in the arithmetic mean series (e.g., 1978 and 1994) are mainly due to isolated large catches. Considering this high variability of catch sizes within years, the geometric mean is the more appropriate indicator of YOY white perch population sizes. It will be the only indicator of abundance presented for the rest of the species/age classes.

YOY white perch abundance indices fluctuated widely about an overall decreasing trend (Fig. 6). Nearly alternating years of relatively high and low abundances characterized the early years of the survey until 1984, when an apparent poor recruitment period began and continued through the early 1990s. A very large spike in the JAI in 1993 indicates a break in the pattern of low recruitment. The following years saw abundances equal to or higher than those that occurred in the early 1980s, except for years of sparse catches of YOY white perch in 1995 and 1999. Since 2000, catches have been moderate.

Fig. 5. White perch JAI time series based on Program 100, Albemarle Sound, 60 ft knitted bag seine samples from June through October.

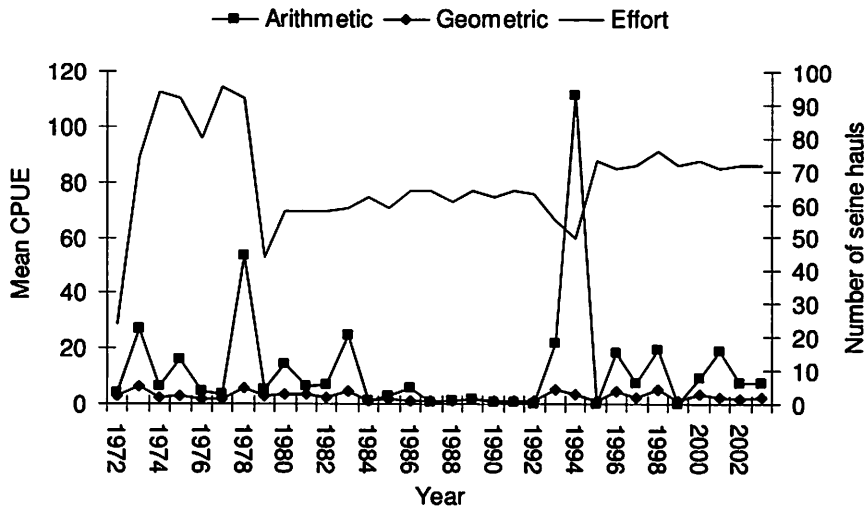
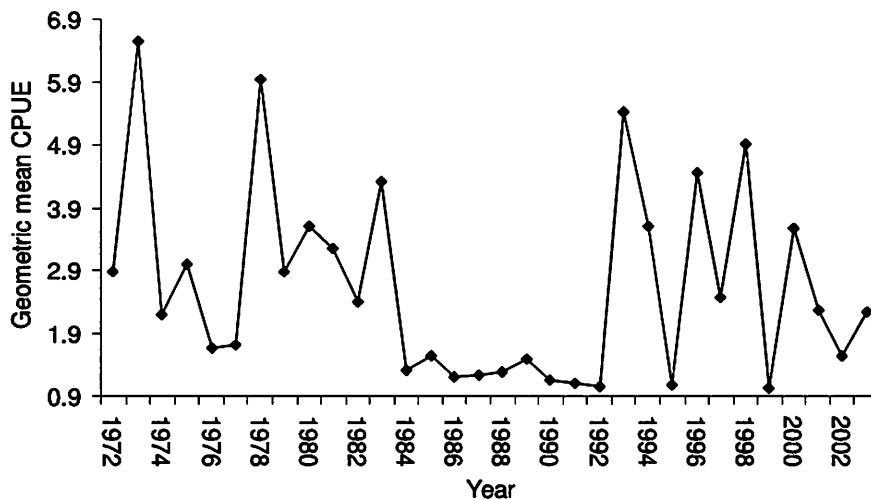


Fig. 6. White perch JAI time series based on Program 100, Albemarle Sound, 60 ft knitted bag seine samples from June through October. Data identical to Fig. 5, but geometric mean only is presented with an expanded y-axis scale.



Age-1 white perch abundance appeared to vary less widely than that of white perch YOY (Fig. 7). The early years of the program were marked by variability, leading up to an unusually large CPUE in 1979. The early 1980s saw a return to average pre-1979 abundances, but those declined to very low levels in the mid 1980s. The abundance

index remained stable at a very low level through 1993, followed by a steep increase in 1994. Over the next two years, abundances again declined, reaching a low point in 1996. A gradual increase in abundance through 1999 preceded a dramatic drop in abundance in 2000. Though 2001 was a better year for age-1 white perch, the remaining years of the series are characterized by low abundances. Taken as a whole, the time series of white perch age-1 abundance indices was trendless.

A visual comparison of abundance indices for YOY and age-1 white perch reveals some similarity in trends from one year to the next within the same cohort. For example, 1978 was a year of relatively high YOY abundance and 1979 was marked by very high age-1 abundance. Poor recruitment in 1999 was followed by a paucity of age-1 white perch in 2000. Low levels of abundance were reflected in abundance indices of both age classes through the late 1980s and early 1990s. The large YOY year class of 1993 was apparent in catches of age-1 in 1994. Geometric mean abundances of white perch YOY were in fact highly correlated with the geometric mean abundances of age-1 white perch in the next year (Pearson correlation coefficient=0.759; $p < 0.0001$; Fig. 8).

Fig. 7. White perch age-1 abundance index time series based on Program 100, Albemarle Sound, 60 ft knitted bag seine samples from June through October.

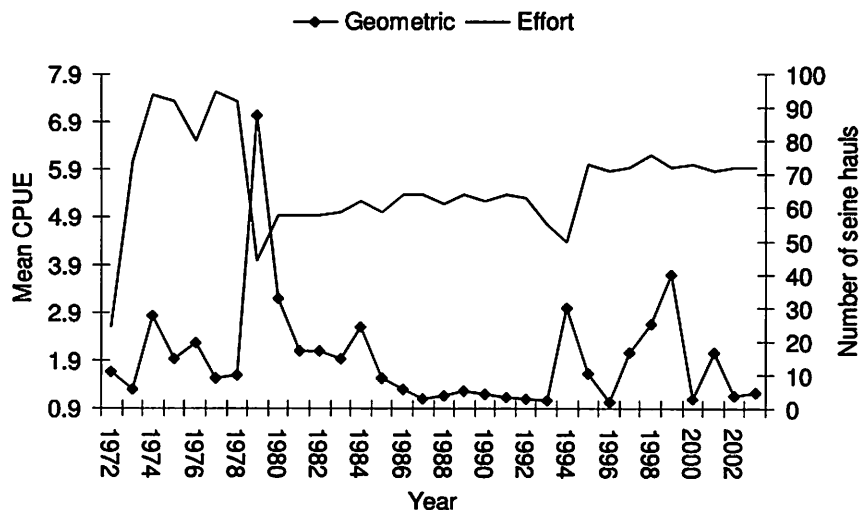
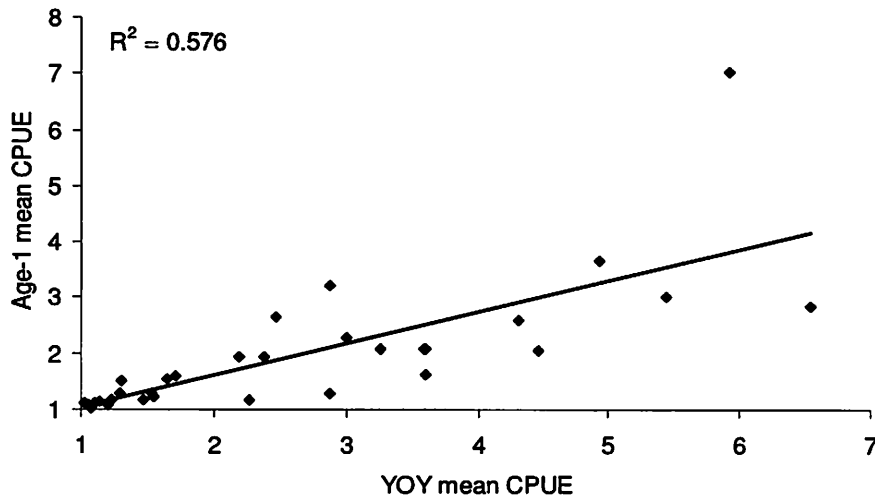
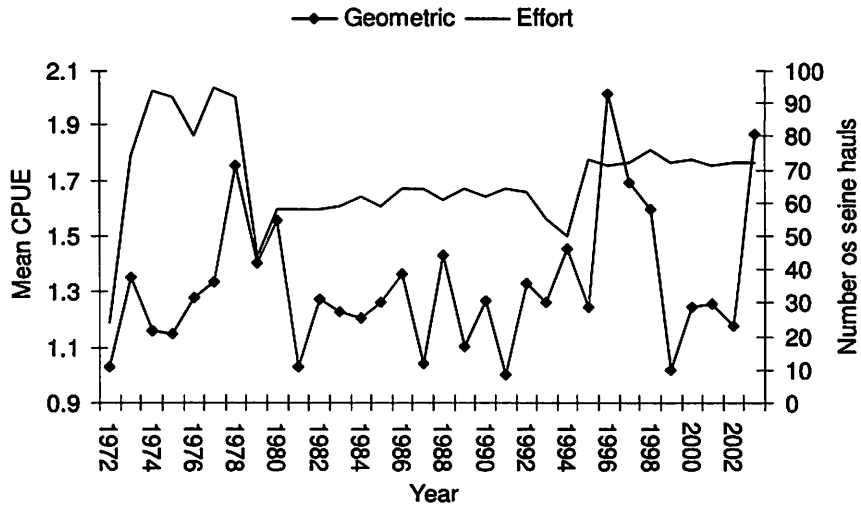


Fig. 8. Correlation between geometric mean CPUEs of YOY and the next year's age-1 white perch.



Yellow perch YOY abundance was particularly low in 1972, 1981, 1987, 1991, and 1999 (Fig. 9). The small cohort of 1981 was preceded by a few years of relatively high abundance, including a peak in 1978. Year class size was stable and average through the early 1980s, but experienced higher variability through the late 1980s and early 1990s, leading into a year of higher than average abundance in 1996. Following poor recruitment in 1999, YOY yellow perch populations were average through 2002, but increased in the last year of the series, 2003. Overall, the time series of geometric mean CPUEs fluctuated about a slightly increasing trend.

Fig. 9. Yellow perch JAI time series based on Program 100, Albemarle Sound, 60 ft knitted bag seine samples from June through October.



Catches of age-1 yellow perch in program 100 were quite low and stable throughout the time period examined (Fig. 10), with the exception of a relatively high mean CPUE in 1979. That peak corresponded to the peak of yellow perch YOY abundance that was recorded in 1978. However, subsequent peaks in YOY abundance did not carry through into the next year's index of age-1 yellow perch. The correlation between yellow perch YOY and the next year's group of age-1 was significant and positive (Pearson correlation coefficient = 0.467; $p = 0.0081$; Fig. 11).

Fig. 10. Yellow perch age-1 abundance index time series based on Program 100, Albemarle Sound, 60 ft knitted bag seine samples from June through October.

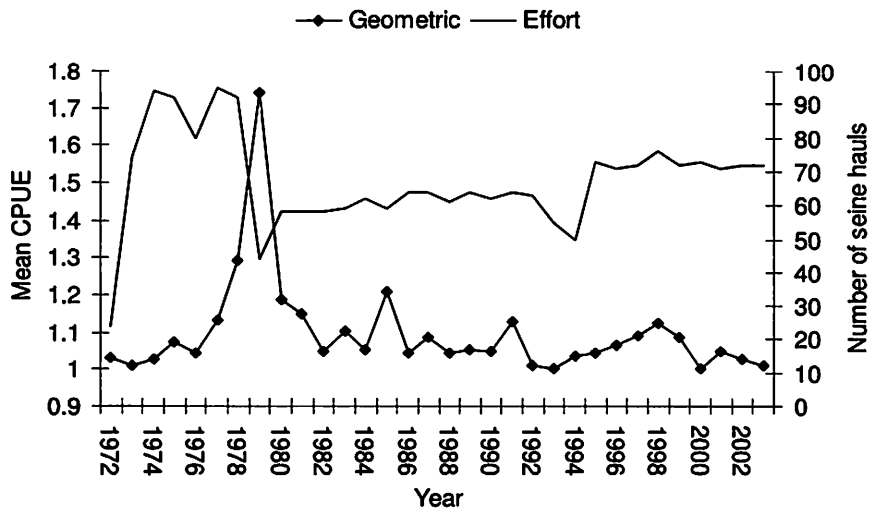
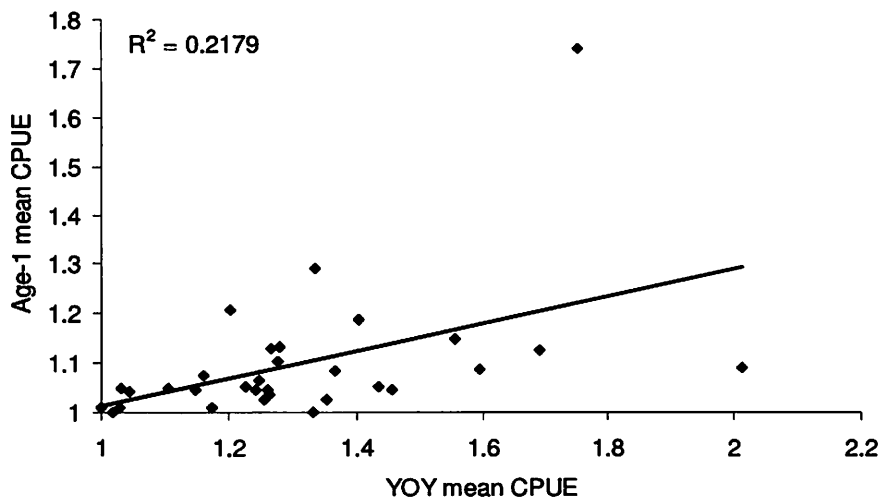


Fig. 11. Correlation between geometric mean CPUEs of YOY and the next year's age-1 yellow perch.



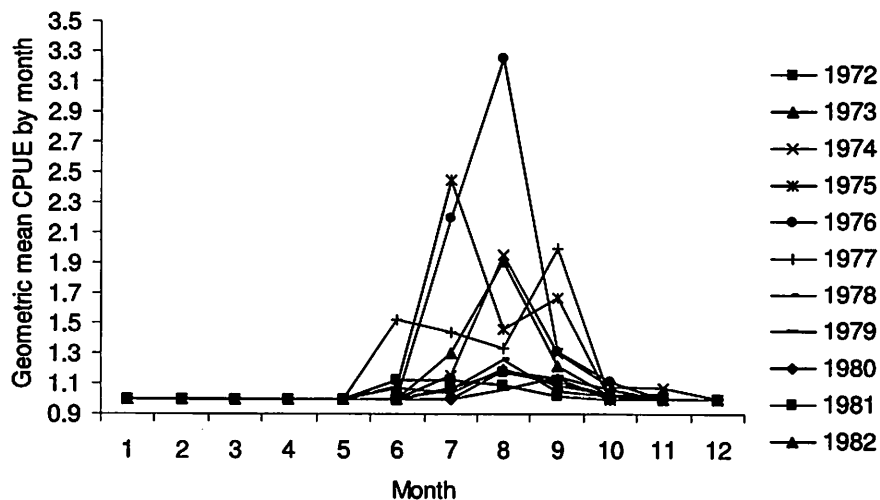
Program 120 spotted seatrout

Spotted seatrout YOY were collected in Program 120. The Estuarine Trawl Survey began in 1970 with a variety of gears and tow times. By the late 1990s, all gears except for the NCDMF stock assessment trawl with 10.5 ft head rope, 0.25 in body mesh and 0.125 in tailbag mesh (*gear 556*) had been dropped. Though this gear was used

throughout the time series, effort was not consistent in the early years. Prior to 1978, total effort was relatively low and variable and consisted of few tows ranging in duration from 30 seconds to 30 minutes. In 1978, tow times were standardized to one minute, and the number of tows conducted rose sharply. Catches in trawls prior to 1978 were standardized to a unit of effort of one minute of trawling.

Pamlico Sound was the primary area in which YOY spotted seatrout were captured. Within the Sound, 64 fixed core sampling stations were consistently sampled with the above specified gear. These stations were the only ones used in the development of the spotted seatrout JAI. Sampling was conducted during most months of the year through 1989, roughly from May through September until 1997, and only in May and June from 1998 on. Because only May and June were consistently sampled throughout the time period, May and June were used to calculate JAIs to be used to describe population trends. However, when the entire year was sampled, the highest catches per unit effort of YOY spotted seatrout often occurred later in the summer (Fig. 12). Thus doubts are cast upon the accuracy of the time series of JAIs for this species.

Fig. 12. Monthly geometric means, by month and year, of YOY spotted seatrout catch per unit effort from core sampling stations within Pamlico Sound. A selection of years in which most months were sampled is presented.



Spotted seatrout YOY were fairly rare in trawl samples of core stations in Pamlico Sound in May and June. None were caught in 17 of the 33 years encompassed by program 120. Spotted seatrout YOY were present in low numbers sporadically throughout the time series (Fig. 13). Apparent peaks in abundance, based on the May/June index, occurred in 1977, 1986, 1995, and 1999. Comparison of abundance indices calculated from only consistently sampled months (i.e., May and June) with those calculated from all available months at the same core stations (Fig. 14) shows that higher CPUEs are obtained with inclusion of more months of sampling. However, the JAIs of later years in the time series are biased because fewer months of sampling were available.

Fig. 13. Spotted seatrout JAI time series based on Program 120, Pamlico Sound, NCDMF stock assessment trawl (10.5 ft headrope), from May and June.

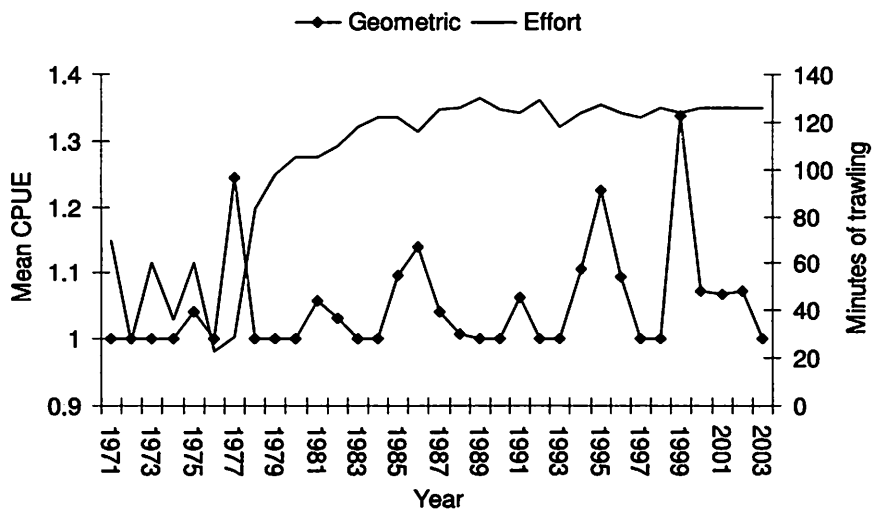
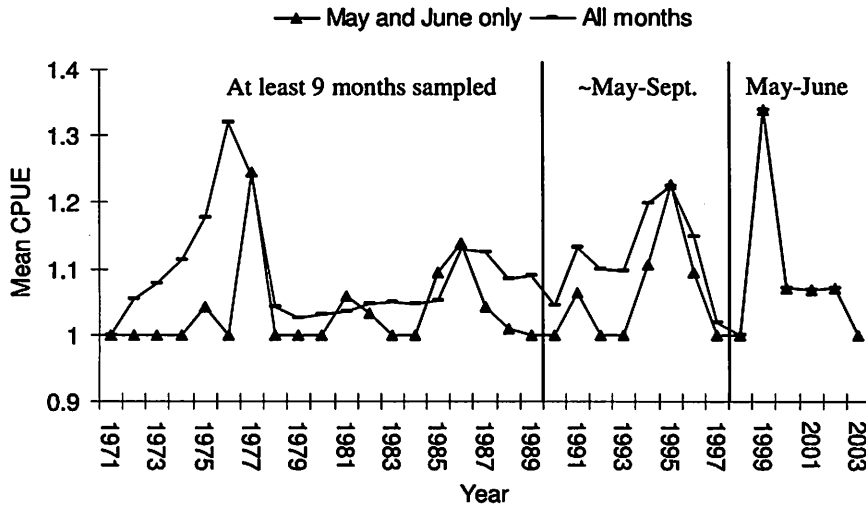


Fig. 14. Spotted seatrout JAI time series (geometric means) based on samples from May and June only (same as Fig. 11), as compared to JAI computed from the same samples, but using all months available.



Program 195 southern kingfish

Quarterly sampling of most of North Carolina's sounds began under the purview of Program 195 in 1987. A mongoose trawl with 30 ft headrope, 1.87 in mesh and 0.75 in tailbag mesh (*gear 539*) was towed for 20 minutes at randomly selected sites within location/depth strata. In 1990, sampling in areas other than Pamlico Sound was discontinued, as was sampling in December. From 1991 to present, sampling occurred only in June and September, with the exception of two years (1999 and 2003) in which sampling was postponed one month due to hurricanes. Abundance indices for YOY and age-1 southern kingfish were computed based on samples taken in Pamlico Sound in June and September (July and October were included for 1999 and October was included for 2003).

Although more plentiful in Pamlico Sound than the congeneric northern and Gulf kingfishes, southern kingfish were rare throughout the sampled time period. The JAI rose above the 1.1 mark in only 1995 and 2000 (Fig. 15). Even fewer age-1 southern kingfish were collected, with abundance indices never rising above 1.05 (Fig. 16). There was no correlation between YOY and age-1 southern kingfish ($p = 0.0854$; Fig. 17).

Fig. 16. Southern kingfish age-1 abundance index time series based on Program 195, Pamlico Sound, mongoose trawls in June and September.

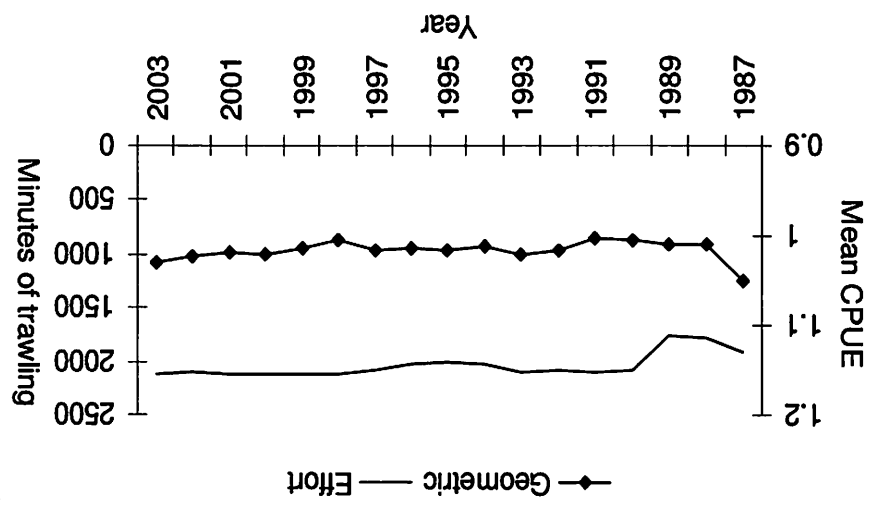


Fig. 15. Southern kingfish JAI time series based on Program 195, Pamlico Sound, mongoose trawls in June and September.

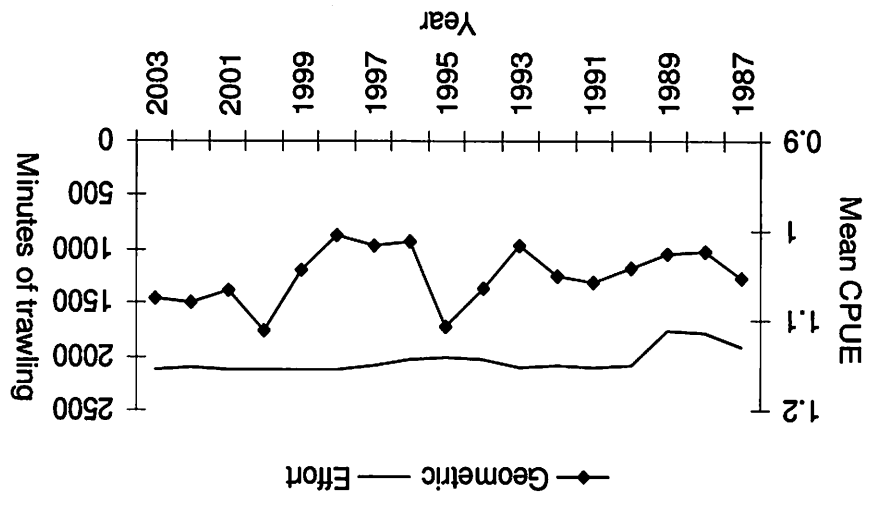
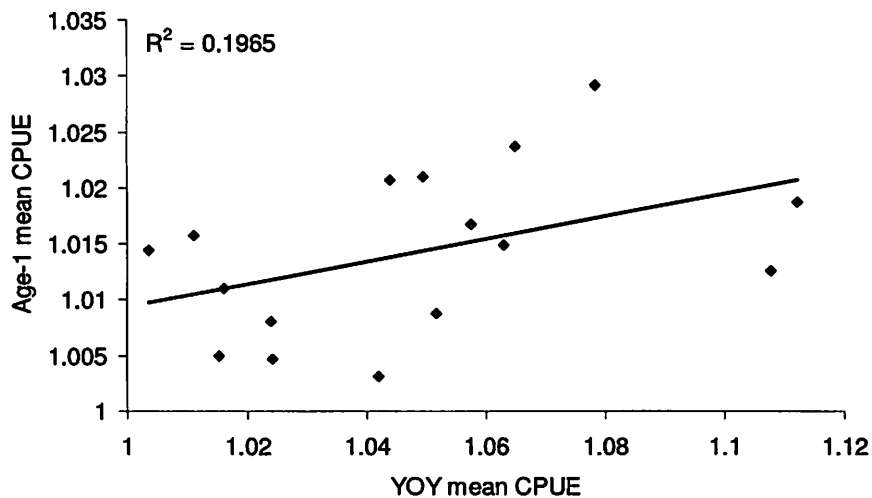


Fig. 17. Correlation between geometric mean CPUEs of YOY and the next year's age-1 southern kingfish.



Discussion/Recommendations

Age determination

Additional data on each of the species considered here would have been extremely helpful in developing JAIs and age-1 abundance indices. In particular, data to aid in the identification of age classes would be invaluable. The primary literature contains no studies on the growth of any of these species with reference to North Carolina populations, but studies of growth in other areas make the necessity of such data clear.

Growth studies have been conducted on white perch from a variety of systems, including Connecticut (Marcy and Richards, 1974), the Hudson River Estuary (Bath and O'Connor, 1982), the Delaware River (Wallace, 1971), and as close as Virginia (St. Pierre and Davis, 1972). These studies show that while length is an acceptable delineator of YOY fish, length is not a good predictor of age by the second summer (Wallace, 1971). Moreover, growth differed even between the James and York Rivers (St. Pierre

and Davis, 1972), indicating that studies of growth in other areas are not likely to be applicable to fish from North Carolina. This is especially true considering Woolcott's (1962) assertion that the Albemarle Sound population of white perch is semi-isolated. While YOY white perch may be easily distinguished from older cohorts through modal progression analysis, any true validation of JAIs will require more accurate age data on those older fish.

Yellow perch have also been the subject of a myriad of growth studies, but these are mostly from lakes (Diana and Salz, 1990; Noble, 1975). While the occasional study was conducted in a brackish environment (Muncy, 1962), yellow perch growth studies in North Carolina estuaries and rivers have not been published. From other studies it is apparent that yellow perch growth is highly variable between populations and from year to year within populations (Noble, 1975; Post and McQueen, 1994). Age data must be taken on a continuing basis in this species for accurate age determination from year to year.

Few studies of spotted seatrout growth were uncovered in the primary literature, and fewer still for any of the kingfish species. Spotted seatrout, in particular, have been noted to comprise several sub-populations in geographically close locations (Iversen and Tabb, 1962). As with the perches, this is strong incentive to conduct area-specific studies. Age determination is further complicated for spotted seatrout and all of the kingfishes because of their prolonged spawning seasons and sexually dimorphic growth (Maceina et al. 1987; Nieland et al., 2002; Smith and Wenner, 1985). The age length keys used to determine ages of the sciaenids included few small fish and were compiled over one restricted period of time. They also should have been applied separately to the different sexes, but sex data were not included for these species in the database.

Ideally, subsets of future collections of all of the species mentioned here will be aged by otolith analysis, and sex will be recorded. Age length keys should be constructed for each species. Fast and variable growth will dictate that age length keys be devised on a monthly or yearly basis for application to short and temporally distinct periods.

Sampling

The strong, positive correlation between YOY and age-1 white perch abundances indicates that the seine survey of program 100 does a good job of indexing YOY abundance for this species. Additional, independent, validation might be obtained if another gear type could be used to collect age-1 white perch. The correlation between YOY and age-1 yellow perch was also positive and significant, but this correlation may become even stronger with an altered sampling design. Although it occurs in brackish waters in North Carolina, the yellow perch is predominantly a fresh water species (Mansueti, 1961; Muncy, 1962). Random sampling, stratified by salinity zones, might provide a more accurate picture of the state of yellow perch populations, and would likely not be detrimental to the indexing of white perch populations.

The scarcity of YOY and age-1 spotted seatrout, southern kingfish, northern kingfish, and Gulf kingfish in fishery independent sampling programs of the NCDMF biological database points to a deficiency in the ability to sample the young of these species. The restriction of sampling in program 120 to only May and June is likely missing the majority of YOY spotted seatrout. New sampling areas (e.g., sea grass beds) might also be tested for their capture, as well as for capture of the kingfishes (e.g., ocean beaches and near-shore shelf habitats). Various species of kingfish YOY have been successfully captured in near-shore ocean trawls (Smith and Wenner, 1985) and in ocean beach seines in New Jersey (Miller et al., 2002) and North Carolina (Ross and Lancaster, 2002).

The NCDMF biological database is a valuable resource for marine and estuarine fisheries researchers in North Carolina. The species examined here were not originally the target species of the various fishery-independent monitoring programs. However, the NCDMF biological database does contain useful information on some of these species. The collection of age data, and additional sampling will improve the accuracy of JAI estimation in the future.

Acknowledgments

The compilation of over 30 years of data required a tremendous amount of work on the part of countless NCDMF personnel. They deserve many thanks. I am particularly grateful to Mike Loeffler, Chip Collier, and John Schoolfield for their time, database explanations, and data. Jeff Buckel of North Carolina State University and Joe Grist of NCDMF provided excellent guidance throughout the preparation of this report, which benefited from their reviews, as well as those of Maury Wolff and Charles Manooch. This work was completed while the author was the North Carolina Marine Fisheries Fellow. The fellowship is supported by North Carolina Sea Grant and NCDMF with funding from NOAA award # NA16FG1220.

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