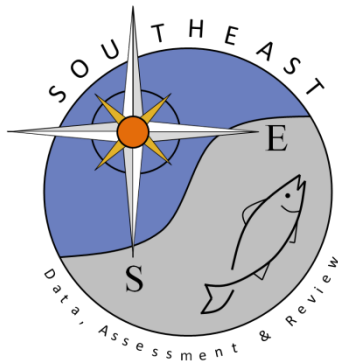


Validation of ages for species of the deepwater snapper/grouper complex off the  
southeastern coast of the United States

SEDAR36-RD07

15 June 2013



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**NOTE - In preparation for SEDAR36, text and figure on last page were appended to the report eight years after it was submitted by P. Harris.**

Final Report

Validation of ages for species of the deepwater snapper/grouper complex off the southeastern coast of the United States

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## **I. Validation of ages for species of the deepwater snapper/grouper complex off the coast of the southeastern United States.**

Patrick J. Harris, South Carolina Department of Natural Resources, Charleston, SC 29412.

**Grant Number:** NA17FF2870

May 2005.

## **II. Abstract**

Radiocarbon ( $^{14}\text{C}$ ) activity in the world's oceans doubled between 1950 and 1970 as a result of the atmospheric testing of nuclear weapons (Campana 1997). Kalish (1993; 1995) identified the utility of using the increase in radiocarbon levels as a tool for validating ages of long-lived fish species by comparing the  $^{14}\text{C}$  activity of otolith cores from hatched during 1950-1970 to a  $^{14}\text{C}$  time-series reconstructed from nearby corals. Campana (1997) performed a similar comparison for haddock from the northwestern Atlantic Ocean, and Baker and Wilson (2001) investigated radiocarbon in the otoliths of red snapper in the Gulf of Mexico. The goal of the study was to validate the annual nature increment counts from otolith sections of tilefish (*Lopholatilus chamaeleonticeps*), snowy grouper (*Epinephelus niveatus*), blackbelly rosefish (*Helicolenus dactylopterus*), and blueline tilefish (*Caulotilus microps*) off the southeastern United States using accelerator mass spectrometry analysis of delta  $^{14}\text{C}$  present in otoliths. Otoliths from thirty of each of the study species hatched between 1950 and 1980 were removed from the archive of otoliths maintained by MARMAP. A 1 mm thin section of an otolith was mounted on a glass slide using tape, and all material outside the first increment of the otolith was ground away using a Dremel tool (Baker and Wilson 2001), and shipped to the Accelerator Mass Spectrometry laboratory of the National Ocean Service (NOSAMS) at the Woods Hole Oceanographic Institute in Massachusetts. After analysis, the AMS laboratory provided a Delta  $^{14}\text{C}$  value for each otolith as defined in Stuiver and Pollach (1977; see <http://www.nosams.who.edu/clients/data.html> for a complete description of the methodological and analytical procedure used). These values were plotted by hatch-date (year) derived from the assumed age of the specimen for each species from increment counts on sectioned otoliths. If the value of Delta  $^{14}\text{C}$  recorded for each otolith and the hatch-date corresponded to the increase in radiocarbon as reflected in the utilized standards, then the increment count would be taken to accurately reflect the true age of the specimen in question. Ages as derived from increment counts obtained from thin sections were validated for snowy grouper and blueline tilefish. However, validation of ages of tilefish and blackbelly rosefish is less certain.

## **III. Executive Summary**

Knowledge of the age of fish is a critical portion of the life history of fish that allows for more complete stock assessments to be conducted for a given species, providing better information about estimates of mortality for that species. While several age and growth studies have been performed for a number of deepwater species in the snapper/grouper complex of the South Atlantic Bight (White et al. 1998; Wyanski et al. 2000; Harris et al. 1998; Harris et al. 2001). However, increments on these otoliths are typically extremely difficult to interpret, and most studies are published without any validation of the periodicity of increment formation. The atmospheric testing of nuclear weapons conducted from approximately 1950 through 1970 caused a large increase in the levels of radiocarbon found in the world's oceans. This 'bomb radiocarbon' is subsequently incorporated into the calcium carbonate matrix of a variety of organisms, including fish otoliths. By comparing the bomb radiocarbon levels contained within the first years' growth of the otolith of a fish hatched during the increase in radiocarbon levels, and comparing the value to a known standard, the age of the fish can be validated.

Age and growth studies have been performed for a number of deepwater species in the snapper/grouper complex of the South Atlantic Bight (White et al. 1998; Wyanski et al. 2000; Harris et al. 1998; Harris et al. 2001). However, increments on these otoliths are typically extremely difficult to interpret, and most studies are published without any validation of the periodicity of increment formation. The goal of the study was to validate the annual nature increment counts from otolith sections of tilefish (*Lopholatilus chamaeleonticeps*), snowy grouper (*Epinephelus niveatus*), blackbelly rosefish (*Helicolenus dactylopterus*), and blueline tilefish (*Caulotilus microps*) off the southeastern United States by comparing the amount of bomb radiocarbon present in otoliths and comparing the value to the year of hatching for that fish as calculated from the otolith derived age.

From the results obtained, the ages of snowy grouper and blueline tilefish derived from otoliths were validated. However, the validation of ages for tilefish and blackbelly rosefish remains uncertain.

#### **IV. Purpose**

A. Age and growth studies have been performed for a number of deepwater species in the snapper/grouper complex of the South Atlantic Bight (White et al. 1998; Wyanski et al. 2000; Harris et al. 1998; Harris et al. 2001). However, increments on these otoliths are typically extremely difficult to interpret, and most studies are published without any validation of the periodicity of increment formation. Stock assessments have been recently been completed for two of the four species studied (tilefish and snowy grouper), and both of these stocks were described as overfished (report available at [http://www.sefsc.noaa.gov/sedar/Sedar\\_Workshops.jsp?WorkshopNum=4](http://www.sefsc.noaa.gov/sedar/Sedar_Workshops.jsp?WorkshopNum=4)). The status of the stocks of blueline tilefish and blackbelly rosefish off the southeastern United States is unclear.

Validation of increment counts was identified as a research recommendation by the stock assessment team for snowy grouper and tilefish, as valid estimates of age are critical if an age-structured model is used for a species. Although marginal increment analyses were performed for snowy grouper (Wyanski et al. 2000) and blackbelly rosefish (White et al. 1998), the sample sizes used for each were rather low (248 for snowy grouper and 294 for blackbelly rosefish). No validation has been attempted for the tilefish or blueline tilefish.

B. The goal of the study was to validate the annual nature increment counts from otolith sections of tilefish (*Lopholatilus chamaeleonticeps*), snowy grouper (*Epinephelus niveatus*), blackbelly rosefish (*Helicolenus dactylopterus*), blueline tilefish (*Caulotilus microps*) and wreckfish (*Polyprion americanus*) off the southeastern United States using accelerator mass spectrometry analysis of delta  $^{14}\text{C}$  present in otoliths.

## V. Approach

A. Radiocarbon ( $^{14}\text{C}$ ) activity in the world's oceans doubled between 1950 and 1970 as a result of the atmospheric testing of nuclear weapons (Campana 1997). Kalish (1993; 1995) identified the utility of using the increase in radiocarbon levels as a tool for validating ages of long-lived fish species by comparing the  $^{14}\text{C}$  activity of otolith cores from hatched during 1950-1970 to a  $^{14}\text{C}$  time-series reconstructed from nearby corals. Campana (1997) performed a similar comparison for haddock from the northwestern Atlantic Ocean, and Baker and Wilson (2001) investigated radiocarbon in the otoliths of red snapper in the Gulf of Mexico.

Otoliths from thirty of each of the study species hatched between 1950 and 1980 were removed from the archive of otoliths maintained by MARMAP. Only specimens that had been aged by two readers from prior age and growth studies, and for which the second otolith had been collected when the fish was sampled, were used. For these specimens, one otolith had already been embedded and sectioned for ageing purposes. The second otolith of selected individuals was embedded in epoxy and sectioned, and a 1 mm thick transverse section was cut for radiocarbon analyses following the protocol described in Baker and Wilson (2001). We were unable to use specimens for which only one otolith had been collected, as proposed, because the thickness of the section cut for aging the specimen did not allow a large enough sample of the otolith core to be collected for reliable radiocarbon analyses. The section was mounted on a glass slide using tape, and all material outside the first increment of the otolith was ground away using a Dremel tool (Baker and Wilson 2001). The portion of the otolith remaining was rinsed in 5% HCl (15 to 30 seconds), ultrasonically cleaned in distilled water, placed in plastic vial, and shipped to the Accelerator Mass Spectrometry laboratory of the National Ocean Service (NOSAMS) at the Woods Hole Oceanographic Institute in Massachusetts.

After analysis, the AMS laboratory provided a Delta  $^{14}\text{C}$  value for each otolith as defined in Stuiver and Pollach (1977; see <http://www.nosams.who.edu/clients/data.html> for a complete description of the methodological and analytical procedure used). These values were plotted by hatch-date (year) derived from the assumed age of the specimen for each species from increment counts on sectioned otoliths. The resultant plot was compared to several published Delta  $^{14}\text{C}$  chronologies, including Floridian and Bermudian corals (Druffel 1989), young haddock from the northwestern Atlantic (Campana 1997) and red snapper from the Gulf of Mexico (Baker and Wilson 2001). If the value of Delta  $^{14}\text{C}$  recorded for each otolith and the hatch-date corresponded to the increase in radiocarbon as reflected in the utilized standards, then the increment count would be taken to accurately reflect the true age of the specimen in question.

B. Dr. Patrick Harris performed all data analyses beyond the derivation of Delta  $^{14}\text{C}$ , and prepared the semi-annual and final reports. Mr. Byron White identified otoliths to used in the study, and prepared samples for submission to the AMS laboratory of the National Ocean Service at the Woods Hole Oceanographic Institute. Analysis of radiocarbon within the otoliths was conducted by the AMS lab at Woods Hole in Massachusetts.

## **VI. Findings**

A. Results were obtained for four deepwater species – snowy grouper, tilefish, blueline tilefish, and blackbelly rosefish.

i. Snowy grouper.

The ages assigned to snowy grouper using otoliths were validated by the analysis of bomb radiocarbon incorporated into the core of the otolith during the first year of life (Figure 1). The increase in radiocarbon in these otoliths showed a slight lag in the uptake of radiocarbon when compared to red snapper, but follow a similar trend to haddock. The haddock standard may represent a better standard as it was derived from known age specimens sampled from the north Atlantic ocean (Campana 1997). The six snowy grouper samples circled on the figure which do not appear to follow the increase in bomb radiocarbon that is implied from their otolith derived hatch dates represent older specimens (age range from 16-21 years old), but not the oldest fish, which was 27. As with many deepwater species, snowy grouper are difficult to age from otoliths (Wyanski et al. 2000), and the slight loss of conformity of these 6 specimens may well be due to under-aging. However, as the trend in radiocarbon within these six specimens appears to be a continuing decrease, it is also possible that this specimens were aged correctly, and that the young-of-the-year were not exposed to increased levels of radiocarbon.

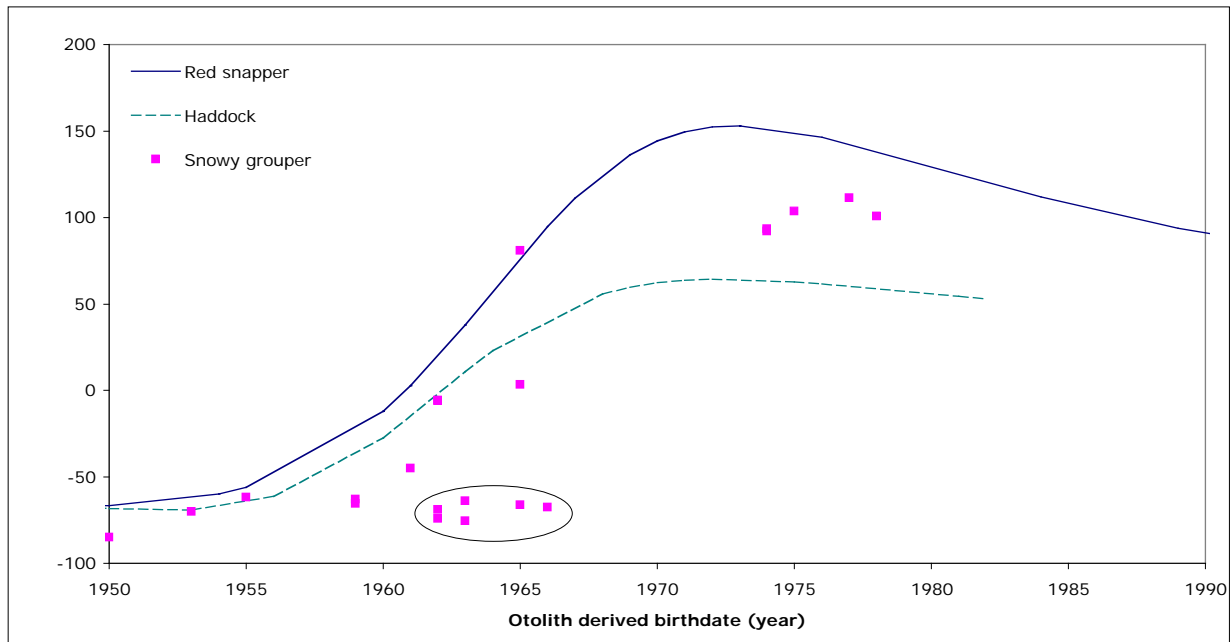


Figure 1. Delta  $^{14}\text{C}$  values by otolith derived hatch-date for snowy grouper captured off the southeastern United States.

ii. Tilefish.

Tilefish otoliths with a more recent hatch-date (1970 on) showed increased levels of radiocarbon (Figure 2). Unfortunately, only one tilefish with an otolith derived hatch-date prior to 1965 was available for analysis. However, that one sample suggests tilefish may have been exposed to increased levels of bomb radiocarbon similar to those demonstrated by the chosen standards, but the levels of exposure did not appear to increase at the same rate, or to the same level, as seen in the standards. A high degree of variability in the amount of bomb radiocarbon present during the first year of otolith growth is apparent (for all species investigated, not just tilefish) and encourages a cautious interpretation of the results. For example, a specimen with an otolith derived hatch date of 1970 had a very similar Delta  $^{14}\text{C}$  value to one hatched in 1958.

The results obtained clearly demonstrate that the levels of radiocarbon in the otoliths of tilefish have increased as a result of atmospheric testing of nuclear weapons. However, whether the lag in the increase relative to the utilized standards is a function of aging error or an effect of the environment in which tilefish spend the first year of their life is unclear. Aging error is the most obvious explanation, but may not be the correct explanation, or may only be part of the explanation. Unfortunately, little is known about the early life history of tilefish, particularly the

duration of the larval phase, where settlement may occur, and how old juvenile tilefish are when they first inhabit tilefish grounds.

The highest levels of radiocarbon recorded in tilefish are approximately 50% lower than the fitted haddock standard, suggesting that the young-of-the-year have a short exposure to bomb radiocarbon, before shifting to an environment with significantly lower radiocarbon levels than might be expected in surface waters. Tilefish habitat off the southeastern United States is primarily mud bottoms in depths ranging from 90 to 140 fathoms (Barans and Stender 1993; Low et al. 1983). The specimens sampled for this study were collected off South Carolina, where tilefish habitat may be subjected to periodic exposure to upwelled waters from the Charleston Bump, a topographic feature that causes a recurring deflection in the Gulf Stream (Bane et al. 2001; Popenoe and Manheim 2001). The water mass found at depth on the Charleston Bump is Antarctic Intermediate Water (AAIW), identified by a unique salinity signal (Atkinson 1983). At the Bump, this water is approximately 900 years old, and as such, has never been directly exposed to atmospheric increases in bomb radiocarbon. Any increases in radiocarbon levels within the AAIW would have to come from diffusion and particulate matter settling out through the water column. Therefore, the exposure of young of the year tilefish to bomb radiocarbon would be limited to the period prior to occupying adult habitat. Subsequent uptake of radiocarbon may be affected by the periodic intrusions of AAIW, resulting in a lower than anticipated signal. Nonetheless, although the paucity of specimens hatched between 1960 and 1970 makes a reliable conclusion difficult to reach, the data available suggest tilefish might have been underaged by up to 10 years.

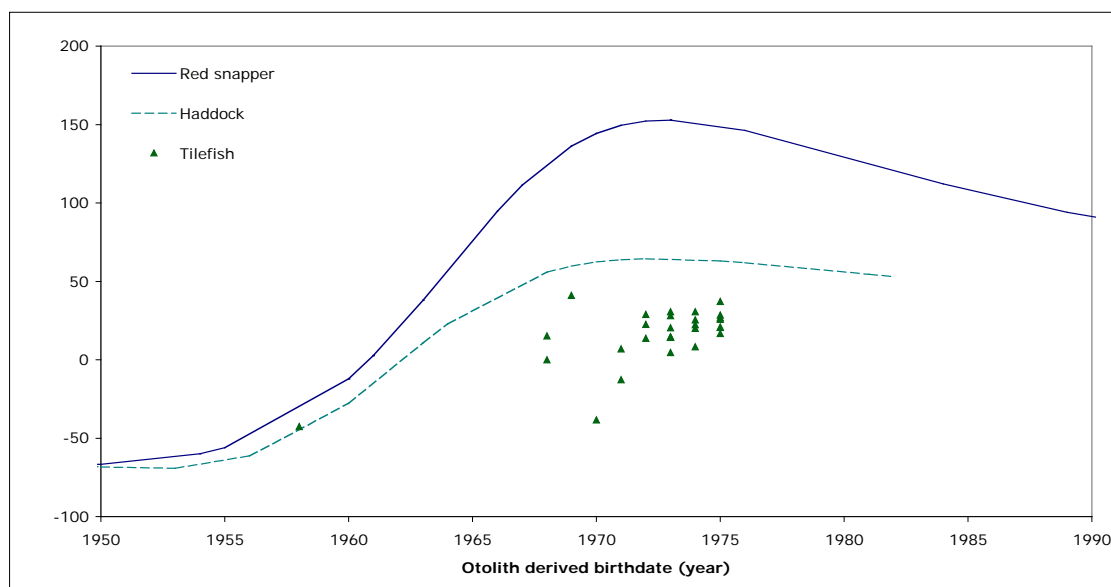


Figure 2. Delta  $^{14}\text{C}$  values by otolith derived hatch-date for tilefish captured off the southeastern United States.



iii. Blueline tilefish.

Blueline tilefish showed an increase from pre-bomb levels of radiocarbon to those reported for haddock in the North Atlantic around 1970 (Figure 3). This suggests that ages have correctly interpreted for this species, although the lack of specimens hatched prior to 1970 makes a concrete determination of when the levels of radiocarbon began increasing somewhat problematic. There is a very high variability in the level of bomb radiocarbon present in otoliths of specimens sampled, even from the same year. For example, the level of Delta  $^{14}\text{C}$  present in specimens estimated from otolith ages to have hatched in 1970 ranged from -34.5 to 75.8 (n=3). Nonetheless, the amount of variability decreases for fish hatched more recently, and the most recent negative value was recorded for a specimen hatched in 1972, suggesting a consistent exposure of young of the year to increased levels of Delta  $^{14}\text{C}$ . The three specimens circled were between 8 and 13 years old, not significantly different from the mean age of the blueline tilefish specimens used in this study. It is unclear why these specimens had such low Delta  $^{14}\text{C}$  values, unless they were underaged by up to 15 years. However, as the remaining specimens do not appear to be underaged, this may not be a likely explanation.

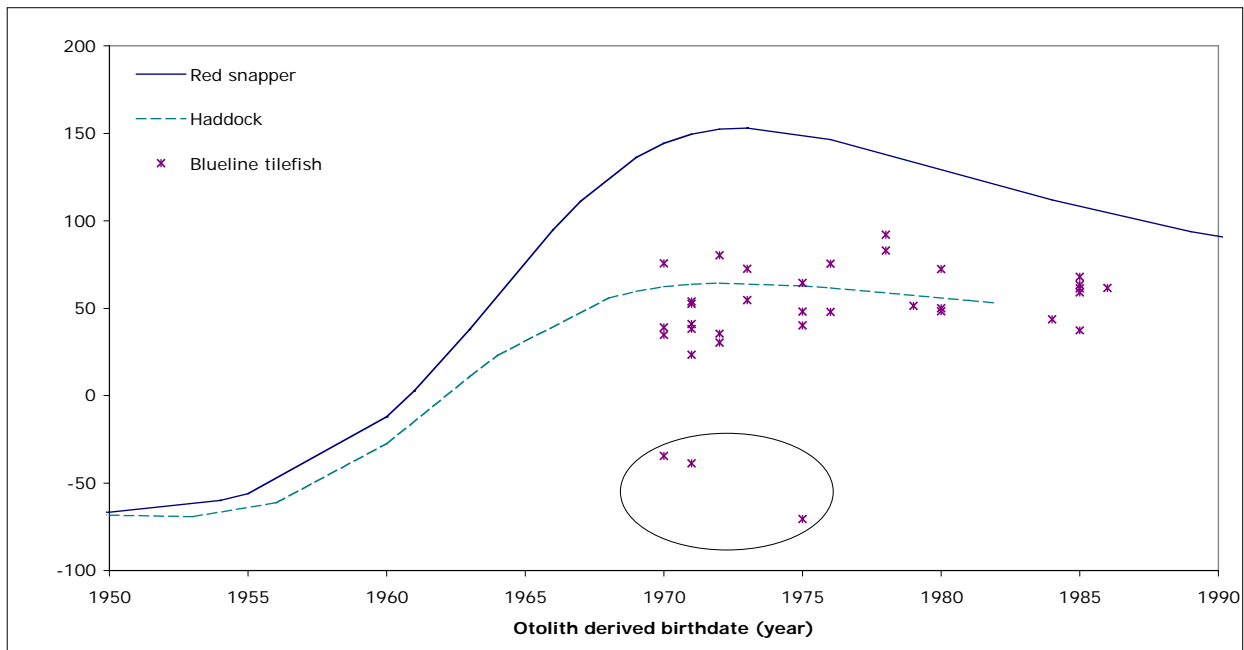


Figure 3. Delta  $^{14}\text{C}$  values by otolith derived hatch-date for blueline tilefish captured off the southeastern United States.

iv. Blackbelly rosefish.

Blackbelly rosefish show a very similar pattern to tilefish, however a more complete pattern of pre- and post-bomb effects is evident, and the increases in bomb radiocarbon in the otoliths are not as pronounced. A

noticeable difference in the pattern from blueline tilefish, and particularly the standards used, is that increased levels of radiocarbon only become evident beginning in 1977. This suggests that blackbelly rosefish have been under-aged by about 10 years. While under-aging is the most likely explanation for the changes in bomb radiocarbon observed, it is possible that, as for tilefish, the delay lag observed in the increase in radiocarbon is due to the environment occupied by young of the year rosefish. Again, a lack of knowledge of the early life history of the species in the region makes interpretation of the results difficult.

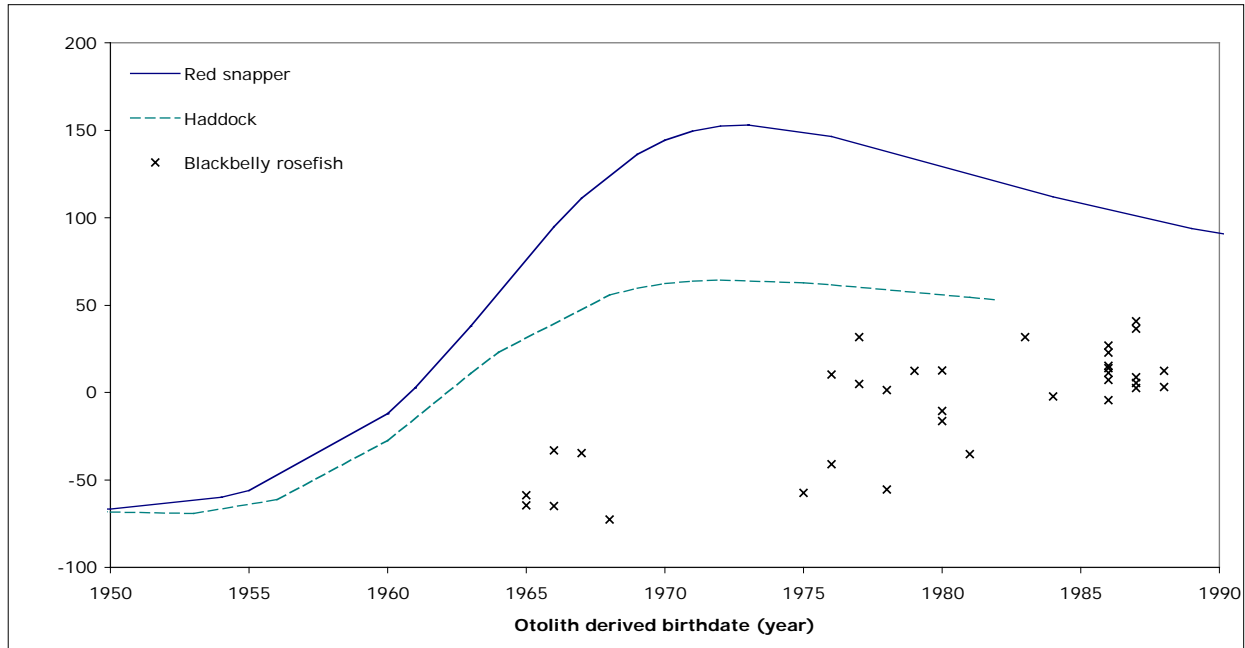


Figure 4. Delta  $^{14}\text{C}$  values by otolith derived hatch-date for blackbelly rosefish captured off the southeastern United States.

#### Conclusions:

The five species selected for age validation in this study were chosen because of their relative importance to the snapper/grouper fisheries off the southeastern United States, and because they were extremely difficult to age. Although a single age reader was not involved in the aging studies for all four species, all species were aged in the same laboratory: one reader was involved in aging snowy grouper and blackbelly rosefish, while a second reader was involved in tilefish and blueline tilefish. These two readers exchanged information and expertise while aging their species, and qualitatively rated all species as equally difficult to interpret. We were unable to include wreckfish in this study because no second otolith could be found for any of the specimens that had been aged.

Of the four species, snowy grouper occupies the shallowest habitat, particularly as younger fish, and the remaining species all typically reside in deeper waters (MARMAP, unpublished data). Based on fishery-

independent sampling data, tilefish tend to be found in the deepest water, although all four species can be captured from the same habitat (Low et al 1983; Barans and Stender 1993). Very little is known about the early life history of any of the species.

The ages of snowy grouper and blueline tilefish appear to have been validated in this study, while the validation of tilefish is less certain. Blackbelly rosefish appears to have been underaged by about 10 years. The same methodologies were used to age all specimens, and snowy grouper was considered to be the most difficult species to age, while tilefish was considered to be the easiest, further confounding the issue of whether two of the four species were underaged. Some potential explanations other than underaging are:

1. The uptake of bomb radiocarbon during the larval and juvenile phases is limited, and may be masked by the radiocarbon absorbed into the otolith once the fish has settled into the adult habitat. This would explain the lag in radiocarbon increase observed in blackbelly rosefish and tilefish. The time of lag in the incorporation of bomb radiocarbon into the otoliths of these four species appears to be loosely correlated to the depth of water in which the species reside. The duration of the larval and juvenile periods, and at what age fish of each species move into adult habitat is a critical variable that is not known for any of these species. It is anticipated that the longer the exposure of young of the year to bomb radiocarbon, the greater the amount incorporated into the otolith.

2. The first increment could have been incorrectly assigned, resulting in either too much material from subsequent years of growth in the adult habitat being incorporated into the sample prepared for analysis, or too little material. If there was an error in correctly indentifying the first increment, it is more likely the increment was made too large, incorporating subsequent years, than too small. The effect of incorporation additional material from years of growth beyond the first year would be similar to the first point – the uptake of bomb radiocarbon during the larval and juvenile phases would be masked by the lower levels of radiocarbon found in adult environment.

It has been documented that the radiocarbon levels of AAIW are up to three times lower than the levels found in surface waters. Therefore, the duration of exposure to increased levels of radiocarbon would be a critical factor in a study such as this. Additionally, the exposure of specimens to radiocarbon limited waters would be an additional critical factor. It seems that the species more commonly found in deeper waters show lower levels of radiocarbon, and the species found in the shallowest waters shows the highest levels. Further study, particularly on the duration of the any pelagic stages of tilefish, blueline tilefish, and blackbelly rosefish would provide a significant addition to our understanding of the aging of these deepwater species.

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B. Our biggest problem was the lack of otoliths available for wreckfish. This is a technique which should work well for wreckfish, as it is well known that they have a prolonged pelagic phase as juveniles. A second problem encountered was the size of the otoliths, particularly blueline tilefish, meant that the core was fairly small, and had to be processed as a small sample by the NOS AMS lab. This increased costs slightly, and meant we were not able to process as many samples as we planned to. This did not have a significant impact on the results, however.

C. Additional efforts, using alternate techniques such as isotope analyses, should be pursued to further attempt to validate ages for tilefish and blackbelly rosefish.

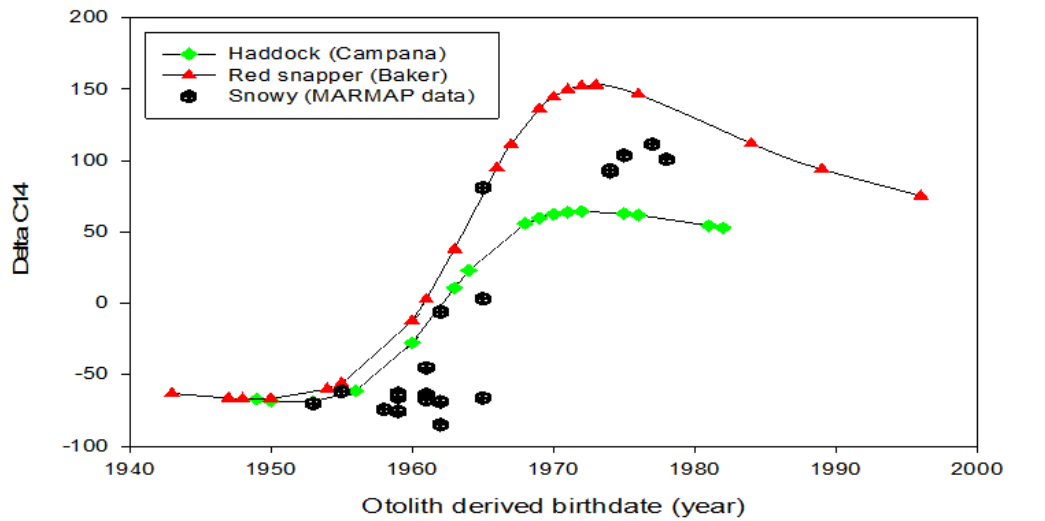
## **VII. Evaluation**

A. 1. The project goals were obtained to the extent possible. The otolith ages of two deepwater species were validated, while potential aging errors were identified for the remaining two.

2. The only modification made to the goals of the study was to eliminate the proposed analysis of wreckfish, due to the unavailability of a second otolith for specimens which had been aged.

B. This final report will be sent to the National Marine Fisheries Service laboratories in Panama City, FL, and Beaufort, NC. Additional copies will be sent to Jack McGovern at the NMFS Regional Office in St. Petersburg, and John Carmichael and Gregg Waugh of the South Atlantic Fishery Management Council. A manuscript will be submitted for review and publication in a peer-reviewed journal (Fishery Bulletin).

Delta C14 values by otolith derived hatch-date for snowy grouper captured off Southeastern United States.



The amended figure above was created by Byron White and added to this report by David Wyanski of the MARMAP program at SCDNR. This figure should be compared to Figure 1 on Page 6. The otolith-derived birthdate of all snowy grouper specimens used for the  $C^{14}$  study was re-assessed independently by two readers (also without knowledge of original value) and then compared to the original value to evaluate precision in assessing age by examination of growth increments in sagittal otoliths of snowy grouper. The birthdate of four specimens was changed, with the result that most of the cluster of specimens with low  $C^{14}$  from the mid 1960s was shifted to an earlier birthdate.