

**Age and Growth of *Rhizoprionodon terraenovae* and the Benefits of Age and
Growth Studies on Conservation Policies**

Jenna Karr, Dr. Bryan Franks

Honors Thesis Research

Florida Southern College

May, 2017

Age and Growth of *Rhizoprionodon terraenovae* and the Benefits of Age and Growth Studies on Conservation Policies

Jenna Karr, Dr. Bryan Franks

Abstract

Age and growth estimates for shark species may be determined through examination of annuli seen in the vertebral cartilage. Age estimates were made for seven male Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) caught in 2014 that ranged in length from 58.5 - 90.8 TL. Vertebrae were analyzed for clear band patterns in order to estimate shark ages and compared to ages calculated using the von Bertalanffy growth equation. A comparison of the calculated and observed age found that there was no significant relationship between the two. A better understanding of the rate of growth within cartilaginous fish species such as *Rhizoprionodon terraenovae* allow for an increased understanding on the potential impacts on population dynamics. As many shark species have an overall slow growth pattern, populations may be unable to recover from detrimental impacts such as overfishing or coastal development. Continuing age and growth studies with shark species will aid in conservation efforts by supporting shark fishing guidelines that would allow healthy population numbers to be maintained based on the growth rate of individuals.

Introduction

Determining the age of Elasmobranchii species is a method important to understanding the population dynamics of sharks, skates and rays (Goldman & Cailliet, 2004). With increasing debate on the state of shark population health, age data is a key component to determining individual longevity, population productivity, and mortality. The increase of human development along coastlines has begun to effect shark nursery habitats as well as adult habitats for some smaller, coastal species. The steady increase of fisheries to sustain human demand has removed prey species for shark populations as well as sharks themselves (Campana, 2014). The increase of negative anthropogenic impact requires an increase of research into population dynamics of Elasmobranch species in order to determine the extent of damage being done to these populations. In order to carry out calculations for shark life history and population dynamics, age data is needed (Age and Growth in Sharks). Age and growth studies for Elasmobranch species are therefore a basis for a higher understanding of overall shark population health.

Age studies for other fish species are more frequent annually due to the regularity of encounters with bony fish populations. Teleost fish samples can be taken from commercial fisheries, markets, research based sampling and individual fishing efforts. This creates a plethora of data in comparison to shark species. Elasmobranch populations are comparably more mobile than teleost fish, leading to a lower amount of population data (Dean & Summers, 2006). Sampling shark populations is higher in cost and effort compared to teleosts, a contributing factor to data gaps in shark population studies (Goldman & Cailliet, 2004).

One of the ways to overcome a lack of physical data is to use mathematical modeling. The von Bertalanffy growth equation is suited for the indeterminate growth of fish species and has been known to accurately calculate an individual's age based on length measurements (Carlson & Baremore, 2002). For sharks, a mathematical model such as the von Bertalanffy growth equation fills in gaps when sampling large populations is difficult. At length measurements may be taken and used to calculate the age of an individual shark in years based on constant growth values within the species (Carlson & Baremore, 2002). The von Bertalanffy growth equation has been known to match with physical ages as observed from age data studies (Campana, 2014).

For age studies in bony fishes, otoliths are one of the major structures used to determine annual growth and overall age. Growth rings appear on these inner ear bones and are deposited at an annual rate (Age and Growth in Sharks). By counting the number of rings on the otolith, an age can be determined for an individual fish. Scales may also be used in a similar way. Cycloid and ctenoid scale types of teleost fish grow with the fish throughout their lifetime. Fish display indeterminate growth, allowing for those species with growing scale types to display their entire lifespan on their scales (Cailliet et al, 1988). Though these strategies prove effective for bony fish species, they are not possible for those within Elasmobranchii. Sharks lack otoliths and their dermal denticle scales are replaced as the individual grows (Age and Growth in Sharks). This generates the issue of determining shark age when they lack the typical 'hard parts' used for aging in other species.

Elasmobranch species, though lacking in ossified structures, have a calcified vertebral column (Ridewood, 1921). Calcified bands within shark vertebrae were first described in the late 1800s. At the time no definite conclusion was made as to the indications of the banded patterns and it was inferred that they may be indicative of taxonomic classification (Ridewood, 1921). A review of shark vertebrae in 1921 brought up the possibility of the calcified bands being defined as “growth zones” (Ridewood, 1921). The possibility that the growth zones could be an indicator of age within individual sharks was brought about two decades later and is now an accepted tool for age determination in Elasmobranchs (Cailliet et al, 1986).

The banding within shark vertebrae appears in pairs of opaque and translucent rings. One pair of bands equals about one year of growth and allows for ages to be determined through ring counts (Age and Growth in Sharks). The width of the band indicates the amount of growth taken place within that particular year and gives insight into the resources available in that time (Hoenig & Gruber, 1990). Elasmobranchs go through more rapid growth within the years leading up to sexual maturity but like other fish species, display indeterminate growth throughout their lifetime. Due to this, their vertebral ring patterns represent total growth within the lifespan.

Rhizoprionodon terraenovae, also known as the Atlantic Sharpnose, is a small requiem shark species that inhabits warm, coastal waters (Loefer & Sedberry, 2003). These sharks rarely grow beyond three and a half feet in length and frequent estuary habitats. Their range extends from the Northeast coast of the United States, down to the east coast of South America (Carlson & Baremore, 2002). With their

proximity to the coast, *R. terraenovae* has a higher encounter rate with humans. Development along coastlines therefore has the potential to negatively effect *R. terraenovae* populations due to habitat loss. Fisheries for *R. terraenovae* do exist along the Atlantic coast of the United States as a part of both large commercial and small fishery sectors (Carlson & Baremore, 2002). The size of *R. terraenovae* do not make the species a typical target for finning operations but the meat is used for human consumption (Carlson & Baremore, 2002). Due to its increased encounter with human activity and its presence in shark fisheries, *R. terraenovae* is a species worth studying in terms of population dynamics and in particular age data studies.

As a species with an established fishery in the United States, it is crucial for fisheries to be aware of impacts on *R. terraenovae* populations in order to maintain a sustainable population. With the slow growth rate of individuals as well as the slow rate of population growth observed in Elasmobranch species, a singular event or improper fishing practices have the potential to devastate population numbers. The possibility of overfishing paired with the impacts of human coastal development in *R. terraenovae* habitat, create a significant purpose for studies involving this species. Age data in particular becomes useful in understanding population growth in order to implement sustainable fisheries and limit negative anthropogenic impact.

The current study examines 24 *R. terraenovae* individuals collected along the Florida coast in the Gulf of Mexico. Growth rings displayed within post-dorsal vertebrae were counted for seven individuals of the sample population and estimated ages were compared to von Bertalanffy growth calculations for *R. terraenovae*. If previous studies concerning growth band patterns within

Elasmobranch vertebrae are true, then the comparison between von Bertalanffy calculations and observed age according to band counts should be significantly similar. By confirming the process by which *R. terraenovae* are aged, population growth data for the use of fishery and coastal development policies may be more accurately reported. Age data for *R. terraenovae* is necessary in order to manage sustainable population levels due to their exposure to human activity. This study contributes to the building of age data for *R. terraenovae* and its implications in management policies.

Methods

Collection

R. terraenovae individuals were captured using rod and reel in September of 2014. Collections were made in Crystal Bay, Florida within the Gulf of Mexico. Once captured, individuals were euthanized using eight milligrams of tricaine methane-sulfonate (MS-222). Samples were measured for precaudal, fork and total length and weighed with a spring scale. All *R. terraenovae* were brought back to the Florida Southern College marine lab and kept frozen until further investigation.

Vertebra Removal and Preservation

Vertebrae removal took place from January to March of 2017. *R. terraenovae* had been kept frozen, though periodically thawed for other research investigations. At the time of vertebrae removal, all samples had a ventral cut from the pectoral girdle to the cloacae and all organs within the main body cavity had been removed.

Each sample was thawed for at least four hours before vertebrae were removed. Precaudal, fork and total lengths were remeasured for all samples and recorded due to a lack of consistency with the 2014 data. Five segments of the vertebral column were sectioned out by going in ventrally from the existing opening made from previous organ removal. Segments located posterior to the dorsal fin were chosen due to the larger size of the vertebrae and were cut out using a scalpel. Excess tissue and muscle was cleaned off using scalpel and scissors. Each vertebral segment was placed in a jar of 70% ethanol and labeled for future use.

Vertebrae Sectioning and Growth Ring Counts

Vertebrae segments for seven samples were removed from ethanol and dried for four hours at room temperature. A High-Tech lapidary saw with two thin-notched diamond saw blades was used with a hard plastic spacer to create vertebrae cuts. Vertebral segments were pushed through the saw blades by hand to make a cut sagittal to the haemal arch of the vertebrae. The process was then repeated in order to generate a bowtie shaped slice of the vertebrae that was less than one millimeter thin. This was repeated for all seven vertebral segments.

Each vertebral section was polished with a fine grain emery board in order to enhance visibility of growth zones. Bowtie cross-sections were observed under a dissecting scope at 2x magnification by two independent observers. Each observer did not know the length of the shark from which the vertebral cross-section was obtained and observed ages were not shared amongst observers until independently recorded. Counts and age observations were made based on the

NOAA technical report stating that a pair of translucent and opaque bands were equal to one year of age and the assumption that the first opaque region was the time of birth (Age and Growth of Sharks). Observed ages were recorded for all seven samples.

Von Bertalanffy Growth Calculations

The von Bertalanffy growth calculation was utilized in order to determine the expected age of each of the seven *R. terraenovae* individuals based on their total length in centimeters.

$$L_t = L_{\text{infinity}}(1 - e^{-k(t-t_0)})$$

The values for L_{infinity} , K and t_0 were used from Carlson and Baremore who ran the same equation for *R. terraenovae* males (2002). With L_t representing length at a given age, the total length of each of the seven *R. terraenovae* samples was put through the equation to calculate an expected age in years.

Comparison of Observed and Expected Ages

To compare the ages observed from growth band counts and calculated from the von Bertalanffy equation, a chi square analysis was run using Excel. Von Bertalanffy calculations represented the expected variable while the growth band data represented the observed variable. Each shark represented an individual category for comparison.

Results

The chi square analysis for the observed growth band age and the calculated von Bertalanffy age generated a p-value of .82.

Figure 1 displays all remeasured lengths taken January-March of 2017. "X" indicates *R.terraenovae* for which heads had been removed for a previous study. Due to this no accurate length measurements could be taken.

Age as observed in growth ring counts were recorded as seen in Figure 2. The number within the observed age column represents the lowest observed number of rings as seen by one or both observers. This number does not include the birth ring seen at the base of the vertebrae slice. Therefore sample 24 represents an individual who may not yet be one year of age. The chi square analysis does not allow number ranges and therefore the lowest observed age was used for the observed data set. The column on Figure 2 displaying "VB age" refers to the van Bertalanffy calculated age for each *R. terraenovae* sample with the total length in the neighboring column for convenience when the calculations were being run.

Using the von Bertalanffy growth equation with *R. terraenovae* constants used from Carlson and Baremore, a growth curve can be established. This curve allows for an individual of *R. terraenovae* at a given length to be aged. This curve can be observed in Figure 3.

A comparison of the observed growth ring age and the calculated von Bertalanffy age can be seen in Figure 4. Each numbered plot point indicates the *R. terraenovae's* sample number as seen in figure 1. Both a calculated and observed

data point are represented by the red square and blue diamond respectively. The chi square p-value of .82 is reflected by Figure 4, where if observed and calculated were the same, points of the same number should display overlap.

Figure 1.

Shark	FL	PCL	TL	Sex
1	65	69	90.8	male
2	57.5	52	69	male
3	76	69	90.8	male
4	73.5	67	87	male
5	72.2	65.5	85	male
6	73.1	68	85.5	male
7	58.2	53	70.8	male
8	70.3	63.6	85.2	male
9	71	65.4	80.6	male
10	57.5	51.5	67	male
11	X	X	X	male
12	X	X	X	male
13	63.5	57.5	76	male
14	75	69	89.5	male
15	67	61.5	82.2	male
16	64.1	69.3	84	male
17	72	65.4	87.2	male
18	X	X	X	male
19	58.4	52.5	70	male
20	69	63	83	male
21	X	X	X	male
22	57.2	51.7	70	male
23	X	X	X	male
24	56.5	50.7	58.5	male

Figure 2.

Shark	Total Length	VB Age	Observed Age
3	90.8cm	4.47	4+
5	85cm	2.32	4+
9	80.6cm	1.73	3+
14	89.5cm	3.56	4+
16	84cm	2.16	2+
17	87.2cm	2.77	4+
24	58.5cm	0.46	0+

Figure 3.

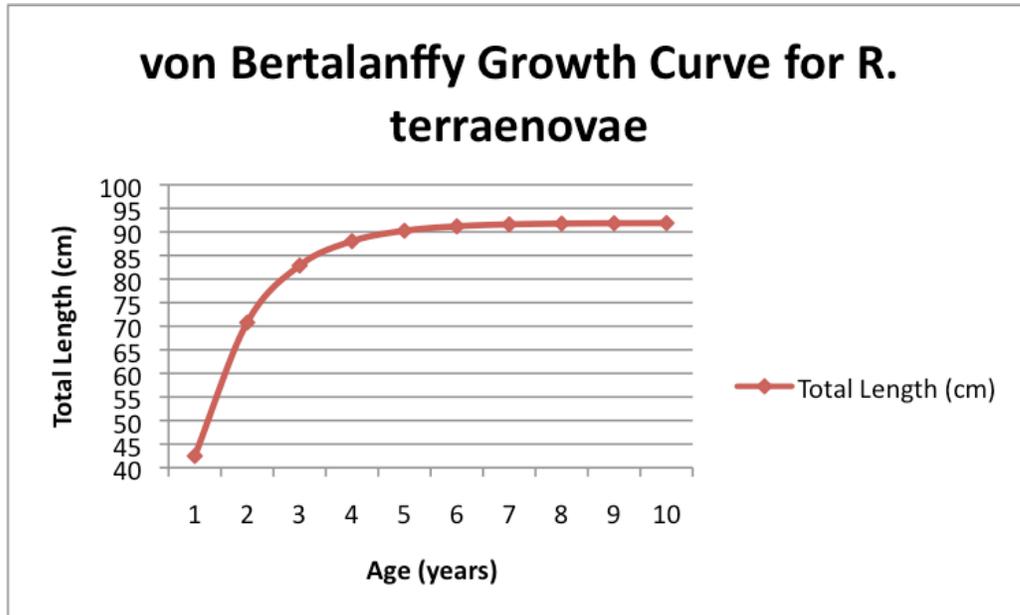
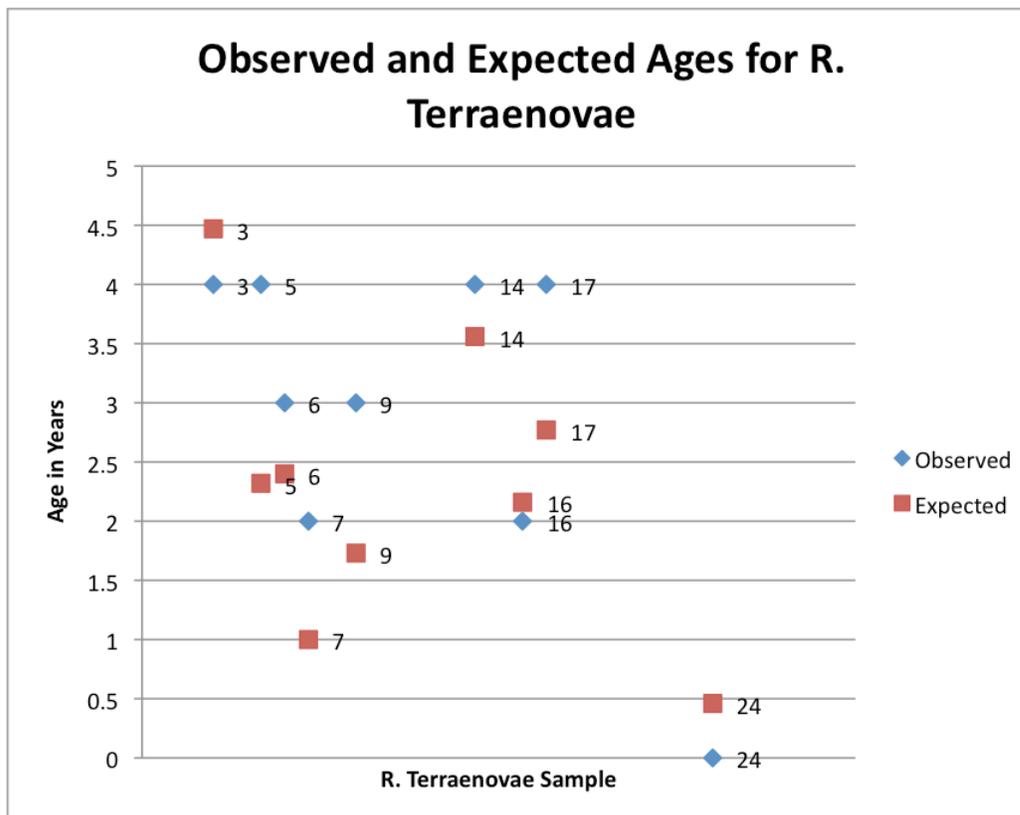


Figure 4.



Discussion

By examining growth ring patterns within the vertebrae of *R. terraenovae*, age data for the species can contribute to population dynamic data. These data sets can aid in determining conservation policies for *R. terraenovae*. It was thought that in comparing the observed growth ring age to the calculated age based on the von Bertalanffy growth equation for individual *R. terraenovae* there would be a significant relationship. However, using a chi square analysis to evaluate the similarities in the calculated and predicted ages, no significance was found between the two data sets.

Looking at Figure 3, none of the data points for the same *R. terraenovae* specimen overlap. Ideally, if the von Bertalanffy calculated age and the observed age were the same, each data point for the same sample would occupy the same point. Visually, this leads to the assumption that the two ages for each sample are not similar. This is confirmed by the chi square analysis that produced a p-value of .82. With the p-value above the significant value of .005, the prediction that both the observed age based off growth ring counts and calculated age from the von Bertalanffy growth equation would be significantly similar goes unsupported.

The lack of significance may be due to inaccurate growth band counts for each of the seven *R. terraenovae* samples. The cutting procedure used to slice the vertebrae segments into observable samples left uneven pieces. The slices produced were wedge-like in that one end was thinner than the opposite side. This unevenness may have caused a warping of the growth band lines and may have

contributed to an addition or loss of observable rings. It is thought that the reason for this uneven cutting was due to the blade spacer used between the saw blades. The spacer may not have been large enough, causing the blades to bend inwards toward each other, causing the uneven cut. This process may have also caused ridges to form on the vertebrae sections. These ridges may have been interpreted as possible growth bands and altered the observed age. A smoother cut would help to avoid this issue and keep growth band counts more accurate. In future use of this saw, a larger spacer is suggested to combat this problem.

The process by which growth rings were counted may also have contributed to inaccurate observed ages. All samples were observed either with the naked eye or with a dissecting scope at 2x magnification. Previous age and growth studies have cited using violet dye or x-ray to enhance growth bands. These methods have been shown to produce clear banding, whereas simple observation leaves the chance for more variability. Though two independent observers were used for this study, it is suggested that three or four be used for future counts to ensure even less bias. The use of higher power magnification or dye paired with an increased number of observers could possibly produce more confident observed ages.

For the van Bertalanffy calculated age, there may be discrepancies within the constants used for L_{∞} , K and t_0 . The values for these variables were taken from Carlson and Baremore who analyzed a population of *R. terraenovae* from within the Gulf of Mexico. Von Bertalanffy standards were formed based off of an all male collection within their study, however, the sample organisms were caught between 1999 and 2001. The time difference between the current study and that run by

Carlson and Baremore leaves room for variation to have occurred within the *R. terraenovae* Gulf of Mexico population. There may have been environmental or molecular changes within the population that could have altered the constant values between 2001 and 2014. In order to achieve the most accurate data set possible, values for L_{∞} , K and t_0 should be calculated based off of the sample population collected for this study.

It is suggested for future use of this study's sample population of *R. terraenovae*, more vertebrae be cut for analysis. A contributing factor to a lack of significance between calculated and observed ages may have been due to the small number of analyzed vertebrae. Only seven of the available 24 vertebrae sections were used due to time constraints and it is suggested that the rest of the available vertebrae be put through the age analysis methods. This would help to either support the current findings that there is no relation between von Bertalanffy calculated age and observed growth ring age for this sample population. In contrast, it may help to determine a significant relationship due to a higher sampling volume.

This study failed to find a significant relationship between calculated age of *R. terraenovae* using the von Bertalanffy growth equation and the observed age through growth ring counts. It is suggested that age data analysis continue with the same sample population in order to determine accurate ages of the *R. terraenovae* samples. Age data for shark populations is a tool necessary for confirming population growth dynamics and for setting conservation policies. It has long been discussed that shark species exhibit slow individual growth, are considered k-selective for reproduction and have slow recruitment rates for populations (Skomal

& Natanson, 2003). With *R. terraenovae* inhabiting shallow, estuarine areas, their possibility of encountering humans is higher in comparison to more pelagic species. *R. terraenovae* also represents a sector of Atlantic coast fisheries. With these factors in mind, sustainability of *R. terraenovae* populations is crucial for fisheries management and ecosystem health (Campana).

The impact that poor management of *R. terraenovae* populations could have on ecosystem function within shallow, coastal waters is monumental. As top predators within the ocean food web, a decrease in populations could have a top-to-bottom cascade of impacts on fish, invertebrates, algae and other marine organisms (Parsons, 1993). In order to maintain a healthy balance within the ecosystem, keeping shark populations, such as *R. terraenovae*, stable is necessary. To form policies that ensure population stability, age data is essential. By determining annual growth of individuals and populations, information on what defines a stable population size is possible (Brown & Gruber, 1988). Studies involving shark age and growth are therefore essential tools for defining healthy population parameters. This aids in setting guidelines for population size within an area and pressures fishery policy in order to meet these goals.

Conclusion

Age data studies for sharks present an opportunity to build upon existing population data for shark species and how populations may fluctuate over time. Aging individuals from a population may establish patterns for annual growth that can be used for fishery and conservation policies later on. In analyzing a sample

population of *Rhizoprionodon terraenovae* from Crystal Bay, Florida it was predicted that a calculated age using the von Bertalanffy growth equation and an observed age from counting vertebral growth rings would produce significantly similar ages for a given individual. However, a chi square analysis left this prediction unsupported. Future age studies concerning this sample population of *R. terraenovae* is highly encouraged due to the species' coastal location, interaction with humans and presence within Atlantic coast fisheries.

Resources

Age and Growth in Sharks. (n.d.). Retrieved April 07, 2017, from <http://nefsc.noaa.gov/nefsc/Narragansett/sharks/age.html>

Brown, C. A., & Gruber, S. H. (1988). Age Assessment of the Lemon Shark, *Negaprion brevirostris*, Using Tetracycline Validated Vertebral Centra. *Copeia*, 1988(3), 747. doi:10.2307/1445397

Cailliet, G. M., Radtke, R. L., & Welden, B. A. (1986). Elasmobranch Age Determination and Verification: A Review .

Campana, S. E. (n.d.). The Campana lab » Ageing Sharks. Retrieved April 08, 2017, from <http://uni.hi.is/scampana/sharks/ageing-sharks/>

Campana, S. E. (2014). *AGE DETERMINATION OF ELASMOBRANCHS WITH SPECIAL REFERENCE TO MEDITERRANEAN SPECIES: A TECHNICAL MANUAL* (Tech.). Rome: FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS.

Carlson, J. K., & Baremore, I. (2002). Changes in Biological Parameters of Atlantic Sharpnose Shark, *Rhizoprionodon terraenovae*, in the Gulf of Mexico: Evidence for Density-dependent Regulation? *Northwest Atlantic Fisheries Organization*.

Dean, M. N., & Summers, A. P. (2006). Mineralized cartilage in the skeleton of chondrichthyan fishes. *Zoology*, 109(2), 164-168. doi:10.1016/j.zool.2006.03.002

- Goldman, K., & Cailliet, G. (2004). Age Determination and Validation in Chondrichthyan Fishes. *Marine Biology* *Biology of Sharks and Their Relatives*, 399-447. doi:10.1201/9780203491317.pt3
- Hoening, J. M., & Gruber, S. H. (1990). Life-history patterns in the elasmobranchs: implications for fisheries management. *NOAA Technical Report . How to Section Large Shark Vertebrae* [Video file]. (2014, September 8). Retrieved from <https://www.youtube.com/watch?v=8timTOnq5YA>
- Loefer, J. K., & Sedberry, G. R. (2003). Life history of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) (Richardson, 1836) off the southeastern United States. *DNR Fishery Bulletin* .
- Parsons, G. R. (1993). Age determination and growth of the bonnethead shark *Sphyrna tiburo*: a comparison of two populations. *Marine Biology*, 117(1), 23-31. doi:10.1007/bf00346422
- Parsons, G. R. (1985). Growth and Age Estimation of the Atlantic Sharpnose Shark, *Rhizoprionodon terraenovae*: A Comparison of Techniques. *Copeia*, 1985(1), 80. doi:10.2307/1444793
- Ridewood, W. G. (1921). On the Calcification of the Vertebral Centra in Sharks and Rays. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 210(372-381), 311-407. doi:10.1098/rstb.1921.0008
- Skomal, G. B., & Natanson, L. J. (2003). Age and growth of the blue shark (*Prionace glauca*) in the North Atlantic Ocean*. *Fishery Bulletin*, 627-639.
- Sminkey, T. R., & Musick, J. A. (1995). Age and Growth of the Sandbar Shark, *Carcharhinus plumbeus*, before and after Population Depletion. *Copeia*, 1995(4), 871. doi:10.2307/1447035