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Pectoral fin ray aging: an evaluation of a non-lethal method for aging gars and its application to a population of the threatened Spotted Gar

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Abstract Spotted Gar (*Lepisosteus oculatus*), a species listed as Threatened under the Canadian Species at Risk Act (SARA) was collected during May and June, 2007 from several sites in Rondeau Bay, a shallow coastal wetland of Lake Erie. The first pectoral fin ray was removed from 78 individuals to age the fish and to determine individual growth characteristics. To assess the validity of using pectoral rays to age Spotted Gar, we compared techniques (otoliths, branchiostegal rays and pectoral rays) for ten individuals captured in southwestern Michigan. Agreement between readers and amongst the three structures was high; thus aging of Spotted Gar using sectioned pectoral rays is an effective method. Rondeau Bay specimens varied in age from 3 to 10 years and from 515 to 761 mm total length. Regression analysis of length vs. age data was calculated to be $y = 19.217x + 491.19\left(R^2 = 0.22\right)$. The low R^2 value is attributed to having males and females, which differ in growth rates, combined. Growth rates of Rondeau Bay specimens were compared to a Louisiana population using ANCOVA. No significant difference was found in the rate of growth between these populations; however, condition was low as compared to a standard weight equation. This may lead to lower fecundity, contributing to the species' rarity in Canada.

Keywords Age · Growth · Species at risk · Pectoral ray · Lake Erie · Spotted Gar

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Introduction

The Spotted Gar (*Lepisosteus oculatus*) is a fish species designated as Threatened under the Canadian *Species at Risk Act* (SARA). The species is distributed throughout the Mississippi River drainage with its northern limit extending into Canada (Fig. 1). In Canada, *L. oculatus* inhabits three coastal wetlands of Lake Erie (Point Pelee, Rondeau Bay and Long Point Bay) with historic records from Lake St. Clair (COSEWIC 2005). The Threatened designation in Canada is due to their limited distribution and possible loss of critical habitat (COSEWIC 2005).

When preparing management strategies for species at risk, information is needed on life history traits, habitat associations, habitat availability and recovery targets (Rosenfeld and Hatfield 2006). This information is lacking for the Spotted Gar in Canada. Much of what is known about the Spotted Gar is based mainly on data gathered in the southern portion of its

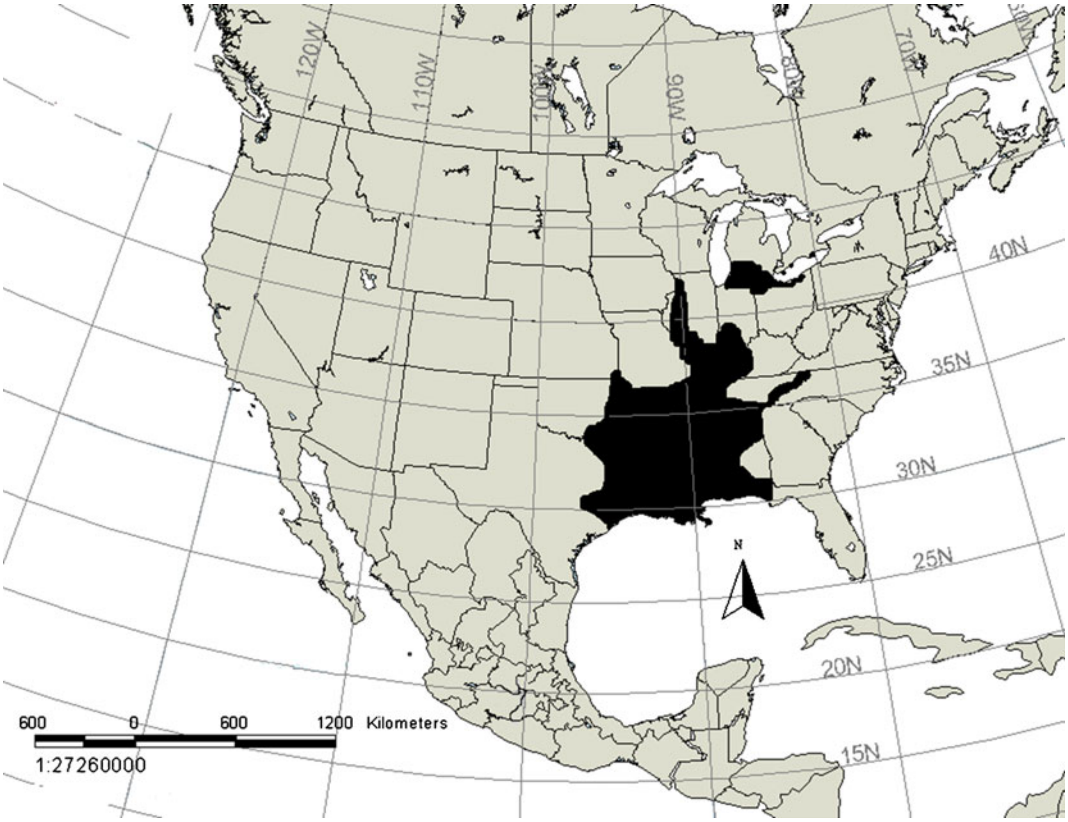

Fig. 1 Range of the Spotted Gar, modified from Page and Burr (1991), to show all areas of known Canadian occurrences

range (Love 2002, 2004). In this study, we attempted to fill in gaps in the life history of this species in Canada by conducting an age and growth study of the Spotted Gar in Rondeau Bay. Rondeau Bay is home to the largest of the known populations of Spotted Gar in Canada (COSEWIC 2005).

Various calcareous structures have been used to determine the age of fish specimens including otoliths, scales, opercula, and fin rays (Ihde and Chittenden 2002). Traditionally, branchiostegal rays have been used to age gar (Love 2004), though otoliths and sectioned scales have also been used (DiBenedetto 2009). The use of branchiostegal rays and otoliths requires sacrifice of the specimen to remove the structures and removal of a section of interlocking ganoid scales would leave the individual prone to infection. For these reasons, we chose a non-lethal method, the use of pectoral fin ray cross sections, to age Spotted Gar specimens. The use of fin ray sections to age specimens is an effective method in several species including Common Carp (*Cyprinus carpio*; Phelps et al. 2007), Lake Whitefish (*Coregonus clupeaformis*; Mills and Beamish 1980), Muskellunge (*Esox masquinongy*; Brenden et al. 2006), Walleye Pollock (*Theragra chalcogramma*), Pacific Cod (*Gadus macrocephalus*), and Albacore (*Thunnus alalunga*; Beamish 1981). In addition, the removal of fin rays for aging has been shown to have no negative effects on the growth and survival of bull trout (*Salvelinus confluentus*; Zymonas and McMahon 2006).

The objectives of this study were to: determine if pectoral fin ray sections are suitable for aging gars; to compare the age and growth of the Spotted Gar population of Rondeau Bay with the age and growth of a Spotted Gar population in Lake Pontchartrain, Louisiana (Love 2004); and to compare the condition of individuals in the Rondeau Bay population with the standard for the species as reported in Bister et al. (2000). Because of a shorter growing season and colder temperatures, we predict that the Spotted Gar of Rondeau Bay will have a slower rate of growth than the Spotted Gar population studied by Love (2004) and be in poorer condition when compared to the standard for the population reported in Bister et al. (2000). Colder temperatures lead to reduced growth rates for many aquatic species (Angilletta et al. 2004).

Methods

Structure comparison

We compared aging structures to test the validity of using pectoral fin rays to age Spotted Gar on 10 specimens collected from southwestern Michigan in October 2008. Five individuals were captured from Loon Lake in Branch County, Michigan (41.8689° N, −84.9427° W) and five were collected from Lake Pleasant in Hillsdale County, Michigan (41.8800° N, −84.5663° W). Michigan samples were collected using boat electrofishing. These individuals were sacrificed and the otoliths, branchiostegal rays, and first pectoral fin rays (clipped as close to the base as possible) were removed for aging. Individuals from these populations were chosen due to the similarity of climate between Michigan and Southern Ontario. The number of individuals used for validation is necessarily low as the species is also at risk (Special Concern) in Michigan. Following the method of Den Haas and Mandrak (2004), pectoral fin rays were embedded in epoxy resin and sectioned with a Buehler-Isomet low-speed saw to a thickness of 0.75 mm. These cross-sections were mounted on microscope slides and examined using a compound microscope at 400× magnification. Growth annuli were counted to estimate the age of the specimens (Fig. 2). Branchiostegal rays were boiled until all flesh was easily removed and then air dried before aging using a dissecting microscope. Otoliths were ground into a thin transverse slice using GatorGrit 120-c waterproof paper (Mastercraft) and polished with 3 M Lapping film, 261×, 30 micron. These thin sections were mounted to microscope slides using Crystalbond 509 (Electron Microscopy Sciences). Otoliths were viewed using magnification of 400× and growth annuli were counted. All structures were viewed and aged independently by two separate readers and an index of precision was determined for each individual structure as in Den Haas and Mandrak (2004). The age as estimated by the first reader of the branchiostegal ray was used as the assigned age for all specimens. For all other age estimations the index of precision was calculated as equation [1].

$$\text{Index of precision} = \frac{|[\text{annuli counted on structure} - \text{assigned age}]|}{\text{assigned age}}$$

Based on the findings of Den Haas and Mandrak (2004), where the bottom 33% of structures had an index of precision score of 0.29 or higher, we will accept the method of aging using pectoral fin ray sections as

Fig. 2 Cross section of pectoral fin ray of 7-year old Spotted Gar, viewed under magnification (400×) showing growth annuli

valid if the average index of precision for the structure is less than 0.29. In addition to the index of precision, a chi-squared test was conducted to compare the observed (pectoral ray) age with the expected (branchiostegal ray) age for each of the two readers of pectoral fin rays.

Aging the Rondeau Bay population

Rondeau Bay is a shallow (<3 m) coastal wetland along the north shore of the central basin of Lake Erie. The bay is characterized by clear water and abundant macrophyte growth. Spotted Gar was collected from 15 sites around Rondeau Bay using fine-mesh fyke nets (1.2 m hoops with 6.35 mm mesh), a non-lethal method of collection, 78 specimens were collected during May and June 2007. Specimens were weighed to the nearest gram and their total length (mm) was measured. The first pectoral fin ray on the right side of each fish was then clipped as close to the base as possible for aging. This technique of aging is non-lethal and all specimens were successfully released after handling. All animals were cared for in accordance with the Canadian Council on Animal Care guide to the care and use of experimental animals and this research was approved by the animal care committees of the University of Windsor and the Canadian Department of Fisheries and Oceans.

The fin rays were then prepared and read in the same manner as those from Michigan. Three sections from each specimen were aged independently of each other by the first reader. A second experienced reader also counted the growth marks, presumed to be annuli, to increase the precision of age estimation of the samples. Where disagreement between samples occurred, the most common age reported was used. The variance among reads of sections from the same individual was calculated.

To describe the growth of the Rondeau Bay population, regression analysis was conducted on the total length vs. age data set. The log-transformed total lengths were substituted into the standard weight equation developed by Bister et al. (2000) to determine the condition of individuals from the Rondeau population as compared to the standard for Spotted Gar.

Length-frequency plots were created for each age class. Upon inspection, each age class was divided into two size classes, based on the length-frequency distributions. As the fish could not be sacrificed to determine sex, and females are larger than males at the same age (Love 2004), the smaller size class individuals were presumed to be the males and the larger size class individuals was presumed to be the females of each age. Log transformations of the lengths, and regression analysis were then conducted for each size class separately. Growth equations were then compared to the growth equations produced by Love (2004) by first extrapolating the raw data from a digital copy of Love's Fig. 3, using the computer software ImageJ (NIH image analysis software, http://rsbweb.nih.gov/ij/) followed by an analysis of covariance.

Results

Structure comparison

There was a relatively good agreement among the various aging structures and readers (Table 1). The combined average index of precision for pectoral ray

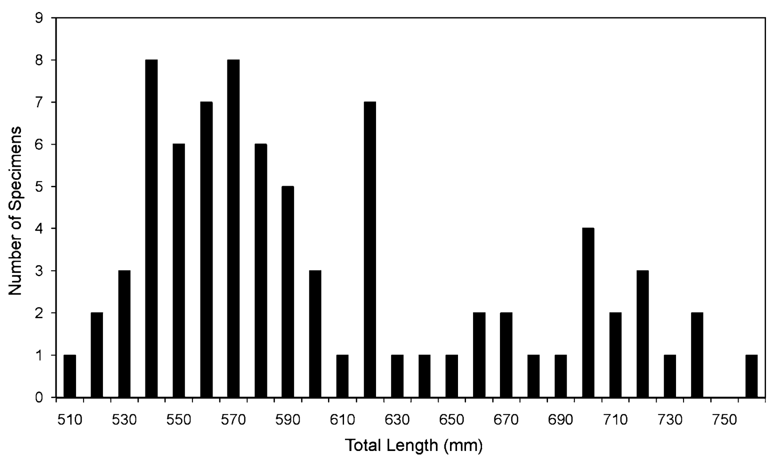

Fig. 3 Length-frequency (total length) histogram for Spotted Gar captured in Rondeau Bay, Ontario, during 2007 sampling

Table 1 Comparison of estimated age for Spotted Gar (*Lepisosteus oculatus*) specimens captured in southwestern Michigan using various structures. Ages in years determined by two separate readers from sectioned pectoral ray, branchiostegal ray and otolith are given for each specimen. The index of precision is shown in brackets for each structure. The age based on branchiostegal reader 1 is taken as the standard, thus index of precision is zero (perfect agreement) for all branchiostegal reader 1 ages

Fish number	Pectoral ray reader 1	Pectoral ray reader 2	Branchiostegal reader 1	Branchiostegal reader 2	Otolith reader 1	Otolith reader 2
118	4 (0.2)	4 (0.2)	5	5 (0)	7 (0.4)	8 (0.6)
120	7 (0)	7 (0)	7	7 (0)	7 (0)	8 (0.14)
121	4 (0.2)	5 (0)	5	5 (0)	5 (0)	5 (0)
122	3 (0)	3 (0)	3	2 (0.33)	3 (0)	4 (0.33)
123	2 (0)	2 (0)	2	2 (0)	3 (0.5)	3 (0.5)
124	6 (0.14)	7 (0)	7	7 (0)	7 (0)	8 (0.14)
125	2 (1)	1 (0)	1	1 (0)	1 (0)	1 (0)
127	4 (0)	4 (0)	4	4 (0)	4 (0)	4 (0)
128	12 (0.14)	14 (0)	14	14 (0)	14 (0)	14 (0)
130	7 (0.13)	7 (0.13)	8	8 (0)	9 (0.13)	9 (0.13)

samples was 0.11, and for the otoliths was 0.14 (Table 1). The average index of precision for branchiostegal rays by the second reader was 0.03. Thus, the accepted standard of branchiostegal rays (lethal technique) is the most precise technique for aging Spotted Gar, followed by the use of sectioned pectoral rays (non-lethal technique) and sectioned otoliths (lethal technique) is the least precise. A Chi-squared test found no difference between the observed age (pectoral fin ray age) and the expected age based on the branchiostegal ray (Chi-square = 0.325, P=0.99).

Aging the Rondeau Bay population

Using non-lethal techniques, we captured, aged, and released 78 Spotted Gar in Rondeau Bay. Specimens collected ranged in age (3 to 10 years), total length (515 to 761 mm) and weight (0.52 to 1.94 kg). The length-frequency distribution appeared bimodal with fewer large than small specimens (Fig. 3). The modal and most common age that was observed was 6 years. Although there was a significant relationship between age and length ($Y = 18.52X + 493.62$; $P<0.0001$), the amount of variation explained was low (R^2=0.22) (Fig. 4).

When the Spotted Gar data from Rondeau Bay were separated into two size classes (presumed males and females), the slopes of the lines representing small size class ($Y = 0.022X + 6.21$; R^2=0.43; $P<0.0001$) and large size class ($Y = 0.041X + 6.25$; R^2=0.64; $P<0.0001$) were significantly different from one another (P=0.004; Fig. 4). A comparison of the age-length data

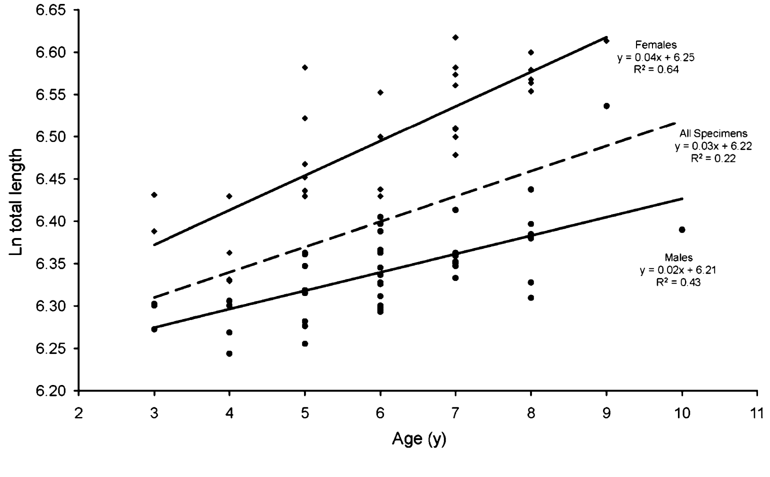

Fig. 4 Natural log total length (mm) vs. age (years) of Spotted Gar captured in Rondeau Bay, Ontario, separated by presumed sex (see text for details). Male specimens are indicated by round dots and female specimens are indicated by diamonds. Dashed line indicates regression line for males and females combined

from Rondeau Bay and Louisiana (Love 2004) showed that growth rates did not differ significantly for Rondeau large size class and Louisiana female (P=0.15) or Rondeau small size class and Louisiana male (P=0.97) Spotted Gar.

Bister et al. (2000) produced a standard weight equation for Spotted Gar, based on data collected from 47 populations of Spotted Gar across eight of the United States. When the log transformed total length for each specimen was substituted into this standard weight equation, we found that 73 of our 78 specimens were below the standard weight. Of the individuals that were over the standard weight for their length, two were 4 years old and the other individuals were 5, 8 and 10 years.

Discussion

The use of sectioned pectoral fin rays as a non-lethal method of aging is useful, particularly when dealing with species at risk or whenever sacrifice of the specimens is undesirable. We found that the method was precise when compared to the accepted standard method of aging using branchiostegal rays. The preparation of pectoral fin ray samples is more time consuming than preparing branchiostegal rays, which only require boiling to remove flesh. Drying of the resin and sectioning of the samples is a fairly lengthy process; however, once the preparation is complete the age estimation is easily accomplished. Crowding of the growth annuli towards the outer edge of the ray, particularly in older specimens, may lead to underestimating the age in some cases. This was evidenced by one reader underestimating the age of our oldest specimen by 2 years compared to the branchiostegal ray. The second reader, however, was able to correctly determine the age of the oldest specimen using the pectoral fin ray; thus we urge caution when aging older specimens.

Growth rates differed by size classes that presumably represented sexes. The large amount of variability in the length-age data from Spotted Gar specimens from Rondeau Bay can be attributed to our inability to directly sex the fish using external characteristics in the field. Because definitive determination of sex in Lepisosteidae requires sacrifice of the fish and examination of the internal sex organs (Ferrara and Irwin 2001), we were unable to determine the sex of our individuals. Love (2004) showed that male and female Spotted Gar had differing length at age and rates of growth, with females growing larger and at a faster rate than males of the same age. Thus, a combined sample of males and females led to little correspondence of length and age. Additionally, it has been shown that there is substantial variation in growth within age cohorts of fish (Post and Parkinson 2001), contributing further to the variation in our length at age relationships.

The grouping of Rondeau Bay specimens based on the best estimation of sex (females larger and males smaller at age) exhibited similar results to the growth curves of Spotted Gar from Lake Pontchartrain, Louisiana (Love 2004). Females from Rondeau Bay grew at a significantly higher rate than males from the same population consistent with the findings of Love (2004) in Louisiana. Interestingly, the rate of growth did not vary among populations for either male or female specimens between the Ontario and Louisiana populations, despite differences in latitude (42°17′N for Rondeau Bay and 30°11′N for Lake Pontchartrain). This is probably due to the high rate of individual variability in both the Rondeau Bay and Louisiana populations. Alternatively, the Rondeau Bay population may have adapted to the shortened growing season by increasing growth rate during the summer months to compensate for an extended winter, as was found by Conover and Present (1990) in the Atlantic Silverside (*Menidia menidia*).

The weight of most of the Rondeau Bay specimens was lower than predicted based on length using the standard length equation proposed by Bister et al. (2000). The reduced robustness of fish at a given length from the Rondeau Bay population may be attributed to the northern location. Many species do not actively feed in the colder months, and thus gar living at the northern latitudes may not have as much time to feed and increase their condition, as compared to a fish of the same size in southern latitudes. This would also lead to a longer inactive season, and thus these individuals would lose relatively more fat content over the winter than those in southern latitudes.

A possible consequence of the reduced weight at length of individuals compared to the southern populations is lower overall fecundity. Ferrara (2001) showed that fecundity of the Spotted Gar was positively correlated with total length and weight, suggesting that individuals in the northern population may produce fewer eggs than individuals of similar length in the southern population. Lower female condition has also been shown to result in smaller egg size in the Atlantic Haddock (*Melanogrammus aeglefinus*) (Trippel and Neil 2004) and smaller larvae which may be less likely to survive. Lower male condition in the Atlantic Haddock resulted in lower fertilization success (Trippel and Neil 2004). Low body condition may also have survival implications. Low condition has been linked to an increase in disease susceptibility and severity of infection as reviewed by Beldomenico and Begon (2009). Thus, the Rondeau Bay population may have lower levels of reproduction and resistance to disease than southern populations.

Love (2004) found that Spotted Gar from Lake Pontchartrain, Louisiana reached sexual maturity at 1 year of age; however, none of the specimens captured from Rondeau Bay was younger than 3 years of age. Because our sampling method specifically targeted individuals moving into the shallows for spawning, the Spotted Gar in Rondeau Bay may delay maturation compared to those of more southern latitudes. Although our sampling method was passive, and smaller individuals may be less likely to be captured if they do not travel as far or frequently as larger individuals, the mesh size used was small enough to capture all but the smallest fishes. Thus, it is likely that smaller gar were not participating in the spawning behaviour that we targeted. Delayed maturity in Rondeau Bay populations can be attributed to the shorter growing season and colder average temperatures in northern than southern latitudes as was demonstrated with the Lake Trout (*Salvelinus namaycush*) by McDermid et al. (2010).

The maximum age of Spotted Gar in Rondeau Bay was found to be 10 years, which was the same as the maximum life expectancy in southern populations reported by Ferrara (2001). This is in contrast to the expectation that individuals in colder climates would have slower growth rates, but a longer lifespan (Angilletta et al. 2004; Charnov and Gillooly 2004). Redmond (1964) however, aged an individual female Spotted Gar from Missouri at 18 years, suggesting that some exceptional individuals may exceed the maximum life expectancy of 10 years.

The high number of individuals in the 5 to 7 year age classes is indicative of strong year classes from the years of 2000 to 2002; however, it is not known at this time what has caused this variation in year classes. More research is needed to determine the long-term viability of the Rondeau Bay population of Spotted Gar, the largest of the Canadian populations. Future research to assure the survival of this top predator in Canada should include a determination of the habitat utilized by the species in Rondeau Bay as well as their diet preference in relation to the southern populations. Protection of the critical habitat as well as the important prey species will be an integral part of a management strategy for this species. Genetic divergence of the northern population from the southern population will also be of interest. Being isolated at the edge of their range, the northern population may have developed unique adaptations to survive. These adaptations will be important to preserve to ensure a future for the species in Canada.

Overall, we found that the Spotted Gar of Rondeau Bay have similar growth rates to those of Louisiana. However, delayed maturity and lower condition, combined with a lifespan that is not extended compared to gar of southern latitudes may lead to lower lifetime reproductive output, possibly contributing to the rarity of the species in Canada, and reinforcing its currently Threatened status.

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