Effects of a Growth Check on Daily Age Estimates of Age-0 Alligator Gar

Richard A. Snow, Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, 500 E. Constellation, Norman, OK 73072

James M. Long, U.S. Geological Survey Oklahoma Cooperative Fish and Wildlife Research Unit, Department of Natural Resource Ecology and Management, 007 Agriculture Hall Oklahoma State University Stillwater, OK 74078

Abstract: Accurate age and growth information is essential for a complete knowledge of life history, growth rates, age at sexual maturity, and average life span in fishes. Alligator gar are becoming increasingly managed throughout their range and because this species spawns in backwater flooded areas, their offspring are prone to stranding in areas with limited prey, potentially affecting their growth. Because fish growth is tightly linked with otolith growth and annulus formation, the ability to discern marks not indicative of annuli (age checks) in alligator gar would give managers some insight when estimating ages. Previous studies have suggested that checks are often present prior to the first annulus in otoliths of alligator gar, affecting age estimates. We investigated check formation in otoliths of alligator gar in relation to growth and food availability. Sixteen age-0 alligator gar were marked with oxytetracycline (OTC) to give a reference point and divided equitably into two groups: a control group with abundant prey and an experimental group with limited prey. The experimental group was given 2 g of food per week for 20 days and then given the same prey availability as the control group for the next 20 days. After 40 days, the gar were measured, sacrificed, and their sagittae removed to determine if checks were present. Checks were visible on 14 of the 16 otoliths in the experimental group, associated with low growth during the first 20 days when prey was limited and accelerated growth after prey availability was increased. No checks were observed on otoliths of the control group, where growth and prey availability were consistent. Age estimates of fish in the control group were more accurate than those in the experimental group, showing that fish growth as a function of prey availability likely induced the checks by compressing daily ring formation.

Key words: sagitta, OTC marking, *Atractosteus spatula*

Journal of the Southeastern Association of Fish and Wildlife Agencies 3:6–10

Accurately estimating age of fish is important for assessing fish populations (Maceina and Sammons 2006). Many structures have been used to determine fish age, but otoliths have shown to be more accurate and precise for most fish species (Maceina et al. 2007). Otoliths do not regenerate or become resorbed during periods of stress, and their layers of calcium carbonate within the otolith matrix act as a long term record of events through the fish's life, including discernable marks classified as checks. For example, larval monk goby (*Sicyopterus japonicus*), New Zealand longfin eel (*Anguilla dieffenbachii*), and short-finned eel (*Anguilla australis*) exhibit a metamorphosis check as they leave the marine environment and enter freshwater (Shen and Tzeng 2002, Tzeng et al. 2007). These checks (sometimes called false annuli or accessory marks) are formed by a temporary cessation in growth caused by factors such as pH change, stress, injury, abrupt growth change, diet switch, and temperature (Hultberg 1977, Ottaway and Simkiss 1977, Wright et al. 2002, Albert et al. 2009). In some cases, checks have been induced on hatchery-reared fish as a reference mark for subsequent identification (Campana and Neilson 1985, Katayama and Isshiki 2007, Volk et al. 1999).

Alligator gar (*Atractosteus spatula*) has become a popular fish species pursued by anglers but is considered imperiled by the American Fisheries Society (Jelks et al. 2008); thus the species is of increased interest to management agencies (Sakaris et al. 2014). However, biologists have only recently begun to obtain vital growth, recruitment, population structure, and mortality data for this species through the examination of otoliths. Several studies have validated otolith-based age estimation techniques for both juvenile and adult alligator gar (Buckmeier et al. 2012, Sakaris et al. 2014, Snow 2014), and Buckmeier et al. (2012) documented checks in the sagittae, especially between early annuli. Although less distinct when illuminated, some checks were described as very similar in appearance to annuli, potentially affecting the accuracy of age estimates. Understanding factors that may influence checks in alligator gar otoliths will lead to a better understanding of life history for this species.

Alligator gar spawn on inundated terrestrial vegetation in backwaters and floodplain areas during periods of high water (Brinkman 2008, Inebnit 2009) which are subject to a wide variety of fluctuating environmental conditions that could affect growth. Inebnit (2009) suggested that 7 days of continuous flooding was needed for larval development and an additional 14 days were required for adequate nursery habitat in the Fourche LaFave River, Arkansas. Backwater areas are subject to isolation by falling water levels in rivers and reservoirs (Junk et al. 1989, Slipke and Maceina 2005), affecting prey availability and ultimately daily ring discernibility in alligator gar due to slowed growth. Daily increment width in otoliths of larval Chinese suckers (*Myxocyprinus asiaticus*) was related to food availability, with starved fish having narrower increment widths than fed fish, and these periods of narrow rings could be interpreted later in life as a check (Song et al. 2009). In Colorado pikeminnow (*Ptychocheilus lucius*), food availability was speculated to affect daily otolith increment clarity, with low contrast rings associated with periods of low food availability or starvation (Bestgen and Bundy 1998). Therefore, we hypothesized that alligator gar could be subject to check formation during early life stages in unstable habitats such as backwater nursery areas, and these check formations could be misinterpreted as annuli later in life. The objectives of this study were 1) to assess the ability of food abundance to create growth checks on otoliths of age-0 alligator gar, 2) determine the effect of growth checks on the accuracy of daily age estimates, and 3) assess the linear relationship between otolith length and fish length between treatment groups.

Methods

Fish Collection and OTC Marking

Fertilized alligator gar eggs were obtained and hatched on 1 and 2 May 2012 at Louisiana State University Agricultural Center Extension, Baton Rouge. Larval gar were transported to the Tishomingo National Fish Hatchery, Tishomingo, Oklahoma, and released into two rearing ponds on 10 May 2012, 8–9 days after hatch. A weekly sample of 10 fish was collected with seines, sacrificed, and preserved in 70% ethanol to evaluate stomach contents. By week three, all alligator gar were piscivorous, which was when we began our experiment.

On 2 June 2012, 60 individuals were collected via seine and given a reference mark by soaking in a solution of 700 mg L^{-1} oxytetracycline (OTC) and 434 mg L^{-1} sodium phosphate (dibasic) buffer for 6 h. This technique produces a glowing yellow to orange mark when viewed under ultraviolet light (Fielder 2002, Kuklinski 2014). Fish were then transported to the Oklahoma Fisheries Research Lab (Oklahoma Department of Wildlife Conservation) and placed into holding tanks.

Tank Experiment and Otolith Preparation

After OTC marking, 16 individual alligator gar were assigned to one of two feeding treatments: experimental and control. We used eight 946-L round fiberglass tanks that were divided into quarters using composite plastic and window screen. Each quarter-tank had one randomly assigned fish and each tank had two treatments, which allowed for 16 replicates total. All tanks had continuous slow water exchange throughout the 40-day experiment. The water source was from a well that was consistently 22.2 to 22.7 C, resulting in water temperatures in each tank ranging from 22.2 to 25.5 C. Dissolved oxygen averaged 7.8 ppm throughout the experiment. The control group was kept with 30–50 prey items at all times throughout the experiment to ensure minimal energy would be spent feeding, thus maximizing growth. The experimental group was fed 2 g of prey a week for 20 days then switched to a feeding schedule identical to the control group for another 20 days. Prey items were received from Tishomingo National Fish Hatchery, Byron State Fish Hatchery, and Manning State Fish Hatchery in Oklahoma. Prey were 15- to 70-mm total length (TL) largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), goldfish (*Carassius auratus*), fathead minnows (*Pimephales promelas*), golden shiners (*Notemigonus crysoleucas*), and mosquitofish (*Gambusia affinis*). All alligator gar were measured for TL (mm) at the beginning of the experiment, after 20 days, and then once per week until completion. At the end of the 40-day experiment, all fish were sacrificed and otoliths (sagittae) removed.

Otoliths were removed using methods described by Snow (2014). The specimen was positioned dorsal side down under a dissecting scope and the head was removed with a transverse incision anterior to the pectoral girdle. Dissection pins secured the head to a dissection platform. The bottom jaw and gill structures were removed with forceps and the ventral side of the braincase was exposed. The parasphenoid was then detached to expose the inner ear structures, located just under the large bulbous portion of the parasphenoid. After removing the parasphenoid, the saccule structure was revealed, allowing the sagittae to be removed.

Otoliths were cleaned and stored dry before viewing. After 24 h, sagittae were measured to the nearest mm, from the dorsal edge to the ventral edge, then browned at 104 C on a hot plate to increase contrast between accretion and discontinuous zones (Secor et al.1992, Snow 2014). After browning, otoliths were embedded in Loctite 349 (Mauck and Boxrucker 2004, Snow 2014) for sectioning with a low speed IsoMet saw (127 x 0.4 mm). Sagittae were sectioned in a transverse plane near the anterior portion of the otolith (Sakaris et al. 2014, Snow 2014). Otolith sections were mounted to glass microscope slides with thermoplastic cement and polished wet with 600-grit sandpaper to enhance visibility of daily rings.

Accessory Check Determination and Data Analyses

Otoliths were examined double-blind at random with no reference to treatment to reduce bias. A check was defined as a broad diffuse band, obviously different in contrast to the daily growth increments immediately prior. To determine if a check was present, otoliths were examined under immersion oil independently by two readers then discussed in concert using a high resolution monitor connected to an optic-mount digital camera attached to an Olympus BH-2 microscope. After check presence was determined, an ultraviolet light was used to verify OTC marks. Otoliths were examined under a power of 100x to 200x depending on the size of the otolith and daily growth increments were counted independently by two readers, discussed in concert, and an age assigned from the OTC mark to the otolith outer edge.

A chi-square test was used to determine differences in proportions of otoliths with a check between treatment and control groups, and number of increments were tested between groups with a t-test using Excel. Finally, the linear relationship between otolith length and fish length was compared between treatment and control groups with analysis of covariance (ANCOVA, SAS Institute 2012). Prior to analyses, data were examined for normality using the software package Statistix (v8, Analytical Software, Tallahassee, Florida). All significance tests were evaluated at $P \leq 0.05$.

Results

Control fish grew steadily throughout the 40-day experiment, whereas growth of experimental fish was minimal during the food ration period and then rapidly increased after prey availability was increased (Figure 1). Although we randomly assigned fish to each group, fish in the control group were larger (mean TL = 113 mm) than fish in the experimental group (mean TL = 99 mm) at the beginning of the study. Proportion of otoliths having a growth check varied between groups (chi-square = 13.08, df = 1, $P = 0.01$), with none of the alligator gar sagittae in the control group having any checks and 14 of 16 otoliths in the experimental group having a check (Figure 2).

Sagittae were successfully marked with 700 mg L^{-1} of OTC and validated daily growth increment formation. We counted twice as many growth increments (mean, 37.4 vs 18.6) from the OTC mark to the edge for fish in the control group compared to fish in the experimental group (T = 29.26, df = 25, $P = 0.01$) (Figure 3), indicating that daily ring counts were more accurate in the control group (40 days from OTC mark to end of experiment). Even though we found differences in daily ring counts between groups, the linear relationship between otolith length and fish length was similar ($F = 1.23$, df = 1.28, $P = 0.28$), demonstrating that otoliths continued to grow in relation to fish size regardless of ration level.

Discussion

Limited food availability compressed daily growth increments and resulted in a growth check on age-0 alligator gar otoliths. This may be important when considering spawning strategy and nursery habitat for alligator gar, where environmental factors may of-

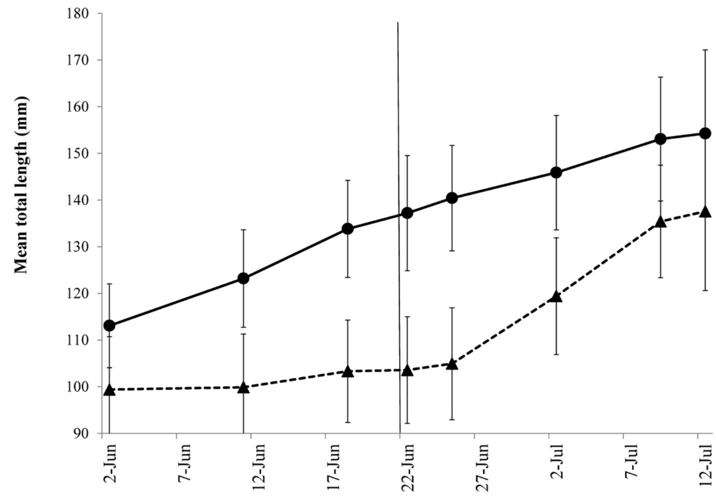

Figure 1. Mean total length of alligator gar fed high ration of prey (control; solid line) and low ration of prey for the first 20 days and then high ration of prey for the subsequent 20 days (experimental; dashed line). Error bars represent 1 SD about the mean. The vertical line represents the change in food rationing for the experimental group.

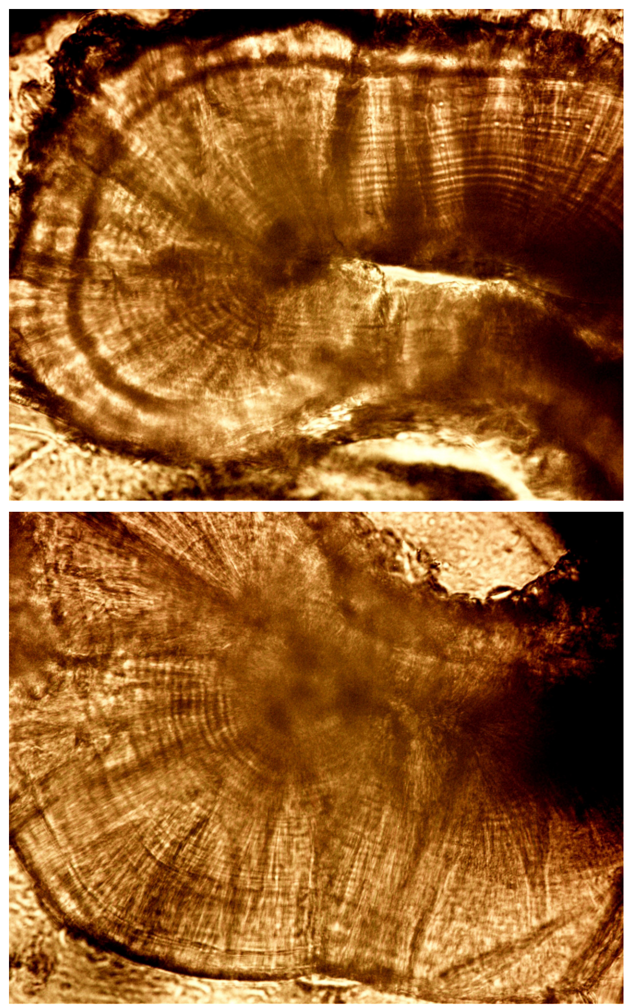

Figure 2. The photo on top illustrates a check created by the compression of daily growth increments due to food rationing in a sagitta of an age-0 alligator gar. The bottom photo is a control fish with no check.

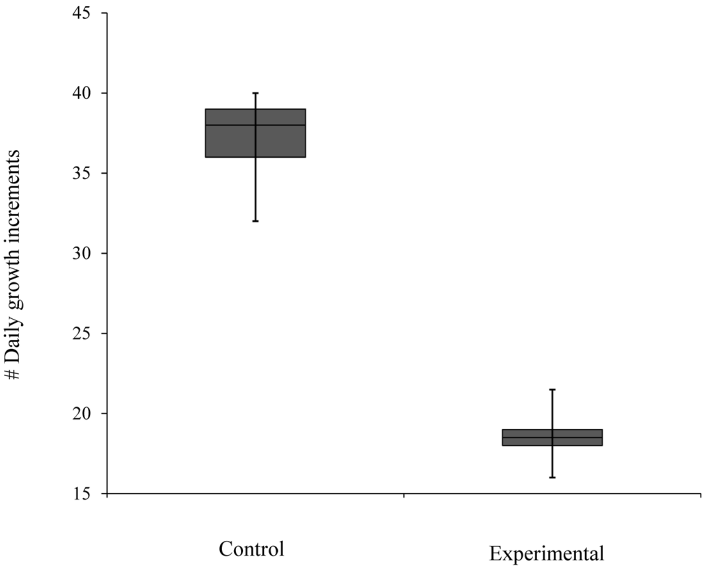

Figure 3. Daily growth increment counts of alligator gar in the control and experimental group. Top and bottom of the boxes represent average range, line inside the box is the median, and the whiskers denote highest and lowest counts within each group.

ten reduce prey availability. These areas may be disconnected and reconnected to adjacent larger water bodies throughout the year (Junk et al. 1989, Slipke and Maceina 2005), causing fluctuations in food supply that may produce multiple checks on otoliths of piscivores such as alligator gar (Robertson et al. 2008). The results of this study should provide a better understanding of detecting checks when estimating age, as unintentional counting of checks could result in error in assigning ages, although to what degree is unknown. Because alligator gar are long lived (Buckmeier et al. 2012), accurately estimating age is important when trying to better understand population dynamics; however, more research should be done to evaluate growth check presence and their effects on age estimates, especially for older fish.

Sagittae from both feeding groups grew in proportion to alligator gar total length, demonstrating that otoliths are good predictors of total length, although differences in daily ring counts clearly indicate that food use can affect daily age estimates. In a daily age validation study, alligator gar formed approximately one ring per day and fish total length was highly related to otolith radius (Sakaris et al. 2014). As a result, Sakaris et al. (2014) concluded there is utility of otolith radius for determining growth history of age-0 alligator gar and that environmental factors would have low influence on this relationship. However, our study found that variable growth rates during the first year of growth can make accurate counting of daily rings more difficult.

On average, our age estimates for control and treatment groups underestimated the known age from the OTC mark by 6.5% and 53.5%, respectively. For fish in the experimental group, this is explained mostly through the inability to discern rings because of extremely reduced growth and the masking presence of a growth check. However, total age of fish in both groups was 72 days, and studies have found that daily ring counts on sagittae of age-0 alligator gar were imprecise and inaccurate past 62 days (Sakaris et al. 2014, Snow 2014). Sagitta develop a concave cross section as they grow, resulting in extra preparation to reveal daily rings near the nucleus and leading to a loss of outer daily rings and underestimates of age. Although we did not examine either of the other two otolith pairs, lapilli were more precise and accurate than sagittae through 91 days post-hatch (Snow 2014), thus use of this otolith for age estimation may overcome some of these limitations. Formation of checks on sagittae of alligator gar in a laboratory setting during their early life history is cause for concern for managers and researchers to accurately interpret them as a long term record of events through a fish's life. Should food become limited in the wild for an extended period of time, checks could form resulting in considerable error in assigning ages. Future research is needed to evaluate other environmental factors that could cause stress resulting in a check and how to objectively identify checks from annuli.

Acknowledgments

The authors thank G. Summers and D. Buckmeier for the collaborative idea and K. Kuklinski, and C. Patterson for help preparing otoliths. K. Kuklinski provided useful comments that improved the manuscript. We thank K. Graves and his staff at Tishomingo National Fish Hatchery for making space available for this study as well as the Byron and Manning State Fish Hatcheries for providing forage. The Oklahoma Cooperative Fish and Wildlife Research Unit is supported by Oklahoma State University, Oklahoma Department of Wildlife Conservation, U.S. Geological Survey, U.S. Fish and Wildlife Service, and Wildlife Management Institute. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Literature Cited

Albert, O.T., M. Kvalsund, T. Vollen, and A.-B. Salberg. 2009. Towards accurate age determination of greenland halibut. Journal of Northwest Atlantic Fisheries Science 40: 81–95.

Bestgen, K.R. and J.M. Bundy. 1998. Environmental factors affect daily increment deposition and otolith growth in young colorado squawfish. Transactions of the American Fisheries Society 127: 105–117.

Brinkman, E.L. 2008. Contributions to the life history of alligator gar, *Atractosteus spatula* (Lacepede), in Oklahoma. Master's thesis. Oklahoma State University, Stillwater.

Buckmeier, D.L, N.G. Smith, and K.S Reeves. 2012. Utility of alligator gar age estimates from otoliths, pectoral fin rays, and scales. Transactions of the American Fisheries Society 141:1510–1519.

Campana, S. E. and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42:1014–1032.

Fielder, D. G. 2002. Methodology for immersion marking walleye fry and fingerlings in oxytetracycline hydrochloride and its detection with fluorescence microscopy. Michigan Department of Natural Resources Fisheries Technical Report 2002-1, Lansing.

Hultberg, H. 1977. Thermally stratified acid water in late winter—A key factor inducing self-accelerating processes which increase acidification. Water, Air, and Soil Pollution 7: 279–294.

Inebnit III, T. E. 2009. Aspects of the reproductive and juvenile ecology of alligator gar in the Fourche Lafave River, Arkansas. Master's thesis. University of Central Arkansas, Conway.

Jelks, H. L., et al. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372–407.

Junk, W. J., P. B. Bayley, and R. E. Sparks. 1989. The flood pulse concept in river-floodplain systems. Canadian Journal of Fisheries and Aquatic Sciences 106:110–127.

Katayama, S. and T. Isshiki. 2007. Variation in otoliths marcostructure of Japanese flounder (*Paralichthys olivaceus*): A method to discriminate between wild and released fish. Journal of Sea Research 57:180–186.

Kuklinski, K. E. 2014. Growth, abundance, and emigration of two *Morone* hybrids in a high flow through Oklahoma reservoir. Journal of the Southeastern Association of Fish and Wildlife Agencies 1:20–25.

Maceina, M. J., J. Boxrucker, D. L. Buckmeier, R. S. Gangl, D. O. Lucchesi, D. A. Isermann, J. R. Jackson, and P. J. Martinez. 2007. Current status and review of freshwater fish aging procedures used by state and provincial fisheries agencies with recommendations for future direction. Fisheries 32:329–340.

——— and S. M. Sammons 2006. An evaluation of different structures to age freshwater fish from a northeastern US river. Fisheries Management and Ecology 13:57–65.

Mauck, P. and J. Boxrucker. 2004. Abundance, growth, and mortality of the Lake Texoma blue catfish population: implications for management. Proceedings of the Southeastern Association of Fish and Wildlife Agencies 58:57–65.

Ottaway, E.M. and K. Simkiss. 1977. A method for assessing factors influencing 'false check' formation in fish scales. Journal of Fish Biology 11:681–687.

Robertson, C. R., S. C. Zeug, and K. O. Winemiller. 2008. Associations between hydrological connectivity and resource partitioning among sympatric gar species (Lepisosteidae) in a Texas river and associated oxbows. Ecology of Freshwater Fish 17:119–129.

Sakaris, P. C, D.L. Buckmeier, and N.G. Smith. 2014. Validation of daily rings deposition in the otoliths of age-0 alligator gar. North American Journal of Fisheries Management 34:1140–1144.

SAS Institute, Inc. 2012. SAS system for linear models. Release 9.4. Cary, North Carolina.

Secor, D. H., J. M. Dean, and E. H. Laban. 1992. Otolith removal and preparation for microstructural examination. Canadian Special Publication of Fisheries and Aquatic Sciences 117:19–57.

Shen, K-N and W-N Tzeng. 2002. Formation of a metamorphosis check in otoliths of the amphidromous goby (*Sicyopterus japonicus*). Marine Ecology Progress Series 228: 205–211.

Slipke, J. W. and M. J. Maceina. 2005. The influence of river connectivity on the fish community and sport fish abundance in Demopolis Reservoir, Alabama. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 59: 282–291.

Snow, R.A. 2014. Documenting utility of otoliths for estimating age and spawning times of alligator gar (*Atractosteus spatula*). Master's thesis. Oklahoma State University, Stillwater.

Song, Z., Z. Fu, C. He, D. Shen, and B. Yue. 2009. Effects of temperature, starvation and photoperiod on otolith increments in larval chinese sucker, *Myxocyprinus asiaticus*. Environmental Biology of Fishes 84:159–171.

Tzeng, Wann-Nian, et al. 2007. Misidentification of the migratory history of anguillid eels by Sr/Ca rations of vaterite otoliths. Marine Ecology Progress Series 348:285–295.

Volk, E.C., S.L. Schhroder, and J.J. Grimm. 1999. Otolith thermal marking. Fisheries Research 43:205–219.

Wright, P.J., D.A. Woodroffe, F. M. Gibb, and J.D. M. Gordon. 2002. Verification of first annulus formation in the illicia and otoliths of white anglerfish (*Lophius piscatorius*) using otolith microstructure. Journal of Marine Science 59:587–593.