Natal origin of Atlantic bluefin tuna (*Thunnus thynnus*) from Canadian waters based on otolith $\delta^{13}$C and $\delta^{18}$O

Ryan W. Schloesser, John D. Neilson, David H. Secor, and Jay R. Rooker

Abstract: Increased knowledge of stock mixing and migration of Atlantic bluefin tuna (*Thunnus thynnus*) is required to properly manage and conserve declining populations. Here, we predicted the nursery origin of giant bluefin tuna ($n=224$) present in samples from Canadian waters using stable $\delta^{13}$C and $\delta^{18}$O isotopes in otoliths. The isotopic composition of milled otolith cores (corresponding to the first year of life) of giant bluefin tuna from three decades (1970s, 1980s, 2000s) and three regions within or adjacent to the Gulf of St. Lawrence was compared with otolith $\delta^{13}$C and $\delta^{18}$O of yearling bluefin tuna collected from eastern (Mediterranean Sea – eastern Atlantic, $n=136$) and western (western Atlantic, $n=103$) nurseries. Maximum likelihood estimates indicated that greater than 99% of bluefin tuna in our Canadian samples originated from the western nursery. No significant differences in estimates of origin for bluefin tuna were detected among decades or among regions, suggesting little to no mixing of eastern and western populations in the Canadian samples examined. These findings justify the use of catch rates from the Gulf of St. Lawrence area as an index of abundance for the oldest members of the western population.

Introduction

Efforts to regulate Atlantic bluefin tuna (*Thunnus thynnus*) populations are headed by the International Commission for the Conservation of Atlantic Tunas (ICCAT). Bluefin tuna are currently managed as two separate stocks in the Atlantic Ocean, divided at 45°W longitude. The two-stock management strategy is based on eastern and western populations spawning in the Mediterranean Sea and Gulf of Mexico, respectively, with the eastern population being 10-fold larger than the western population (ICCAT 2006). Recent estimates from ICCAT suggest that the western spawning stock biomass (SSB) has fluctuated between 18% and 27% of the population level in the 1970s, and the eastern SSB is at least 40% (ICCAT 2008). In response, ICCAT recommends substantially reducing fishing mortality in the near future to reduce the risk of fishery collapse and rebuild stocks (ICCAT 2008). Despite being managed by ICCAT for several decades, bluefin tuna populations have not recovered, leading many to question current stock assessments and the biological data used in population models.

Uncertainty regarding the population structure and degree of stock mixing and migration is a primary concern for the conservation of Atlantic bluefin tuna. The conservation status of Atlantic bluefin tuna has been of increasing concern, with the population at less than 40% (ICCAT 2008). In response, ICCAT recommends substantially reducing fishing mortality in the near future to reduce the risk of fishery collapse and rebuild stocks (ICCAT 2008). Despite being managed by ICCAT for several decades, bluefin tuna populations have not recovered, leading many to question current stock assessments and the biological data used in population models.
of mixing between eastern and western populations is a major concern of assessment scientists. Evidence indicates that mixing occurs off the North American continental shelf from the Mid-Atlantic Bight to the Scotian Shelf and possibly extending to the Flemish Cap (Block et al. 2005; Rooker et al. 2007). However, the degree of mixing over time in this broadly defined foraging zone, which includes important fishing grounds of bluefin tuna from the Gulf of St. Lawrence to the Mid-Atlantic Bight, is unresolved. It is possible that contribution rates of eastern and western migrants are region- and time-specific within the western Atlantic, and thus population composition may vary spatially and temporally. Contemporary assessments of the Gulf of St. Lawrence fishery suggest that the region is comprised primarily of bluefin tuna from the western population (Rooker et al. 2008b); however, estimates of fidelity or stock structure over longer time periods are needed to assess natural variation in contribution rates to this region.

The Gulf of St. Lawrence has supported a rod-and-reel fishery for giant bluefin tuna for over 40 years (Mather et al. 1995), and catches from this region represent approximately 15% of the total allowable catch for the western stock (ICCAT 2006). The long-term consistency of catches dominated by giant bluefin tuna in the Gulf of St. Lawrence fishery has led to its use by ICCAT as an index of western stock abundance (ICCAT 2006). However, data from the Gulf of St. Lawrence fishery may not accurately capture population trends of the western stock if contribution rates of eastern and western bluefin tuna vary over time. Therefore, information on temporal or spatial changes in the stock

Fig. 1. Collection areas of yearling (baseline sample) and giant Atlantic bluefin tuna (Thunnus thynnus) in the (a) western and (b) eastern Atlantic Ocean and associated seas. Inset in western Atlantic plot shows the five ports from which otoliths of giant bluefin tuna were obtained from Canadian waters. Regional groupings (eastern, western, and exterior) of ports within and adjacent to the Gulf of St. Lawrence (GSL) are also denoted.
structure of bluefin tuna in the Gulf of St. Lawrence is critically needed, particularly if this region represents an essential foraging ground of the vulnerable western population.

This study used stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope ratios in otoliths as markers of natal origin for bluefin tuna from Canadian waters within or adjacent to the Gulf of St. Lawrence. Stable isotope signatures in milled otolith cores of giant bluefin tuna were compared with yearling (age-1) signatures from eastern and western nurseries (established baseline in part from Rooker et al. 2008a) to determine whether individuals originated from spawning grounds in the Mediterranean Sea or the Gulf of Mexico. Interdecadal and spatial variations in contribution rates to the Gulf of St. Lawrence fishery were also assessed to investigate temporal changes in mixing by predicting the origin of bluefin tuna collected in the 1970s, 1980s, and 2000s among multiple regions. Our working hypothesis is that the western population shows fidelity to the Gulf of St. Lawrence foraging ground, and thus contribution rates will be markedly higher for the western population over all three decades.

### Materials and methods

#### Otolith collecting and processing

Otoliths from yearling bluefin tuna (approximately 50–80 cm fork length) were collected from eastern (eastern Atlantic and Mediterranean Sea, $n = 23$) and western (western Atlantic, $n = 22$) nurseries from 2006 to 2007 and combined with samples from 1999 to 2004 (eastern, $n = 113$; western, $n = 81$; Rooker et al. 2008a). Archived otoliths of giant bluefin tuna (mature adults $>141$ kg) collected in the Gulf of St. Lawrence (GSL) and St. Margaret’s Bay (Scotian Shelf) from 1975 to 1984 were provided by Fisheries and Oceans Canada ($n = 193$; Fisheries and Oceans Canada, St. Andrews Biological Station, St. Andrews, New Brunswick). In addition, giant bluefin tuna were collected from the Gulf of St. Lawrence fishery out of North Lake, Prince Edward Island, in 2006 and 2007 (Rooker et al. 2008b; $n = 31$). Samples were representative of five fishing ports, which were grouped into three regions (western GSL (Caraquet and Tignish), eastern GSL (Havre Boucher and North Lake), exterior GSL (St. Margarets Bay); Fig. 1; Table 1). Sagittal otoliths were removed from all bluefin tuna, and fork length (cm) and weight (kg) were recorded for each specimen. Approximately 98% of measured tuna were 250–300 cm fork length (>15 years of age) for archived samples, and 90% were 230–270 cm fork length (>12 years of age) for modern samples (ages based on Rooker et al. 2007).

A single sagittal otolith (right or left) was randomly selected from each pair for analysis. Selected otoliths were cleaned of excess tissue and rinsed with deionized water (dH$_2$O). For yearling bluefin tuna (1999 to 2004), the entire otolith was powdered using a mortar and pestle to obtain material deposited during the first year of life. Otoliths from adults (and 2006 to 2007 yearlings) were embedded in Struers epoxy resin (Struers A/S, Ballerup, Denmark) for further processing and milling. Comparisons of whole versus milled otoliths were examined with otolith pairs from the same individuals, and no effect was detected (paired $t$ tests, $p > 0.05$), indicating that core signatures did not differ between direct powdering and embedding–milling techniques (Rooker et al. 2008b). A 1.5 mm thick section of resin containing the otolith core was cut along a transverse plane using a Buehler IsoMet saw (Buehler, Lake Bluff, Illinois) and then attached to a sample plate using Crystalbond thermoplastic glue (SPI Supplies/Structure Probe Inc., West Chester, Pennsylvania). The region corresponding to the first year of growth (identified from measurements of transverse sections of otoliths from yearling bluefin tuna; Fig. 2) was isolated and powdered using a New Wave Research Micromill (Fremont, California). A series of drill passes was run over a preprogrammed drill path by a 500 μm diameter Brasseler carbide bit (Brasseler USA, Medical L.L.C., Ventura, California) until a depth of approximately 750 μm was reached. Powder was collected with a microspatula and loaded into sample trays. All sampling equipment was

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### Table 1. Number of Atlantic bluefin tuna (*Thunnus thynnus*) otoliths collected at each sampling location each year from Canadian waters. All samples were from the Gulf of St. Lawrence, with the exception of those from St. Margaret’s Bay (Nova Scotia).

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**Fig. 2.** Transverse section of otolith from a yearling Atlantic bluefin tuna (*Thunnus thynnus*) with preprogrammed drill path used to mill core material (age-1 period): continuous lines represent the micromill drill path to be powdered; broken lines highlight the otolith material collected within the approximate 250 μm distance that the drill bit will reach.
cleaned with 70% ethanol between samples to prevent cross contamination.

Otolith powders were analyzed for $\delta^{13}$C and $\delta^{18}$O using an automated carbonate preparation device (KIEL-III; Thermo Fisher Scientific, Inc., Waltham, Mass.) coupled to a gas chromatograph – isotope ratio mass spectrometer (Finnigan MAT 252; Thermo Fisher Scientific, Inc.) at the University of Arizona. Powdered samples were reacted with dehydrated phosphoric acid under vacuum at 70°C. Analytical precision of the mass spectrometer was ±0.1‰ for $\delta^{18}$O and ±0.06‰ for $\delta^{13}$C (1 standard deviation, SD). Isotope ratios were calibrated based on repeated measurements of NBS-19 and NBS-18 and reported relative to the Pee Dee Belemnite (PDB) standard.

**Data analysis**

Analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were used to determine whether $\delta^{13}$C and $\delta^{18}$O in the otoliths of yearling and giant bluefin tuna differed among sampling regions and decades. Otolith $\delta^{13}$C and $\delta^{18}$O from yearling bluefin tuna were classified by quadratic discriminant function analysis (QDFA) and used as a baseline set for predicting the origin of giants from nursery signatures (in part from Rooker et al. 2008a). Shaded region shows gap between 1 standard deviation (SD) from the mean otolith $\delta^{18}$O of yearlings from each nursery (−1.19 to −1.21).

**Results**

Isotope concentrations ($\delta^{13}$C or $\delta^{18}$O) from otoliths of yearling bluefin tuna were distinct between eastern (eastern Atlantic and Mediterranean Sea) and western (western Atlantic) nurseries (MANOVA, $p < 0.05$; Fig. 3). Separation between nursery signatures was due to a significant $\delta^{18}$O enrichment of 0.66‰ (ANOVA, $p < 0.05$) in eastern yearling otoliths (mean ± SD = −0.94‰ ± 0.25‰, $n = 136$) over their western counterparts (mean ± SD = −1.60‰ ± 0.39‰, $n = 136$) (Table 2). No significant differences were detected in otolith $\delta^{13}$C between nurseries, with only a 0.02‰ enrichment in otolith $\delta^{13}$C from western yearlings (mean ± SD = −8.46‰ ± 0.40‰, $n = 103$) over their eastern counterparts (mean ± SD = −8.44‰ ± 0.42‰, $n = 136$). Despite similar

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**Note:** Values for giant bluefin tuna are displayed according to the decades (1970s, 1980s, and 2000s) and regions (eastern, exterior, and western) from which samples were obtained. SD, standard deviation.

![Fig. 3. Otolith core $\delta^{13}$C and $\delta^{18}$O of yearling Atlantic bluefin tuna (*Thunnus thynnus*) collected from the eastern (open circle, $n = 136$) and western Atlantic (solid circle, $n = 103$) nurseries (MANOVA, $p < 0.05$; Fig. 3). Separation between nursery signatures was due to a significant $\delta^{18}$O enrichment of 0.66‰ (ANOVA, $p < 0.05$) in eastern yearling otoliths (mean ± SD = −0.94‰ ± 0.25‰, $n = 136$) over their western counterparts (mean ± SD = −1.60‰ ± 0.39‰, $n = 136$) over their western counterparts (mean ± SD = −1.60‰ ± 0.39‰, $n = 136$) over their western counterparts (mean ± SD = −1.60‰ ± 0.39‰, $n = 136$). Despite similar.
Otolith δ¹³C between nurseries, differences in δ¹⁸O were great enough that cross-validated classification success from DFA was 84% among all year classes, with a 92% and 75% classification success for eastern and western yearlings, respectively.

No significant differences in adult otolith core δ¹³C or δ¹⁸O were detected among decades (MANOVA, \( p > 0.05 \)) or regions (MANOVA, \( p > 0.05 \)), which was expected given that interdecadal variation was accounted for in our adjustment. Mean otolith core δ¹³C ranged from –8.59‰ to –8.32‰ across the three decades and did not differ significantly (ANOVA, \( p > 0.05 \)) among the regions examined within or outside the Gulf of St. Lawrence (GSL): eastern GSL (mean = –8.53‰), exterior GSL (mean = –8.41‰), and western GSL (mean = –8.51‰) (Table 2; Fig. 4). Likewise, otolith core δ¹⁸O was also similar among decades (ANOVA, \( p > 0.05 \)), with means ranging from –1.6‰ to –1.5‰ in 1970 and 2000, respectively (Table 2; Fig. 4). No significant difference in otolith δ¹⁸O was detected among regions (ANOVA, \( p > 0.05 \)), with mean values ranging between –1.6‰ and –1.5‰.

MLE for all decades combined indicated that 100% of giant bluefin tuna in our Canadian samples originated from the western nursery, with no contribution from the eastern nursery (Table 3). The standard deviation around the western stock estimated proportion was <0.1% for all decades combined, meaning that there was a 68% probability that the actual contribution of the western nursery to the Gulf of St. Lawrence area was 100%. High classification (± SD) to the western nursery remained relatively consistent across decades: 1970s (100% ± 0.2%), 1980s (100% ± 0.1%), 2000s (99.6% ± 0.2%). Results from classifications of unadjusted values were nearly identical to those of adjusted values (Table 3).

Estimates of origin by region were also assessed using MLE, and the percentage of giants in our Canadian samples originating from the western nursery was greater than 99% in each of the three regions (Table 3). Western population contribution (± SD) was lowest in the exterior GSL region but still very high (99.3% ± 2.1%), indicating that only a small fraction (0.7%) of bluefin tuna in this region originated from the eastern nursery. Given that the standard deviation around the estimated proportions was 2.1%, there was a 68% probability that the eastern contribution of bluefin tuna in our sample ranged from 0.0% to 2.8%. Similarly, the contribution (± SD) of eastern Atlantic migrants to the other regions was also low: western GSL (0.0% ± 0.1%), eastern GSL (0.0% ± 0.2%). Necessary adjustments for decadal trends in isotope concentrations had little effect on classifications, with unadjusted signatures actually increasing the proportion of bluefin tuna classified to the western stock in the 2000 decade and exterior GSL region (Table 3).

**Discussion**

Otolith core δ¹³C and δ¹⁸O showed little variation among decades and regions. The lack of differences in stable isotope signatures among decades suggests that mixing rates of bluefin tuna migrating to the Gulf of St. Lawrence area have not changed during the time period covered. With over 99% of the bluefin tuna in the Gulf of St. Lawrence from the western population, it appears that a specific migratory contingent of the western population relies on this area as a feeding ground (Rooker et al. 2007). Furthermore, otolith δ¹³C and δ¹⁸O were similar among northern regions, suggesting that the bluefin tuna fishery examined in the Gulf of St. Lawrence is targeting a single contingent that migrates from the Gulf of Mexico spawning ground to forage in this area.
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... through the majority of the western Atlantic, including important foraging grounds from the Mid-Atlantic Bight to throughout the majority of the western Atlantic, including important foraging grounds from the Mid-Atlantic Bight to the Scotian Shelf (Block et al. 2005; Rooker et al. 2007). Evidence from tagging studies has shown mixing forces for movements after spawning are poorly understood. Fromentin and Powers (2005) concluded that bluefin tuna from Canadian waters indicated that these individuals were almost exclusively of western origin, which differs from estimates of exchange in other parts of the western Atlantic. Therefore, the use of catches from the Gulf of St. Lawrence fishery as an indicator of western population abundance in assessments by ICCAT appears valid.

Isotopic signatures in the otolith cores of giant bluefin tuna from Canadian waters indicated that these individuals were almost exclusively of western origin, which differs from estimates of exchange in other parts of the western Atlantic. Evidence from tagging studies has shown mixing throughout the majority of the western Atlantic, including important foraging grounds from the Mid-Atlantic Bight to the Scotian Shelf (Block et al. 2005; Rooker et al. 2007). Additionally, otolith chemistry results from Rooker et al. (2008b) report that trans-Atlantic movement and population mixing is significant and size-dependent, with a substantial number of adolescent bluefin tuna collected in the US Atlantic originating from the Mediterranean Sea. These authors also reported that trans-Atlantic movement became more limited at the onset of sexual maturity. Thus, our finding of nearly 100% western bluefin tuna in the Gulf of St. Lawrence was not surprising given that all individuals were exclusively older, mature fish rather than adolescents. Our results suggest that mixing of giant bluefin tuna of eastern origin with their western counterparts at the northern extent of their range in the western Atlantic was exceptionally low for all three decades investigated.

Multiple studies have described migration routes of fishes and linked movement to specific environmental cues. It has commonly been hypothesized that physicochemical variables (i.e., temperature) influence migration pathways of bluefin tuna (Marsac 1999; Ravier and Fromentin 2004). In addition, Dodson (1988) hypothesized that bluefin tuna may intrinsically follow migration routes as a result of imprinting (natal homing) or spatial learning through schooling experiences (repeat homing). Repeated returns of bluefin tuna to spawning grounds supports natal homing (Rooker et al. 2008b) and spawning site fidelity (Teo et al. 2007), but driving forces for movements after spawning are poorly understood. Fromentin and Powers (2005) concluded that bluefin tuna are theoretically likely to perform repeat homing, with multiple studies showing that fish are capable of learning migration paths (Helfman and Schultz 1984; Mazeroll and Montgomery 1995) and experienced individuals can entrain behaviors in more numerous naïve fish (Laland and Williams 1997; Rees 2000). Therefore, returns of giant bluefin tuna to the Gulf of St. Lawrence over the period investigated may be due to familiarity of the migration route by older, experienced individuals. If this hypothesis is true, declines in the numbers of adults making return trips to foraging grounds may alter migratory pathways of inexperienced bluefin tuna and ultimately influence where these individuals forage.

There are two documented accounts of extirpating bluefin tuna from regional fishing grounds without mass returns since, the Nordic fishery and the Brazilian episode (Fromentin and Powers 2005; MacKenzie and Myers 2007), both of which collapsed after large catches in the 1960s. The removal of bluefin tuna that were accustomed to Nordic or Brazilian migrations resulted in a loss of these migration routes. Removing key components of a population may also result in population bottlenecks that can jeopardize recovery efforts (Gardmark et al. 2003). Reduced population numbers are of particular concern for the smaller western population because there are only a few known feeding grounds for western bluefin tuna, and it appears likely that the Gulf of St. Lawrence serves as essential habitat for the western population. Consequently, the Gulf of St. Lawrence, as well as other critical habitats of bluefin tuna (i.e., Gulf of Mexico), must continue to be protected to ensure the sustainability of this fishery and the western population.

**Acknowledgments**

This work was supported by grants from the University of New Hampshire Large Pelagic Research Center and the National Oceanic and Atmospheric Administration Southeast Fisheries Science Center. In addition, support from the Fisheries and Oceans Canada International Governance Strategy and the International Commission for the Conservation of Atlantic Tunas (ICCAT) Bluefin Year Program assisted with recent sampling efforts in the Gulf of St. Lawrence. We are grateful to Fisheries and Oceans Canada for provid-

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**Table 3.** Summary data for maximum likelihood classifications of giant Atlantic bluefin tuna (*Thunnus thynnus*) collected from Canadian waters.

<table>
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<tr>
<th>Year</th>
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**Note:** Values are displayed according to the decades (1970s, 1980s, and 2000s) and regions (eastern, exterior, and western Gulf of St. Lawrence) from which samples were obtained. SD, standard deviation.

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The lack of differences among regions also indicates that catches from the Gulf of St. Lawrence fishery are primarily composed of the western population, with little to no mixing with the eastern population. Therefore, the use of catches from the Gulf of St. Lawrence fishery as an indicator of western population abundance in assessments by ICCAT appears valid.

**Isotopic signatures in the otolith cores of giant bluefin tuna from Canadian waters indicated that these individuals were almost exclusively of western origin, which differs from estimates of exchange in other parts of the western Atlantic.** Evidence from tagging studies has shown mixing throughout the majority of the western Atlantic, including important foraging grounds from the Mid-Atlantic Bight to the Scotian Shelf (Block et al. 2005; Rooker et al. 2007). Additionally, otolith chemistry results from Rooker et al. (2008b) report that trans-Atlantic movement and population mixing is significant and size-dependent, with a substantial number of adolescent bluefin tuna collected in the US Atlantic originating from the Mediterranean Sea. These authors also reported that trans-Atlantic movement became more limited at the onset of sexual maturity. Thus, our finding of nearly 100% western bluefin tuna in the Gulf of St. Lawrence was not surprising given that all individuals were exclusively older, mature fish rather than adolescents. Our results suggest that mixing of giant bluefin tuna of eastern origin with their western counterparts at the northern extent of their range in the western Atlantic was exceptionally low for all three decades investigated.

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ing samples to assist this research. We also thank David Dettman and Jay Kaufman for analytical support.

References


