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Nitrogen isotopes in otoliths reconstruct ancient trophic position

Kirsten Rowell · David L. Dettman · Robert Dietz

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Abstract The ratio of $^{15}N/^{14}N$ ($\delta^{15}N$) from consumer and prey tissue is commonly used in ecological studies to determine trophic level, food web structure, and mean trophic level in aquatic ecosystems. There is a predictable positive relationship between the $\delta^{15}N$ values in tissue and trophic level, caused by the bioaccumulation of ^{15}N in tissues of consumers with each step up the food chain. Reconstructing trophic structure or food chain length over time may provide resource managers with insights about ecosystem biodiversity and resilience. Yet, in many marine systems the absence of baseline information before anthropogenic disturbances makes comparative studies addressing ecosystem responses extremely difficult. Here we attempt to retrospectively reconstruct trophic position in four species of fish from the upper Gulf of California, Mexico before perturbations such as overfishing or the damming of the Colorado River. We first validated if otolith $\delta^{15}N$ approximates the $\delta^{15}N$ observed in fish tissue. We then used the $\delta^{15}N$ encapsulated in ancient fish otoliths that are between 1,000 and 5,500 years old to define the food web structure. Our results suggested that $\delta^{15}N$ in otoliths has slightly more positive $\delta^{15}N$ than soft tissue. The $\delta^{15}N$ values from ancient otoliths appropriately defined the fishes' relative trophic position. We found significant differences in $\delta^{15}N$ between functional groups, apex predator versus intermediate predators. Juveniles and adult fishes displayed trophic separation between functional groups. Our findings advocate the application of $\delta^{15}N$ analysis of prehistoric otoliths for establishing pre-disturbance ecological benchmarks.

Keywords Otoliths · Trophic reconstruction · Gulf of California · Totoaba · Corvina · Colorado River

K. Rowell (✉)
Department of Biology, University of Washington,
Seattle, WA 98195, USA
e-mail: rowellk@u.washington.edu

D. L. Dettman · R. Dietz
Department of Geosciences, University of Arizona,
Tucson, AZ 85721, USA

Introduction

Finding historical ecological information is difficult for most marine ecosystems (Dayton et al. 1998; Jackson et al. 2001). Without such archives, managers and biologists have no point of reference for measuring change over time. In the upper portion of Gulf of California, Mexico, the combination of intense habitat alterations (shrimp farming and diverting and damming the Colorado River) and aggressive fishing practices have reportedly altered the ecosystem, yet empirical evidence of system-wide response to these pressures is lacking. In part, this is the direct result of no preexisting ecological records of the upper Gulf prior to anthropogenic alterations (Kowalewski et al. 2000; Glenn et al. 2001; Rowell et al. 2005, 2008a; Glenn et al. 2007). In this paper, our objective is to lay

the methodological foundation for an examination of potential changes in trophic structure resulting from these anthropogenic changes. Specifically we test the hypothesis that nitrogen stable isotope ratios embedded in otolith aragonite can record the relative trophic position of fish that lived thousands of years in the past, before large human influences occurred in the upper Gulf of California. The relationship between nitrogen stable isotope ratios in muscle tissue and trophic position have been previously shown to be positive (Cabana and Rasmussen 1994; Vander Zanden et al. 1997; Post 2002; Wolf et al. 2009), thus the nitrogen in protein within otoliths should also characterize trophic position. We first validated that the stable isotope ratio in otoliths is comparable to values observed in the muscle tissue of the same fish. We then used ancient otoliths to determine if the nitrogen stable isotope ratio values correctly estimate their relative trophic level. Ultimately we aim to establish a time averaged $\delta^{15}N$ baseline for key species in the upper Gulf of California. Establishing such baselines is an essential first step for documenting ecosystem change in response to anthropogenic alterations.

Isotopes and trophic position

The ratio of ^{15}N to^{14} N ($\delta^{15}N$) increases as one moves from lower to higher levels in a food chain. This bioaccumulation of ^{15}N (Post 2002; Karasov and Martinez del Rio 2007) appears to result in an average increase in $\delta^{15}N$ of about 3.4‰ as nitrogen passes up the food chain from prey to consumer (Post 2002). Because of this predictable shift in isotopic ratio, $\delta^{15}N$ in tissue is commonly used in ecological studies to determine trophic level, trophic structure, and food chain length (Cabana and Rasmussen 1994; Vander Zanden et al. 1997; Post 2002; Wolf et al. 2009). The increase in $\delta^{15}N$ from one trophic level to the next depends on the fractionation factor (simply defined as the change in $\delta^{15}N$ from prey to consumer), and even though many have successfully used Post's (2002) fractionation factor 3.4‰, the amount of fractionation between consumer $\delta^{15}N$ and food source $\delta^{15}N$ is not fixed and seems to vary considerably between species, tissue, type of consumer (detritivore, carnivore, or herbivore) and habitat type (marine, freshwater, or terrestrial) (Vander Zanden et al. 1997; Vanderklift and Ponsard 2003). In addition, the $\delta^{15}N$ in tissues is strongly influenced by the protein turnover rates in tissues reflecting the $\delta^{15}N$ assimilated during that time period (Wolf et al. 2009 and references therein). In the present study we test if $\delta^{15}N$ in the otoliths of fish can approximate the $\delta^{15}N$ observed in muscle tissue of fish. The advantage to using fish otoliths over soft tissue is that they do not have any material turnover. Instead of reworking material, as is seen in muscle, fish add material to otoliths over time, providing a chronological geochemical log. This property makes it possible to use otolith isotopic chemistry to track environmental and dietary changes over the lifespan of the fish (Koch 1998; Tutken et al. 2006; Schwarzhans 2007).

Here we ask the following three questions: 1) Can $\delta^{15}N$ in otolith aragonite be used to approximate values of $\delta^{15}N$ in soft tissue of individuals?; 2) Do $\delta^{15}N$ values of fish otoliths reflect the trophic position of fish that lived thousands of years ago?; 3) Do $\delta^{15}N$ values increase from juvenile to adult fish, reflecting the ability of adult fish to consume larger prey that are higher on the food chain? We conducted two complementary investigations to address these questions. First, we conducted a validation study to determine if fingerling *Totoaba macdonaldi* grown under known controlled conditions have comparable $\delta^{15}N$ values in muscle tissue and otolith aragonite. Second, we conducted a comparative analysis of $\delta^{15}N$ values in otoliths from four species of fish (*Totoaba macdonaldi*, *Cynoscion othonopterus*, *Cynoscion parvipinnis* and *Micropogonias megalops*), all taken from aboriginal middens along the edge of the Gulf of California. Because the basic trophic rank of these four species can be established a-priori based on diet (*T. macdonaldi* should be higher than the other three species, see below), we used otolith $\delta^{15}N$ to assess the trophic position of these species. These studies are the initial steps to reconstruct the historical trophic structure of the upper Gulf of California and investigate how human influences impacted the marine ecosystem in the upper Gulf of California.

Material and methods

Background

We use otoliths recovered from archeological sites dated 1,000–5,000 years before present (Rowell et al. 2008b), along the Sonoran, and Baja California Norte

coast to investigate the historical trophic position for these four species. All the otoliths retrieved from middens belonged to the sciaenidae family and were easily identified to species. All but *Cynoscion parvipinnis* are endemic to the Gulf of California (Fig. 1). Here we summarize the known basic parameters (geographic range, size, and diet) for each species in this study.

Totoaba macdonaldi (totoaba) is a long-lived, apex predator (Morales-Zarate et al. 2004; Sala et al. 2004; Diaz-Uribe et al. 2007; Lercari and Chavez 2007) that can reach up to 2 m in length (Flanagan and Hendrickson 1976). Totoaba were fished heavily from the 1940s until they were listed as endangered in 1975 (Flanagan and Hendrickson 1976; Cisneros-Mata et al. 1995). Since that time, totoaba has been protected by the Convention on International Trade in Endangered Species of Fauna and Flora (CITES), as well as the U.S. and Mexican endangered species act. Despite 30 years of protection, totoaba populations have still not recovered (Cisneros-Mata et al. 1995; Lercari and Chavez 2007). Many cite the lack of river flow

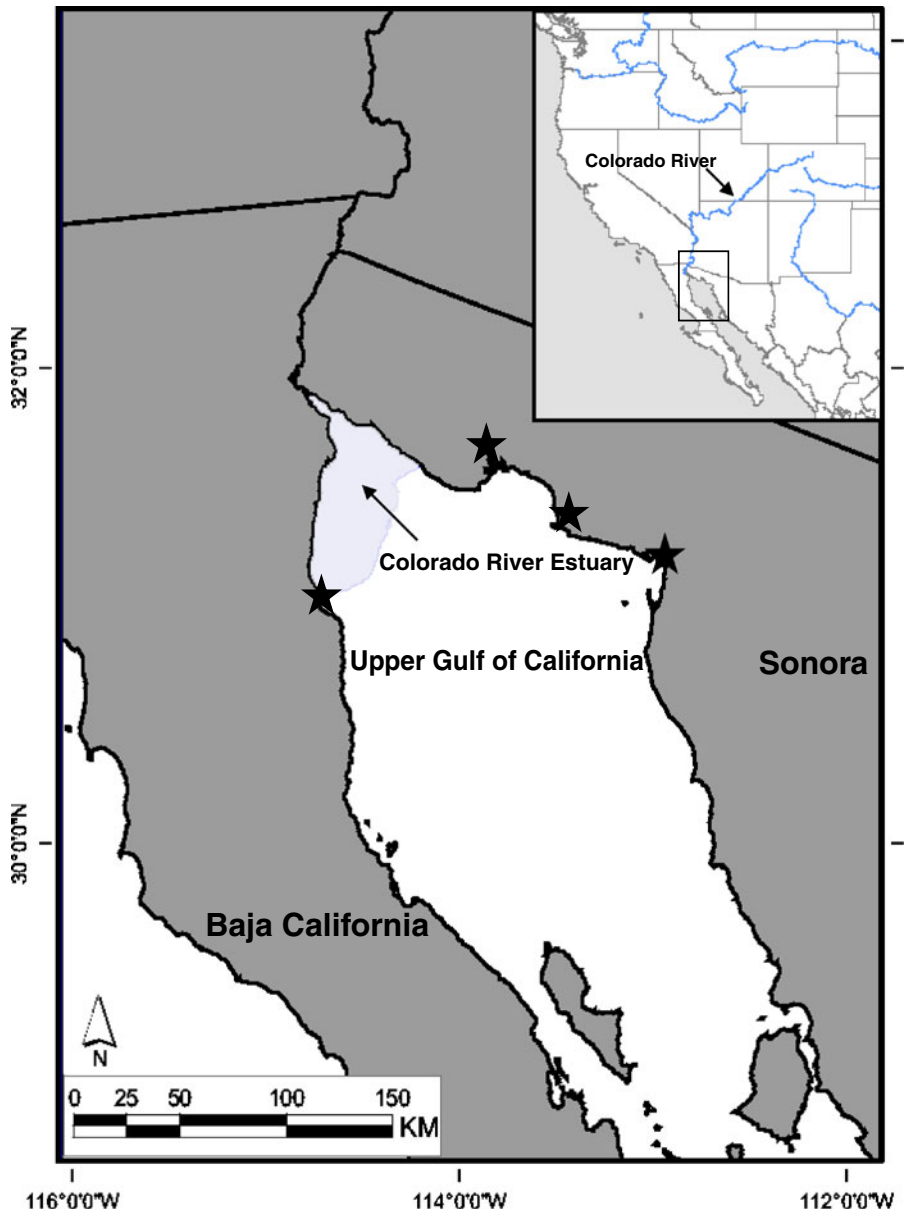

Fig. 1 Map of the Upper Gulf of California and mouth of the Colorado River mouth. Stars denote collection sites for ancient otoliths

as contributor to lack of recovery (Flanagan and Hendrickson 1976; Barrera-Guevara 1990; Lercari and Chavez 2007; Rowell et al. 2008a, b).

Cynoscion othonopterus (Gulf corvina) grows to about 1 m in length and lives up to 9 years (Roman-Rodriguez 2000; Rowell et al. 2005). It has a diet that consists of mainly of sardine (*Cetengraulis mysticetus*), but crab, shrimp, octopus and copepods are also commonly found in their stomachs (Roman-Rodriguez 2000). *C. othonopterus* has ben listed by the American Fisheries Society as a vulnerable species because of overfishing and reduced Colorado River flow—its spawning and nursery grounds (Musick et al. 2000; Rowell et al. 2005); however, it still supports a lucrative fishery in the region.

Cynoscion parvipinnis (corvina) grows to be up to 69 cm in length (Chao 1995). There is little detailed information on *C. parvipinnis* diet, except that it consists of octopus and small fishes (Chao 1995). *C. parvipinnis* currently makes up an important fishery in the Gulf of California.

Micropogonias megalops (chano) can reach up to 49 cm in length and is known to have an omnivorous diet consisting of bivalves, gastropods, decapods and polychetes, and small fish, (Roman-Rodriguez 2000). *M. megalops* is commonly found in *T. macdonaldi* stomachs (Roman-Rodriguez 2000). The fishery for *M. megalops* began in the 1980's and now supports a fairly consistent subsistence fishery in the region.

Laboratory comparison of Otolith and Muscle $\delta^{15}N$

The validation study was conducted with the endangered *Totoaba macdonaldi,* grown-out from larvae on a prescribed diet. There are several reasons why we use this species: 1) *T. macdonaldi* plays a key role in the upper Gulf of California—it has conservation importance (internationally listed as endangered) and is ecologically significant (apex predator and endemic); 2) we knew *T. macdonaldi* was one of the species in the midden otoliths; and 3) a brood stock and hatchery already existed. We conducted these tests at the totoaba hatchery at Universidad Autonoma de Baja California (UABC). Because totoaba are federally protected, experiments that involved manipulation of environmental parameters that may put individuals at risk were not permitted, thus laboratory conditions and diet were not altered for this study (for specifics on aquaculture facilities and growing methods see Rowell et al. 2008b). Our objective for this study was to document that the $\delta^{15}N$ in otolith material approximates the $\delta^{15}N$ in muscle tissue, which can be used to infer trophic position or mean trophic level.

Sample preparation and analysis

Sagittal otoliths from 6 incidental deaths of fingerling totoaba ranging from 34 to 37 days old (1.8 cm–4.3 cm standard length) were donated from Universidad Autonoma de Baja California, Ensenada, Baja California to the University of Arizona fish collections (UAZ2005–07 to -12). Otoliths were thoroughly cleaned of tissue, given a final rinse in with deionized water in a sonicator for 3 min and dried at room temperature overnight. Since fingerling otoliths were small (2 mm), we used the entire otolith for analysis. Individual weights were too small to analyze otolith powder independently, thus otoliths were grouped into two separate batches and ground whole with mortar and pestle. The resulting powdered otolith samples weighed 19 and 24 mg.

We collected three $\delta^{15}N$ measurements of each food type used in the aquaculture diet: live *Artemia nauplii* (crustacean); larval maintenance diet; rotifers; and Moreclark™ 250–300 μ. In addition to food samples, we took muscle samples from the same 6 fish mentioned above to validate the assimilated $\delta^{15}N$ from the diet. All organic samples were freeze-dried, pulverized, and homogenized (see Rowell et al. 2008b). Samples weighed between 0.5 and 1.0 mg.

Stable isotope measurement

All nitrogen stable isotope measurements were performed on a continuous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL) at the University of Arizona's Environmental Isotope Laboratory. Samples were combusted using an elemental analyzer (Costech) coupled to the mass spectrometer. Standardization is based on acetanalide for elemental concentration, NBS-22 and USGS-24 for $\delta^{13}C$, and on IAEA-N-1 and IAEA-N-2 for δ15N. Precision for organic samples was typically ± 0.2 for $\delta^{15}N$ based on repeated internal standards. Precision for carbonate otolith samples was lower because of the small amounts of nitrogen measured. The repeated standards run during otolith analyses had repeatabilities of 0.2 to 0.35‰ (1 sigma). Nitrogen content for

successfully run otolith samples ranged from 13 micrograms to 45 micrograms N in the total sample. N% ranged from 0.05% to 0.31% (by weight). Because these samples were unusually small, they were analyzed with two sets of internal standards—one to establish the basic calibration of the individual run and a second series across a range of sizes matched to the expected size of the unknowns. This second series was used to correct the $\delta^{15}N$ ratio for the effect of sample size.

To measure trace amounts of organic matter encapsulated in carbonate, otolith samples were ground to a very fine powder. We do not acidify samples because it can lead to physical loss or volatilization loss of organic matter during the acidification process. When samples are finely powdered, samples appear to burn well, with sharp peaks for both N2 and CO2 from the EA detector, and no tailing apparent after the CO2 peak. This method was tested with artificial mixtures of powdered calcite mixed with trace amounts of the internal acetanilide standard and no difference was observed between mixed samples and standards (unpublished data Dettman). To prevent the possibility of sample cross-contamination due to incomplete combustion empty tin capsules were combusted between each sample and before standards.

Trophic position reconstruction

We assessed the relative trophic position of our four species by measuring $\delta^{15}N$ in a total of 48 otolith spread across the four species: *T. macdonaldi* (n=16); *C. othonopterus* (n=1); *C. parvipinnis* (n=9) and *M. megalops* (n=23). Fourty-four of the 48 otoliths were collected from archeological sites along the Sonoran Coast (Foster et al. 2008) that have been carbon dated between 1m000 and 5,500 calyr BP by shells, otoliths and charcoal (Foster et al. 2008; Rowell et al. 2008a). Four additional otoliths were collected in 1955, near San Felipe, Baja California (Fig. 1), from a midden dated between 805 and 1,280 years before present using ^{14}C from marine shell and charcoal dates (see Rowell et al. 2008b).

Otoliths were thoroughly scrubbed with deionized water and nylon brush to remove any debris before sampling. They were cross sectioned (exposing the natal portion) using a low-speed ISOMET saw with diamond wafer blade, polished using 3 and 30 micron aluminum oxide lapping film, and ultrasonically rinsed with deionized water. To test for ontogenetic differences in trophic level, we took two spot samples from otoliths: one from the core to the end of the first year of growth (here after referred to as juvenile stage) and a second from the outer edge of the otolith (adult, 3+ years in age). Not all otoliths had an adult portion. We used a dental drill and a 0.3 mm diameter drill bit to collect samples. Samples weighed between 14–20 mg. We used the same methods for isotopic analysis of the otolith material as explained in the laboratory validation (see above).

Statistical analyses

We did not perform statistical tests on the data from the validation experiment due to the small sample size and need to bulk sample material across individuals. To test if $\delta^{15}N$ values in otoliths can detect trophic differences between species and within species (from juvenile to adult), we separated data collected from the otolith core (representing the first year of growth; n=40 otoliths from 4 species) from data collected at the otolith margin from 3+ year old individuals (representing adult growth periods; n=20 otoliths from 4 species). We ran separate analyses on each dataset to control for differences in life stage, avoid pseudo-replication (some otoliths were sampled both at the core and the perimeter) and determine if any patterns in trophic position are consistent across life stages. For each dataset, we use general linear models with least square fits and a-priori contrasts to test our primary hypotheses (below). In addition, we use paired t-tests to test a second set of hypotheses regarding the shift in trophic position from juvenile to adult life-stages. All analysis was conducted using program R version 2.10.0 (R Development Core Team 2008).

We test the following primary hypotheses: First, if $\delta^{15}N$ in otolith tracks the trophic level of fish, *T. macdonaldi,* the apex predator, should have a higher trophic position ($>\delta^{15}N$) than the three intermediate predator species (*C. othonopterus, C. parvipinnis,* and *M. megalops*). Second, this effect should be greater in adult fish than in juviniles. Third, we test the hypotheses that samples taken from the adult portion of otoliths (perimeter sample 3+ years in age) show higher trophic position ($\delta^{15}N$) than paired samples taken from the core of the same otoliths (n=18 pairs).

Results

Laboratory validation

The otolith $\delta^{15}N$ of laboratory raised fish approximated the $\delta^{15}N$ measured in muscle tissue of the same fish. The mean $\delta^{15}N$ value of laboratory diet was 8.4‰; the mean value tissue was 12.0‰; and the mean otolith value was 11.3‰ (Table 1). We observed a 3.6‰ increase between mean diet and mean soft tissue $\delta^{15}N$ values and a 2.56‰ increase in $\delta^{15}N$ between mean diet values and mean otolith $\delta^{15}N$ values. The difference between otolith and tissue $\delta^{15}N$ values was- 0.76‰.

Trophic position reconstruction

The $\delta^{15}N$ values of ancient otoliths correctly depict the trophic position of these four fish in both juvenile (F=3.08, P=0.03; apex predator vs. intermediate predators t=2.96, P=0.004; Table 2) and adult stages (F=7.28, P=0.02; apex predator vs. intermediate predators t=4.16, P=0.0007), supporting our first prediction, and the much stronger differences between the apex predator and the intermediate predators in adult life stages supports our second prediction (Fig. 2, Table 2). In addition, we found no differences between any of the intermediate predators as juveniles (t's<1.7, p's>0.11) or as adults (t's<1.1, p's>0.29, see Fig. 2.). In contrast, we found no support for a consistent increase in $\delta^{15}N$ when we compared juvenile to adult life stages within otoliths (paired $t_{1,17}$=0.8, p=0.43).

Discussion

Laboratory validation

Our results indicate that like muscle tissue, otolith $\delta^{15}N$ seems to record information on the organisms' diet and trophic position. In our validation study, we observed a 2.56‰ increase in $\delta^{15}N$ between mean diet and mean otolith $\delta^{15}N$ values, which is within the appropriate range of prey to consumer fractionation (Vander Zanden et al. 1997; Post 2002). To our knowledge, only one ecological study has compared tissue and otolith aragonite $\delta^{15}N$ values, and they also found otoliths to be on average slightly lighter than muscle tissue of fish (Vandermyde and Whitledge 2008). Vandermyde and Whitledge (2008) reported that on average otoliths are 1.1‰ depleted in ^{15}N than muscle tissue. Our results are not too different from what Vandermyde and Whitledge (2008) report—our laboratory raised fish were 0.76‰ depleted in ^{15}N compared to their tissue. Similarly for the aragonite portion of bivalve shells, the tissue to shell $\delta^{15}N$

Table 1 Data from laboratory validation of $\delta^{15}N$ in prescribed diet, muscle tissue from totoaba, and otolith carbonate from the same totoaba individuals

Diet			$\delta^{15}N$ ‰
Artemia			10.9
Larval maintenance			9.5
Moreclark™			13.8
Rotifers			-0.6
Mean diet			8.4
Individual ID	Days old	sl (cm)	Tissue $\delta^{15}N$ ‰
UA 2005–07	34	3	12.0
UA 2005–08	37	4.3	12.1
UA 2005–09	37	1.6	12.1
UA 2005–10	37	3.8	12.1
UA 2005–11	37	3.8	12.0
UA 2005–12	37	3.9	lost
Mean tissue			12.0
Individual ID for lumped otolith sample	Otolith samples $\delta^{15}N$ ‰		
UA 2005–07, 08, 09, 10, 11			12.4
UA 2005–08, 12			10.2
Mean otolith			11.3

Table 2 The $\delta^{15}N$ values ancient otoliths from *T. macdonaldi, C. othonopterus, C. parvipinnis, and M. megalops* (juvenile and adult portion of the otolith)

Species	Life stage	$\delta^{15}N$
T. macdonaldi n=16 individuals		
T01	adult	18.40
T02	juv	16.09
T02	adult	19.70
T03	adult	19.67
T07	juv	20.01
T07	adult	21.12
TM2005—OHFS	juv	18.88
TM2006—OH1A	juv	19.13
TM2006—OH2A	juv	19.56
TM2007—EM25A	juv	19.35
TM2007—LFOH1A	juv	19.77
TM2007—LFOH1B	juv	18.57
TM2007—LFOH2a	juv	19.02
TM2007—OH2a	juv	16.90
TM2007—OH4A	juv	18.50
TM2007—OHFSA	juv	18.72
TM2007—OYHA	juv	15.25
TM2007—OYHB	juv	16.79
C. othonopterus n=1		
CORVINA 25	adult	16.34
CORVINA 25	juv	14.05
C. parvipinnis n=9		
CP2007—EM25	juv	16.27
CP2007—EM25b	juv	18.99
CP2007—EM25J	juv	17.44
CP2007—LFOH1a	juv	18.72
CP2007—LFOH1c	juv	16.87
CP2007—LFOH2G	juv	14.29
CP2007—LFOH2I	adult	17.32
CP2007—LFOH2I	juv	17.49
CP2007—OH2d	juv	12.21
CP2007—OHPSA	adult	17.94
CP2007—OHPSA	juv	17.47
M. megalops n=23		
mm CHANO 2	juv	16.99
mm CHANO 3	adult	16.66
mm CHANO 3	juv	16.90
mm CHANO 4	juv	13.31
mm2007—LFOH1A	adult	18.22
mm2007—LFOH1A	juv	17.41
mm2007—LFOH1AG	adult	18.15
mm2007—LFOH1AG	juv	16.69
mm2007—LFOH1AI	juv	17.46
mm2007—LFOH1AO	adult	17.45
mm2007—LFOH1AO	juv	20.38
mm2007—LFOH1AV	juv	18.57
mm2007—LFOH1BK	adult	15.97
mm2007—LFOH1BK	juv	19.50
mm2007—LFOH1CC	adult	18.50
mm2007—LFOH1CC	juv	17.25
mm2007—LFOH1CN	adult	17.22
mm2007—LFOH1CN	juv	14.89
mm2007—LFOH1D	juv	15.69
mm2007—LFOH1DC	juv	16.57
mm2007—LFOH1E	adult	16.29
mm2007—LFOH1E	juv	13.07
mm2007—LFOH1G	adult	15.50
mm2007—LFOH1G	juv	14.70
mm2007—OH1P	juv	16.34
mm2007—OH2A	adult	18.35
mm2007—OH2A	juv	18.16
mm2007—OH2P	juv	18.13
mm2007—OH5A	adult	17.96
mm2007—OH5A	juv	20.28
mm2007—OH5I	juv	17.52
mm2007—OH5P	adult	17.32
mm2007—OH5P	juv	17.66
mm2007—OYH1C	juv	16.64
mm2007—OYHA	adult	16.76
mm2007—OYHA	juv	18.05

comparisons suggest that shells are depleted in ^{15}N (2.3‰ to 2.5‰) relative to their soft tissues (Carmichael et al. 2008; Delong and Thorp 2009). Our muscle tissue to otolith $\delta^{15}N$ comparisons support others findings that there is a slight depletion in ^{15}N between the biogenic aragonite and soft tissue within an organism. These validation results are necessarily limited because it was conducted with an endangered species. The combination of small numbers of individuals and the necessity of lumping samples of otoliths into two batches limits our inference, and suggests the need for a more comprehensive validation study. Still, our findings add to the growing support for using $\delta^{15}N$ in aragonite skeletal remains

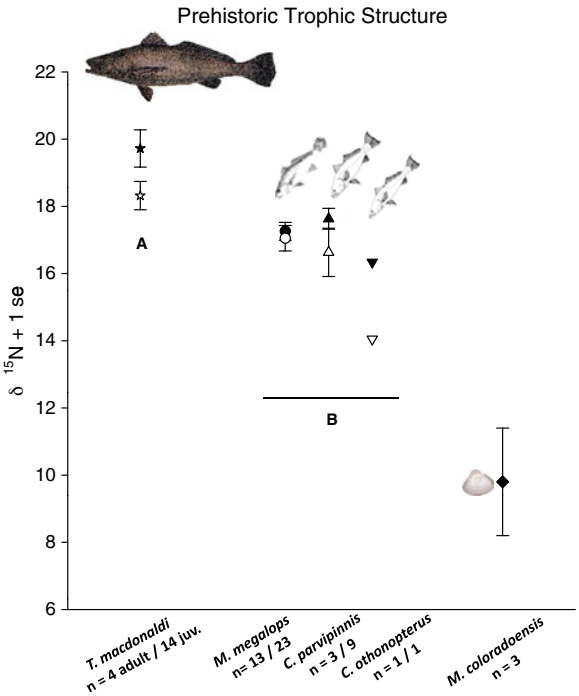

Fig. 2 The $\delta^{15}N$ values of ancient otoliths from middens (1,000–5,500 ybp) along the coast of the upper Gulf of California. Filled symbols for fish represent adult values and open symbols represent the first year of life. Sample sizes for each species are listed below x-axis (adult n / juvenile n)

of aquatic organisms for investigating dietary information and trophic position (O'Donnell 2003; Vandermyde and Whitledge 2008; Carmichael et al. 2008; Delong and Thorp 2009).

Otolith to tissue validation experiments are important, because Otoliths provide some obvious benefits over the use of tissue in ecological studies. First, otoliths preserve diet information over the life span of an individual, while tissues have protein turnover rates that vary within an individual, limiting the temporal scale of investigations (Karasov and Martinez del Rio 2007). Second, otoliths also preserve this lifetime diet information over thousands of years with little diagenesis, providing opportunities to study environmental and ecological conditions of marine ecosystems before major human alterations (Koch 1998; Tutken et al. 2006; Schwarzhans 2007).

Trophic position reconstruction

The $\delta^{15}N$ embedded in otoliths have correctly portrayed the trophic functional groups of these four species of fishes from the upper Gulf of California. The $\delta^{15}N$ values of *T. macdonaldi* (apex predator) were significantly different from the intermediate predators' (*C. othonopterus, C. parvipinnis,* and *M. megalops*) values in both life stage samples types (adult and juvenile). In other words, juvenile *T. macdonaldi* feed higher on the food chain compared to juvenile *C. othonopterus, C. parvipinnis,* and *M. megalops* and this trophic separation strengthens with maturity. There is no significant difference in $\delta^{15}N$ values between the intermediate predators; however, we suspect that because these samples represent time averages, there may be subtle differences between species that we are unable to detect. Even though we observed a stronger difference in $\delta^{15}N$ values with maturity between the apex predator and the intermediate predators, there is no difference between the $\delta^{15}N$ values from paired juvenile and adult samples. Assuming that fish length scales with trophic level in fishes (Badalamenti et al. 2002), young of the year *T. macdonaldi* should have $\delta^{15}N$ values that are more similar to intermediate predators. In a trophic study using $\delta^{15}N$ in fish muscle tissue, Jardine and Curry (2006) found that size was a better indicator than age at predicting $\delta^{15}N$ values, and larger fish had higher $\delta^{15}N$ values. We suspect that with a larger sample size that any differences between the adult and juveniles may become more apparent, especially for *T. macdonaldi* because of the large difference in size between juvenile and adults (Rowell et al. 2008a). Rowell et al. (2008a), estimated that prehistoric *T. macdonaldi* were between 53–120 cm standard length after 1 year of growth, while mature totoaba were 130–200 cm.

For comparative purposes, we have included data from ancient shells of *Mulina coloradoensis,* an endemic clam in the upper Gulf of California (Dietz 2008). As expected, $\delta^{15}N$ values in these shells are much lower than what we observe in fish aragonite (mean=9.8 ‰±0.6 se). Because these shells were analyzed using similar methods, the comparison is informative and helps to anchor the base of the food chain, as *M. coloradoensis* is a filter feeder. While there were no validation studies done with *M. coloradoensis*, and there is substantially more variation in the $\delta^{15}N$ of shells compared to fish (Watanabe et al. 2009), others have confirmed that shell and tissue $\delta^{15}N$ track the dietary $\delta^{15}N$ (Carmichael et al. 2008; Delong and Thorp 2009). And yet without a

multi-trophic experiment addressing the degree to which both shell and otolith aragonite are recording diet and trophic position, it is not certain that the stable nitrogen ratios observed in shells and otoliths are truly comparable. Our results, when compared with those of Dietz (2008), suggest considerable promise for the use of shell and otolith in tandem to evaluate past trophic structure.

Several features of the upper Gulf of California system combine to make it a particularly good place to evaluate changes in trophic structure using otolith isotopic chemistry. First, while many large fish undergo large-scale migrations that involve diet shifts, the $\delta^{15}N$ values from these four species is likely to represent a defined biogeographic region, because *T. macdonaldi* is the only species known to migrate long distances over the course of its life (Flanagan and Hendrickson 1976; Cisneros-Mata et al. 1995; Cisneros Mata et al. 1997). Mature *T. macdonaldi* supposedly undergo a winter migration south following schools of sardine, returning in early spring to spawn in the Colorado River estuary (Flanagan and Hendrickson 1976), and thus the $\delta^{15}N$ values in the adult stage should include the record of *T. macdonaldi* diet during migration. However, there is been no evidence of *T. macdonaldi* changing its feeding habits during migration, and is widely recognized as an apex predator in its adult stage (Morales-Zarate et al. 2004; Sala et al. 2004; Diaz-Uribe et al. 2007; Lercari and Chavez 2007).

Second, this system appears to be robust to interannual variation in source $\delta^{15}N$ values. $\delta^{15}N$ values can vary both within and between years, and because humans have been harvesting these species for thousands of years (Foster et al. 2008), the opportunity for interannual variation in $\delta^{15}N$ source values is large. In the present study, we sampled 48 otoliths which undoubtedly came from different years, and thus the variation we are reporting among otoliths includes this year-to-year variation in $\delta^{15}N$ source values, but the variation in $\delta^{15}N$ was quite low, and the differences between apex predators and intermediate predators appears quite robust to this variation. Seasonal variation can also influence isotopic otolith signatures but in the present study, all our samples were collected to represent at least 1 year of growth, creating time averaged $\delta^{15}N$ values of specific life stages that are unlikely to be strongly influenced by seasonal variation in $\delta^{15}N$.

This study is the first step in exploring how $\delta^{15}N$ in otoliths can establish trophic structure for ecosystems where baseline information is absent. Looking forward, $\delta^{15}N$ in otoliths and shells has the potential to provide important information about pre-disturbance conditions in altered ecosystems. Such information is increasingly important in many systems, where empirical data on historical baselines is needed to justify restoration goals (Dayton et al. 1998; Rodriguez et al. 2001). With the rapidly growing field of stable isotope ecology, new methods, such as compound-specific analyses of amino acids, are expanding the resolution and application of these tools (McClelland and Montoya 2002; Montoya 2007; Popp et al. 2007; Wolf et al. 2009), and the use of more specific analyses with ancient remains may provide more resolution to trophic reconstructions and increase the sensitivity of past/present comparisons.

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