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Rapid Communication

Can amino acid racemization be utilized for fish age validation?

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Abstract

We investigated the relationship between aspartic acid D:L ratios and otolith-derived age estimates in Gulf of Mexico red snapper, *Lutjanus campechanus* (ages 1–26 years; $R^2 = 0.89$) and Caribbean yellowtail snapper, *Ocyurus chrysurus* (ages 2–17 years; $R^2 = 0.84$). The estimated racemization rate was 0.61×10^{-3} year^{-1} for red snapper and 1.28×10^{-3} year^{-1} for yellowtail snapper, reflecting temperature differences between study regions. Mean jackknifed error in ages predicted from aspartic acid D:L was 1.70 ± 0.39 years for red snapper and 1.57 ± 0.41 years for yellowtail snapper. Results suggest amino acid racemization may be an effective tool for direct age estimation and potentially age validation in fishes.

Key words: amino acid racemization, age validation, reef fish, eye lens

Introduction

Age estimation is a critical process in the study of population dynamics. Additionally, age-structured stock assessments require accurate and precise estimates of the age composition of landings and discards. A variety of methods have been employed to validate fish age estimates, such as chemically marking ageing structures followed by mark-recapture of treated fish. However, these approaches are often limited by small sample sizes and long experiment durations required to collect sufficient recaptures (Campana 2001). An alternative approach, the bomb ^{14}C chronometer has been widely applied to validate age estimates of freshwater and marine fishes, but its utility in freshwater or estuarine systems can be limited due to high levels of variability driven by differential inputs or exposure to waters with dissolved inorganic carbon that is enriched or depleted in ^{14}C independent of the surface marine bomb ^{14}C signal (Campana 2001; Davis-Foust et al. 2009). Additionally, marine systems that experience upwelling of deep ^{14}C-depleted water typically impart greater interannual variability in aragonite $\Delta^{14}C$ signatures (Kilbourne et al. 2007; Andrews et al. 2021), thus reducing the utility of the bomb ^{14}C chronometer for age validation.

We hypothesized that amino acid racemization (AAR) within eye lens cores may provide an alternative process for directly estimating or validating fish age, including for freshwater and estuarine fishes. Amino acids exist in two optical isomers, levorotatory (L) and dextrorotatory (D) enantiomers. Living tissues are composed of only the L enantiomer. However, in tissues that lack metabolic activity, the L enantiomer converts to the D enantiomer, and vice versa, through a process called racemization (Bada and Schroeder 1975), which occurs until the enantiomers have equal concentrations. Eye lenses are an ideal structure for AAR analysis because they begin forming prior to hatching, and eye lens proteins are metabolically inert once formed (Dahm et al. 2007; Wride 2011).

AAR has been widely applied as a geochronological tool (Miller et al. 2013), as well as applied to estimate age in terrestrial and marine mammals via its analysis in eye lens cores that are formed in early life (George et al. 1999). AAR is a temperature-dependent process, thus animal age estimation with AAR has only been applied to homeotherms to date. However, the effect of temperature on racemization rates has been well described for fossils (Williams and Smith 1977). Therefore, it may be possible to develop AAR as an age validation tool in fishes if the temperature effect on AAR in eye lens cores could be quantified.

Several questions must be addressed to develop eye lens AAR as an age validation tool. Here, we investigated the first two of these, which are (1) does AAR have significant relationships with species-specific age estimates, hence time? and (2) Is species-specific fish age accurately predicted with AAR? Our study species were northern Gulf of Mexico (nGOM) red snapper, *Lutjanus campechanus* and Caribbean yellowtail snapper, *Ocyurus chrysurus*, two economically and ecologically important marine reef fishes. Both species are moderately long-lived (>50 years for red snapper and >25 years for yellowtail snapper), have had otolith-derived age estimation validated via the bomb ^{14}C chronometer, and typically have moderate-to-high between-reader ageing precision (Barnett et al. 2018; Zajovits 2021). Due to the lower temperature variability in the surface waters of the tropical Caribbean relative to the subtropical to temperate nGOM, we hypothesized that there would be less variance in the relationship between Caribbean yellowtail snapper D:L$_{asp}$ and otolith-derived age estimates. Furthermore, we expected a priori that yellowtail snapper eye lens cores would exhibit a higher rate of racemization, given the warmer waters of the Caribbean versus the nGOM. Results are discussed in the context of using AAR as a tool for direct age estimation and potentially developing AAR as a tool for fish age validation.

Methods

Otoliths and eyes were extracted from red snapper ($n = 29$) sampled on fishery-independent surveys in the nGOM (on the continental shelf off Texas through west Florida) and yellowtail snapper ($n = 24$) sampled in the US Caribbean (Puerto Rico, St. Thomas, and St. Croix). Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals under protocols approved by the University of Florida Institutional Animal Care and Use Committee (Protocol #201810404) and the University of South Carolina Aiken Institutional Animal Care and Use Committee (Protocol #USCA-IACUC-005). Otoliths were rinsed of adhering tissue and stored dry in coin envelopes. Eyeballs were placed in plastic bags, frozen, and stored at −20 °C (yellowtail snapper) or −80 °C (red snapper) until cores were extracted for AAR analysis. Cold storage is necessary to slow, or effectively halt AAR. Each otolith was embedded in epoxy, a 0.5 mm-thick transverse section was cut through its core, and its opaque zones were enumerated under a dissecting microscope with transmitted light.

Eye lens samples were treated similarly between species except for one departure. Yellowtail eye lenses were dried at room temperature prior to shipping to the University of Florida to be processed for AAR analysis. Eye lenses for both species were freeze-dried for 12 h, and the layers of the eye lens or lamellae were removed until the target eye lens core diameter of 2 mm was reached, based on a mean lens diameter of 120 mm, age-0 red snapper (Patterson et al. 2021). Prior to AAR analysis, eye lens cores were weighed to the nearest 0.01 mg and stored at 4 °C in borosilicate vials that had been baked at 525 °C for 24 h. A t test was computed to test for differences in eye lens core mass between species and to determine whether eye lens core extraction was consistent between species, and the target lens core diameter was consistently achieved for both.

Eye lens core D:L$_{asp}$ was analyzed at the Amino Acid Geochronology Laboratory at Northern Arizona University with high pressure liquid chromatography following the method of Kaufman and Manley (1998). Among potential amino acids to investigate, aspartic acid (asp) was chosen because it is abundant in protein and has the fastest AAR rate (Goodfriend and Meyer 1991). Aspartic acid has also been shown to be useful in predicting age of marine mammals (George et al. 1999). Briefly, eye lens cores were hydrolyzed for 6 h at 110 °C in 100 μL of 6 mol L^{-1} HCl per milligram of eye lens core, dried under vacuum, and then rehydrated in 100 μL of 0.01 mol L^{-1} HCl, 1.5 mmol L^{-1} sodium azide (to inhibit bacterial growth), and 0.03 mmol L^{-1} L-homoarginine (an internal standard) per milligram of eye lens core. Sample solutions were derivatized online with an HP1100 autoinjector prior to injection into a reverse phase chromatography column. The derivatized D and L amino acid isomers were separated in the column and enantiomer peaks were detected with an HP (Agilent) G1321A programmable fluorescence detector to quantify concentrations relative to the internal spike. Analytical precision for aspartic acid enantiomers was <3%. The rate of aspartic acid racemization, k_{asp} was estimated separately for red and yellowtail snappers by fitting a linear regression between transformed D:L$_{asp}$ ratios and otolith-derived age estimates using the linearized equation for a first-order reversible reaction (e.g., Masters et al. 1977):

$$\ln\left(\frac{1+(D:L)}{1-(D:L)}\right) = 2k_{asp} \times a + \ln\left(\frac{1+(D:L)_0}{1-(D:L)_0}\right) \tag{1}$$

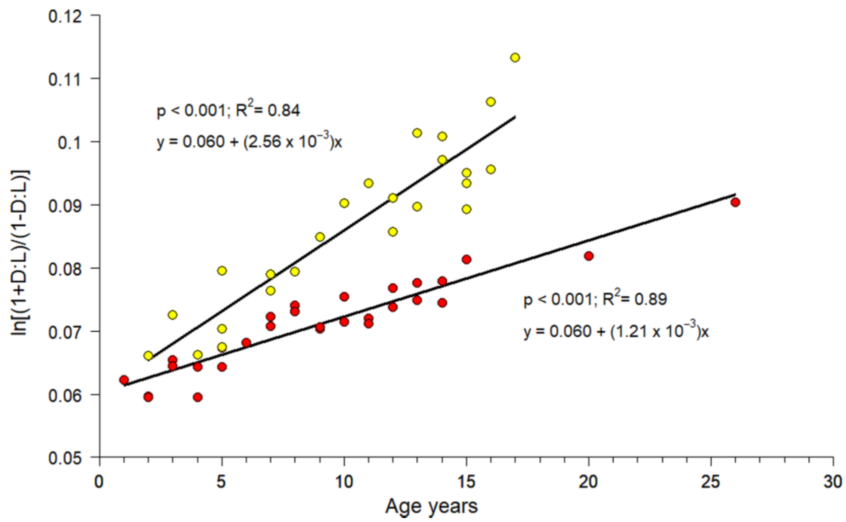
where D:L is the measured ratio of D$_{asp}$ to L$_{asp}$, k_{asp} (year^{-1}) is the characteristic rate of racemization, a is age in years, and D:L$_0$ is the D:L value at age 0, which includes a small amount of racemization induced during laboratory hydrolysis and is estimated based on linear regression.

All statistical analyses were performed in R (R Core Team 2021). Species-specific regressions were fit to transformed D:L$_{asp}$ versus otolith-derived age estimates and the root mean square error (RMSE) was calculated for each model. A jackknifed regression analysis was performed to estimate the predicative capability and mean error in age prediction from transformed lens core D:L$_{asp}$ ratios in each snapper species. For each sample, a regression equation was computed between transformed D:L$_{asp}$ and age estimates excluding the sample of interest, and then the regression was used to predict the sample's age from its transformed D:L$_{asp}$ ratio. The difference between the otolith-derived age estimate and the jackknifed predicted age was computed for each sample, and mean error ±95% confidence interval (CI) was estimated as the mean of the absolute value of the residuals for each species. Linear regressions were computed to determine the relationship between the D:L$_{asp}$ predicted age and otolith-derived age estimates. A student's t test was used to test whether the intercept was significantly different from 0 and the slope was significantly different from 1 for each species.

Results

Red snapper age estimates ranged from 1 to 26 years (Fig. 1; Table S1). Mean (±95% CI) mass of their extracted eye lens cores was 1.13 (±0.05) mg. The estimated rate of racemization was 0.61×10^{-3} year^{-1} for red snapper, and there was a strong relationship between otolith-derived age and the transformed D:L$_{asp}$ ratio (RMSE = 0.002; $R^2 = 0.89$; Fig. 1). The jackknifed regression analysis resulted in a mean error in predicted age of 1.70 ± 0.39 years. The slope and y-intercept (±SE)

Fig. 1. Least squares linear regressions fit to transformed eye lens core D:L$_{asp}$ ratios versus the otolith-derived age estimates for northern Gulf of Mexico red snapper (red symbols; $n = 29$) and Caribbean yellowtail snapper (yellow symbols; $n = 24$).



for the regression of the D:L$_{asp}$ predicted ages versus otolith-derived age estimates were 0.87 ± 0.06 and 1.11 ± 0.69, respectively. The slope was not significantly different than 1 ($p = 0.06$) and the y-intercept was not significantly different than 0 ($p = 0.12$) (Fig. 2A).

Yellowtail snapper age estimates ranged from 2 to 17 years (Fig. 1; Table S1). Mean (±95% CI) mass of the extracted yellowtail snapper eye lens cores was 1.07 (±0.07) mg; there was no significant difference between eye lens core mass between species (t test, $p = 0.29$). Yellowtail snapper exhibited a higher rate of racemization, 1.28×10^{-3} year^{-1} than red snapper, with less of the variance in the transformed D:L$_{asp}$ ratios explained by the otolith-derived age (RMSE = 0.006; $R^2 = 0.84$; Fig. 1). The jackknifed regression analysis resulted in a mean error in predicted age of 1.57 ± 0.41 years. The slope and y-intercept (±SE) for the regression of D:L$_{asp}$ predicted age versus otolith-derived age estimates were 0.85 ± 0.08 and 1.60 ± 0.85, respectively. The slope was not significantly different than 1 ($p = 0.08$) and the y-intercept was not significantly different than 0 ($p = 0.10$) (Fig. 2B).

Discussion

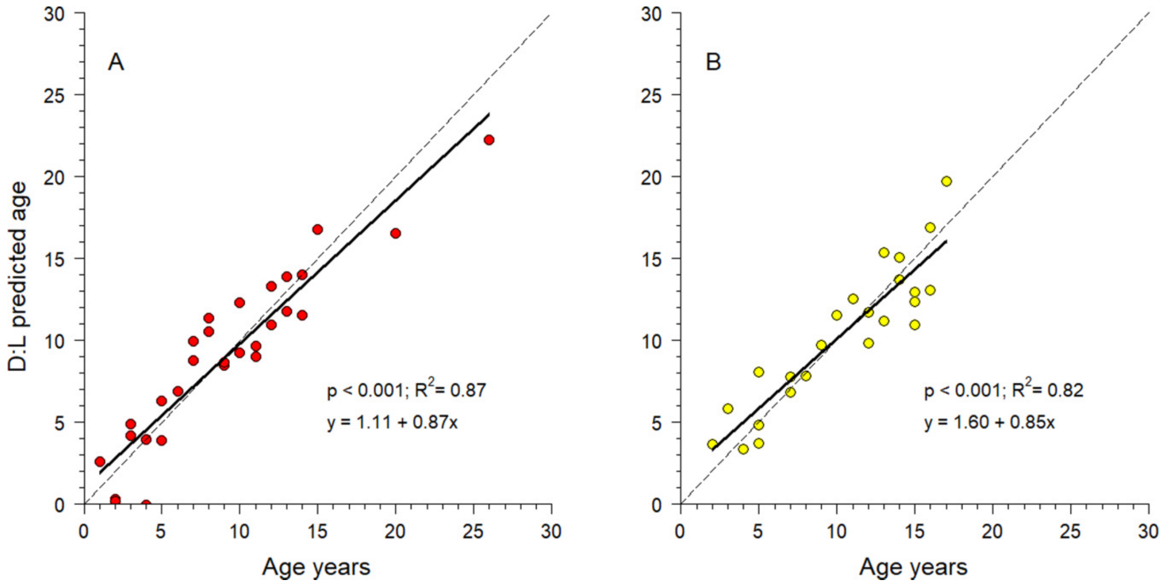
Significant relationships between transformed eye lens core D:L$_{asp}$ and otolith-derived age estimates for red and yellowtail snappers suggest AAR may serve as an effective tool for fish age estimation and potentially for age validation. A priori, we expected there to be less variance in the yellowtail snapper AAR data given lower intra-annual water temperature variability in the northern Caribbean versus GOM, but the red snapper data had lower variance. However, regressions fit to the data for both species had R^2 values of ≥0.84, indicating strong relationships between eye lens core D:L$_{asp}$ and age in these snapper species from tropical versus subtropical to temperate systems.

The estimated rate of aspartic acid racemization for yellowtail snapper was approximately twice that of red snapper, supporting the hypothesis that yellowtail snapper would exhibit a faster rate of racemization due to the warmer waters inhabited by them in the Caribbean. In mammals, the rate of racemization is estimated to increase linearly with body temperature (Rosa et al. 2013). There are interspecific differences in the rate of aspartic acid racemization in mammals, but this is due to differences in core body temperature in mammals. For example, core body temperature is approximately 36.1 °C for fin whales, 33.8 °C for bowhead whales, 34.7 °C for minke whale, and 37 °C for humans, which yields different eye lens AAR rates for aspartic acid among these species. There is a strong relationship between body temperature and the rate of racemization in mammals ($R^2 = 0.999$), which leaves little room for factors other than temperature explaining variance in mammal eye lens AAR rate.

The snappers examined here display the expected increase in estimated k_{asp} with increasing water temperature. However, with a sample of only two species, it is not possible to determine the shape of the relationship between aspartic acid AAR rate and temperature in fish eye lens cores. We also do not have direct measurements of the temperature history of the fish whose eye lenses were analyzed in this study, but indirect estimation of mean temperatures the species experienced in the two study regions is possible. For example, we estimated a mean water temperature experienced by yellowtail snapper of approximately 28 °C, which is based on their depth of capture (<30 m), regional sea surface temperature records from the US National Data Buoy Center (1971) (https://www.ndbc.noaa.gov/), and the vertical temperature structure of the water column in the US Caribbean (Seijo-Ellis et al. 2019). For red snapper, the estimated mean temperature they experienced is approximately 22 °C, which is based on three-dimensional telemetry of red snapper on nGOM reefs (Bohaboy et al. 2022) and CTD casts in the region (https://data.gulfresearchinitiative.org/).

Additional sampling across a broader range of species found in habitats or regions with a greater range of ambient

Fig. 2. Age predicted from transformed eye lens core D:L$_{asp}$ ratios for (A) northern Gulf of Mexico red snapper (n = 29) and (B) Caribbean yellowtail snapper (n = 24). Solid black lines are linear regressions fitted to the data, and the dashed black lines indicate lines of 1:1 agreement between D:L$_{asp}$ predicted age and otolith-derived age estimates.



temperatures would be necessary to build and test a general model of the effect of temperature on the AAR rate in fish eye lenses (e.g., Allen et al. 2013). AAR-based age estimation could then be applied across fish species without estimating a species-specific AAR rate, given the mean lifetime temperature of fish is known or estimable. This assumes that eyeball vitreous fluid pH does not affect the rate of racemization between species, which is reasonable given the rate of racemization is independent of pH in the pH range of 5–8 (Bada 1971). One way to estimate temperature histories in bony fishes would be via measurement of otolith δ^{18}O in otoliths, given the tight coupling between δ^{18}O fractionation in otoliths and water temperature (Patterson et al. 1993). This could be accomplished by bulk analysis of homogenized otoliths or potentially by milling transects across otolith sections to be analyzed with stable isotope mass spectrometry, or by microsampling across otoliths with secondary ionization mass spectrometry.

To directly estimate fish age from AAR measurements would require not only an accurate model (that accounts for temperature), but also a precise one, as ageing precision is a key component to minimizing ageing error (Campana 2001). Among the limited data presented here for red and yellowtail snappers, the mean error in predicting age from transformed D:L$_{asp}$ was 1.6–1.7 years, which is within the range of precision reported for age prediction from otolith mass (e.g., Pacheo et al. 2021) or Fourier transform near-infrared analysis (e.g., Passerotti et al. 2020). Accounting for temperature effects within and among species or systems, as well as greater sample size, may increase the precision of predicting age from transformed eye lens core D:L$_{asp}$, but it is unknown whether predicting fish age from eye lens AAR would be adopted for routine age estimation applications. However, it may prove useful in predicting age or longevity in exceptional circumstances, or for fishes that live in environments ill-suited to application of the bomb ^{14}C chronometer or whose longevity is greater than the bomb ^{14}C chronometer can resolve (i.e., born prior to the 1960s).

Overall, our results demonstrate the utility of using eye lens AAR to predict fish age, as well as the potential utility of eye lens AAR to be utilized in age validation. The two main challenges to operationalizing the latter are developing a general model of the effect of temperature on fish eye lens aspartic acid AAR rate and estimating the temperature history of fish, both of which appear to be tractable. If these two challenges could be solved, then there could be several advantages to eye lens AAR-based age validation relative to other methods. Nearly all fish have eyes and it is relatively easy to remove and extract their lens cores, and the biogeochemistry of eye lens protein has already been shown to be useful for fish age validation, albeit with bomb ^{14}C (Patterson et al. 2021; Shervette and Rivera Hernández 2022). Metabolic activity is restricted to the outer epithelial monolayer of eye lenses, thus the inner laminae are metabolically inert once formed (Dahm et al. 2007; Wride 2011). Therefore, the eye lens core acts as a natural biogeochemical tag in a closed system (similar to otoliths), and the D:L$_{asp}$ ratio should only be affected by time and temperature. Unlike the bomb ^{14}C chronometer whose utility can be affected by various sources of ^{14}C-enriched or -depleted waters in different habitats or regions, the only factor likely to affect the eye lens AAR rate is temperature. Additionally, all fish have D:L$_{asp}$ signatures in their eye lens cores that reflect the racemization, which has occurred since birth, eliminating the sample size and experimental duration limitations inherent in some methods of age validation. Therefore, AAR-based age estimation or validation may have the potential to be widely applicable in fisheries science and in fish population ecology more broadly.

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Data availability

Data generated or analyzed during this study are provided in full within the published article and its supplementary material.

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Competing interests

The authors declare there are no competing interests.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2022-0161.

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