Age Validation of Red Snapper, *Lutjanus campechanus*, and Red Drum, *Sciaenops ocellatus*, from the Northern Gulf of Mexico Using $^{210}Po/^{226}Ra$ Disequilibria in Otoliths

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ABSTRACT

Radiometric analysis of naturally occurring ^{226}Ra and its daughters within otoliths was used to validate age estimates derived from sectioned otoliths of red snapper and red drum from the northern Gulf of Mexico. Ages of most fish species are often estimated by counting presumed annual growth zones in otoliths. Radiometric age validation provides an independent alternative to conventional methodology by removing reader interpretation variability from the age estimation process. Red snapper and red drum are two moderately long-lived species inhabiting the Gulf of Mexico which are currently under intense state and federal management. Despite the poor counting statistics and the high backgrounds incurred with the technique used in this study, radiometric age estimates closely approximated otolith section ages for both red snapper and red drum, thus validating the continued use of otolith sections as the most accurate way to estimate ages for these species.

KEY WORDS: Otoliths, radiometric, validation

INTRODUCTION

Most fishes are aged by counting growth increments on hard-part structures such as otoliths. For most fishes, each alternating opaque and translucent zone together in the otolith represents one year; however, it is possible that some fishes deposit variable growth zones each year. Therefore, in order for fisheries management to be successful, each species should be carefully examined to determine if the zones present in an otolith are being deposited annually. Two species that have warranted this consideration in the past are the reef-dwelling red snapper, *Lutjanus campechanus*, (family Lutjanidae) and the estuarine dependent red drum, *Sciaenops ocellatus*, (family Sciaenidae). Validation of the age determination methods used to estimate age for these species is critical to management strategies.

Methods previously used to estimate ages for red snapper have been largely inconsistent and have included presumed annulus counts from scales, whole otoliths, and otolith sections (Moseley 1966, Wade 1981, Nelson and Manooch 1982, Nelson et al. 1985, Szedlmayer and Shipp 1994, Wilson et al. 1994, Wilson et al. 1998). Over the past 30 years, these life history studies have resulted in increasing estimates of longevity (Table 1). As a result, age estimation of red snapper has been prohibitive. Unlike red snapper, age estimates of red drum from the Gulf of Mexico have easily been validated throughout its entire life history using traditional methodologies (Beckman et al. 1988, Beckman et al. 1988, Murphy and Taylor 1991). Annuli on sagittal otolith sections are now considered the best estimator of age for red snapper, as well as most fish species, from the northern Gulf of Mexico (Szedlmayer and Shipp 1994, Shirripa and Burns 1997, Wilson et al. 1994, Wilson et al. 1998). Independent validation of the otolith section technique used to age red snapper and red drum is critical for the proper management of these economically important fish species (Beamish and McFarlane 1983, Wilson et al. 1998).

Age estimation through radiometric analysis of the disequilibria of radium-226 (^{226}Ra) and its daughter elements in otoliths provides that virtually any marine fish species can be analyzed effectively over any time period up to 100 years (Bennett et al. 1982). Radiometric analysis of the naturally occurring ^{226}Ra and its daughters within otoliths has been used to validate age estimates derived from otoliths in a number of species (Bennett et al. 1982, Campana et al. 1990, Fenton et al. 1991, Smith et al. 1991, Fenton et al. 1992, Campana et al. 1993, Kastelle et al. 1994, Fenton and Short 1995, Milton et al. 1995, Stewart et al. 1995). Most of these studies however have focused on slow growing, long-lived species inhabiting deep, cold water environments with little emphasis on temperate and tropical species.

Our objective was to determine with radiometric analysis whether ages derived from sectioned otoliths indicate true (radiometric) age for red snapper, *Lutjanus campechanus*, and red drum, *Sciaenops ocellatus*, from the northern Gulf of Mexico.

Table 1. Von Bertalanffy growth parameters of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico (O = otoliths, S = scales, F = female, M = male, MaxAge = maximum age, L_∞ = maximum length reported as fork length (FL) or total length (TL), k = growth coefficient, t_0 = time at age-0).

Method	Max. Age	L_∞	K	t_0	Source
S	4+	-	-	-	Moseley, 1966
S	9	-	-	-	Wade, 1981
S,O	13	941 TL	0.17	-0.10	Nelson and Manooch, 1982
O	10	925 TL	0.14	-0.00	Nelson et al., 1985
O	42	1025 TL	0.13	-	Szedlmayer and Shipp, 1994
O	53	772 $FL^{(F)}$	0.18	0.00	Wilson et al., 1994
		859 $FL^{(M)}$	0.09	2.21	
O	49	913 $FL^{(F)}$	0.16	0.72	Wilson, et al., 1998
		842 $FL^{(M)}$	0.17	0.58	

MATERIALS AND METHODS

The red snapper and red drum otoliths utilized in this study were sampled from the northern Gulf of Mexico commercial and recreational harvests (1986 -1998). Fork length in mm and eviscerated body weights in g were recorded for each fish. Both sagittal otoliths were removed and weighed to the nearest milligram. Sex of each specimen and location of capture was recorded when possible. Otolith preparations and ageing protocols for both red snapper and red drum followed standard procedures previously described elsewhere (Beckman et al. 1988, Wilson et al. 1994, Render 1995).

Otoliths used in the radiometric analyses were cored to the age class size of 1 to 4 years, depending on the species and the number of samples available. Since radiometric procedures used in this study require a minimum of 100 mg otolith material per sample, otolith cores were pooled for most age categories. As many as 20 red snapper sagittae (age 0.4 years) were pooled together to establish the minimum sample weight. Red drum otolith cores were larger than those of red snapper and were analyzed individually. Cores were obtained by embedding whole otoliths in araldite epoxy resin and progressively removing visible layers with a Buehler low speed saw and variable speed sanding wheel. Care was taken to clean the sanding wheel with a bristle brush and distilled water after each otolith was processed. Otolith cores were rinsed with distilled water, dried, weighed to the nearest milligram.

Radiometric analysis of ^{226}Ra and its daughters in otolith cores was conducted with a Photon - Electron Rejecting Alpha Liquid Scintillation (PERALS®) spectrometer. Alpha measurements were quantified with a Dell 450/L personal computer fitted with Maestro software, Model A65-B1, Version 3.06. Polonium-210 (half-life, 138 days) was assumed to be in secular equilibrium with ^{210}Pb (half-life, 22.3 years) in all samples since all otolith samples were collected at least 1 year prior to analysis. Otolith sample weights ranged from 0.153 to 0.733 g (mean = 0.388 g, n = 8). The mean reagent blank for 210Po was 0.056 ± 0.011 disintegration per minute (dpm). Recovery of the ^{209}Po standard was highly variable and ranged from 10% to 47%. Polonium samples and standards were analyzed for a minimum of 0.7 days and a maximum of 4.6 days (mean = 3 days, n = 8). The mean reagent blank for ^{226}Ra was 0.049 ± 0.019 dpm. Recovery of the ^{224}Ra standard was also highly variable and ranged from 14% to 60%. Radium samples and standards were analyzed for a minimum of 1 day and a maximum of 4.9 days (mean = 3.3 days, n = 7).

Otolith cores within an age group were placed in a 20 ml borosilicate glass scintillation vial and spiked with a known aliquot of ^{224}Ra and ^{209}Po. The otoliths were dissolved in 12 N HCl, reduced to 3 ml over heat, and cooled to room temperature in an ice bath. The solution was brought to neutrality by addition of concentrated NH_4OH. Before simultaneous extraction of ambient

^{226}Ra and the standard ^{224}Ra from the dissolved otolith solution, an extractive scintillator for radium (Radaex®) was converted from the acid phase to a salt in order to activate the solution. This procedure was performed by equilibrating the desired quantity of acid-form Radaex with an approximately equal volume of 0.35 M $NaNO_3$ and 0.2 M NaOH. Gentle swirling of the two-phase solution by hand for 5 minutes activated the solution. Approximately 3 g of the extractive scinillator was extracted from the equilibration vial with a 5 ml disposable syringe and transferred to the vial containing the otolith solution. The two-phase solution was capped and swirled vigorously for 15 minutes to allow for transfer of radium into the activated Radaex®. One gram of Radaex was removed from the vial by a 5 ml disposable syringe and transferred to a 10 x 75 mm borosilicate glass (or silicon) culture tube.

The solution was sparged (with a disposable Pasteur pipette as a sparging lance) with dry, toluene-saturated ultra-pure argon gas for 5 minutes and capped with a silicone stopper. To ensure no loss of solution, the top of the culture tube including the cap was sealed with parafilm. The otolith sample solution was separated from the unused radium extractive scintillator solution with a 30 mL separatory funnel. Polonium-210 and the standard ^{209}Po were extracted from the sample solution in exactly the same manner but with an extractive scintilallor for polonium (Polex®). Blanks were prepared in the exact manner as the sample/standard combinations.

Activity ratios ($^{210}Po/^{226}Ra$) were calculated for each red snapper (n = 6) and red drum (n = 2) sample. Lead-210 and its daughter product ^{210}Po were assumed to be in secular equilibrium in all samples. The ratio of $^{210}Po/^{226}Ra$ in otolith cores was used to predict a radiometric age using the equation:

$$t = \frac{-1}{\lambda_p}\left[\frac{\ln(1 - S)}{1 - Ro}\right]$$

where t = age (yrs), λ_p = decay constant for ^{210}Pb (-0.031/yr), S = $(^{210}Po/^{226}Ra)_t$ = activity at time t assuming insignificant ^{222}Rn loss, and $R_0 = (^{210}Po/^{226}Ra)_t$ = initial activity ratio at time t = 0.0 (Baker 1999). To compare the radiometric ages with the section ages of the same otoliths, the time between collection and analysis was added to the otolith section ages. A paired two-sample t-test comparison was used to test for differences between radiometric and otolith section age estimates.

RESULTS

In all red snapper otolith cores, the $^{210}Po/^{226}Ra$ activity ratio increased as presumed annuli count increased (Table 2). Radium-226 activity was highest in the 0.4 year core sample (0.975 (0.433 dpm/) and lowest in the three year core sample (0.208 (0.052 dpm/g). For each core sample, ^{210}Po activity (dpm/g (1σ) was proportional to ^{226}Ra activity and otolith section age. The initial activity ratio (R_0), calculated by radiometric analysis of the 0.4 year age group, was used to propagate a radiometric age estimate of -1.5 years. The negative value was assumed be a result of measurement error associated with the radiometric technique; therefore, $R_0 = 0.0$. Mean radiometric age estimates including storage time were 1.6, 6.8, 6.3, 14.1, 21.1, and 36.8 years (Figure 1a). A paired 2-sample t-test comparison indicated that radiometric ages were not significantly different from otolith section ages ($t = 0.763$, $p = 0.479$).

As with red snapper, the mean activity ratio in both red drum core samples corresponded to otolith section age (Table 2). The initial activity ratio R_0 was assumed to be 0.0 and ^{222}Rn loss from within otolith cores was assumed to be insignificant. Mean radiometric age estimates including storage time were 19.0 and 32.1 years (Figure 1b).

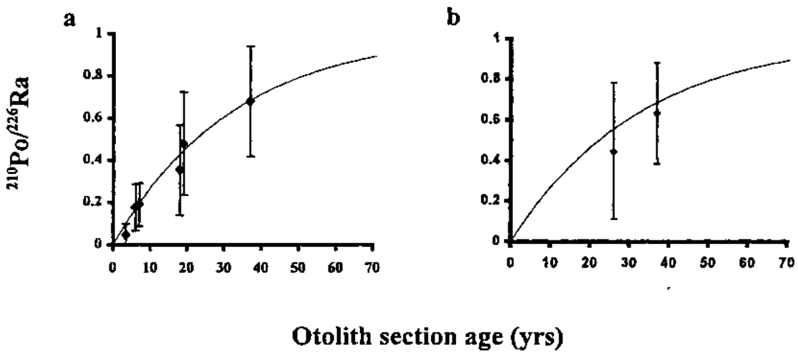

Figure 1. Observed $^{210}Po/^{226}Ra$ ratios of red snapper (a) and red drum (b) otolith cores plotted against theoretical $^{210}Po/^{226}Ra$ ratio with respect to time (—). All age estimates assume that the initial activity ratio (R_0) = 0.0 and $^{222}R_n$ loss from otoliths is negligible. Errors are expressed as ± 1σ.

Table 2. Results of the $^{210}Po/^{226}Ra$ age validation of red snapper and red drum otolith section age estimates from the northern Gulf of Mexico. The radiometric ages calculated include the time between collection and analysis (storage time). Otolith sample weights ranged from 0.153 to 0.733 g (mean = 0.388 g, n = 8).

Species	Otolith age (incl. storage time in years)	Cure Size (years)	^{226}Ra (dpm g^{-1} ± 1 s)	^{210}Po (dpm g^{-1} ± 1 s)	Radiometric age in years
Red snapper	3.4 (3)	0.4	0.975 ± 0.433	0.046 ± 0.046	1.6 ± 1.7
	7 (2)	2	0.447 ± 0.196	0.085 ± 0.026	6.8 ± 3.5
	6 (1)	1	0.058 ± 0.249	0.103 ± 0.047	6.3 ± 3.8
	18 (8)	2	0.375 ± 0.217	0.133 ± 0.023	14.1 ± 7.7
	19 (9)	3	0.208 ± 0.052	0.009 ± 0.044	21.1 ± 7.7
	37 (7)	2	0.708 ± 0.228	0.481 ± 0.099	36.8 ± 9.7
Red drum	26 (6)	1	0.651 ±0.040[1]	0.221 ± 0.186	19.0 ± 13.1
	39 (7)	1	0.757 ± 0.267	0.478 ± 0.054	32.1 ± 9.1

[1]Mean ^{226}Ra activity of a 70g, ~1 yr old red drum pooled otolith sample determined with gamma spectrometry (Baker 1999).

DISCUSSION

The results of this study indicated that mean radiometric age estimates closely approximated age estimates derived from otolith sections of both red snapper and red drum from the northern Gulf of Mexico. The findings confirm that red snapper deposit one opaque and one translucent zone each year at least up to 30 years of age. This study was the second instance where relatively young lutjanids (10 years of age) were successfully aged using analyses of the $^{210}Po/^{226}Ra$ radioisotopic pair (Milton et al. 1995). Although red drum age estimation was successful using radiometric dating, more sample analyses over a broader age range are needed. These results however, support the continued use of counting annuli on sagittal otolith sections as the most effective age determination method for red snapper and red drum from the Gulf of Mexico.

Radiometric dating of otoliths requires that three assumptions be satisfied:

i) uptake of ^{226}Ra into the otolith occurs at rate constant to the mass increase of the otolith

ii) the initial activity ratio (R_0) in otoliths is close to zero or at least known

iii) the otolith remains a chemically-closed system in terms of no loss or gains of radionuclides with respect to time.

The first assumption concerning constant ^{226}Ra uptake was largely circumvented in this study due to our use of cores instead of whole otoliths. In addition, individual ^{226}Ra activities for each sample were used instead of an average ^{226}Ra activity. The second assumption was confirmed in this study through successful radiometric analysis of the young-of-the-year (YOY) age group of red snapper. Otolith microchemical analyses of Pb and Ba, stable elements which serve as proxies for ^{210}Pb and Ra, have also indicated that $R_0 = 0.0$ is applicable for both red snapper and red drum YOY (Baker, 1999). The possibility of ^{222}Rn loss is important and must be considered. Significant loss of ^{222}Rn, a noble gas, would lead to less ^{210}Po than expected and thus a reduction in the radiometric age estimate. We believe that ^{222}Rn loss is possible, given the characteristics of the gas and the internal composition of the otolith; however, the radiometric technique used in this study produced age estimates with high variability which probably masked any detectable loss of ^{222}Rn if in fact ^{222}Rn loss did occur. Nevertheless, mean radiometric and otolith section age estimates of red snapper were determined to be in close agreement.

The observed activities within red snapper otolith cores indicated that 226Ra activity decreased as otolith mass (age) increased. There are two possibilities as to why ^{226}Ra was higher in young cores rather than older ones. Radium is deposited onto the otolith at a nearly constant ratio to that of Ca (Moore et al. 1973). Red snapper exhibit a strong linear relationship between otolith weight and age until approximately 10 years of age when the relationship ceases to be significant (Wilson et al. 1998). The otolith weight-to-age relationship could explain the initial high values of ^{226}Ra in very young fish and why ^{226}Ra activities decrease with increasing age of the otolith core. Secondly, ^{226}Ra activity in general is significantly higher in the riverine and estuarine waters of the coastal shelf than areas offshore (Moore and Scott, 1986; Kraemer and Curwick 1991). Red snapper exhibit a major shift in habitat from the time when they are young (≤ 2 years) to the time of maturity, thus encountering variable concentrations of ^{226}Ra. Juvenile red snapper are commonly found on the nearshore shrimping grounds and adults are generally associated with offshore environments and areas containing hard substrate such as natural reefs and oil and gas platforms (Render 1995). Habitat preference by life history stage may play a key role in ^{226}Ra accumulation.

Although the results of this study were satisfactory to accomplish the objective of age validation, analyses of radionuclides in fish otoliths was difficult with the PERALS system. Reagent blanks used in this study were excessively high compared to those used in traditional, ultra-low level alpha spectrometry techniques: however, the significance of the high values was reduced by the high 226Ra activities observed in otolith cores. Highly variable recoveries from dissolved otolith solutions was also a significant problem and is most likely attributable to Ca interference during the radionuclide extraction procedures. The radiometric dating method should prove successful to other fishes of the Gulf of Mexico provided that these species have ^{226}Ra activities similar to the high values observed in this study. Radiometric age validation of short to long-lived marine fishes holds great promise, provided that further research is undertaken to improve existing methodology.

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