

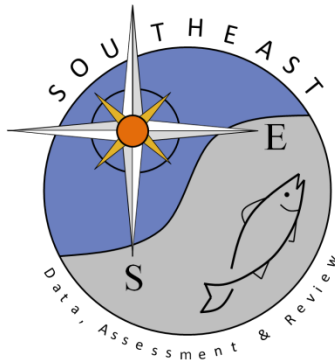
# Radiocarbon Age Validation for Caribbean Parrotfishes

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# RADIOCARBON AGE VALIDATION FOR CARIBBEAN PARROTFISHES

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## **Radiocarbon age validation for Caribbean parrotfishes**

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### **Abstract**

For management efforts to succeed in Caribbean fisheries, local fishers must support and be willing to comply with fishing regulations. This is more likely when fishers are included in a stock assessment process that utilizes robust scientific evidence, collected in collaboration with fishers, to evaluate the health of fish stocks. Caribbean parrotfishes are important contributors to coral reef ecosystem health while also contributing to local fisheries. Scientifically robust stock assessments require regional species-specific information on age-based key life history parameters, derived from fish age estimates. Evaluation of the accuracy of age estimation methods for fisheries species is a critical initial step in managing species for long-term sustainable harvest. The current study resulted from a collaborative research program between fish biologists and local fishers investigating age, growth, and reproductive biology of the seven parrotfish species landed in U.S. Caribbean fisheries. It is the first to directly validate age estimation for parrotfishes through analysis of  $\Delta^{14}\text{C}$  from eye lens cores. Our results show that enumeration of opaque zones from thin sections of sagittal otoliths of *Sparisoma* and *Scarus* species provides accurate age estimates. The oldest stoplight parrotfish *Sparisoma viride* and queen parrotfish *Scarus vetula* in the  $\Delta^{14}\text{C}$  validation series were 14 y and 16 y, respectively.

## Introduction

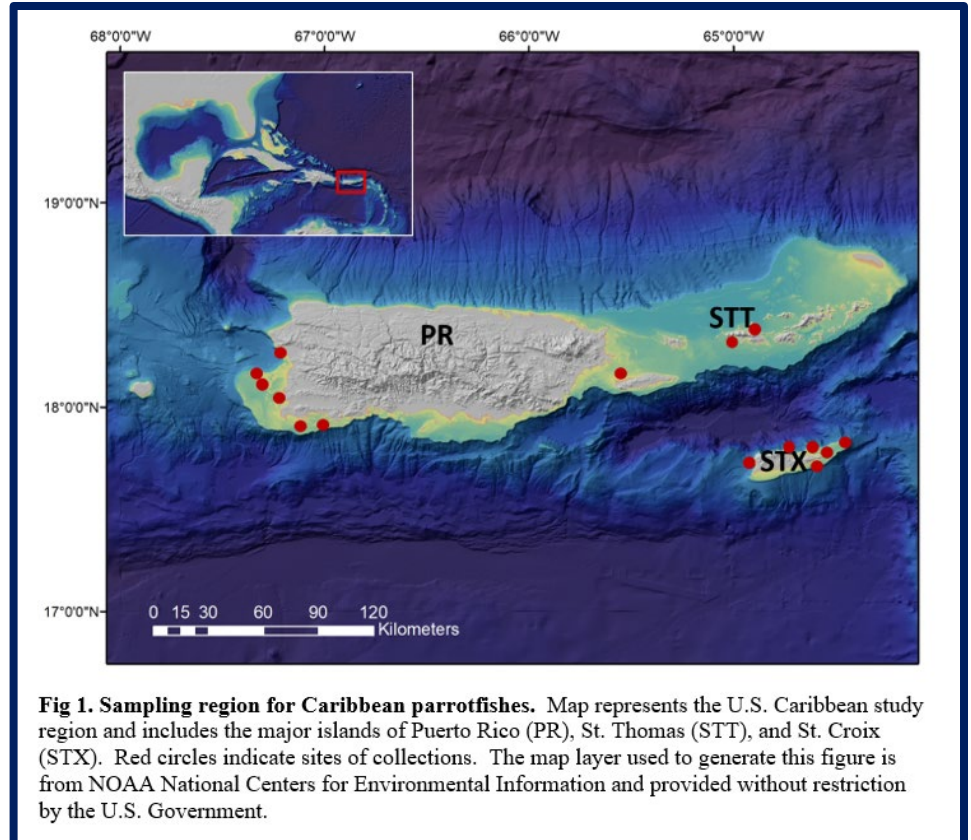
Parrotfishes are integral contributors to the ecosystem function and maintenance of shallow water coral reefs due to their roles as algal consumers and recyclers of coral skeletal material into copious amounts of sediment [1-4]. Parrotfishes also are important food fishes targeted by artisanal (small-scale) commercial fisheries throughout much of the Caribbean [5-8]. Despite their importance in Caribbean reef fisheries, parrotfishes are considered data-deficient/data-poor in terms of fisheries management due to a lack of species-specific information regarding population demographics. In the U.S. Caribbean, there is a critical need for documenting basic life history parameters of Caribbean parrotfishes so that fisheries scientists, in collaboration with local fishers and local resource managers, can conduct scientifically rigorous stock assessments and then implement relevant management strategies to ensure the long-term sustainable harvest of parrotfishes. The determination of population age structure is an essential step in addressing life history information gaps for Caribbean parrotfishes. Age estimates of individuals in a population are used with corresponding fish length data and information on sex to calculate growth rates, mortality, age/size at sexual maturity, and for sequential hermaphrodites such as parrotfish species, age/size at sexual transition; all integral life history parameters used in fisheries management [6, 9-12].

Many coral reef fish species have complex sexual ontogenies which can influence size and sex specific growth patterns [13-17]. Parrotfishes in particular exhibit a combination of complex life history patterns and reproductive strategies [13, 18-21]. Additionally, several studies have noted a decoupling of size and age in parrotfish species emphasizing that size can be a poor estimate of age in terms of understanding demographic patterns related to life history [15, 19, 22, 23]. Ultimately, age data, preferably derived from a validated age estimation method, are essential to document the biology and ecology of parrotfish populations.

The most common means of ageing marine bony fishes is by enumeration of growth increments in otoliths. However, otolith increments in tropical reef fishes can be relatively difficult to visualize or interpret due to a combination of lower environmental variability among seasons and complex life histories [24-26]. Therefore, age estimation validation is critically important to establish the accuracy of an ageing method [27, 28]. Annual growth zone formation in otoliths has been validated through numerous approaches; however, the one best-suited to validate age estimates of medium to long-lived species is application of a regional bomb radiocarbon ( $\Delta^{14}\text{C}$ ) time series [28-32]. The time-specific  $\Delta^{14}\text{C}$  records from shallow marine waters provide regional reference chronologies that are used to evaluate fish age estimates through comparison of fish  $\Delta^{14}\text{C}$  measured in otolith core material that formed during early life. Fish otoliths are composed principally of aragonite, which is metabolically inert once formed, with  $^{14}\text{C}$  incorporated into otoliths from dissolved inorganic carbon from the surrounding seawater and dietary sources [33, 34]. Bomb radiocarbon time series have been applied extensively to validate age estimation for reef fishes that had birth years in the 1950s and 1960s during the period of rapid rise in oceanic  $\Delta^{14}\text{C}$  [30, 35-40] and more recently the post-peak  $\Delta^{14}\text{C}$  decline period (since the 1980s) has been applied to validate bony fish age estimates of younger and more recently collected fishes [32, 41, 42].

Precise extraction of otolith core material from parrotfish otoliths can be difficult due to the structural nature of their small, thin, fragile otoliths, which can lead to contamination of the target core region by material from subsequent growth zones. However, several studies recently demonstrated that eye lens cores contain archived chemical isotopic signatures from early life [26, 43-46] and have been successfully used to determine the radiocarbon signature a fish

experienced during early life which enabled age estimation [47] and age validation for populations of several tropical marine fish species [25, 26, 48, 49]. Eye lens cores can be used as a source of “birth year” (hatch year) carbon signatures in bomb radiocarbon age validation efforts because eye lenses begin formation prior to hatching [50, 51], grow throughout the life of a fish [43, 50, 51], and consist of carbon-rich optical proteins that become metabolically inert shortly after formation and are deposited in successive, concentric layers [45, 46, 50, 51].



In the U.S. Caribbean (Figure 1), seven parrotfish species are landed in the reef fish fisheries of Puerto Rico (PR) and the U.S. Virgin Islands (USVI): stoplight parrotfish *Sparisoma viride*, redband parrotfish *Sp. chrysopterygus*, redtail parrotfish *Sp. aurofrenatum*, yellowtail parrotfish *Sp. rubripinne*, princess parrotfish *Scarus taeniopterus*, striped parrotfish *Sc. iseri*, and queen parrotfish *Sc. vetula* [5, 7, 52-55]. In general, Caribbean parrotfishes are described as sequential hermaphrodites displaying protogyny; an individual is first a female and then transforms into a male but does not function simultaneously as both [13, 20, 21]. However, in some species, primary males (male not derived from females) have been observed (males that transitioned from females are called secondary males) [13]. Additionally, presumed gonochoristic-like females (those that do not appear to transform to males) may occur in some populations [56], but this must be confirmed with age data, since the presence of large females does not necessarily equate to old females. Most parrotfish species also exhibit a complex sequence of ontogenetic changes in color patterns associated with sexual identities; all but one of the West Atlantic *Scarus* and *Sparisoma* species (midnight parrotfish *Sc. coelestinus*) exhibit two distinct color patterns as adults: initial color phase and terminal color phase [56].

Parrotfish species in the U.S. Caribbean need to undergo stock assessments; past attempts to assess parrotfish populations were incomplete due to a lack of basic life history information [55]. Thus, the overall goal of this study was to utilize  $\Delta^{14}\text{C}$  to validate the age estimation method of enumerating growth zones from sagittal otoliths of parrotfishes from the north Caribbean as a first step toward documenting population demographics and investigating species-specific life history strategies. Validation of the age estimation methods for parrotfishes

ensures that accurate ages are used to compute population parameter estimates. Specific objectives were primarily to utilize bomb radiocarbon from eye lens cores to evaluate age estimation accuracy for stoplight parrotfish and queen parrotfish through utilization of the north Caribbean  $\Delta^{14}\text{C}$  reference time series [32]; and secondarily to provide an updated summary of longevity estimates for parrotfish species from the western Atlantic documented in recent studies.

## Methods

### Collection and Initial Processing

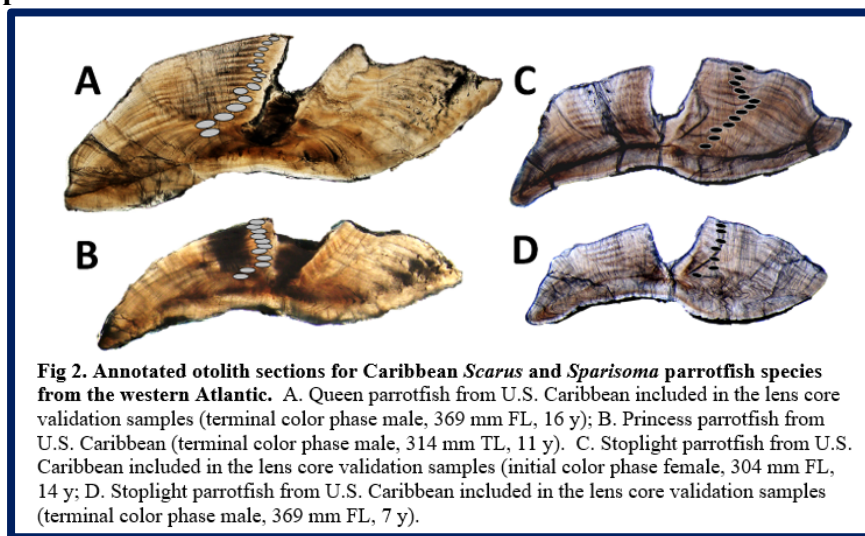
A collaborative research program, between fish biologists and local fishers, to study age, growth, and reproductive biology of the seven parrotfish species landed in U.S. Caribbean fisheries began in 2015. Detailed information on the study area and sample collection design for our parrotfish samplings efforts is described in Jones et al. [20]. Briefly, fish samples collected throughout the U.S. Caribbean (PR, St. Thomas/St. John [STT/J], and St. Croix [STX]; Figure 1) were obtained from a combination of fishery-dependent and fishery-independent collections with a variety of gear types (spear, trammel-net, traps). All fish were kept on ice until processing occurred. Initial processing included weighing each fish ( $\pm 1$  g) and measuring ( $\pm 1$  mm) for standard length (SL), fork length (FL), and total length (TL).

To obtain information on the sex of each fish sample, gonads were carefully removed and preserved for histological evaluation using the methods described in Jones et al. [20]. Sagittal otoliths were carefully extracted, cleaned gently with water, dried, and placed in plastic vials for later age determination.

Samples utilized for age estimation validation required additional collection and processing of eyes from fish specimens; therefore we began collecting fish eyes from each parrotfish sample starting in 2018. Eyes were dissected from fish, wrapped in foil, and stored in labeled plastic bags at  $-5^{\circ}\text{C}$  until further processing.

### Age estimation for Caribbean parrotfishes

Sagittal otoliths were processed for age estimation following the methods described previously in Jones et al. [20]. Briefly, one sagittal otolith from each fish sample was embedded in epoxy, then otoliths were sectioned transversely through the nucleus to a thickness of  $\sim 0.3$  mm using a low-speed saw with a diamond-edged blade. Otolith sections were mounted on glass slides and covered with a clear mounting media.



**Fig 2. Annotated otolith sections for Caribbean *Scarus* and *Sparisoma* parrotfish species from the western Atlantic.** A. Queen parrotfish from U.S. Caribbean included in the lens core validation samples (terminal color phase male, 369 mm FL, 16 y); B. Princess parrotfish from U.S. Caribbean (terminal color phase male, 314 mm TL, 11 y). C. Stoplight parrotfish from U.S. Caribbean included in the lens core validation samples (initial color phase female, 304 mm FL, 14 y); D. Stoplight parrotfish from U.S. Caribbean included in the lens core validation samples (terminal color phase male, 369 mm FL, 7 y).

Ages for all otoliths were determined based on the number of increments counted by a primary reader using a stereoscope with transmitted light at a magnification of 20-40x. Each increment consisted of a set of one translucent band and one opaque band (Figure 2). A second

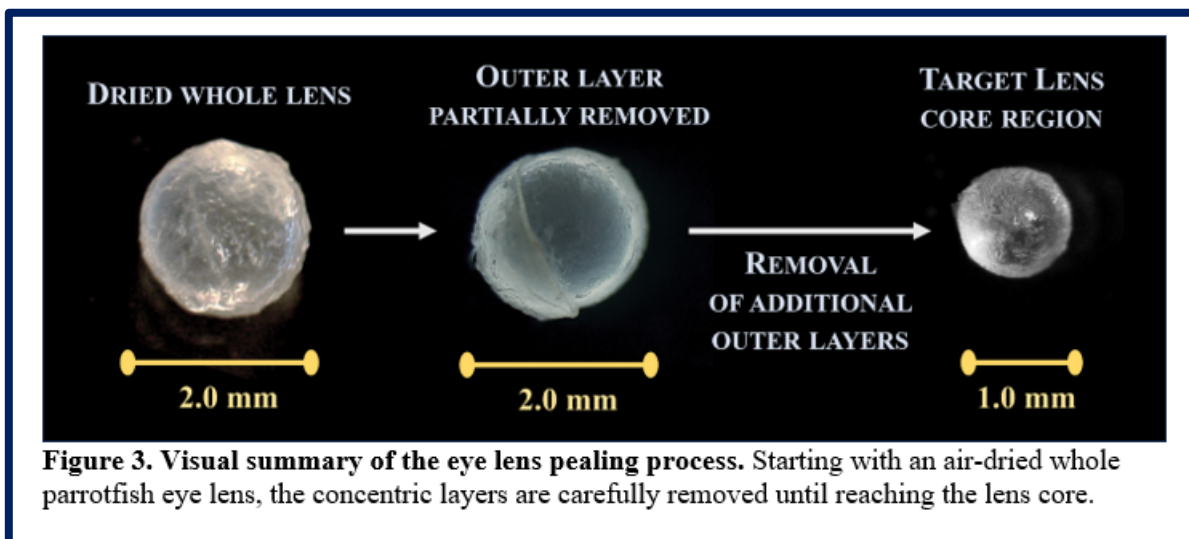
reader evaluated otolith slides and only fish samples with perfect agreement between the two readers were considered for this validation study. Increment counts were assessed without knowledge of fish size or time of year that the sample was collected.

Samples for age estimation validation were selected from fish collected during 2018-2019 and the selection process for each species incorporated random selection of individuals from the following groupings: youngest fish with eyes; initial color phase males with eyes; oldest female with eyes, oldest males with eyes, individuals binned in age class groups with eyes.

### Bomb radiocarbon age estimation validation

We set the target diameter of the eye lens core region (hereafter referred to as “lens core”) that we analyzed for the  $\Delta^{14}\text{C}$  signature an individual fish experienced during the first ~ 6 months of life at 1 mm for the two parrotfish species based on the following: analysis of age-0 stoplight parrotfish ( $n = 4$ ; mean SL = 73 mm) eye lenses indicated a mean dry eye lens diameter of 0.9 mm and mean mass of 0.8 mg. Analysis of age-0 queen parrotfish ( $n = 2$ ; mean SL = 91 mm) indicated a mean dry eye lens diameter of 1.0 mm and mean mass of 0.9 mg.

Forceps and glassware used to obtain and store eye lens cores for  $\Delta^{14}\text{C}$  analysis were pretreated to prevent carbon contamination as described in Shervette and Rivera Hernández [26]. For each fish sample selected for age estimation validation, whole eyes were thawed at room temperature then eye lenses extracted. Next, lenses were dried in pre-treated glass petri dishes and then peeled by removing the concentric layers until the target core diameter was reached (Figure 3). Each lens core was weighed to the nearest 0.1 mg. In some fish, a single core did not provide sufficient mass for robust isotopic analysis (minimum mass for high precision results = ~0.6 mg), in which case we obtained the equivalent-sized core from the second eye lens of the same fish and combined the two eye lens cores [45]. Lens cores were placed in pre-treated glass vials for shipment and cores were analyzed for  $\Delta^{14}\text{C}$  via accelerator mass spectrometry at the National Ocean Sciences Accelerator Mass Spectrometry facility at Woods Hole Oceanographic Institute ([www.whoi.edu/nosams/radiocarbon-data-calculations](http://www.whoi.edu/nosams/radiocarbon-data-calculations)). The  $\Delta^{14}\text{C}$  value for each of the lens cores analyzed was assumed to represent the  $\Delta^{14}\text{C}$  present during the first six months of life of the fish.

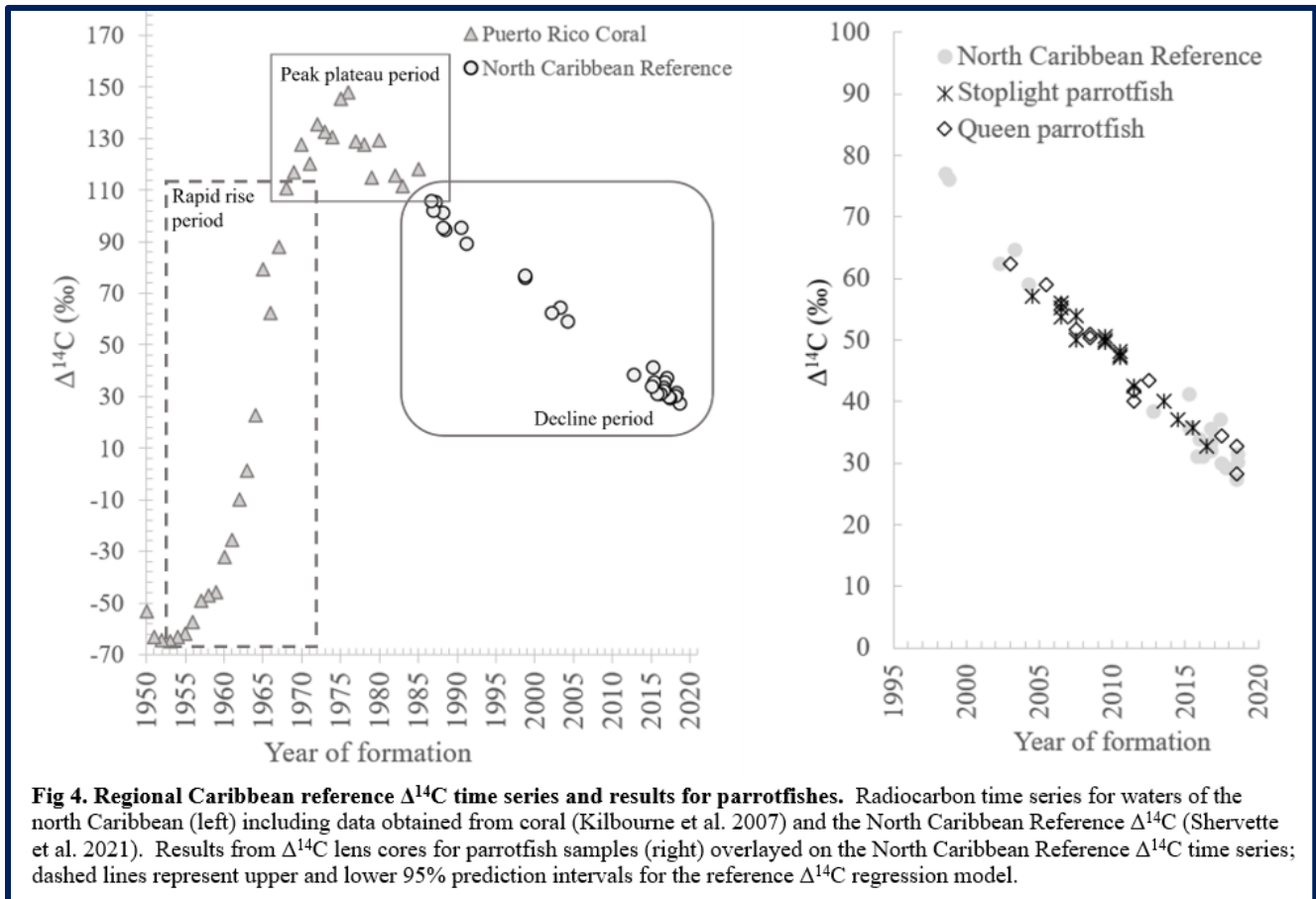


“Birth year” (hatch year/time period of formation of eye lens core) for each sample was computed by subtracting the otolith-based estimated age of a sample from the year of sample collection. For example, a fish caught in 2019 with an estimated age of 14 y (based on the



otolith section increment count) would have a birth year of 2005. Stoplight parrotfish spawn year round [17], with peak spawning documented from February-April for the north Caribbean [21]. Peak spawning month for queen parrotfish, based on histological analysis of gonads from mature females collected from 2015-2022 (as part of our ongoing parrotfish life history work) was also February-April. We adjusted the birth year value for each sample by +0.5 to incorporate the timing of peak spawning and the midpoint in time that the lens core region formed [26].

We used separate sum of squared residuals (SSR) bias analysis [26, 32, 40] to evaluate the age estimation accuracy for each species. Estimated birth years and corresponding  $\Delta^{14}\text{C}$  of stoplight and queen parrotfish eye lens cores were overlaid on the north Caribbean reference  $\Delta^{14}\text{C}$  time series (Figure 4) [32]. Birth years derived from original age estimates represented an age bias of 0 (null model), age bias models of +1, +2, +3 shifted age estimates older, and age bias models of -1, -2, -3 shifted age estimates younger. For each model, using the north Caribbean reference  $\Delta^{14}\text{C}$  time series linear regression equation ( $y = 4680 - 2.30x$ ) [32], we computed the sum of squared residuals (SSR) from observed eye lens core  $\Delta^{14}\text{C}$  minus predicted values. The model with the lowest SSR is considered the most parsimonious prediction of birth years; if the null model for a species produces the lowest SSR, then the age estimation method is considered accurate [40].



## Results

For stoplight parrotfish,  $\Delta^{14}\text{C}$  eye lens core results were obtained from 17 samples with an estimated age range of 1-14 y and a corresponding birth year range of 2005-2017 (Table 1). A total of 12 queen parrotfish samples had  $\Delta^{14}\text{C}$  eye lens core results and ranged in age from 1-16 y with corresponding birth years of 2002-2018 (Table 1). The oldest stoplight parrotfish included in the validation series was an initial color phase female (14 y; 304 mm FL) collected from waters of St. Croix, USVI (Table 1; Figure 2). Female stoplight parrotfish analyzed for  $\Delta^{14}\text{C}$  ranged in size from 127 – 334 mm FL and age from 1-14 y. Terminal color phase males ranged in size from 292 – 371 mm FL and age from 5-12 y. The youngest male in the validation series was an initial color phase individual with a length of 307 mm FL and age of 4 y (Table 1). The oldest queen parrotfish included in the validation series was a terminal color phase male (16 y; 369 mm FL) collected from St. Croix, USVI (Table 1; Figure 3). Females analyzed for  $\Delta^{14}\text{C}$  ranged in size and age from 181 – 225 mm FL and 2-5 y (Table 1). Terminal color phase males ranged in size and age from 274 – 384 mm FL and 5-16 y. Initial color phase queen parrotfish with gonads transitioning from female to male ranged in size and age from 140-322 mm FL and 1-14 y.

Table 1. Stoplight and queen parrotfishes eye lens core validation samples.

Species	FishID	Size (mm)	Color phase Sex	Year Caught	Age	Birth year	$\delta^{13}\text{C}$	$\Delta^{14}\text{C}$	$\sigma$
Stoplight	STX-SPF01	257 SL/304 FL	Initial Female	2019	14	2005.5	-15.88	57.21	2.00
Stoplight	STX-SPF02	321 SL/371 FL	Terminal Male	2018	12	2006.5	-15.54	53.74	2.30
Stoplight	STX-SPF03	285 SL/334 FL	Initial Female	2018	11	2007.5	-15.93	55.25	2.10
Stoplight	PR-SPF01	297 SL/355 FL	Terminal Male	2019	11	2008.5	-13.85	55.93	2.20
Stoplight	STX-SPF04	285 SL/327 FL	Terminal Male	2018	10	2008.5	-16.31	50.07	2.00
Stoplight	STT-SPF01	316 SL/365 FL	Terminal Male	2019	10	2009.5	-16.48	50.50	2.60
Stoplight	PR-SPF02	273 SL/330 FL	Terminal Male	2019	10	2009.5	-13.49	49.96	2.30
Stoplight	STX-SPF05	265 SL/310 FL	Initial Female	2018	9	2009.5	-16.85	54.02	2.10
Stoplight	PR-SPF03	247 SL/292 FL	Initial Female	2018	8	2010.5	-12.06	49.63	2.30
Stoplight	STT-SPF02	306 SL/360 FL	Terminal Male	2018	8	2010.5	-16.65	47.55	2.00
Stoplight	STT-SPF03	302 SL/357 FL	Terminal Male	2018	7	2011.5	-14.89	48.11	2.50
Stoplight	STT-SPF04	322 SL/369 FL	Terminal Male	2019	7	2012.5	-15.26	47.17	2.20
Stoplight	PR-SPF04	275 SL/329 FL	Terminal Male	2019	6	2013.5	-13.99	39.99	2.10
Stoplight	PR-SPF05	262 SL/299 FL	Terminal Male	2018	5	2013.5	-13.20	42.56	2.10
Stoplight	STT-SPF05	273 SL/310 FL	Terminal Male	2019	5	2014.5	-14.27	37.05	2.00
Stoplight	PR-SPF06	263 SL/307 FL	Initial Male	2019	4	2015.5	-13.88	35.75	2.00
Stoplight	STT-SPF06	103 SL/127 FL	Initial Female	2018	1	2017.5	-14.06	32.73	2.10
Queen	STX-QPF01	309 SL/369 FL	Terminal Male	2018	16	2002.5	-13.96	62.38	2.20
Queen	STX-QPF02	262 SL/322 FL	Initial Transition	2019	14	2005.5	-12.01	58.97	2.30
Queen	STX-QPF03	311 SL/384 FL	Terminal Male	2018	11	2007.5	-12.13	51.65	2.20
Queen	STX-QPF04	282 SL/340 FL	Terminal Male	2018	10	2008.5	-11.68	55.89	2.30
Queen	STX-QPF05	321 SL/395 FL	Terminal Male	2018	10	2008.5	-14.62	51.02	2.10
Queen	STX-QPF06	330 SL/382 FL	Terminal Male	2018	8	2010.5	-13.66	50.46	2.20
Queen	STX-QPF07	310 SL/378 FL	Terminal Male	2019	7	2012.5	-11.86	41.77	2.10
Queen	STX-QPF08	224 SL/274 FL	Terminal Male	2019	5	2014.5	-15.50	40.16	2.10
Queen	STX-QPF09	182 SL/225 FL	Initial Female	2018	5	2013.5	-13.80	43.45	2.80
Queen	PR-QPF01	172 SL/211 FL	Initial Female	2019	4	2015.5	-15.04	34.50	2.60
Queen	STX-QPF10	148 SL/181 FL	Initial Female	2019	2	2017.5	-14.40	32.75	2.10
Queen	STX-QPF11	115 SL/140 FL	Initial Transition	2019	1	2018.5	-12.52	28.28	2.20

Results for  $\Delta^{14}\text{C}$  ranged from 32.73 – 57.21‰ for stoplight parrotfish, and 28.28 – 62.38‰ for queen parrotfish (Table 1; Figure 4). Age estimates for all the stoplight parrotfish and queen parrotfish samples included in the validation series had corresponding birth year estimates versus eye lens core  $\Delta^{14}\text{C}$  that fit within the 95% prediction intervals of the north Caribbean reference  $\Delta^{14}\text{C}$  time series regression relationship during the  $\Delta^{14}\text{C}$  decline period (Figure 4). The SSR ageing bias analysis results for stoplight parrotfish indicated that the original, unadjusted age estimates (null model) provided accurate ages because they had the lowest SSR at 81 (Table 2). The SSR results for the biased age models ranged from 129 to 953 (Table 2). The SSR ageing bias analysis results for queen parrotfish indicated that the null model (unadjusted age estimates) utilized accurate ages because it had the lowest SSR at 62 (Table 2). The SSR results for the biased age models ranged from 115 to 664 (Table 2).

Table 2. Results from ageing bias analysis. Birth year estimates were purposefully biased by +/- 1 to 3 years for each species and then the squared residuals from the predicted north Caribbean reference  $\Delta^{14}\text{C}$  time series regression were computed and summed for each age model.

Age Model	Bias applied (y)	Stoplight parrotfish SSR	Queen parrotfish SSR
Null	0	81	62
-1	-1	203	115
-2	-2	493	295
-3	-3	953	603
+3	+3	733	664
+2	+2	346	336
+1	+1	129	136

Application of the age estimation method validated for stoplight parrotfish and queen parrotfish to other parrotfish species collected from the U.S. Caribbean with samples that have been aged as of December 2023 resulted in the following age ranges for each species: 0-13 y for redband parrotfish, 0-11 y for red band parrotfish, 0-14 y for yellowtail parrotfish, 0-11 y for princess parrotfish, and 0-10 y for striped parrotfish. Additional samples aged for stoplight parrotfish from our collections aged using the validated age estimation method included an individual with an age of 20 y. Additional samples aged for queen parrotfish from our collections included an individual from Bermuda with an age of 21 y.

## Discussion

The current study was the first to directly validate age estimation for parrotfish species through analysis of  $\Delta^{14}\text{C}$  from eye lens cores and the first study to validate age estimation for Caribbean parrotfishes utilizing  $\Delta^{14}\text{C}$ . Our results show that enumeration of opaque zones from thin sections of sagittal otoliths of *Sparisoma* and *Scarus* species provides accurate age estimates. Utilization of  $\Delta^{14}\text{C}$  from fish eye lens cores has several advantages compared to otolith cores, especially for species with small, fragile otoliths that are generally inhospitable to precise core material extraction. Fish eye lenses are relatively easy to obtain and to process for lens cores compared to obtaining otolith core material which requires the use of a computerized

micromill system. Additionally, many fish species have otoliths that are so small they do not contain enough otolith core material representing birth year  $\Delta^{14}\text{C}$  signatures required for precise  $\Delta^{14}\text{C}$  analysis [25] or are morphologically shaped such that the otolith core cannot be extracted without contamination from subsequent years beyond the birth year [26].

### **Radiocarbon validation of age estimation for parrotfish species**

For the purpose of age estimation validation, a  $\Delta^{14}\text{C}$  reference time series must provide region-specific trends in  $\Delta^{14}\text{C}$  for shallows marine waters (< 100 m depth). Shervette et al. [32] emphasized that successful application of  $\Delta^{14}\text{C}$  reference time series for validation efforts of reef fisheries species includes adherence to three important considerations. The first is ensuring that the original goal/objectives and study design of potential  $\Delta^{14}\text{C}$  reference time series data are applicable for the use of age validation. This is because several studies that report  $\Delta^{14}\text{C}$  data from coastal and marine systems were designed to answer questions beyond documenting temporal trends of  $\Delta^{14}\text{C}$  present in shallow marine waters. The current study fully complied with this since the North Caribbean  $\Delta^{14}\text{C}$  reference time series used to validate age estimation for Caribbean parrotfishes was specifically developed for use as an age validation tool and previously has been utilized successfully to validate age estimation methods for red hind *Epinephelus guttatus*, mutton snapper *Lutjanus analis*, white grunt *Haemulon plumierii* [32], queen snapper *Etelis oculatus* [25], yellowtail snapper *Ocyurus chrysurus* [49], and hogfish *Lachnolaimus maximus* [48]. The second important consideration is to ensure the applicability of potential  $\Delta^{14}\text{C}$  reference time series data to the region/location where fish samples under evaluation were obtained. This is because temporal  $\Delta^{14}\text{C}$  trends vary regionally [57]. The current study fully complied with this; the fish samples analyzed were collected from the same sampling areas in Puerto Rico and USVI (Figure 1) where samples were obtained to establish the  $\Delta^{14}\text{C}$  reference time series [32]. The third critical consideration is that the timing and magnitude of the  $\Delta^{14}\text{C}$  reference time series applied to fish age estimation validation reflects the  $\Delta^{14}\text{C}$  experienced by study species in the habitats where juveniles reside. This is because many marine species exhibit ontogenetic shifts in habitat use within a regional seascape and  $\Delta^{14}\text{C}$  gradients have been documented in association with freshwater input [58, 59], upwelling [57, 60], and depth [61-64]. Our study also fully complies with this last consideration; juvenile habitats of stoplight and queen parrotfishes occur within the same reef systems where adults reside [16, 65-67].

Two studies from waters of the Pacific utilized  $\Delta^{14}\text{C}$  in age validation efforts for three parrotfish species and a wrasse species [19, 68]. Andrews et al. [68] had the stated goal of determining the feasibility of using  $\Delta^{14}\text{C}$  to date the otoliths from adult humphead wrasse *Chelinus undulatus* (n = 7) and bumphead parrotfish *Bolbometopon muricatum* (n = 5) and to evaluate the accuracy of age estimates obtained via increment counts in thin sections of sagittal otoliths for the two species. That study focused on large adults in hopes of obtaining fish old enough so that the birth year otolith core  $\Delta^{14}\text{C}$  signatures aligned with the  $\Delta^{14}\text{C}$  rapid rise period (1950s-1960s; general time period illustrated in Figure 4) of the regional reference time series because that period would provide an extremely narrow range of predicted age estimates ( $\pm 1-2$  y) for the samples based on the  $\Delta^{14}\text{C}$  results. However, all seven humphead wrasse and two of the five bumphead parrotfish samples analyzed had estimated birth year  $\Delta^{14}\text{C}$  results that when overlaid on the regional  $\Delta^{14}\text{C}$  reference series occurred during the 15-20 y  $\Delta^{14}\text{C}$  plateau period (1970s-early 1980s; general time period illustrated in Figure 4). Andrews et al. [68] noted “this result is not ideal in terms of validation age” essentially acknowledging the results could not be used to conclusively validate the accuracy of the ageing method. But Andrews et al. (2015)

concluded that for humphead wrasse “it is likely that growth zone counting is an accurate method” for age estimation of this species since sagittal otolith sections contained “clean and clearly visible series” of growth zones. For bumphead parrotfish, three of the five samples selected for age validation had estimated birth year- $\Delta^{14}\text{C}$  values that did occur during the rise period of the reference time series. However, two of the samples fell outside of the loess curve prediction intervals for the reference  $\Delta^{14}\text{C}$  time series possibly indicating that both fish may have been underage by a few years or that the otolith core samples analyzed for  $\Delta^{14}\text{C}$  were contaminated with otolith material that formed later in life. Andrews et al. [68] may not have fully accomplished the goal of validating the age estimation method, but the study demonstrated that bumphead parrotfish can attain ages in excess of 30 y and it also emphasized that it could be challenging to obtain parrotfish otolith core material, even with a computerized micromill system, because of otolith fracturing during the milling process.

The second study from the Pacific that applied  $\Delta^{14}\text{C}$  to validate age estimation in parrotfish species focused primarily on documenting growth rates and longevity for five species of parrotfishes that contributed to Hawaiian fisheries [19]. Two of the five species were evaluated for age validation via  $\Delta^{14}\text{C}$ : redlip parrotfish *Scarus rubroviolaceus* (n = 23 for  $\Delta^{14}\text{C}$ ) and spectacled parrotfish *Chlorurus perspicillatus* (n = 21 for  $\Delta^{14}\text{C}$ ). The estimated birth year- $\Delta^{14}\text{C}$  values from micromilled otolith cores for both species occurred during the  $\Delta^{14}\text{C}$  decline period (general time period illustrated in Figure 4) of the regional reference time series. DeMartini et al. [19] utilized ANCOVA to evaluate if the overall linear trends of the two species differed significantly from the  $\Delta^{14}\text{C}$  reference decline trend and since no significant results were indicated, the study concluded that the ageing method used for all five parrotfish species provided accurate age estimates. Both redlip parrotfish and spectacled parrotfish had maximum ages of 19 y. From the combined results of our study and DeMartini et al. [19], enumeration of growth increments in sagittal otolith sections from Pacific and West Atlantic parrotfish species appear to provide accurate age estimates.

### **Observed trends for stoplight parrotfish and queen parrotfish**

The oldest stoplight parrotfish from the eye lens validation samples was 14 y. Using the validated ageing method in this study, we have documented several stoplight parrotfish from the U.S. Caribbean collected prior to 2018 (which was the year we started saving eyes for age estimation validation) that were older than 14 y including an initial color phase female (319 mm FL) collected from STX with an age of 20 y. Van Rooij and Videler [69] utilized repeated visual censuses that included marked fish to estimate growth and mortality rates for stoplight parrotfish across multiple monitoring sites in waters of Bonaire. Results from that study indicated that 10% of stoplight parrotfish >250 mm FL attained an estimated age of 17 y and greater with an estimated life span of over 25 y [69]. A maximum age of 20 y for stoplight parrotfish from the U.S. Caribbean seems to further support the work of Van Rooij and Videler [69] in that the species can attain a maximum age of two decades or more. Also, since the oldest stoplight parrotfish documented in our efforts was an initial phase female suggests that some presumed gonochoristic-like females may occur in the U.S. Caribbean population of this species. The oldest queen parrotfish analyzed in the current study for  $\Delta^{14}\text{C}$  was a terminal color phase male with an age of 16 y. Interestingly, the oldest queen parrotfish we have aged using the validated ageing method from this study was a 21 y old terminal color phase male (410 mm FL) from Bermuda. A maximum age of 21 y for queen parrotfish is similar to the maximum age of 20 y noted for this species in Comoros-Raynal et al. [70].

Sexual identities associated with parrotfish color phases include: female initial phase, primary male initial phase, secondary male initial phase, primary male terminal phase, secondary male terminal phase, and sexually transitioning initial color phase. Robertson and Warner [13] speculated that primary males and large initial phase males may channel more energy into growth in early life; differences in growth rates may exist among the various sexual identities within a population, but ultimately this can only be verified with size-at-age information combined with gonad histological analysis [20]. For many parrotfish species in the Caribbean, male sexual identities are associated with particular mating behaviors and reproductive strategies. For example, males in the terminal color phase from stoplight, redband, yellowtail, princess, striped, and queen parrotfish species are mostly territorial, form harems, and utilize pair spawning [13, 17, 71-73]. Initial color phase males do not appear to hold territories, but rather employ several spawning behaviors and mating strategies that relate to interfering with pair spawning of territorial males and also display group spawning [13, 74-76]. These divergent male strategies may correlate with differences in growth rates due to variations in energetic investment towards reproductive output versus somatic growth. With validated ageing methods combined with histological analysis of gonads, we can further evaluate these complex parrotfish sexual ontogenies and document sex-specific growth patterns. Moreover, the age distribution of reproductive effort in parrotfishes is not well understood, but could be examined more fully, which will ultimately aid in evaluating population health of individual species and in employing management strategies geared towards sustainable fisheries practices in the U.S. Caribbean and elsewhere. Analyses of population age structure, growth, and reproductive biology for each of the seven U.S. Caribbean parrotfish species sampled as part of our on-going collaborative life history research program is underway and will ultimately provide a more detailed understanding of species-specific differences in growth related to sexual identities and reproductive strategies.

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