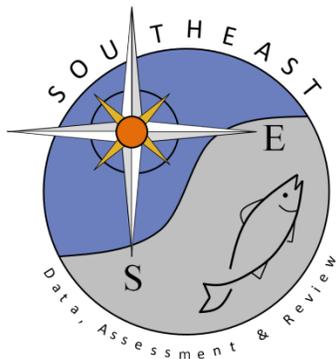


Age and growth of bluespine unicornfish (*Naso unicornis*): a half-century life-span  
for a keystone browser, with a novel approach to bomb radiocarbon dating  
in the Hawaiian Islands

Allen H. Andrews, Edward E. DeMartini, Jeff A. Eble, Brett M. Taylor, Dong Chun Lou, and  
Robert L. Humphreys

SEDAR50-RD24

18 July 2016



# Age and growth of bluespine unicornfish (*Naso unicornis*): a half-century life-span for a keystone browser, with a novel approach to bomb radiocarbon dating in the Hawaiian Islands

Allen H. Andrews, Edward E. DeMartini, Jeff A. Eble, Brett M. Taylor, Dong Chun Lou, and Robert L. Humphreys

**Abstract:** Bluespine unicornfish (*Naso unicornis*) from Hawaii were aged to >50 years using cross-sectioned sagittal otoliths. Fish length was a poor indicator of age because of rapid and variable early growth, exemplified by fish aged to be 4 years near maximum length. Growth was deterministic with adult ages decoupled from body length. Otolith mass and thickness were evaluated as proxies for age and both were encouraging; thickness explained more variance but mass was easier to measure. An age estimation protocol was validated through ontogeny using bomb radiocarbon ( $^{14}\text{C}$ ) dating. Use of the postbomb  $^{14}\text{C}$  decline period from a regional reference chronology enabled age validation of young fish — a novel approach for the Pacific Ocean. A probabilistic procedure for assigning bomb  $^{14}\text{C}$  dates (CALIBomb) was used for the first time to determine fish birth years. The age-reading protocol was generally validated, and it was possible to describe length-at-age despite difficulties in counting otolith annuli beyond 30–40 years. Growth curves differed between the sexes, and a four-parameter generalized von Bertalanffy growth function provided the best fit.

**Résumé :** Il a été établi, à la lumière de coupes d'otolithes sagittaux, que les nasons à éperons bleus (*Naso unicornis*) d'Hawaii atteignent plus de 50 ans d'âge. La longueur des poissons constitue un mauvais indicateur de l'âge en raison d'une croissance rapide et variable tôt durant la vie, comme en témoignent des poissons dont l'âge a été établi à 4 ans, mais dont la longueur s'approche de la longueur maximum. La croissance est déterministe, l'âge des adultes n'étant pas associé à la longueur du corps. L'utilisation du poids et de l'épaisseur des otolithes comme variables substitutives de l'âge a été évaluée, et les deux variables se sont avérées prometteuses; l'épaisseur explique mieux la variance, mais le poids est plus facile à mesurer. La longueur selon l'âge a été validée au long de l'ontogenèse par la datation au radiocarbone ( $^{14}\text{C}$ ) issu de bombes. L'utilisation de la période de baisse du  $^{14}\text{C}$  après les explosions tirée d'une chronologie de référence régionale a permis de valider les âges de jeunes poissons, ce qui constitue une nouvelle approche pour l'océan Pacifique. Une procédure probabiliste d'affectation des âges au  $^{14}\text{C}$  issu de bombes (CALIBomb) a été employée pour la première fois dans le but de déterminer l'année de naissance des poissons. Le protocole de lecture de l'âge a été validé de manière générale, et il a été possible de décrire la longueur selon l'âge malgré les difficultés que présente le dénombrement des zones de croissance d'otolithes au-delà de 30–40 ans. Les courbes de croissance varient selon le sexe, et une fonction de croissance de von Bertalanffy généralisée à quatre paramètres produit le meilleur ajustement. [Traduit par la Rédaction]

## Introduction

Bluespine unicornfish (*Naso unicornis*), also known as “kala” in Hawaiian, is a large-bodied herbivore that plays a key role in structuring the algal assemblage of nearshore benthic communities on tropical coral reefs and functions as a principal species among browsing fishes (Bellwood et al. 2003; Hoey and Bellwood 2009; Edwards et al. 2014). It is a mostly inshore species that regularly moves into very shallow water on reefs to access fleshy macroalgae, such as *Sargassum* spp. (Randall 2001). Bluespine unicornfish has widespread importance economically and culturally as an extracted resource throughout the tropical Pacific (Houk et al. 2012), and its commercial landings in Hawaii have increased dramatically since 2010 (Division of Aquatic Resources 2013). There is no clear relationship between the abundance of bluespine unicornfish and fishing pressure or other factors related to human

population density in Hawaii and elsewhere in the tropical central and western Pacific Ocean (Williams et al. 2008, 2011). However, a recent evaluation of the stock status of 19 resource species has flagged bluespine unicornfish as one of six species most likely to be experiencing overfishing in Hawaii (Nadon et al. 2015). The latter study used length-based methods and provided an important preliminary stock status for major fishery species; however, stock assessments should be age-based to the extent that data on size- and age-at-maturity, growth rate, and longevity are available and suitable. Body lengths at sexual maturity have been described for bluespine unicornfish in Hawaii (DeMartini et al. 2014) and at Guam and Pohnpei in Micronesia (Taylor et al. 2014). While length-at-age data exist for this species on the Great Barrier Reef of Australia (Choat and Axe 1996) and in Micronesia (Taylor et al. 2014), with preliminary estimates of growth in Hawaii (Eble et al.

Received 13 January 2016. Accepted 31 March 2016.

A.H. Andrews, E.E. DeMartini, and R.L. Humphreys. NOAA Fisheries – Pacific Islands Fisheries Science Center, 1845 Wasp Boulevard, Honolulu, HI 96818, USA.

J.A. Eble. University of West Florida, Center for Environmental Diagnostics and Bioremediation, 11000 University Parkway, Pensacola, FL 32514, USA.

B.M. Taylor. Joint Institute of Marine and Atmospheric Research, School of Ocean and Atmospheric Sciences, University of Hawaii, Honolulu, HI 96822, USA.

D.C. Lou. College of Marine & Environmental Sciences, James Cook University, Townsville, Queensland 4811, Australia.

**Corresponding author:** Allen H. Andrews (email: [allen.andrews@noaa.gov](mailto:allen.andrews@noaa.gov)).

Copyright remains with the author(s) or their institution(s). Permission for reuse (free in most cases) can be obtained from [RightsLink](https://www.copyright.com).

2009), the age and growth of this species have not been validated anywhere.

The age of some tropical reef fishes can be estimated using visual growth zone counts in cross-sectioned otoliths (ear stones) despite difficulties that can be encountered for fishes of aseasonal environments at equatorial latitudes (Fowler 2009). Age validation, although it may be unnecessary for all fish populations (Choat et al. 2009), is still needed when first describing growth or when no validation otherwise exists for that or related species. Validating the age of fishes with small otoliths can be complicated or impossible because of growth-related or technological limitations, but recent developments in bomb radiocarbon ( $^{14}\text{C}$ ) dating have led to new opportunities to validate the age of tropical reef fishes (Andrews et al. 2015).

Bomb  $^{14}\text{C}$  dating is an effective tool for gaining an understanding of life history traits for tropical marine fishes (Kalish 1993; Andrews et al. 2011). The approach relies on the time specificity of the bomb-produced  $^{14}\text{C}$  signal — typically reported as  $\Delta^{14}\text{C}$  or  $\text{F}^{14}\text{C}$  in reference to a pre-nuclear standard (Stuiver and Polach 1977; Reimer et al. 2004) — created by atmospheric testing of thermonuclear devices in the 1950s and 1960s (Broecker and Peng 1982). Correlation of  $^{14}\text{C}$  measurements from otolith carbonate with regional hermatypic coral  $^{14}\text{C}$  records can provide estimates of age that are independent of other traditional forms of age estimation (i.e., otolith growth zone counting). A case study of Hawaiian pink snapper (*Pristipomoides filamentosus*), or “opakapaka” in Hawaiian, was its inaugural application to fishes of Hawaii (Andrews et al. 2012). While the relatively large otoliths of opakapaka are representative of deep-water eteline snappers, many tropical reef fishes have otoliths that are much smaller and would provide a challenge for the usual protocol used for bomb  $^{14}\text{C}$  dating. To use this method, the otolith core (earliest growth) must be mechanically extracted by either grinding the exterior to the core or micromilling the core region and extracting it as powder, which usually results in sample masses of ~3–5 mg to ~10–20 mg (Kalish 1993; Andrews et al. 2012). Recent advances made in both micromilling technology and accelerator mass spectrometry have enabled more accurate core extraction and more precise measurement of  $^{14}\text{C}$  in smaller quantities of otolith carbonate (Andrews et al. 2015). In addition, bomb  $^{14}\text{C}$  dating recently progressed in an age validation study of young and recently collected fish in the Gulf of Mexico (Andrews et al. 2013). This novel approach used the postpeak  $^{14}\text{C}$  decline period (typically more recent than ~1980 in the tropical Northern Hemisphere) and is also feasible for fish of the Hawaiian Islands based on observations presented in this study.

The specific goals of this study were to (i) estimate the age of bluespine unicornfish by counting growth zones visible in otolith sections, (ii) apply bomb  $^{14}\text{C}$  dating to the small otoliths of this species using the  $^{14}\text{C}$ Chrono method (CALIBomb) in determining probabilistic dates of formation for core samples relative to an existing coral  $^{14}\text{C}$  record, (iii) utilize the full coral bomb  $^{14}\text{C}$  record to provide validated estimates of age from largest to smallest (oldest to youngest) fish, and (iv) estimate comprehensive age and growth parameters from validated length-at-age data while exploring complications introduced by sexual dimorphisms (DeMartini et al. 2014, 2016) and considering ages predicted from otolith morphometrics and validated growth zone counts. These goals aim to thoroughly characterize bluespine unicornfish demographics in Hawaii for potential input to a formal stock assessment.

## Materials and methods

### Sample selection and age estimation

A total of 597 bluespine unicornfish were considered for age estimation, and a subset ( $n = 15$ ) was used for bomb radiocarbon age validation. Larger (LRG) fish that were older than young-of-year (YOY) fish ( $n = 555$ ) were collected in haphazard fashion by either Eble et al. (2009) or provided by fishers and regional mar-

kets that sourced fish from off the windward (northeastern) coast and North Shore of Oahu in the main Hawaiian Islands (Fig. 1). These samples were complemented by an additional 42 YOY (32 “early” YOY: 2- and 4-month-olds collected in July and September 2014, plus 10 “late” YOY: 11-month-olds collected in May 2015) that were throw-netted from the leeward coast (southern shore) of Oahu by a cooperating State of Hawaii fisheries biologist. All fish specimens were fresh-measured using fork length (FL,  $\pm 0.1$  cm) and weighed using whole body mass (nearest gram). The sex of all LRG fish was identified in a prior study of sex-specific length at maturity (DeMartini et al. 2014); identical histology methods were unsuccessful at identifying the sex of late YOY. Sagittal otoliths were obtained by removing the brain case of fresh or frozen fish using either a mallet and butcher knife or hand saw (LRG fish) or scalpel (YOY), excising both otoliths under a dissecting microscope, cleaning them of attached tissues, and then washing and rinsing with 95% ethanol and air-drying them before storage in individually labeled vials. The dried otoliths were weighed ( $\pm 0.1$  mg) prior to preparation for age-reading.

Otoliths of all LRG fish were prepared and aged using the following series of steps. Whole otoliths were initially mounted on their edge to a glass microscope slide with Crystalbond and sanded to expose the focus (transverse orientation). The sanded otolith was then dismounted, its orientation reversed, and remounted such that the sanded side was flat against the slide. The otolith was then further sanded to produce a thin, transverse cross-section (~200–300  $\mu\text{m}$ ). The cross-section was then covered in Crystalbond to reveal annuli and (or) polished using a progression of lapping film (9.0, 3.0, and 0.3  $\mu\text{m}$  grit) to expose daily growth increments (DGI). Otolith growth zone structure was interpreted and aged with a dissecting microscope and transmitted light at an average magnification of  $\times 20$  (Fig. 2).

Initial age-readings were conducted using the otoliths of 111 LRG fish collected from Oahu during 2009–2011 (Eble et al. 2009). This initial set of otoliths provided the baseline for further exploration and made the original discovery that the longevity of this species may exceed 50 years. These specimens were supplemented with otoliths from an additional 382 LRG fish collected during 2011–2014 and sourced from the same region. An age-reader experienced in tropical fish age estimation ultimately read all of the LRG otoliths, with some refinement to the age-reading protocol from age-validated otolith sections (presented later). Three independent and nonconsecutive age-readings were made for each otolith section, and the mean of three final reads was used in determining age and growth parameters.

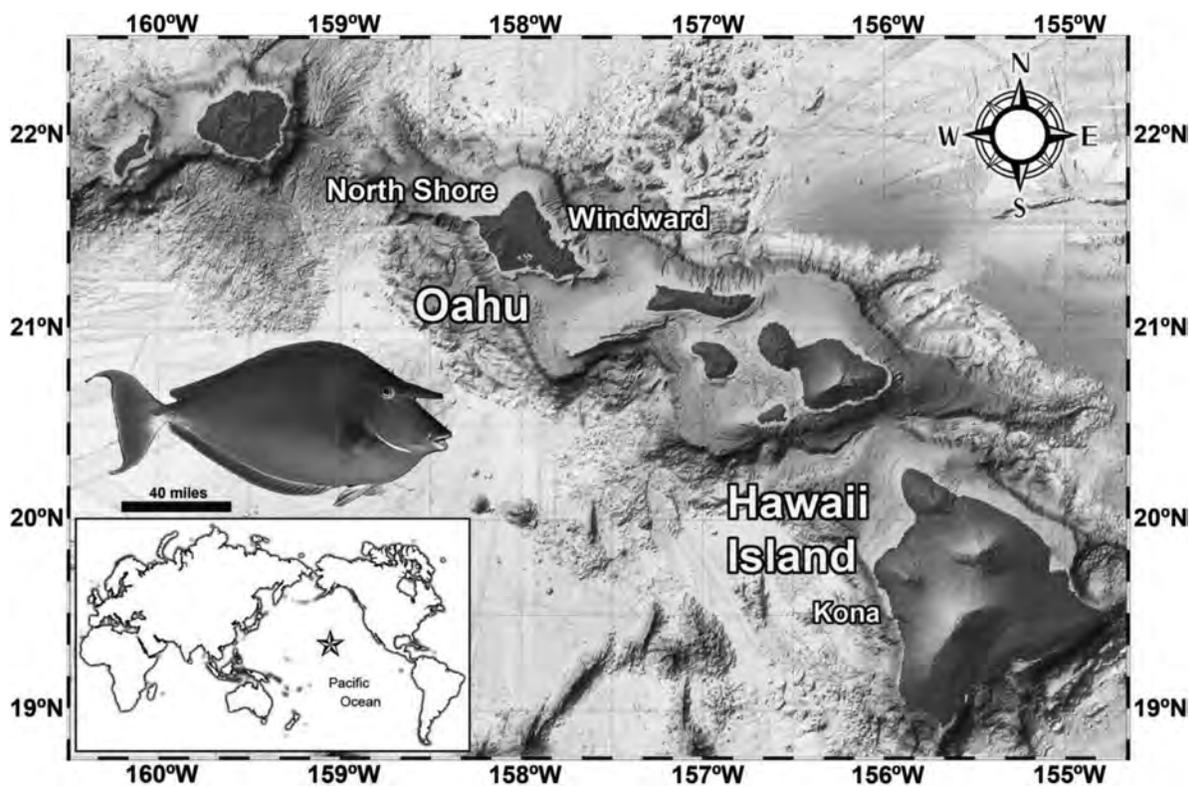
DGIs were investigated for the YOY specimens. Age in DGIs beyond the settlement check (Wilson and McCormick 1999) was estimated as the mean of three independent and nonconsecutive age-readings. A subset of these preparations was used to estimate mean and median age at formation of the settlement check (planktonic larval duration, PLD). Mean presettlement age was added to postsettlement age to estimate the total age of each specimen. Median length at settlement was back-calculated by direct proportion from length and age at capture.

The various age-reading results led to a total of 534 fish that were used in an age and growth analysis that included length-at-age estimates through ontogeny. In addition, approximately 50 otoliths from fish with incomplete collection data were included in the evaluation of otolith morphometrics.

### Bomb radiocarbon dating

Specimens were selected for bomb  $^{14}\text{C}$  dating from across the length distribution of collected fish and spanned the estimated age range (Table 1). Early bomb  $^{14}\text{C}$  investigations were based on the initial age estimates made by Eble et al. (2009). These findings were supplemented later with bomb  $^{14}\text{C}$  dating of otoliths from fish that filled in age or length gaps (Table 1). Selection of otoliths for the entire bomb  $^{14}\text{C}$  dating process was not random. Rather, it

**Fig. 1.** Regional map of the main Hawaiian Islands with an inset photograph of a bluespine unicornfish (*Naso unicornis*; photo credit: Richard Field). Location of the coral used as a  $\Delta^{14}\text{C}$  reference was Kona, Hawaii Island, and fish specimens were collected off the windward coast and North Shore of Oahu. Base bathymetric map was modified from a regional map available from the Pacific Islands Benthic Habitat Mapping Center, School of Ocean and Earth Science and Technology, University of Hawaii at Manoa. (Scale: 1 mile = 1.609 km.)



was more opportunistic and selective and had a focus on spanning length-at-age through ontogeny.

To apply bomb  $^{14}\text{C}$  dating to the whole otoliths of bluespine unicornfish, the dimensions and masses of several whole juvenile otoliths, coupled with observed dimensions of a clearly visible opaque region at the core, were used to delineate a targeted core region within adult otolith specimens. An otolith from a 12.0 cm FL juvenile measured 2.8 mm length (L)  $\times$  1.7 mm wide (W)  $\times$  0.5 mm thick (Th) and weighed 2.6 mg (Kala 05). This otolith provided clear guidance on creating a well-centered extraction design within these dimensions to minimize the possibility of extracting more recently formed material. Using a New Wave Research (ESI-NWR Division, Fremont, California) micromilling machine, a core extraction of 1.9 mm L  $\times$  1.1 mm W  $\times$  0.25 mm Th was achieved using a 23-point line scan with a 300  $\mu\text{m}$  Brasseler (Savannah, Georgia) bur in two successive and overlapping scans at a depth of 125  $\mu\text{m}$  for each pass. Extraction mass was near 0.8 mg of  $\text{CaCO}_3$ , which was  $\sim 8 \mu\text{mol C}$  and required small sample handling for  $^{14}\text{C}$  analysis (2–9  $\mu\text{mol C}$ ).

The extracted otolith samples were submitted as carbonate to the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS), Woods Hole Oceanographic Institution in Woods Hole, Massachusetts, for small-sample  $^{14}\text{C}$  analysis using accelerator mass spectrometry. Radiocarbon measurements were reported by NOSAMS as the fraction modern, the measured deviation of the  $^{14}\text{C}/^{12}\text{C}$  ratio from a “modern” sample. This reference is defined as 95% of the  $^{14}\text{C}$  concentration of the National Bureau of Standards oxalic acid I standard (SRM 4990B) normalized to  $\delta^{13}\text{C}_{\text{VPDB}}$  (–19‰) in 1950 AD (VPDB = Vienna Pee Dee Belemnite geological standard; Coplen 1996). Results were normalized to –25‰ for fractionation correction using a reported  $\delta^{13}\text{C}$  value and is reported here

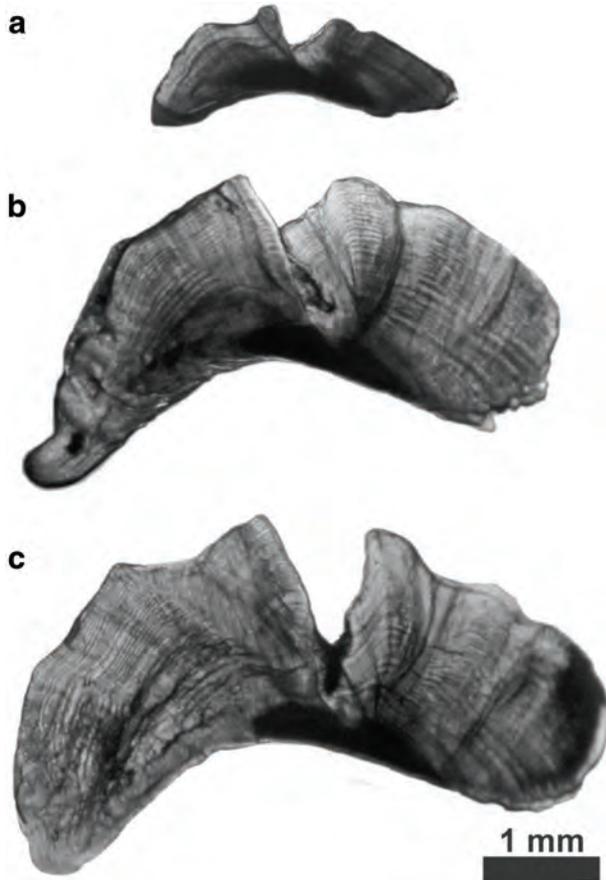
as  $F^{14}\text{C}$  (Reimer et al. 2004). Each  $F^{14}\text{C}$  value was ultimately corrected for age to the time of formation (birth year).

An existing coral  $^{14}\text{C}$  reference record from Kona, Hawaii Island, was selected for bomb  $^{14}\text{C}$  dating of the fish otoliths because of its proximity to the fish collections on Oahu (Fig. 1). The  $^{14}\text{C}$  reference series spans the bomb  $^{14}\text{C}$  chronology (1950s to 1990s; Roark et al. 2006) and was accessed through  $^{14}\text{Chrono}$  at Centre for Climate, the Environment, and Chronology (Queen’s University Belfast, United Kingdom) using the CALIBomb interface ([www.calib.qub.ac.uk/CALIBomb/](http://www.calib.qub.ac.uk/CALIBomb/)). The CALIBomb interface provided the opportunity to submit measured  $^{14}\text{C}$  values from otoliths that were corrected for fractionation ( $F^{14}\text{C}$ ) to be statistically analyzed in a moving-average probability fit to the coral core  $F^{14}\text{C}$  record, similar to the approach reported for calibration of  $^{14}\text{C}$  samples using an atmospheric  $F^{14}\text{C}$  reference record (Reimer et al. 2004). Because a well-defined peak ( $\sim 1970$  to 1980) is reached in the  $F^{14}\text{C}$  reference record from the Kona coral core, fish could be aged using not only the rise period ( $\sim 1955$  to 1970), but also the decline period (after  $\sim 1980$ ). In each case, a range of dates were determined by the CALIBomb analysis routine by projecting the measured  $F^{14}\text{C}$  values, with corresponding measurement uncertainty (1 or 2 standard deviations, SD), through time for an alignment with the  $F^{14}\text{C}$  reference (see Reimer et al. 2004). In addition, a smoothing function made the averaging effects of the sample formation period ( $\sim 0.5$  years of otolith growth) a factor in determining the year of formation (Fig. 3).

#### Otolith proxies for age

Both whole otolith mass and thickness (measured in the mounted otolith cross-section) were evaluated as potential proxies for age. Thickness was measured using a calibrated eyepiece micrometer

**Fig. 2.** Succession of otolith sections from young to old (*a–c*) bluespine unicornfish (*Naso unicornis*) from Hawaii showing the growth pattern used as an age-reading protocol in estimating age (*a* = 3 years, *b* = 20 years, *c* = 32 years). Note the well-defined zones with complicated interweaving that can lead to inaccurate (reader-biased) estimates. This complication increased markedly for fish approaching 50 years of age.



(one eyepiece unit = 25  $\mu\text{m}$  at  $\times 60$  magnification) and a compound microscope. Rank correlations among otolith mass, otolith thickness, decimal age (based on growth zone counts), and fish body size (length and mass) were first explored using Proc CORR in PC SAS version 9.3 (SAS Institute, Inc. 2006, 2012). Interrelationships among otolith metrics were next evaluated by simple linear regression (Proc REG). Seemingly good fits between growth zone ages and otolith mass and between age and otolith thickness suggested that either or both otolith metrics might be used to predict age in the absence of age data (e.g., Boehlert 1985). This possibility was further explored using stepwise (forward selection, backward elimination) multiple regression in Proc REG, with a probability of *F* to enter and be retained set at  $\leq 0.10$ , with ages regressed on otolith mass, as well as otolith thickness, as predictor variables. Age and both otolith metrics were log-transformed to linearize relationships. For this series of analyses only, growth zone counts for the 15 bomb  $^{14}\text{C}$  specimens were left uncorrected for validation to restrict interpretation to growth zone counting. Finally, estimates of otolith metric-adjusted ages, for comparison with empirical growth zone ages, were predicted by nonlinear regression using Proc NLIN. Preliminary model fits using full data inputs to these and other regressions described herein were trimmed for statistical outliers by deleting observations whose Studentized residuals exceeded an absolute value of 3.0. Because age-frequency distributions are the currency used in age-based fisheries stock assessments, the relative performance of the best otolith-adjusted

ages versus initial ages based solely on growth zone counts was evaluated by comparing the age-frequency distributions of the two series of age estimates by two-sample Kolmogorov–Smirnov (K-S) test (Siegel and Castellan 1988).

#### Age and growth analyses

The estimated ages of all LRG fish were first approximated based on growth zone counts for all otolith specimens with at least one growth zone (i.e., otoliths whose cross-section contained at least one full opaque and translucent zone). These age estimates were expressed in decimal years with reference to a birthdate of 1 June (DeMartini et al. 2014) versus date of fish collection. The ages of YOY specimens were estimated using DGI counts expressed in decimal years. Precision of the age estimates was evaluated by the mean coefficient of variation (CV; Campana 2001) among the sets of three nonconsecutive age-readings for YOY and LRG fish separately. Growth zone counting criteria were validated for LRG fish using the subset of otoliths that were aged with bomb  $^{14}\text{C}$  dating.

Length-at-age was initially described using the standard three-parameter von Bertalanffy growth function (VBGF):

$$L_t = L_{\text{INF}} \cdot \{1 - [e^{-k(t-t_0)}]\}$$

where  $L_t$  is length (FL, in cm) at age  $t$  (in years),  $L_{\text{INF}}$  is mean asymptotic length,  $t_0$  is estimated age at zero length, and  $k$  is the growth coefficient representing the rate at which asymptotic length is attained.

In addition, generalized (four-parameter: Richards 1959; five-parameter: Schnute and Richards 1990) versions of the VBGF were also evaluated. The four-parameter formulation evaluated was

$$L_t = L_{\text{INF}} \cdot \{1 - [e^{-K(1-m)(t-t_0)}]^{1/(1-m)}\}$$

where  $L_t$ ,  $L_{\text{INF}}$ , and  $t_0$  are the same as those of the standard VBGF,  $K$  is the growth coefficient representing the rate at which  $L_t$  is attained in the four-parameter model, and  $m$  is a parameter further controlling curvature (Richards 1959; see Tjorve and Tjorve 2010).

Specification of the final growth model was justified by examination of residuals (lower variation) and likelihood ratio (LR) test comparisons of model fits (Kimura 1980). The potential necessity of sex-specific models was also evaluated using LR tests, which were evaluated excluding unsexable YOY fish. All growth curves were modeled using SAS Proc NLIN.

Mean maximum body length ( $L_{\text{MAX}}$ ) was estimated by mean length of fish in the uppermost 10% of the length distribution of specimens. Longevity was approximated by mean maximum age ( $T_{\text{MAX}}$ ), defined as the mean age of fish in the uppermost 10% of the age distribution (Trip et al. 2008, 2014). These metrics were estimated separately for each by sex because of perceived sexual differences in growth rates (verified in Results). Mean and maximum lengths and ages of specimens were compared between the sexes by nonparametric median tests (Siegel and Castellan 1988).

## Results

### Bomb radiocarbon dating

The otoliths from 15 bluespine unicornfish were analyzed for  $^{14}\text{C}$  in the extracted core samples and used to provide a validated basis for age estimates (Table 1). The length of specimens used for  $^{14}\text{C}$  analysis ranged from a juvenile of 12.0 cm FL to a series of adults approaching maximum size up to 55.1 cm FL. All fish were collected in the years 2009 to 2012 with age estimates of 0.5 to 58 years and corresponding birth years of 1951 to 2008. Measured  $F^{14}\text{C}$  values ranged as predicted from near prebomb levels, through to peak values that were slightly above the coral  $F^{14}\text{C}$

**Table 1.** Fish collection data with extracted otolith sample mass and resultant radiocarbon and age data determined from otoliths of bluespine unicornfish (*Naso unicornis*).

Sample No. (WHOI No.)	Length (cm FL)	Collection year	Est. age (years)	Mass (mg)	F <sup>14</sup> C ± 2 SD	δ <sup>13</sup> C (‰)	Birth year	F <sup>14</sup> C age (years)	Birth year period
Kala 01 (OS-104354)	51.6	2009.47	32, 35	0.8	1.1677±0.0089*	-6.96	1968.8–1972.4 <sup>‡</sup>	38.8±1.8	Peak
Kala 02 (OS-104711)	50.5	2009.47	24, 24	0.8	1.1191±0.0055	NM	1985.6–1991.9	20.7±3.1	Decline
Kala 03 (OS-104709)	51.5	2009.46	20, 19	1.0	1.1102±0.0049	NM	1988.1–1992.2 <sup>§</sup>	19.3±2.0	Decline
Kala 04 (OS-107693)	32.3	2009.48	NA, 5	0.7	1.0466±0.0052*	-8.26	2005.7–2009.7	1.7±2.5	Decline–interpolate
Kala 05 (OS-104713)	12.0	2009.09	NA, 0.5	0.6	1.0435±0.0067*	-6.82	2008.5	0.5	Reference
Kala 06 (OS-104340)	51.6	2012.39	NA, 42	0.8	1.0289±0.0107	NM	1960.4–1962.1	51.1±0.9	Rise
Kala 07 (OS-104712)	51.8	2012.02	NA, 25	0.8	1.1105±0.0068*	-4.26	1987.6–1992.2 <sup>§</sup>	22.1±2.3	Decline
Kala 08 (OS-104705)	50.0	2012.02	NA, 17	0.9	1.0981±0.0048	NM	1992.1–1996.1	18.0±2.5	Decline–interpolate
Kala 09 (OS-107687)	46.5	2011.96	NA, 9	0.8	1.0622±0.0035	NM	2001.6–2005.6	8.4±2.5	Decline–interpolate
Kala 10 (OS-107681)	41.1	2011.25	NA, 5	0.8	1.0540±0.0033	NM	2003.8–2007.8	5.5±2.5	Decline–interpolate
Kala 11 (OS-84466)	52.8	2009.47	58, 48	1.1	1.0073±0.0055 <sup>†</sup>	NM	1959.6–1961.1	49.1±0.7	Rise
Kala 12 (OS-84461)	49.8	2009.46	51, 48	0.9	0.9627±0.0070 <sup>†</sup>	NM	1952–1958	54.5±3.0	Rise
Kala 13 (OS-84467)	55.1	2009.48	38, 37	1.1	1.1475±0.0145 <sup>†</sup>	NM	1966.3–1987.0	32.8±10.4	Near peak
Kala 14 (OS-84462)	52.2	2009.46	35, 37	0.7	1.1618±0.0089 <sup>†</sup>	NM	1967.6–1977.2	37.1±4.8	Peak
Kala 15 (OS-84463)	52.0	2009.47	35, 38	0.9	1.1742±0.0075*	-6.51	1970.4–1972.0 <sup>‡</sup>	38.2±0.8	Peak

**Note:** Estimated age is initial age-reading (Eble et al. 2009) followed by subsequent and finalized age-reading. NM, not measured; online accelerator mass spectrometry (AMS) measurement of δ<sup>13</sup>C was used for fractionation correction; no value reported. NA, not available.

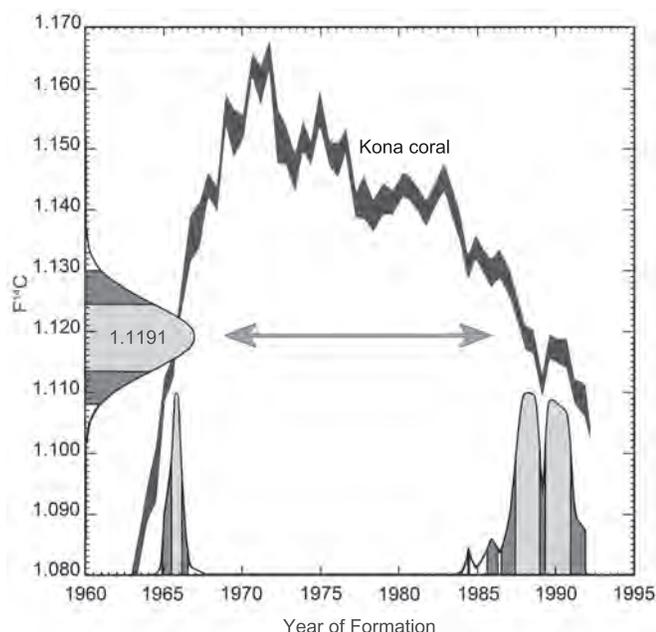
\*Post-AMS correction from sample-specific δ<sup>13</sup>C value provided.

<sup>†</sup>Not online δ<sup>13</sup>C corrected: post-AMS δ<sup>13</sup>C correction from mean of measured δ<sup>13</sup>C values (-6.56‰).

<sup>‡</sup>F<sup>14</sup>C ratio reduced to fall within peak maximum for the coral reference (F<sup>14</sup>C ~ 1.16) for CALIBomb year determination with lower 2 SD limit conserved.

<sup>§</sup>Most recent date estimated as the last value in the coral F<sup>14</sup>C reference data (1992.2).

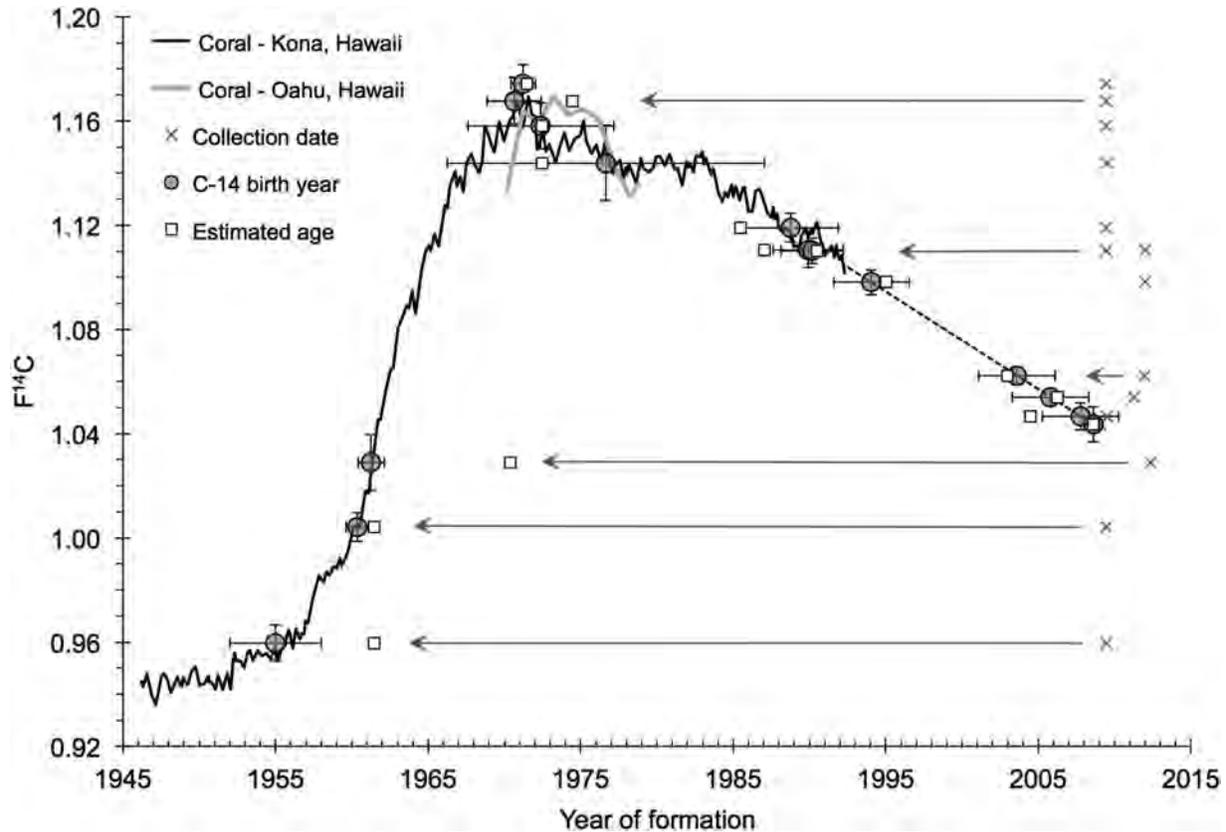
**Fig. 3.** Example of CALIBomb output for F<sup>14</sup>C data from one of the otolith samples analyzed in this study (Kala 02) of bluespine unicornfish (*Naso unicornis*) from Hawaii. The F<sup>14</sup>C reference data from a coral core at Kona, Hawaii Island (Fig. 1), shows the bomb-produced <sup>14</sup>C signal as a jagged distribution across time (Roark et al. 2006), with a smoothing function input of 0.5 years (compensation for the growth period of the otolith core extraction). Hence, the measured F<sup>14</sup>C value of 1.1191‰ ± 0.0055‰ is shown as a 1 SD and 2 SD peak on the y axis (light and dark shaded zones, respectively) that was projected to the F<sup>14</sup>C coral reference to obtain probable dates of formation (2 SD = 1964.9–1966.4 or 1985.6–1991.9; seen as irregular peaks along the x axis with corresponding 1 SD and 2 SD distributions, light and dark shaded zones, respectively). Based on other age-related information, the older age scenario was not considered valid, and the most probable date range was 1985.6–1991.9 (>99% of the young-age-scenario probability).



reference record, into the decline period, and ended with the F<sup>14</sup>C reference point established by a juvenile fish (Kala 05). The fish with greatest estimated age were both under- and overestimated relative to the dates provided by CALIBomb, whereas ages up to ~20 years tended to be relatively accurate based on the <sup>14</sup>C decline period (Fig. 4). Fish with intermediate ages and corresponding birth years near the peak period were less definitive owing to the inherent uncertainty of dates near peak levels. Validated ages range from near ~2–5 years for fish 30–40 cm FL to 54.5 ± 3.0 years for a fish 49.8 cm FL, with confirmation of rapid early growth to near maximum size in <10 years.

The potential dates of formation from CALIBomb for each sample had variable levels of precision that were dependent on the characteristics of the Kona coral F<sup>14</sup>C reference (Table 1). Highest precision was during the F<sup>14</sup>C rise period where age was within ±1 year. Second-best precision was on the postpeak decline period where age was resolved to within approximately ±2.5 years (range of years provided by CALIBomb). The lowest precision was from a few values near the F<sup>14</sup>C peak with uncertainty up to ±10 years. Two measurements exceeded the maximum F<sup>14</sup>C value in the Kona coral record, but the uncertainty for each measurement overlapped the coral record. Hence, the F<sup>14</sup>C values for these samples were reduced artificially to reside within the coral peak, while maintaining the lower limit of measurement uncertainty, to allow a CALIBomb date determination (Table 1). This adjustment functions strictly as a rough proxy for age and is not a robust age determination because of the inherent variability in peak F<sup>14</sup>C across the region (e.g., Andrews et al. 2016). Because the Kona F<sup>14</sup>C coral record ended in 1992 (year of coral core extraction), a continuation of the decline trend to the known-age juvenile otolith (Kala 05) was used to estimate birth year and age (Fig. 4). Uncertainty was estimated to be ±2.5 years based on the F<sup>14</sup>C decline variability stated above. The original age estimates from growth zone counting were also plotted with the coral F<sup>14</sup>C record and CALIBomb date ranges to show the extent of age-reader agreement (Fig. 4). Note that estimates of age for the oldest fish near 50 years, despite refinement of the age-reading protocol from reviewing the age-validated otolith sections, were more variable and typically underaged.

**Fig. 4.** Plot of the measured  $F^{14}C$  values from bluespine unicornfish (*Naso unicornis*) otolith cores relative to the Kona coral  $F^{14}C$  reference (Roark et al. 2006). Shown is the alignment of the most probable dates from CALIBomb ([www.calib.qub.ac.uk/CALIBomb/](http://www.calib.qub.ac.uk/CALIBomb/)) from the 2 SD uncertainties for the  $F^{14}C$  values (see Fig. 3 for example). Coral reference was extended beyond 1992 (coral core collection year) as a continuation of the  $F^{14}C$  decline to the known-age juvenile otolith (Kala 05). The growth zone counting results are plotted to highlight early agreement (decline period) and increased difficulty at ages approaching 50 years. The short  $F^{14}C$  record from an Oahu coral is presented here to illustrate potential variability of peak levels in the main Hawaiian Islands (Druffel 1987).



#### Otolith proxies for age

Both otolith mass and otolith thickness were strongly related to age estimated using growth zone counts (Figs. 5a, 5b) — not unexpected given the strong relation between otolith mass and thickness (refer to online supplementary material, Fig. S1<sup>1</sup>; Table S1<sup>1</sup>). Age was more strongly related to otolith thickness than otolith mass for females, but the opposite was true for males (Table S2<sup>1</sup>). After correction for bomb  $^{14}C$  validation, age was highly correlated with both otolith metrics despite the low sample size (Table S3<sup>1</sup>). Unlike the otolith metrics, fish body size (length, mass) was poorly related to age (Table S4<sup>1</sup>). Preliminary regression analyses relating fish size and the cross-product of otolith mass and thickness to age were not informative, and these variables were not subsequently considered. The age-to-otolith mass relationship differed between males and females (Fig. 5a), but that of age-to-otolith thickness did not (Fig. 5b; Table S5<sup>1</sup>) — likely because the three-dimensional shapes of otoliths differed between males and females of a given body size and otolith mass. Otolith metric-adjusted ages and initial growth zone count ages produced equivalent age-frequency distributions for females (Fig. 6a) and males (Fig. 6b; two-sample K-S tests, both  $p > 0.1$ ).

#### Age and growth analyses

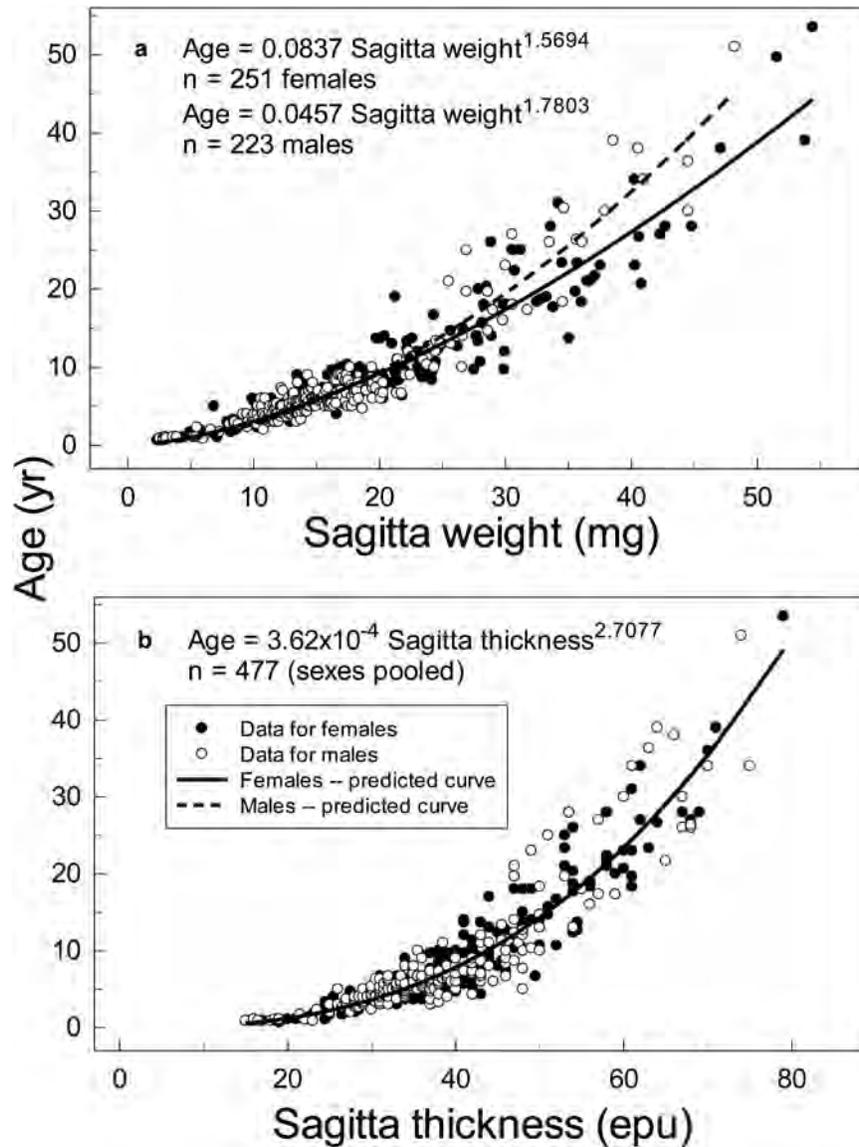
Otoliths of bluespine unicornfish were difficult to read, but ages could be estimated with acceptable accuracy and precision and were ultimately used for determining growth parameters.

Estimated ages spanned 1 to 58 years in the initial age-reading (Eble et al. 2009), but were later refined by the experienced age-reader to span 0 to 50 years overall. Because of the dampening effect of greater age on proportionate variation, the precision of age estimates overall was better when age was  $>30$  years ( $CV = 3.7\%$ ,  $n = 31$ ), relative to when age was  $<30$  years ( $CV = 7.2\%$ ,  $n = 513$ ;  $t$  test with Satterthwaite's approximation for unequal variances:  $t = -6.3$ ,  $P < 0.0001$ ). The mean  $CV$  for age-readings of DGIs in early and late YOY was  $4.5\%$  ( $n = 41$ ). Ages used for the growth curve fitting were from finalized growth zone counts for LRG fish, coupled with bomb  $^{14}C$  validated length-at-age data, plus aged YOY using a validated form of age estimation, DGI counting. Because of the valid ages determined from DGI counting, growth curves were well anchored in small fish at early ages. The median lengths and ages of the early YOY specimens collected (i) in July ( $n = 20$ ) were 7.3 cm FL at 87 days and (ii) in September ( $n = 11$ ) were 8.4 cm FL at 114 days, with a mean of 17 and 36 days of postsettlement, respectively. Overall median length-at-settlement was 5.9 cm at a median PLD of 71 days. For the late YOY specimens collected in May ( $n = 10$ ), the median length was 11.8 cm FL at 216 days.

The best growth curve fitted to all of the finalized length-at-age data described above was a four-parameter generalized VBGF model. An exploration of the five-parameter Schnute and Richards (1990) model, with extensive iterations, failed to converge on meaningful growth parameter estimates. The four-parameter model, along

<sup>1</sup>Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2016-0019>.

**Fig. 5.** Scatterplots and predicted fits of estimated age versus (a) otolith mass (mg) and (b) otolith cross-section thickness (epu, eyepiece unit) for female and male bluespine unicornfish (*Naso unicornis*) from Hawaii. Best model:  $Y = a \cdot X^b$  for both relationships. Note congruence of the female and male curves in panel b.



with the standard three-parameter model (to provide readily interpretable growth coefficient estimates), was applied to both sexes-pooled and sex-specific cases (Table 2). The growth curve was best described for each sex and both sexes pooled (LR tests; reject  $H_0$ ; three-parameter VBGF = four-parameter VBGF; all two-tailed  $p < 0.001$ ) by the four-parameter VBGF.

Growth curves differed overall between males and females (LR test; accept  $H_a$ : growth of males > females;  $\chi^2 = 5.24$ , 1 df, one-tailed  $p < 0.025$ ; Fig. 7). Approximate values for the  $k$  parameter (from the standard VBGF) were not disparate:  $0.46\text{-year}^{-1}$  for males versus  $0.43\text{-year}^{-1}$  for females (Table 2). Early growth rate appeared to be faster for males, but females on average attained a slightly larger maximum size ( $L_{INF} = 48.4$  cm FL) than males ( $L_{INF} = 47.8$  cm). Among all aged LRG fish, the median length of males (42.8 cm FL) was smaller than that of females (44.1 cm; median test:  $2 \times 2$  contingency  $\chi^2 = 3.02$ ,  $0.05 > p > 0.025$ ).  $L_{MAX}$  for females ( $53.3 \pm 0.4$  (standard error, SE) cm FL,  $n = 27$ ) was greater than that for males ( $51.9 \pm 0.2$  cm,  $n = 27$ ;  $t$  test:  $t = 3.14$ ,  $p < 0.01$ ). The respective median age of males (5.7 years) was younger than that of females (6.3 years; median test:  $\chi^2 = 8.0$ ,  $p < 0.005$ ).  $T_{MAX}$  esti-

mates for males ( $33.6 \pm 1.4$  (SE) years,  $n = 26$ ) and females ( $32.4 \pm 1.4$  years,  $n = 27$ ) were indistinguishable ( $t$  test:  $t = 1.59$ ,  $p = 0.56$ ).

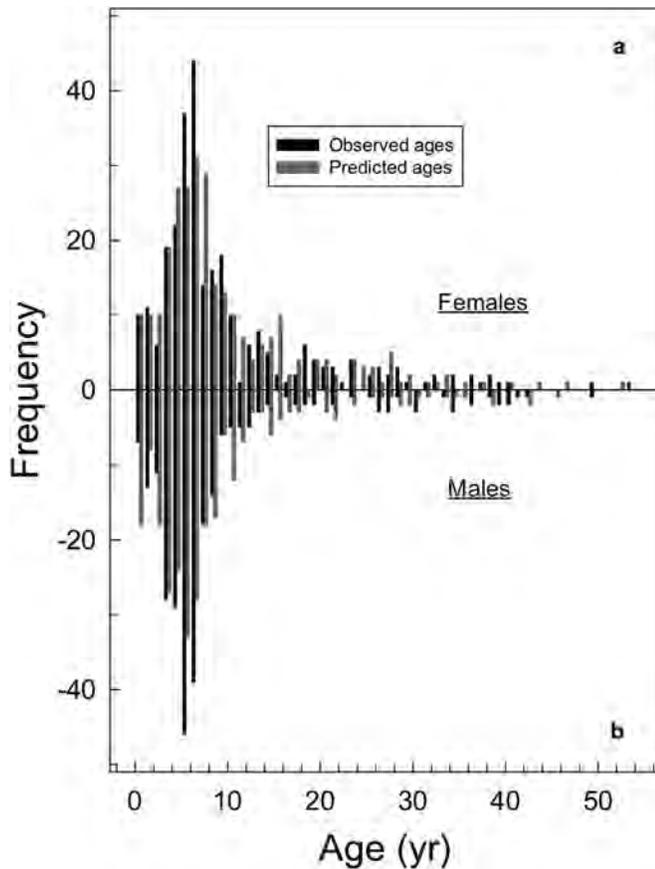
## Discussion

### Bomb radiocarbon dating

This study represents the first age validation for a surgeonfish using bomb  $^{14}\text{C}$  dating and has shown that bluespine unicornfish can live for half a century. These findings join many other such demonstrations for diverse groups of fishes, with consequent importance to fishery sustainability (e.g., Ewing et al. 2007; Cailliet and Andrews 2008; Andrews et al. 2012). To date, only two other studies have validated length-at-age relationships for acanthurids. These were based on field mark-recaptures of oxytetracycline-labeled fish of four species in three genera (not including *Naso* spp.) on Great Barrier Reef of Australia (Choat and Axe 1996) and mark-recaptures of similarly characterized *Naso lituratus* at Guam (Taylor et al. 2014).

The utility of bomb radiocarbon dating was expanded in this study to more recent birth years by using the decline period,

**Fig. 6.** Plotted frequency distributions of estimated ages for bluespine unicornfish (*Naso unicornis*) as determined directly from otolith growth zone counts (solid bars) with ages predicted from a regression relating growth zone age to both otolith mass and otolith cross-section thickness (open bars) for (a) female (above x axis) and (b) male (below x axis).



which demonstrates that younger and more recently collected fish can be age-validated in the Hawaiian Islands. This is similar to the work performed on speckled hind (*Epinephelus drummondhayi*) in the Gulf of Mexico (Andrews et al. 2013), but use of the decline period was more extensive in the present study. Seven fish were aged from near 2–5 years to 20–22 years with good precision ( $\pm 2.5$  years). Use of the decline rate to age fish with birth years more recent than the Kona coral collection year was supported by the known age otolith from a recent recruit aged at  $\sim 0.5$  years (Kala 05). This monotonic decline has also been documented for  $^{14}\text{C}$  in dissolved inorganic carbon of the mixed surface layer of the north Central Pacific and is consistent with the interpolated relationship presented in the present study (e.g., Druffel et al. 2008; Andrews et al. 2016). Ages estimated for bluespine unicornfish were within the margin of error or slightly outside when not in full agreement. Hence, the age estimation protocol used for bluespine unicornfish was accurate up to ages of at least 20 years based on the close correlation with the coral  $F^{14}\text{C}$  decline. While the margin of age error for estimates based on the coral reference is greater on the decline, the utility of describing successively older specimens that fit this trend lends support to accurate age determination of the oldest fish from  $^{14}\text{C}$  levels measured in otolith core material. In addition, this information indicates that the validated ages from bomb  $^{14}\text{C}$  dating provide valid length-at-age information that is independent of growth zone derived age estimates, as was the case for opakapaka and speckled hind (Andrews et al. 2012, 2013).

The independence of bomb  $^{14}\text{C}$  dating from growth zone counting becomes apparent when considering two of the three oldest bluespine unicornfish. The  $^{14}\text{C}$  values measured for these fish were the most diagnostic in terms of validated age resolution because of the rapid  $F^{14}\text{C}$  rise. Two of these fish were aged to 49 and 51 years with an uncertainty of less than 1 year. While this conclusion relies on the assumptions that (i) the  $^{14}\text{C}$  in otolith core material is in temporal agreement with the coral record and (ii) the coral chronometer is a validated time series, there is evidence the uncertainty would not be much greater. As stated previously from observations of the decline period otoliths, the temporal correlation of otolith material with coral material is supported from those age-related observations, but is limited to the precision provided by the birth year calibrations ( $\pm 2.5$  years). At present, the validity of the Kona coral record remains unresolved and is currently based on band counting interpretation of the coral core. In either case, a temporal offset of more than 2–3 years is unlikely and is further supported by coral  $F^{14}\text{C}$  records from French Frigate Shoals and Kona, Hawaii (Druffel 1987; Druffel et al. 2001; Andrews et al. 2016), which is far lower than the uncertainty demonstrated for otolith age-reading of the oldest fish.

The least diagnostic ages from bomb  $^{14}\text{C}$  dating were from near prebomb levels and the peak period because of leveling of  $F^{14}\text{C}$  over time. The oldest fish in this study was close to prebomb levels, but CALIBomb provided an opportunity to objectively estimate the validated birth years. In previous studies, a Loess curve fit provided a centralized distribution with random levels of variability around the weighted mean, leading to use of calculated prediction intervals to describe the uncertainty in birth year and consequently age. Prediction intervals were less conservative than those using the CALIBomb approach because they included the greatest variability while smoothing over regions of reduced variability. Hence, birth year determinations were likely to be less precise than they could have been and near prebomb fish could not be aged, except to a minimum age (i.e., older than the earliest alignment of the measured  $F^{14}\text{C}$  value). Use of a probability distribution with CALIBomb was better because it took into consideration the fine-scale variability of the  $F^{14}\text{C}$  reference for a given value, leading to well-discriminated birth years. Hence, when the  $F^{14}\text{C}$  value for the oldest fish was considered relative to the coral reference, minor variations in prebomb levels created an opportunity to age this fish by finding it improbable that this fish had a birth year prior to 1952 and that the most likely age was  $54.5 \pm 3.0$  years.

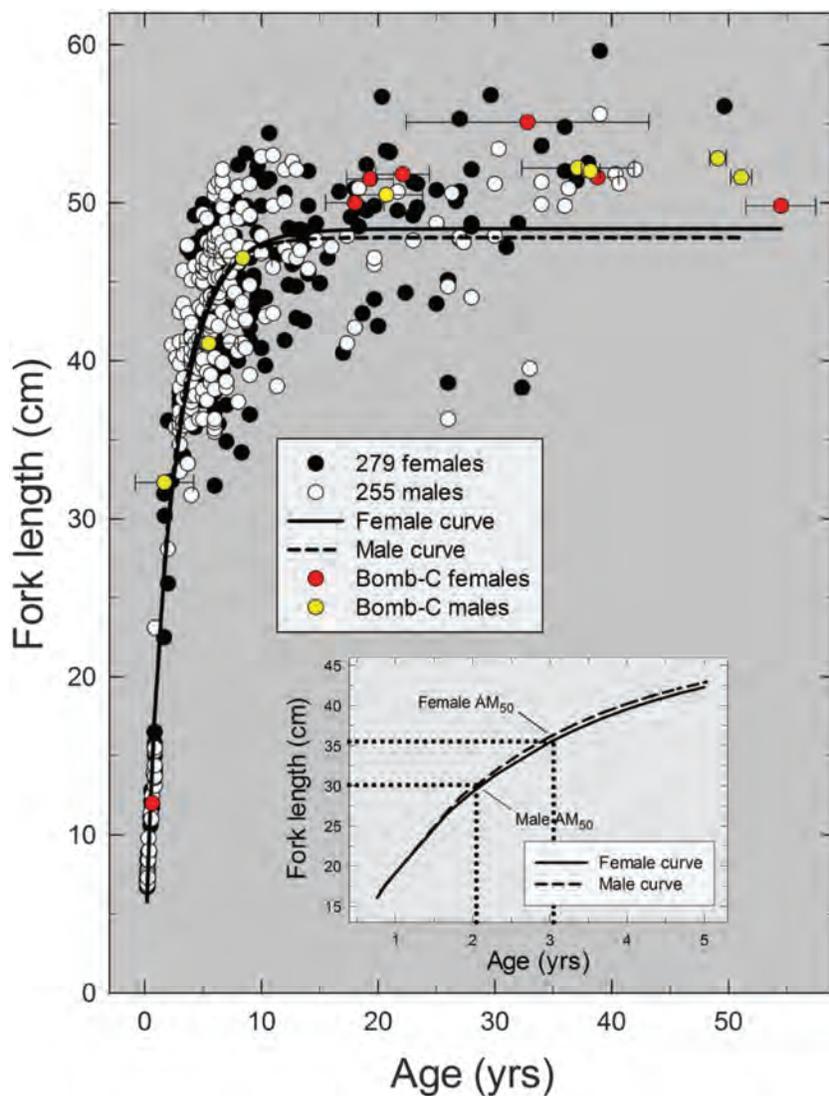
Four fish otoliths provided  $^{14}\text{C}$  measurements that were the least diagnostic because birth years were in the peak period of the  $F^{14}\text{C}$  coral reference. One  $F^{14}\text{C}$  measurement crossed the widest part of the plateau and also happened to have the lowest  $F^{14}\text{C}$  measurement precision (Kala 13). These circumstances led to what could be the lowest possible birth year precision from a measured  $F^{14}\text{C}$  value, an age of  $\sim 33$  years with an uncertainty span of 20 years. Use of CALIBomb under these circumstances provided an objective way to age this specimen when use of other more subjective methods may have been quite confusing. However, this sample was not considered further in other analyses because of its poor temporal resolution. Three other specimens were closer to the peak and could be constrained to a narrower level of uncertainty, but measured  $F^{14}\text{C}$  values for two specimens exceeded the maximum  $F^{14}\text{C}$  value in the coral reference. Because the  $F^{14}\text{C}$  measurement error encompassed the peak levels, each measurement was artificially reduced to  $F^{14}\text{C} = 1.16$ , while maintaining the lower limit of measurement error, to allow a determination of birth year from the CALIBomb analysis routine. This assumes that  $F^{14}\text{C}$  levels during this peak period were not variable across the region, but one fish was apparently aged a few years younger than could be accounted for by the calculated CALIBomb birth years. Because the ocean circulation around the Hawaiian Islands is a mix of

**Table 2.** Summary statistics for nonlinear regressions relating fork length (FL, cm) to age.

A. Three-parameter VBGF: $L_t = L_{INF} \cdot \{1 - [e^{-k(t-t_0)}]\}$												
Sex	$L_{INF}$	$SE_{LINF}$	$k$	$SE_k$	$t_0$	$SE_{t_0}$	$R^2$	$F$	Prob >F	$n$		
Female	48.0	0.4	0.43	0.02	-0.14	0.06	0.89	1121	<0.0001	279		
Male	47.6	0.4	0.46	0.02	-0.11	0.05	0.90	1086	<0.0001	255		
Pooled	47.8	0.3	0.44	0.02	-0.12	0.04	0.89	2213	<0.0001	534		
B. Four-parameter VBGF: $L_t = L_{INF} \cdot \{1 - [e^{-K(1-m)(t-t_0)}]^{1/(1-m)}\}$												
Sex	$L_{INF}$	$SE_{LINF}$	$K$	$SE_K$	$t_0$	$SE_{t_0}$	$m$	$SE_m$	$R^2$	$F$	Prob >F	$n$
Female	48.4	0.5	0.49	0.04	0.05	0.10	0.26	0.12	0.99	8033	<0.0001	279
Male	47.8	0.5	0.48	0.06	-0.02	0.14	0.14	0.19	0.99	7821	<0.0001	255
Pooled	48.1	0.3	0.49	0.06	0.02	0.08	0.20	0.11	0.99	15907	<0.0001	534

**Note:** Input to both models A and B included 41 young of year (YOY) (unsexable by histology, sex assigned randomly) as well as LRG fish. Models were fitted by maximum likelihood using Proc NLIN in PC SAS version 9.3. VBGF, von Bertalanffy growth function.

**Fig. 7.** Modeled length-at-age curves for bluespine unicornfish (*Naso unicornis*) from Hawaii, with age-reader estimates and bomb  $^{14}C$  ages (with 99% confidence limits), using a four-parameter generalized von Bertalanffy growth function. Input includes both unsexed YOY and LRG fish of both sexes. Data and curve for females are indicated by solid circles and solid line and those for males by open circles and dashed line. The inset of zoomed growth curves illustrates estimated ages at median sexual maturity ( $AM_{50}$ ) for females and males with reference to the respective body lengths at median maturity (35.5 cm FL for females, 30.1 cm FL for males; DeMartini et al. 2014).



sources from the North Pacific Gyre and the North Equatorial Current, it is likely that the CALIBomb birth year range was underestimated because the analysis was limited to one coral  $F^{14}C$  record. A short  $F^{14}C$  coral record from Oahu provides evidence

that peak  $F^{14}C$  levels were greater for a longer period (Druffel 1987; Fig. 4). This can be explained by the influence of equatorial waters that were  $^{14}C$ -depleted owing to  $CO_2$  saturation states and would likely result in minor variations in the  $F^{14}C$  signal across

the main Hawaiian Islands (Broecker and Peng 1982; Andrews et al. 2016). The additional  $F^{14}C$  data were not incorporated in CALIBomb at the time of analyses, but could explain the apparent underestimation of age from otoliths whose growth zones may have been accurately counted.

### Otolith proxies for age

Less costly alternatives to direct age-reading of otoliths are in great need for data-poor tropical fish fisheries. Compared with temperate zone fishes, few coral reef fishes have been aged because of a lack of local funding, laboratory expertise, and equipment availability (Choat et al. 2009). Similarly, few studies of reef fishes have explored the use of otolith metrics to predict age, with otolith mass the most frequently used (Worthington et al. 1995; Lou et al. 2005) and other metrics evaluated to a lesser extent (e.g., otolith thickness; Choat and Axe 1996). In a study on gray angelfish (*Pomacanthus arcuatus*) in the Florida Keys, otolith cross-section thickness had particularly encouraging results (Steward et al. 2009); however, there has not been a routine application of this relatively simple technique.

Although otolith mass is easier to measure and may be a satisfactory proxy for age, otolith cross-section thickness explained more variance in age for bluespine unicornfish than otolith mass, but was more costly to obtain. The final choice of whether to age by proxy versus direct age estimation and choosing between alternative proxies should be based on a rigorous cost–benefit analysis (Francis and Campana 2004). Such was beyond the scope of the present study, but in this case the cost savings in using otolith mass would likely surpass the observed slight increase in predictive power using otolith thickness. Age proxies should be routinely considered when age-reading tropical reef fishes with the following caveat: because otolith metric-to-age relationships often differ with growth rate, the relationship between an otolith proxy and age likely differs among geographic populations that differ in growth rates or within a population whose growth rates vary over time. Hence, the relationship between an otolith metric and age needs to be recalibrated when studying different geographic populations of a species or when temporal changes in growth rate are likely for a given population (Pilling et al. 2003; Francis and Campana 2004; Lou et al. 2005).

### Age and growth analyses

The initial age-reading represents a first-look for some of the LRG bluespine unicornfish specimens for this species and were instrumental in recognizing the potential utility of bomb  $^{14}C$  dating. Development of an age-reading protocol leading from the initial estimates by adding more specimens, coupled with refinement of the age-reading protocol from age-validated otoliths, led to a series of length-at-age estimates spanning most of the length and age distributions. However, it is clear that distinguishing early-growth annual growth from among accessory growth checks that typically occur in the otoliths of reef fishes during the period of prematurational growth is important (Fowler 2009). In this study, the earliest growth was quantified with validated DGI counting, which was consistent with the length-at-age for the youngest growth zone counting. In addition, discrimination of the fine growth zone structure late in otolith formation can also require considerable effort. For bluespine unicornfish, the age-reading protocol may need further investigation for larger and older individuals. Section preparation or viewing methods may need to be altered for the oldest sections, such as thinner versus thicker sections and use of transmitted versus reflected lighting. The rarity of very old fish complicates matters.

It has been stated that the best of all measures of age-reading precision is the CV, and published studies for fishes with moderate longevity (decades) and otoliths of average difficulty had an observed overall mean CV of 7.6% (Campana 2001). Hence, the CV of 7.0% attained for LRG fish in this study was reasonably good.

Based on the growth-zone-derived age estimates for the age-validated otoliths of bluespine unicornfish, age-reading of the oldest fish provided the greatest challenge for accuracy.

Bluespine unicornfish in the Hawaiian Islands grow rapidly and are long-lived. The growth rate is initially high and then slows shortly before, and continues subsequent to, sexual maturation, after which there is a prolonged period of slow growth. The curve exhibited by this species is typical for acanthurids by having a flat-topped or “square” functional growth form (Choat and Axe 1996; Hart and Russ 1996; Craig et al. 1997; Choat and Robertson 2002; Trip et al. 2008). Large fish of both sexes, ranging in length from 40 to 50 cm FL, exemplify this growth pattern by spanning ages of ~4 to ~50 years. This age and growth relationship was well described by a four-parameter VBGF, which effectively accommodated length-at-age through ontogeny (YOY to LRG fish). While many large and old fish whose ages were bomb  $^{14}C$ -validated had positive residuals, this is not unexpected because the selection of these specimens was not random, initially targeted fish with the oldest age estimates, and was focused on filling length or age discrepancies. Growth differed between females and males in typical fashion, with males exhibiting slower growth following an earlier maturity at smaller body size (DeMartini et al. 2014) and consequently a smaller average maximum size. The importance of sexual variation in bluespine unicornfish depends on the specific biological trait and its potential use in a stock assessment.

In the Hawaiian Islands, bluespine unicornfish mature at lengths of approximately 30 and 36 cm FL for males and females, respectively (DeMartini et al. 2014). This equates to median age-at-maturity near 2 years for males and near 3 years for females (Fig. 7 inset). It is not unusual for surgeonfishes to differ sexually in body size (Robertson 1985) or size at maturity (DeMartini et al. 2014), but bluespine unicornfish, unlike most other long-lived bony fishes (Froese and Binohlan 2000), mature at a relatively young age. This early-maturation trait is shared with many other acanthurids (Choat and Robertson 2002) and is similar to that of the long-lived banded morwong (*Cheilodactylus spectabilis*), an unrelated but also shallow-reef dwelling species of temperate waters, for which the ecological importance of this trait was first questioned (Ewing et al. 2007). Data are lacking with which to resolve this issue for bluespine unicornfish, but it is suggested that early maturity coupled with great longevity likely reflects uncertainty in reproductive success in any given year (Cailliet and Andrews 2008). Hence, it is reasonable that species with this type of life history would have highly episodic recruitment resulting from extremely variable early mortality (Doherty et al. 2004).

Bluespine surgeonfish in the Hawaiian Archipelago are at the far northeastern edge of their geographic distribution. Therefore, it is not surprising that the species matures at a larger size, attains greater maximum size, and lives longer in Hawaii than at lower latitudes near the center of its distribution. Robertson et al. (2005a, 2005b) and Trip et al. (2008) have described analogous patterns for confamilial surgeonfishes (*Acanthurus bahianus* and *Ctenochaetus striatus*) in the Atlantic Ocean and across the Pacific and Indian oceans, respectively. The phenomenon of larger body size is generally true for many organisms at their high latitude extremes (Atkinson and Sibly 1997). Estimated life history milestones of bluespine unicornfish in the western central Pacific empirically support this contention. In Micronesia, the species matures at ~27–30 cm FL when ~2–4 years old, maximum lengths are <50 cm FL, and observed maximum ages are considerably lower at 16 and 23 years for Guam and Pohnpei, respectively (Taylor et al. 2014). Whether such latitudinal differences include planktonic and early postsettlement growth is unresolved for bluespine unicornfish; however, the estimate of median body length at settlement from the plankton onto reefs at 5.9 cm with a median PLD of 71 days from the present study partly aligns with the values reported for the species at Moorea, French Polynesia (settlement at 4–5 cm after 75 days; Doherty et al. 2004).

It is important to close this section with a caveat on estimating mortality when a growth curve is square. It has been clearly demonstrated that the decoupling of size with increasing age in fishes with acanthurid-like growth greatly complicates, if not invalidates, the estimation of mortality rates for adults using standard VBGF-based techniques (Trip et al. 2014). Adult body size is attained so quickly that even a catch curve restricted to immature ages would not be informative.

In summary, this study provides important new information describing the demography of a functionally and economically important surgeonfish. It represents one of the few comprehensive studies of age and growth for any acanthurid and validates length-at-age estimates through ontogeny with longevity exceeding 50 years. Furthermore, this study represents the first application of bomb  $^{14}\text{C}$  validation using the decline period of an age reference chronology in the Pacific Ocean and the second such application anywhere. This effectively paves the way for future analogous applications to other species in the region. It also represents the first application of the CALIBomb interface for a probabilistic improvement to the resolution of bomb  $^{14}\text{C}$  age estimates for fishes. Lastly, it is a case study that demonstrates the potential of otolith metrics (mass and cross-section thickness) as proxies for age. Such relationships could be very useful for reef fishes, especially species for which age is decoupled from body length but whose otoliths continue to accrete material at large body sizes and old ages. The method should be more broadly evaluated and applied if growth rates are known or can be safely assumed to be temporally or spatially stable.

## Acknowledgements

Thank you to Toby Daly-Engel, Michelle Gaither, Randall Kosaki, Matt Ross, and Zoltan Szabo for assistance with collecting juvenile and adult specimens; Neal Hazama of the Department of Land and Natural Resources, Hawaii, for providing most of the YOY specimens; Jeff Sampaga for extracting otoliths from most fish specimens; Ron and Paula Reimer at  $^{14}\text{Chrono}$ , Belfast, Northern Ireland, for access to the CALIBomb interface for analysis of otolith  $^{14}\text{C}$  data; Tom Guilderson of Lawrence Livermore National Laboratory for permission to use the Kona coral  $\text{F}^{14}\text{C}$  data via the  $^{14}\text{Chrono}$  CALIBomb interface; and Joe O'Malley for a thorough manuscript review and assistance with evaluating the four-parameter VBGF. Funding was provided in part by the Hawaii Division of Aquatic Resources, Dingell-Johnson Sportfish Restoration award to Brian Bowen (University of Hawaii), and by the National Marine Fisheries Service Bio-Sampling Initiative.

## References

- Andrews, A.H., Kalish, J.M., Newman, S.J., and Johnston, J.M. 2011. Bomb radiocarbon dating of three important reef-fish species using Indo-Pacific  $\Delta^{14}\text{C}$  chronologies. *Mar. Freshw. Res.* **62**: 1259–1269. doi:10.1071/MF11080.
- Andrews, A.H., DeMartini, E.E., Brodziak, J., Nichols, R.S., and Humphreys, R.L. 2012. A long-lived life history for a tropical, deepwater snapper (*Pristipomoides filamentosus*): bomb radiocarbon and lead-radium dating as extensions of daily increment analyses in otoliths. *Can. J. Fish. Aquat. Sci.* **69**(11): 1850–1869. doi:10.1139/f2012-109.
- Andrews, A.H., Barnett, B.K., Allman, R.J., Moyer, R.P., and Trowbridge, H.D. 2013. Great longevity of speckled hind (*Epinephelus drummondhayi*), a deep-water grouper, with novel use of postbomb radiocarbon dating in the Gulf of Mexico. *Can. J. Fish. Aquat. Sci.* **70**(8): 1131–1140. doi:10.1139/cjfas-2012-0537.
- Andrews, A.H., Choat, J.H., Hamilton, R.J., and DeMartini, E.E. 2015. Refined bomb radiocarbon dating of two iconic fishes of the Great Barrier Reef. *Mar. Freshw. Res.* **66**: 305–316. doi:10.1071/MF14086.
- Andrews, A.H., Siciliano, D., Potts, D.C., DeMartini, E.E., and Covarrubias, S. 2016. Bomb radiocarbon and the Hawaiian Archipelago: Coral, otoliths, and seawater. Radiocarbon [online ahead of print]. doi:10.1017/RDC.2016.32.
- Atkinson, D., and Sibly, R.M. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends Ecol. Evol.* **12**: 235–239. PMID:21238056.
- Bellwood, D.R., Hoey, A.S., and Choat, J.H. 2003. Limited functional redundancy in high diversity ecosystems: resilience and ecosystem function on coral reefs. *Ecol. Lett.* **6**: 281–285. doi:10.1046/j.1461-0248.2003.00432.x.
- Boehlert, G. 1985. Using objective criteria and multiple regression models for age determination in fishes. *Fish. Bull.* **83**: 103–117.
- Broecker, W.S., and Peng, T.-H. 1982. Tracers in the sea. Lamont–Doherty Geological Observatory, Columbia University, Palisades, New York.
- Cailliet, G.M., and Andrews, A.H. 2008. Age-validated longevity of fishes: Its importance for sustainable fisheries. In *Fisheries for global welfare and environment*. Edited by K. Tsukamoto, T. Kawamura, T. Takeuchi, T.D. Beard, and M.J. Kaiser. 5th World Fisheries Congress 2008. TERRAPUB, Tokyo, Japan. pp. 103–120.
- Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish Biol.* **59**: 197–242. doi:10.1111/j.1095-8649.2001.tb00127.x.
- Choat, J.H., and Axe, L. 1996. Growth and longevity in acanthurid fishes: an analysis of otolith increments. *Mar. Ecol. Prog. Ser.* **134**: 15–26. doi:10.3354/meps134015.
- Choat, J.H., and Robertson, D.R. 2002. Age-based studies. In *Coral reef fishes: dynamics and diversity in a complex ecosystem*. Edited by P. Sale. Academic Press, San Diego, Calif. pp. 57–80.
- Choat, J.H., Kritzer, J.P., and Ackerman, J.L. 2009. Ageing in coral reef fishes: do we need to validate the periodicity of increment formation for every species of fish for which we collect age-based demographic data? In *Tropical fish otoliths: information for assessment, management and ecology*. Reviews: methods and technologies in fish biology and fisheries 11. Edited by B.S. Green, B.D. Mapstone, G. Carlos, and G.A. Begg. Springer, New York. pp. 23–54.
- Coplen, T.B. 1996. New guidelines for reporting stable hydrogen, carbon, and oxygen isotope-ratio data. *Geochim. Cosmochim. Acta.* **60**: 3359–3360. doi:10.1016/0016-7037(96)00263-3.
- Craig, P.C., Choat, J.H., Axe, L.M., and Saucerman, S. 1997. Population biology and harvest of the coral reef surgeonfish *Acanthurus lineatus* in American Samoa. *Fish. Bull.* **95**: 680–693.
- DeMartini, E.E. 2016. Sexual dimorphisms in the bluespine unicornfish, *Naso unicornis* (Acanthuridae): external metrics for movement ecology and life history. *Copeia*, **104**(2): 498–505.
- DeMartini, E.E., Langston, R.C., and Eble, J.A. 2014. Spawning seasonality and body sizes at sexual maturity in the bluespine unicornfish, *Naso unicornis* (Acanthuridae). *Ichthyol. Res.* **61**: 243–251. doi:10.1007/s10228-014-0393-z.
- Division of Aquatic Resources. 2013. Commercial marine landings summary trend report. Department of Land and Natural Resources, State of Hawaii, Honolulu, Hawaii.
- Doherty, P.J., Dufour, V., Galzin, R., Hixon, M.A., Meekan, M.G., and Planes, S. 2004. High mortality during settlement is a population bottleneck for a tropical surgeonfish. *Ecology*, **85**: 2422–2428. doi:10.1890/04-0366.
- Druffel, E.R.M. 1987. Bomb radiocarbon in the Pacific: annual and seasonal time-scale variations. *J. Mar. Res.* **45**: 667–698. doi:10.1357/00222408788326876.
- Druffel, E.R.M., Griffin, S., Guilderson, T.P., Kashgarian, M., Southon, J., and Schrag, D.P. 2001. Changes of subtropical north Pacific radiocarbon and correlation with climate variability. *Radiocarbon*, **43**: 15–25.
- Druffel, E.R.M., Bauer, J.E., Griffin, S., Beupré, S.R., and Hwang, J. 2008. Dissolved inorganic radiocarbon in the North Pacific Ocean and Sargasso Sea. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **55**: 451–459. doi:10.1016/j.dsr.2007.12.007.
- Eble, J.A., Langston, R., and Bowen, B.W. 2009. Growth and reproduction of Hawaiian Kala, *Naso unicornis*. Final Report prepared for the Division of Aquatic Resources, Department of Land and Natural Resources, Honolulu, Hawaii.
- Edwards, C.B., Friedlander, A.M., Green, A.G., Hardt, M.S., Sala, E., Sweatman, H.P., Williams, I.D., Zgliczynski, B., Sandin, S.A., and Smith, J.E. 2014. Global assessment of the status of coral reef herbivorous fishes: evidence for fishing effects. *Proc. R. Soc. B Biol. Sci.* **281**: 20131835. doi:10.1098/rspb.2013.1835.
- Ewing, G.P., Lyle, J.M., Murphy, R.J., Kalish, J.M., and Ziegler, P.E. 2007. Validation of age and growth in a long-lived temperate reef fish using otolith structure, oxytetracycline and bomb radiocarbon methods. *Mar. Freshw. Res.* **58**: 944–955. doi:10.1071/MF07032.
- Fowler, A.J. 2009. Age in years from otoliths of adult tropical fish. In *Tropical fish otoliths: information for assessment, management and ecology*. Reviews: Methods and technologies in fish biology and fisheries 11. Edited by B.S. Green, B.D. Mapstone, G. Carlos, and G.A. Begg. Springer, New York. pp. 55–92.
- Francis, R.I.C.C., and Campana, S.E. 2004. Inferring age from otolith measurements: a review and a new approach. *Can. J. Fish. Aquat. Sci.* **61**(7): 1269–1284. doi:10.1139/f04-063.
- Froese, R., and Binohlan, C. 2000. Empirical relationships to estimate asymptotic length, length at first maturity and length at maximum yield per recruit in fishes, with a simple method to evaluate length frequency data. *J. Fish Biol.* **56**: 758–773. doi:10.1111/j.1095-8649.2000.tb00870.x.
- Hart, A.M., and Russ, G.R. 1996. Response of herbivorous fishes to crown-of-thorns starfish *Acanthaster planci* outbreaks. III. Age, growth, mortality and maturity indices of *Acanthurus nigrofuscus*. *Mar. Ecol. Prog. Ser.* **136**: 25–35. doi:10.3354/meps136025.
- Hoey, A.S., and Bellwood, D.R. 2009. Limited functional redundancy in a high diversity system: single species dominates key ecological processes on coral reefs. *Ecosystems*, **12**: 1316–1328. doi:10.1007/s10021-009-9291-z.
- Houk, P., Rhodes, K., Cuetos-Bueno, J., Lindfield, S., Fread, V., and McIlwain, J.L.

2012. Commercial coral-reef fisheries across Micronesia: a need for improving management. *Coral Reefs*, **31**: 13–26. doi:10.1007/s00338-011-0826-3.
- Kalish, J.M. 1993. Pre- and post-bomb radiocarbon in fish otoliths. *Earth Planet. Sci. Lett.* **114**: 549–554. doi:10.1016/0012-821X(93)90082-K.
- Kimura, D.K. 1980. Likelihood methods for the von Bertalanffy growth curve. *Fish. Bull.* **77**: 765–766.
- Lou, D.C., Mapstone, B.D., Russ, G.R., Davies, C.R., and Begg, G.A. 2005. Using otolith weight–age relationships to predict age-based metrics of coral reef fish populations at different spatial scales. *Fish. Res.* **71**: 279–294. doi:10.1016/j.fishres.2004.09.003.
- Nadon, M.O., Ault, J.S., Williams, I.D., Smith, S.G., and DiNardo, G.T. 2015. Length-based assessment of coral reef fish populations in the Main and Northwestern Hawaiian Islands. *PLoS ONE*, **10**(8): e0133960. doi:10.1371/journal.pone.0133960. PMID:26267473.
- Pilling, G.M., Grandcourt, E.M., and Kirkwood, G.P. 2003. The utility of otolith weight as a predictor of age in the emperor *Lethrinus mahsena* and other tropical fish species. *Fish. Res.* **60**: 493–506. doi:10.1016/S0165-7836(02)00087-5.
- Randall, J.E. 2001. Surgeonfishes of Hawaii and the world. Mutual Publishing and Bishop Museum Press, Honolulu, Hawaii.
- Reimer, P.J., Brown, T.A., and Reimer, R.W. 2004. Discussion: reporting and calibration of post-bomb <sup>14</sup>C data. *Radiocarbon*, **46**(3): 1299–1304.
- Richards, F.J. 1959. A flexible growth function for empirical use. *J. Exp. Bot.* **10**: 290–301. doi:10.1093/jxb/10.2.290.
- Roark, E.B., Guilderson, T.P., Dunbar, R.B., and Ingram, B.L. 2006. Radiocarbon-based ages and growth rates of Hawaiian deep-sea corals. *Mar. Ecol. Prog. Ser.* **327**: 1–14. doi:10.3354/meps327001.
- Robertson, D.R. 1985. Sexual size dimorphism in surgeonfishes. In *Proceedings of the Fifth International Coral Reef Congress, Tahiti*. Vol. 5. pp. 403–408.
- Robertson, D.R., Ackerman, J.L., Choat, J.H., Posada, J.M., and Pitt, J. 2005a. Ocean surgeonfish *Acanthurus bahianus*. I. The geography of demography. *Mar. Ecol. Prog. Ser.* **295**: 229–244. doi:10.3354/meps295229.
- Robertson, D.R., Choat, J.H., Posada, J.M., Pitt, J., and Ackerman, J.L. 2005b. Ocean surgeonfish *Acanthurus bahianus*. II. Fishing effects on longevity, size and abundance? *Mar. Ecol. Prog. Ser.* **295**: 245–256. doi:10.3354/meps295245.
- SAS Institute, Inc. 2006. Base SAS 9.1.3 Procedures guide. 2nd ed. Vols. 1, 2, 3, and 4. SAS Institute, Inc., Cary, N.C.
- SAS Institute Inc. 2012. Base SAS® 9.3 Procedures guide: statistical procedures. 2nd ed. SAS Institute, Inc., Cary, N.C.
- Schnute, J.T., and Richards, L.J. 1990. A unified approach to the analysis of fish growth, maturity, and survivorship data. *Can. J. Fish. Aquat. Sci.* **47**(1): 24–40. doi:10.1139/f90-003.
- Siegel, S., and Castellan, N.J. 1988. Nonparametric statistics for the behavioral sciences. 2nd ed. McGraw-Hill, New York.
- Steward, C.A., DeMaria, K.D., and Shenker, J.M. 2009. Using otolith morphometrics to quickly and inexpensively predict age in the gray angelfish (*Pomacanthus arcuatus*). *Fish. Res.* **99**: 123–129. doi:10.1016/j.fishres.2009.05.011.
- Stuiver, M., and Polach, H.A. 1977. Discussion: Reporting of <sup>14</sup>C data. *Radiocarbon*, **19**: 355–363.
- Taylor, B.M., Rhodes, K.L., Marshall, A., and McIlwain, J.L. 2014. Age-based demographic and reproductive assessment of orangespine *Naso lituratus* and bluespine *Naso unicornis* unicornfishes. *J. Fish Biol.* **85**: 901–916. doi:10.1111/jfb.12479. PMID:25082347.
- Tjorve, E., and Tjorve, K.M.C. 2010. A unified approach to the Richards-model family for use in growth analyses: why we need only two model forms. *J. Theor. Biol.* **267**: 417–425. doi:10.1016/j.jtbi.2010.09.008.
- Trip, E.L., Choat, J.H., Wilson, D.T., and Robertson, D.R. 2008. Inter-oceanic analysis of demographic variation in a widely distributed Indo-Pacific coral reef fish. *Mar. Ecol. Prog. Ser.* **373**: 97–109. doi:10.3354/meps07755.
- Trip, E.L., Craig, P., Green, A., and Choat, J.H. 2014. Recruitment dynamics and first year growth of the coral reef surgeonfish *Ctenochaetus striatus*, with implications for acanthurid growth models. *Coral Reefs*, **33**: 879–889. doi:10.1007/s00338-014-1182-x.
- Williams, I.D., Walsh, W.J., Schroeder, R.E., Friedlander, A.M., Richards, B.L., and Stamoulis, K.A. 2008. Assessing the importance of fishing impacts on Hawaiian coral reef fish assemblages along regional-scale human population gradients. *Environ. Conserv.* **35**: 261–272. doi:10.1017/S0376892908004876.
- Williams, I.D., Richards, B.L., Sandin, S.A., Baum, J.K., Schroeder, R.E., Nadon, M.O., Zgliczynski, B., Craig, P., McIlwain, J.F., and Brainard, R.E. 2011. Differences in reef fish assemblages between populated and remote reefs spanning multiple archipelagos across the Central and Western Pacific. *J. Mar. Biol.* **2011**: Article ID 826234. doi:10.1155/2011/826234.
- Wilson, D.T., and McCormick, M.I. 1999. Microstructure of settlement marks in the otoliths of reef fishes. *Mar. Biol.* **134**: 29–41. doi:10.1007/s002270050522.
- Worthington, D.G., Doherty, P.J., and Fowler, A.J. 1995. Variation in the relationship between otolith weight and age: implications for the estimation of age of two tropical damselfish (*Pomacentrus moluccensis* and *P. wardi*). *Can. J. Fish. Aquat. Sci.* **52**(2): 233–242. doi:10.1139/f95-023.