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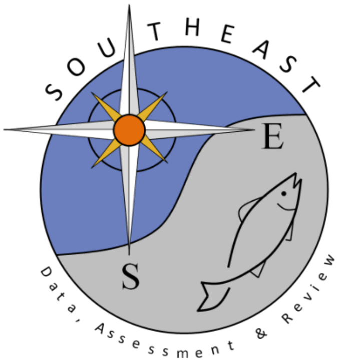

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Refined bomb radiocarbon dating of two iconic fishes of the Great Barrier Reef

Allen H. Andrews[A,D], *John H. Choat*[B], *Richard J. Hamilton*[C]
and Edward E. DeMartini[A]

[A]NOAA Fisheries – Pacific Islands Fisheries Science Center, 1845 Wasp Boulevard,
Honolulu, HI 96818, USA.
[B]School of Marine and Tropical Biology, James Cook University, Townsville, Qld 4811, Australia.
[C]The Nature Conservancy – Asia-Pacific Division, 245 Riverside Drive,
West End, Qld 4101, Australia.
[D]Corresponding author. Email: allen.andrews@noaa.gov

Abstract. Refinements to the methodology of bomb radiocarbon dating made it possible to validate age estimates of the humphead wrasse (*Cheilinus undulatus*) and bumphead parrotfish (*Bolbometopon muricatum*). Age for these species has been estimated from presumed annual growth zones in otoliths at ~30 and ~40 years respectively. The validity of these estimates was tested using bomb radiocarbon dating on the small and fragile otoliths of these species, and provided an opportunity to refine the method using advanced technologies. A regional Δ^{14}C reference record from hermatypic coral cores from the Great Barrier Reef was assembled and Δ^{14}C measurements from extracted otolith cores of adult otoliths were successful. Validated ages supported the accuracy of growth zone derived ages using sectioned sagittal otoliths.

Additional keywords: Australia, *Bolbometopon muricatum*, bumphead parrotfish, carbon-14, *Cheilinus undulatus*, humphead wrasse, Labridae, micromilling, otolith.

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Introduction

There is concern for the conservation and management of two iconic fishes of Indo-Pacific coral reefs, the humphead wrasse (F. Labridae, *Cheilinus undulatus*) and bumphead parrotfish (F. Labridae, *Bolbometopon muricatum*). Whereas the reasons for concern are numerous (Sadovy *et al.* 2003; Russell 2004; Chan *et al.* 2012), an important aspect of effective management is the use of age validated life history information, especially as many species achieve greater ages than previous work had suggested. Age based demography provides information on longevity, age at maturity and mortality rates, all of which are integral to comprehensive fishery assessments. Assumptions about age estimate validity have led to inaccurate life history parameters, a problem that is acute when estimating longevity and the age composition of the older adult portion of the population (Beamish *et al.* 2006; Cailliet and Andrews 2008).

Age and growth estimates for *C. undulatus* and *B. muricatum* have been estimated from visual counts of presumed annual growth zones in otoliths with longevity estimated at ~30 and ~40 years for the respective species (Choat and Robertson 2002; Choat *et al.* 2006; Hamilton and Choat 2012). Although estimates of age have been validated to a limited extent using oxytetracycline marking of captive *C. undulatus* (Choat *et al.* 2006), estimates for *B. muricatum* remain unvalidated. Attempts to validate age for both species using conventional means in the wild have been unsuccessful because of the practical difficulties in the capture, tagging and recapture of these large, rare and mobile reef fishes (Hamilton 2004; Choat *et al.* 2009).

A recent method that can address questions of adult fish age and longevity is bomb radiocarbon dating – an approach that has the ability to provide valid age estimates for individual fish. Successful use of this method has either led to support for existing (sometimes contested) adult age estimates or drastic adjustments to longevity and other life history parameters, of which several studies were from tropical and sub-tropical marine environments (i.e. Baker and Wilson 2001; Neilson and Campana 2008; Cook *et al.* 2009; Andrews *et al.* 2011, 2012, 2013*a*).

Bomb radiocarbon dating generally relies on a conserved record of increasing radiocarbon that occurred in the oceans as a result of atmospheric testing of thermonuclear devices in the 1950s and 1960s (Broecker and Peng 1982). This bomb produced radiocarbon signal is reported as delta carbon-14 (Δ^{14}C, per thousand, ‰) in reference to changes relative to a prenuclear standard (Stuiver and Polach 1977). Numerous bomb radiocarbon records have been recovered from shallow water corals and the initial marine uptake of this signal was virtually synchronous in the mixed layer of tropical seas (Grottoli and Eakin 2007). However, the characteristics of the Δ^{14}C rise and peak are regionally defined and can differ significantly in both amplitude and timing, differences that are typically attributed to diffusion of radiocarbon (in the form of atmospheric CO_2) at the sea surface, including atmospheric and oceanographic circulation (Druffel and Suess 1983; Guilderson *et al.* 2000; Glynn *et al.* 2013).

The efficacy and precision of bomb radiocarbon dating of fishes has evolved considerably from the time of its first application to a New Zealand porgy (Family Sparidae, *Pagrus auratus*; Kalish 1993). Its utility as an age validation tool for other marine organisms is widespread, ranging from calcareous algae (Frantz *et al.* 2005; Darrenougue *et al.* 2013), mollusks (Kilada *et al.* 2007; Andrews *et al.* 2013*b*) and deep sea coral (Roark *et al.* 2006; Sherwood *et al.* 2008), to sharks (Campana *et al.* 2002; Passerotti *et al.* 2014) and cetaceans (Stewart *et al.* 2006). Prior to the initial application of bomb radiocarbon dating to marine fishes, evidence of the bomb radiocarbon signal was measured as a time series in hermatypic corals from tropical and sub-tropical marine environments (Knutson and Buddemeier 1973; Druffel and Linick 1978; Nozaki *et al.* 1978). Because the annual periodicity of growth banding in hard corals can be validated with other methods (i.e. annual temperature cycles by $\delta^{18}O$ or Sr/Ca proxy), its utility as a regional marine reference for the bomb radiocarbon signal is well substantiated (Grottoli and Eakin 2007).

Relative to teleost fishes, otoliths also provide a direct measure of environmental chemistry and the conserved otolith record includes dissolved inorganic carbon, of which radiocarbon is an isotopic component. Hence, a direct comparison of measured radiocarbon levels in the earliest otolith growth (otolith core) of tropical and sub-tropical fishes to a coral reference record can provide validated age estimates that are well defined (Neilson and Campana 2008; Andrews *et al.* 2011). Given an appropriate $\Delta^{14}C$ reference record for the region of study, the period of $\Delta^{14}C$ rise can be used to determine validated age estimates. Age estimates acquired in this manner are independent of observations of growth or growth zone structure for the organism under study, an unprecedented advancement for the study of the age and growth of fishes.

The otoliths of *C. undulatus* and *B. muricatum* present a series of logistical challenges to bomb radiocarbon dating. Although each species can attain a large body size and length, the otoliths are relatively small (maximum otolith mass = 100–150 mg). Consequently, the earliest growth for these fishes may be represented by only a few milligrams of calcium carbonate. Kalish (1993) estimated the first year of otolith growth consisted of 25–30 mg for *P. auratus*, whose otoliths can reach 1 g in adults. Precision of the radiocarbon measurement is also important relative to potential sample mass. The extracted 1-year cores for *P. auratus* were analysed for $\Delta^{14}C$ with measurement precision at ±6–12‰ (s.d.). Accelerator mass spectrometry of radiocarbon has improved considerably since that time and a precision of ±3–4‰ (s.d.) is attainable for 1–2 mg of carbonate. Given this precision for such a low sample mass, *P. auratus* could be sampled to a temporal resolution on the order of months of otolith growth, but there are practical problems with extracting an otolith core this size. Hand grinding or Dremmel tool extraction has been the standard, but to accurately extract a 1 mg core sample in this manner is unlikely and would be difficult if not impossible to verify. Recent advances utilising computerised micromilling machines allow precise extraction of targeted otolith material and verification is possible because most of the otolith remains intact post-coring (e.g. Andrews *et al.* 2013*a*).

The objective of this study was to refine the limits of bomb radiocarbon dating on the small otoliths of *C. undulatus* and *B. muricatum* and determine the validity of age estimates for these species on the Great Barrier Reef. The specific goals of this study were to: (1) assemble a regional $\Delta^{14}C$ reference record from hermatypic coral cores on the Great Barrier Reef (GBR); (2) determine the feasibility of using the bomb radiocarbon technique to date the small and fragile adult otoliths for each species; (3) measure $\Delta^{14}C$ in extracted otolith cores of adult otoliths and calibrate age using the regional $\Delta^{14}C$ reference; and (4) use validated age estimates to evaluate the accuracy of age estimates based on visual ring counts for sectioned sagittal otoliths.

Materials and methods

GBR bomb radiocarbon dating reference

Existing $\Delta^{14}C$ reference records from coral cores were assembled from three locations to prepare for bomb radiocarbon dating of the fish specimens. The applicable bomb radiocarbon records for the GBR were all from the southern end, where hermatypic coral cores were collected at Abraham Reef, Heron Island and Lady Musgrave Island (Druffel and Griffin 1995; Fig. 1). Collectively, the coral $\Delta^{14}C$ records continuously covered a period ranging from well before the bomb radiocarbon pulse to the peak in the 1970s and ending during the post-bomb decline period (Fig. 2). Other $\Delta^{14}C$ records were sought to corroborate use of the combined record for fishes collected farther north in the GBR and to investigate the $\Delta^{14}C$ decline period, some support was provided from $\Delta^{14}C$ measurements on the inorganic component of an alga collected in 1973 (Polach *et al.* 1978, 1981) and a juvenile *C. undulatus* otolith (this study). A study of the regional oceanic currents provides additional support for a common bomb $\Delta^{14}C$ record for the GBR (Fig. 1).

Specimen selection and core extraction

Specimens for bomb radiocarbon dating and for comparative otolith size analysis and visual age counting of annuli were selected to increase the likelihood of success (Table 1). Otoliths of *C. undulatus* were from specimens collected by Choat *et al.* (2006). Fly and Day Reefs are located at 14°S on the GBR. The remaining sample locations lie between 18 and 19°S. The juvenile *C. undulatus* used as a reference in this study was collected from the central GBR (Fig. 3; 228 mm TL). For *B. muricatum*, all adult specimens used were collected from northern reefs of the GBR at 14°S. Juveniles of this species were not encountered over the adult ranges on the GBR (Bellwood and Choat 2011) and otoliths used as a mass and dimensional reference in this study were collected from Cocos Keeling Island of the Indian Ocean (Fig. 4; 96 and 226 mm TL). Procedures used to cross-section otoliths (sagittae) and enumerate annuli were described by Choat *et al.* (1996, 2006, 2009).

The feasibility of bomb radiocarbon dating was based on the ability to locate and extract the core (within the first year of growth) from the otoliths of adult *C. undulatus* and *B. muricatum*.

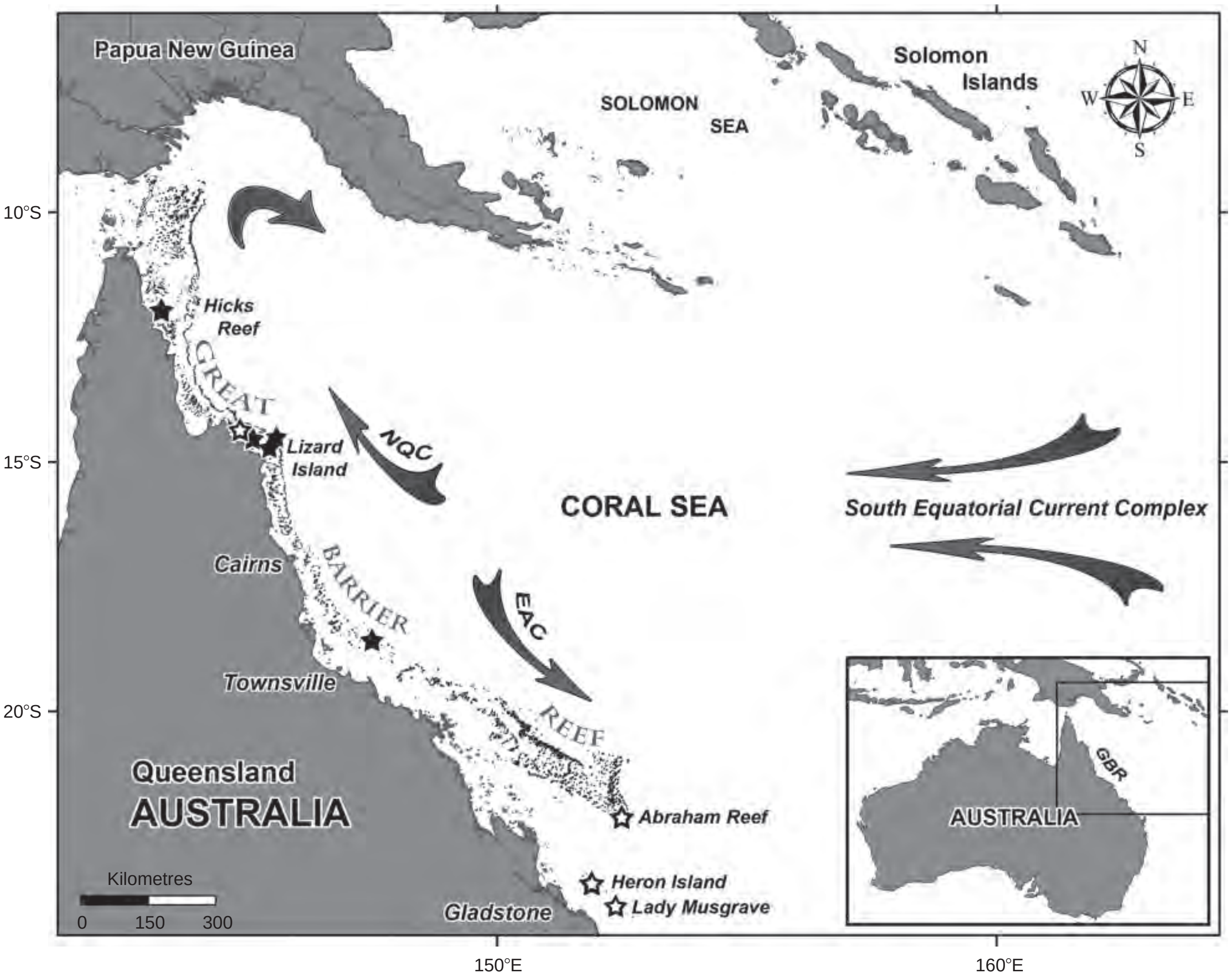

Fig. 1. Map of the region covered by the Great Barrier Reef (GBR) along Queensland, Australia, with locations of fish specimen collections (indicated by black stars) and radiocarbon reference locations (open stars). Fishes were collected from locations along the northern (Hicks, Day and Fly Reef and Lizard Island) and central GBR (general location off Townsville). Complete bomb radiocarbon references were available from the southern GBR (Abraham Reef, Heron Island and Lady Musgrave Island; Druffel and Griffin 1995). An alga carbonate $\Delta^{14}C$ record from the northern GBR provided additional information (near Lizard Island; Polach *et al.* 1978, 1981). The oceanic current system associated with the GBR is typically a westerly flow across the Coral Sea to a divergence (Eastern Australian Current, EAC and North Queensland Current, NQC; Ganachaud *et al.* 2007), lending support for a common bomb $\Delta^{14}C$ reference for the GBR.

This study was challenged by not only the small size and mass of adult otoliths, but also the fragile nature of the otolith structure. This was especially true for *B. muricatum* otoliths. Otoliths from juvenile fish were analysed to provide guidelines in the extraction of otolith core material. These otoliths were measured and weighed to provide a target sample size. The location of the earliest growth within adult otoliths was determined based on microscopic observations of whole otoliths (juvenile to adult) together with observations made in transverse cross-sections of adult otoliths. In addition, otolith thickness and weight were evaluated for predicting fish age for *C. undulatus*; this was not possible for *B. muricatum* because of otolith fragility and mass loss during extraction and handling.

Selected otoliths were prepared for core extraction on a micromilling machine in a series of preliminary steps. Individual otoliths were cleaned using alternating steps with deionised water and mild detergent. Sonication was not used because the otoliths were too fragile. Whole otoliths were mounted on glass slides with the sulcus side down, making the distal surface accessible for core extraction by micromilling. Cytoseal60 (Thermo Scientific, www.thermoscientific.com) was used as a low viscosity adhesive in several harden-reapply applications, and no media entered the extraction area. This was necessary to fully penetrate and ultimately support the fragile otolith structure of each species. The final mount was allowed to cure for a week and was heat treated (20 min at 45°C) to solidify the mount before further preparation. Because adult otoliths of *C. undulatus* accrete a small amount of otolith material onto the distal side of the otolith (Fig. 3*a*), wet hand grinding using 320- to 1000-grit, wet–dry carbide sandpaper was performed to expose the earliest otolith growth. The first few years of growth were usually visible as grinding proceeded. The concentric growth zone structure and radial lines emanating outward from the nucleus were used as a guide in exposing the core (Fig. 3*b*).

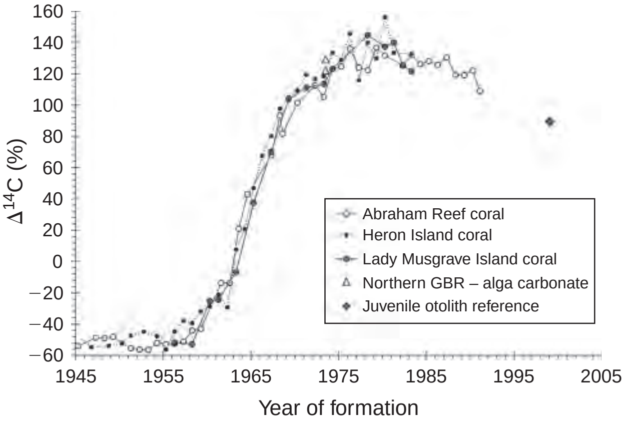

Fig. 2. Bomb radiocarbon reference records for the Great Barrier Reef (GBR) obtained from coral cores at Abraham Reef, Heron Island and Lady Musgrave Island (Druffel and Griffin 1995). The common Δ^{14}C rise and peak characteristics are an indication that these records can provide a temporal correlation for age determination of reef fishes throughout the GBR. In support of this notion is a Δ^{14}C record from an alga carbonate in the northern GBR (Polach *et al.* 1978). A juvenile *C. undulatus* otolith provided a more recent Δ^{14}C record and evidence of a regional decline for the bomb Δ^{14}C signal.

The distal surface preparation was not necessary for *B. muricatum* otoliths because there was no accretion to the distal margin in adult otoliths (Fig. 4*a*). Juvenile otoliths and a change in the otolith growth structure guided the location of core extraction (Fig. 4*b*). It was evident that the core is solid (first year of growth), but that a branched and crenulated pattern of mass growth develops with increasing age. This outer otolith structure was very fragile because of interstitial spaces among the crenulations. Minor mass loss between the time of otolith extraction to cleaning and coring was a constant problem and can be clearly seen in the whole otolith cross-section (Fig. 4*a*). In addition, estimated otolith weight for adult fish will typically be less than the *in vivo* whole otolith mass and it is uncertain how much mass was lost from each adult otolith used in this study. To stabilise the whole otolith for core extraction, the mounting medium was allowed to penetrate the interstitial spaces of the outer otolith. Milling for each species proceeded as an extraction of the core dimensions from the distal surface of the prepared and properly positioned otolith (Fig. 5).

Extraction of the otolith core utilised the computer automated capabilities of a New Wave Research (ESI-NWR Division; Fremont, CA, USA) micromilling machine. A 300-μm diameter Brasseler (Savannah, GA, USA) bur was used to drill an overlapping surface scan within the spheroidal dimensions of a juvenile otolith. The surface scan was a guided extraction that conformed to the uneven surface structure of each otolith. The extracted samples were submitted to the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at Woods Hole Oceanographic Institution (WHOI) in Woods Hole, MA, for routine radiocarbon analysis.

Table 1. Data for selected *C. undulatus* (HHW) and *B. muricatum* (BHP) collected from the Great Barrier Reef (GBR) with analysis results

Laboratory and collection numbers are listed with the collection date and location on the GBR and fish length (fork length, FL) and sex, whole otolith weight (plus thickness for *C. undulatus* only). Extracted sample mass and the resultant radiocarbon measurements are provided with age determinations. Δ^{14}C values were corrected for fractionation (δ^{13}C values are robust) and for estimated time of formation. Count age was based on growth zone counting in otolith sections (performed at James Cook University). Bomb age is given as a range and was based on the difference in time from collection date to Δ^{14}C coral reference date. The applicable region of the bomb radiocarbon curve is qualitatively noted as either 'Rise' or 'Peak.' Collection locations are indicated below and were either from the northern (14°S) or central (18–19°S) Great Barrier Reef (Fig. 1)

Laboratory number (Collection number)	Collection date Location	Length (FL) Sex	Otolith weight (g) (thickness, mm)	Mass (mg)	Fraction modern	Δ^{14}C (‰)	δ^{13}C (‰)	Count age (years)	Bomb age (years)
HHW-1(A4178)	25-Jan-1999 Townsville[A]	588 mm Imm. (F?)	0.0325 (0.90)	2.5	1.0958	89.3 ± 2.8	−2.19	9	Ref[B]
HHW-3(A4224)	10-Jan-2000 Day Reef[C]	1100 mm M	0.0886 (1.58)	2.2	1.1280	123.9 ± 3.3	−3.59	22	Peak (12–26)
HHW-6(A4917)	22-Jul-2001 Central GBR[A]	1180 mm M	0.0816 (1.47)	1.6	1.1423	138.2 ± 2.8	−3.67	22	Peak (20–26)
HHW-7(A3581)	26-Jan-1999 Fly Reef[C]	831 mm F	0.0847 (1.49)	2.0	1.1414	137.3 ± 3.2	−2.34	22	Peak (18–24)
HHW-8(A3197)	14-Apr-1998 Townsville[A]	864 mm F	0.0738 (1.54)	1.7	1.1284	124.3 ± 6.5	−2.65	19	Peak (11–25)
HHW-9(A4908)	16-Apr-2001 Townsville[A]	940 mm F	0.0947 (1.92)	2.0	1.1254	121.3 ± 3.1	−4.17	28	Peak (11–30)
HHW-10(A2888)	1-Nov-1999 Yankee Reef[A]	1420 mm M	0.0911 (1.63)	1.8	1.1344	130.3 ± 2.8	−2.26	21	Peak (12–26)
HHW-12(A2374)	1-Nov-1999 Yankee Reef[A]	940 mm M	0.0790 (1.57)	1.5	1.1331	129.0 ± 2.8	−2.83	19	Peak (12–26)
BHP-1(A1000)	21-Dec-1994 Hicks Reef[C]	710 mm F	0.0560	2.1	1.1305	126.4 ± 3.5	−8.34	13	Peak (7–22)
BHP-2(A0675)	19-Jan-1994 Day Reef[C]	724 mm F	0.0579	2.1	1.1484	144.2 ± 2.8	−7.05	17	Peak (13–18)
BHP-3(A1003)	22-Dec-1994 Hicks Reef[C]	830 mm F	0.1535	2.3	0.9906	−11.0 ± 2.6	−6.13	33	Rise (32–34)
BHP-4(A0999)	21-Dec-1994 Hicks Reef[C]	768 mm F	0.0924	2.4	1.0211	19.5 ± 2.5	−6.05	29	Rise (30–32)
BHP-5(A4893)	15-Feb-2001 Lizard Island[C]	981 mm F	0.1402	2.4	0.9802	−21.3 ± 2.4	−6.38	37	Rise (39–41)

[A] Mid-shelf off Townsville.

[B] The rostral tip of the otolith from this fish was used as known-age reference material (collection date).

[C] Northern GBR reefs.

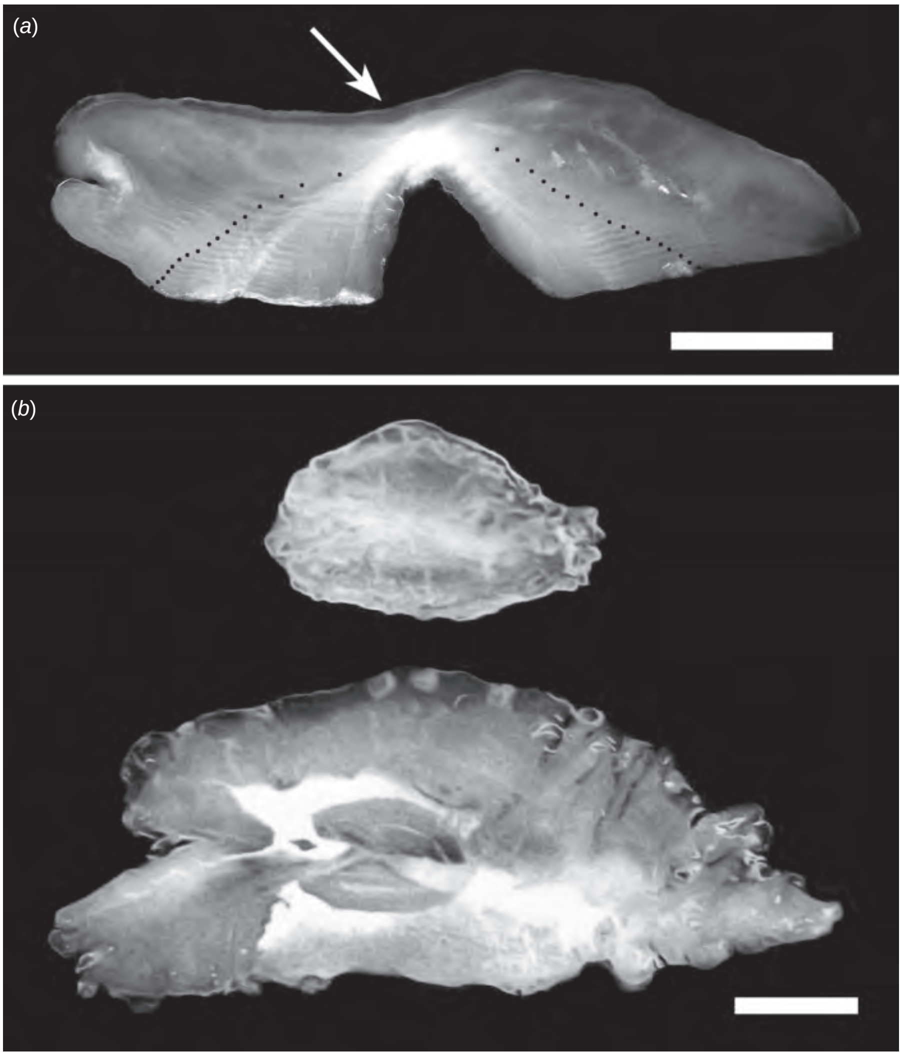

Fig. 3. Transverse section of adult *C. undulatus* otolith viewed with reflected light (*a*). The estimated age of this fish was 17 years and counts were similar on either side of the sulcus (black dots). This section revealed an accretion of otolith material on the distal side as a thin translucent margin (arrow), which was removed to expose the core region before core extraction (Scale bar = 1 mm). Images of whole otoliths from *C. undulatus* (*b*). The top otolith was from a 1-year-old juvenile (228 mm fork length (FL), otolith weight = 8.6 mg). The bottom otolith was from an adult male (1100 mm FL) with an estimated age of 22 years (HHW-3). The extracted core can be seen as a recess in the smoothed and reflective surface (younger distal surface removed). Note that the core was smaller than the 1-year-old otolith (Scale bar = 2 mm).

Radiocarbon analysis

Radiocarbon measurements were reported from NOSAMS as the Fraction Modern (Fm), which was used to calculate Δ^{14}C with a correction for isotopic fractionation (Stuiver and Polach 1977). Fraction Modern is the measured deviation of the ^{14}C/^{12}C ratio from a 'modern' sample. This internationally agreed upon reference is defined as 95% of the radiocarbon concentration of the NBS Oxalic Acid I standard (SRM 4990B) normalised to $\delta^{13}C_{VPDB}$ (−19‰) in 1950 AD (VPDB = Vienna Pee Dee Belemnite geological standard; Coplen 1996). Sample Fm values were corrected for fractionation using a measured δ^{13}C value that is robust and useful in understanding trophic relationships for the carbon sources to the otolith.

The calculated Δ^{14}C values reported in this study were corrected for age (time of formation) based on an approximate birth year. A retrospective estimate was generated based on the initial Δ^{14}C value and its known proximity in time relative to the coral Δ^{14}C reference records. The year used in the corrections was based on a Δ^{14}C criterion as follows:

- Pre-bomb period = 1950 for <-35‰
- Rise period = 1963 for −35 to 110‰
- Near peak period = 1980 for >110‰

The reason for such corrections is to provide a Δ^{14}C value that takes into account the decay that occurred between the approximate year of birth and the time of

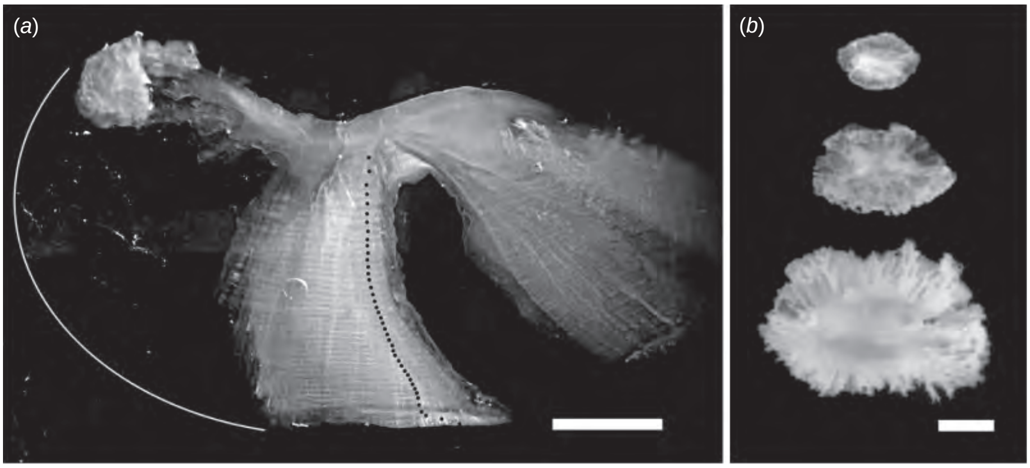

Fig. 4. Transverse section of adult *B. muricatum* otolith (BHP-5) with distal side facing upward (*a*). Note lack of additional accreted material opposite sulcus (top edge), unlike the otoliths of adult *C. undulatus* (Fig. 3*a*). Sulcal ridges were more stable than the fragile branched crenulations lost during handling and processing. The arc drawn on left side roughly encompasses the missing portion of the otolith (ventral). Age was estimated for this specimen by counting the zones marked with black dots. 42 zones were marked, although the resolved age among readers was originally 37 years (scale bar = 1 mm). Series of *B. muricatum* otoliths from juvenile to adult showing the formational changes through ontogeny (*b*). The top otolith was from a 0.3-year-old juvenile (96 mm fork length (FL); otolith weight = 3.2 mg). The middle otolith was from a juvenile (226 mm FL) estimated to be 1 year old. The bottom otolith was from a mature 17-year-old female (724 mm FL) that was later cored (BHP-2; scale bar = 2 mm). This series was used to determine otolith core extraction parameters for the micromilling machine.

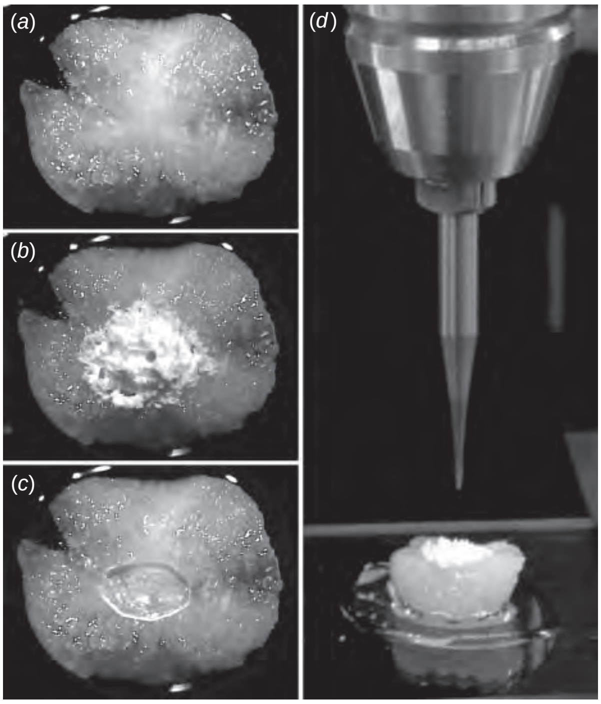

Fig. 5. Image series of a mounted *B. muricatum* otolith (BHP-5) undergoing the process of core extraction with the micromilling machine (*a–d*). Mounting medium within the interstitial spaces of the outer otolith create shiny surfaces around the coring region. Image series is from the mounted pre-coring (*a*), post-coring with sample powder on top (*b*) and after sample collection showing the extracted core (*c*). Milling bur was a 300-μm Brasseler USA carbide cutter (*d*) driven by a New Wave Research (ESI) micromilling machine system. Extracted sample mass was 2.4 mg and the width of the whole otolith was 8.0 mm for scale (*a–c*).

measurement, as is the case with all coral Δ^{14}C reference records.

Bomb radiocarbon dating

The unified coral Δ^{14}C reference was used to provide validated age estimates for *C. undulatus* and *B. muricatum*. Estimates of age were determined by projecting the measured and corrected Δ^{14}C values from the fish collection date back in time to the coral Δ^{14}C reference series. Levels measured near the Δ^{14}C peak would be assigned an age range that could be attributed to the time the region held those Δ^{14}C levels. For levels attributed to the Δ^{14}C rise period (most diagnostic), the Δ^{14}C data from the coral records were combined to form a generic regional Δ^{14}C reference described with a Loess curve fit (spline interpolation smoothing parameter = 0.2, 2-parameter polynomial; SigmaPlot 11.2). Age of an individual fish was estimated based on its measured Δ^{14}C value and its fit to the Loess curve, with an uncertainty in birth year (fish age) from estimated prediction intervals. Pre-bomb levels would be given a minimum birth year and age based on the fit of the value to the Loess curve of the coral Δ^{14}C data.

Results

Specimen selection and core extraction

Selection of adult otolith specimens from the GBR for *C. undulatus* and *B. muricatum* was primarily based on estimated age from growth zone counting and the calculated birth year from collection year (Table 1). Otolith age estimates were made previously at James Cook University using transverse thin sections; archived otolith records provided the opportunity to select specimens. Specimens available for consideration from the GBR were not numerous for either species.

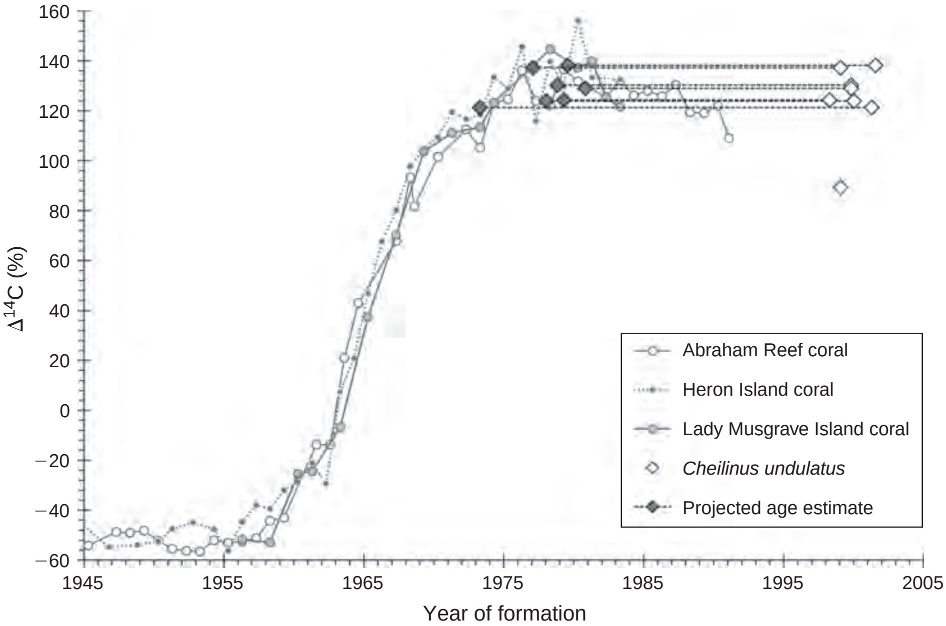

Fig. 6. Bomb radiocarbon plot of Δ^{14}C reference records and measured Δ^{14}C values from *C. undulatus* otolith cores from fish collected on the Great Barrier Reef (GBR). Open diamonds represent measured Δ^{14}C value for that particular fish on the collection date (Table 1), which was then projected back in time to an estimated birth year (grey diamond). Agreement between the Δ^{14}C reference and estimated age was good (although widely quantified by near peak Δ^{14}C values) and provided general support for the growth-zone derived age estimates. One juvenile sample (isolated open diamond) provided an indication of the amount of Δ^{14}C decline and a limit to how young the adult fish could have been.

For *C. undulatus* there were 20 archived otolith specimens collected between 1996 and 2001 for which body lengths of fish ranged from 560 to 1420 mm fork length (FL) and estimated ages from 6 to 30 years, but otoliths from the 30-year-old fish could not be located. Only five specimens were available for *B. muricatum*. Four were collected in 1994 and one in 2001 from fish with lengths of 710–981 mm FL and ages from 13 to 37 years. Fish length and otolith weight and thickness were also considered to discriminate among specimens that may have been older than estimated (e.g. smaller fish with an unusually heavy or thick otolith). The primary goal was to select specimens that had the greatest potential for a birth year on the Δ^{14}C rise, but some younger fish were also selected to provide a progression of Δ^{14}C values through the peak period and possibly into the Δ^{14}C decline period.

Bomb radiocarbon dating

One juvenile and seven adults were selected from the samples available for *C. undulatus* to span several different age estimate scenarios (Table 1). The juvenile fish was chosen as a temporal reference for the Δ^{14}C decline period (HHW-1). This fish was sampled by taking the otolith rostrum tip (2.5 mg) as a known age reference point (assuming a mean of ~6 months of deposition for the Δ^{14}C measurement). The Δ^{14}C value was consistent with the decline rate observed from the Abraham Reef coral core Δ^{14}C record and confirmed the lower limits to age ranges determined for near peak levels measured in otoliths. The remaining seven adults were large fish ranging from lengths near 800–900 mm FL to near maximum size at ~1400 mm FL. Otolith core extraction was consistent at 1.5–2.2 mg, weighing much less than the smallest juvenile otolith available as a reference (8.6 mg and estimated to be 1 year old; Fig. 3*b*).

Measures of Δ^{14}C for the adult *C. undulatus* otolith series were all consistent with GBR peak bomb radiocarbon levels (Δ^{14}C greater than ~120‰). Hence, alignment of the measured values with the coral Δ^{14}C reference series provided a pattern of general agreement with estimated age (Fig. 6). Moderate peak Δ^{14}C levels provided the greatest uncertainty for a validated age range of ~12–26 years. Near peak Δ^{14}C values led to greater precision because of the limits of the peak date-range when Δ^{14}C values approach 140‰ (~19–25 years). The fish with the oldest age estimate had the greatest age range potential because the Δ^{14}C value (121.3 ± 3.1‰) was approaching either a Δ^{14}C rise or decline level. Hence, the age range crosses the broadest part of the peak at 1971–1990 (~11–30 years of age), but significant age underestimation can be ruled out for this specimen. Counts of the growth zones visible in an example otolith section from *C. undulatus* showed growth zones that are easily enumerated, hence it is likely that the conventional age estimation procedures are accurate (Fig. 3*a*). In the sample selection process, otolith weight and thickness were also used as discriminating factors, considering that the potential age estimates from the growth zone counting (19–28 years) might be manifested in otolith growth. Otolith thickness (OtoTh) and weight (OtoWt) thus might function as a proxy for fish age ($n = 8$):

$$\text{Age} = 19.88(\text{OtoTh}) - 9.82 \ (R^2 = 0.892)$$

$$\text{Age} = 260.4(\text{OtoWt}) \ (R^2 = 0.993)$$

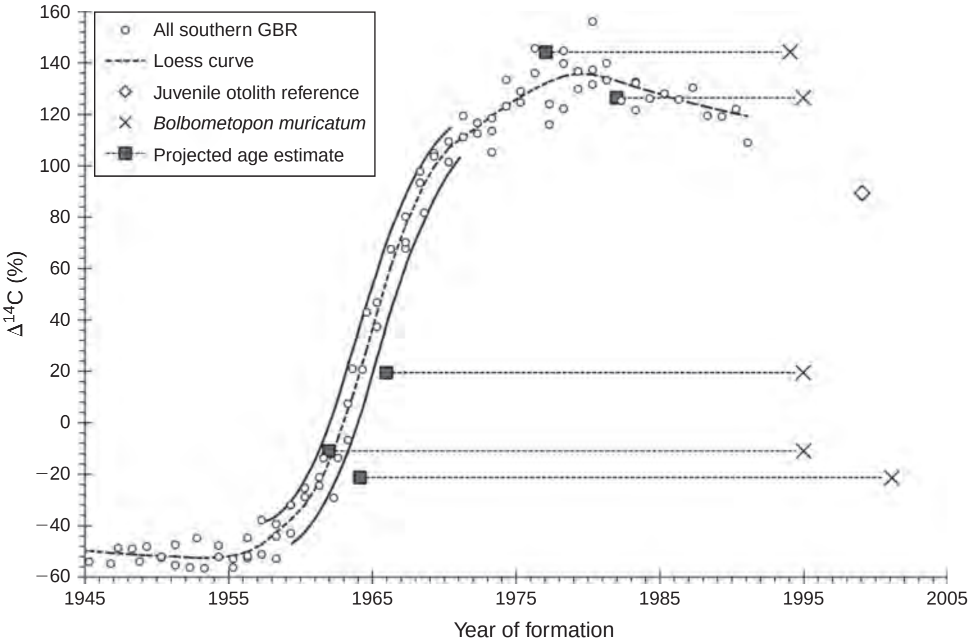

Fig. 7. Bomb radiocarbon plot of $\Delta^{14}C$ reference records and measured $\Delta^{14}C$ values from *B. muricatum* otolith cores of fish collected on the Great Barrier Reef (GBR). X symbols represent the measured $\Delta^{14}C$ value for a particular fish on the collection date (Table 1), which was then projected back in time to an estimated birth year (grey square). Agreement between the $\Delta^{14}C$ reference and growth-zone derived age estimates was good with minor underestimation of age for two specimens in the rise period, and more limited agreement for the near peak $\Delta^{14}C$ values.

where age is in years, otolith thickness (OtoTh) is in millimetres and otolith weight (OtoWt) is in grams (Table 1). However, these findings assume linear relationships and require further investigation using larger sample sizes.

Although otoliths of *B. muricatum* from the GBR were few, the series of samples available provided a fortuitous coincidence of estimated birth years within the rise of $\Delta^{14}C$. Five specimens were selected with estimated ages of 13–37 years and lengths of 710–981 mm FL (Table 1). Otolith weight ranged from ~0.06 g for the smallest fish to ~0.15 g for the second largest fish. Otolith coring was consistent because of the success with the extraction design at 2.1–2.4 mg (Fig. 5), weighing less than the smallest juvenile otolith available as a reference (3.2 mg and estimated to be 0.3 years old; Fig. 4*b*). Measures of $\Delta^{14}C$ for the otolith series ranged as expected from a value near the base of the $\Delta^{14}C$ rise (−21.3 ± 2.4‰) to values comparable to $\Delta^{14}C$ peak levels (144.2 ± 2.8‰). Alignment of the measured values with the coral $\Delta^{14}C$ reference series revealed some general agreement and minor disagreement with estimated age (Fig. 7). The smallest fish had $\Delta^{14}C$ values at peak period levels as expected from estimated birth years. Minor differences indicated age was underestimated by a few years for two of the oldest fish (BHP-3 and 4; Table 1). The largest fish was the oldest fish at 39–41 years (BHP-5) based on an alignment of the $\Delta^{14}C$ value with the prediction intervals. A subsequent count of the growth zones visible in the cross-sectioned otolith of this fish revealed growth zones that could be quantified up to 42 years (Fig. 4*a*).

Discussion

The bomb radiocarbon records that were available for fish age validation on the GBR were limited to three hermatypic coral cores from the southern end of the GBR. It is likely these $\Delta^{14}C$ records are a good representation of the bomb radiocarbon signal that has propagated throughout the GBR for the following reasons. The oceanic current system associated with the GBR is typically from a westerly flow across the Coral Sea to a divergence area, which separates into a southern component (Eastern Australian Current, EAC) and a northern component (North Queensland Current, NQC; Ganachaud *et al.* 2007). The influx of water to the Coral Sea is predominantly from the South Equatorial Current in a complex of jets running north and south of Vanuatu and New Caledonia (Ganachaud *et al.* 2007), providing a common source of oceanic water for the length of the GBR. In addition, some support was provided from $\Delta^{14}C$ measurements on the inorganic component of a green macroalga (*Halimeda* sp.) collected in 1973 from Berwick Reef, a location near Lizard Island on the northern GBR (Polach *et al.* 1978, 1981). The purpose of the collection was to check contemporary ocean bicarbonate radiocarbon levels among prehistory radiocarbon samples. The measured $\Delta^{14}C$ values (122.2 ± 6.6‰ and 129.2 ± 7.7‰) from these samples were consistent with the trend in (and on the upper edge of) the near peak distribution described from the three southern GBR coral $\Delta^{14}C$ records. However, it is important to note that there are numerous terrigenous and reef dissolution carbon sources (^{14}C fortified or depleted) to the GBR that may complicate more regional bomb radiocarbon signals, similar to prehistory radiocarbon records of the region (Gillespie and Polach 1979) and as observed for fishes with an estuarine life history phase (Campana and Jones 1998). Hence, comprehensive documentation of the bomb radiocarbon record is necessary for the northern GBR and careful consideration must be given to the early life history of the fish species under study when using this compiled GBR coral $\Delta^{14}C$ record.

The collective $\Delta^{14}C$ records continuously covered a period ranging from well before the bomb radiocarbon signal to the peak in the 1970s and ending near the beginning of the post-bomb decline period. Both Heron Island and Lady Musgrave Island records end in 1983 during a time when $\Delta^{14}C$ levels plateaued and had remained elevated. The Abraham Reef $\Delta^{14}C$ record provided evidence of the post-bomb $\Delta^{14}C$ decline and ended in 1991. The additional juvenile *C. undulatus* otolith further reinforced this observation with a continuation of the $\Delta^{14}C$ decline by an additional 20‰ in 8 years. The extended period for peak $\Delta^{14}C$ levels of 15–20 years is typical for marine bomb radiocarbon signals that are intermediate in amplitude and in the southern hemisphere (e.g. Guadalcanal; Guilderson *et al.* 2004; Schmidt *et al.* 2004). This duration is greater than typical for existing north Pacific $\Delta^{14}C$ records (e.g. Japan and Hawaiian Islands; Grottoli and Eakin 2007), a factor that is unfortunate for fish age determination from otolith cores that contain the plateaued $\Delta^{14}C$ levels. Even where the post-peak $\Delta^{14}C$ decline is strong, the $\Delta^{14}C$ peak is not useful for well defined age estimates (e.g. Andrews *et al.* 2012). However, the combined $\Delta^{14}C$ record from these coral cores exhibited good temporal continuity and age estimate resolution for the most informative period, the $\Delta^{14}C$ rise. Between *c.* 1958 and *c.* 1970 the $\Delta^{14}C$ levels across this region rose by ~140‰ (from ~–40 to ~100‰). Determination of age for otoliths with measured $\Delta^{14}C$ values in the rise period led to age estimates that are accurate to ~±1 year based on the prediction intervals.

Specimen options for *C. undulatus* otoliths from the GBR were greater than was available for *B. muricatum*, but in each case the measurement of $\Delta^{14}C$ from the otolith cores provided some form of age estimate confirmation. For *C. undulatus*, the initial age estimates from growth zone counting indicated that birth years might not fall within the informative $\Delta^{14}C$ rise period. If age estimates were correct, $\Delta^{14}C$ values would all be at or after the $\Delta^{14}C$ peak in the 1970s to 1980s. However, ages underestimated by only a few years would have been clearly defined as reduced $\Delta^{14}C$ values equivalent to the upper part of the $\Delta^{14}C$ rise period (<100‰). No $\Delta^{14}C$ value from *C. undulatus* otoliths was below peak levels and birth years calculated from growth-zone derived age estimates for these fish were consistent with the broad peak period. This result is not ideal in terms of validating age, but none of these fish were older than 30 years of age and it is likely that growth zone counting is an accurate method for age estimation of this species on the GBR. Otolith sections provided a clean and clearly visible series of growth zones and it is possible that otolith thickness or weight might provide a proxy for age; however, rigorous evaluation of otolith section age and the age proxies would require additional specimens to adequately describe the relationship.

Whereas few *B. muricatum* otoliths were available, these importantly provided validated age determinations from bomb radiocarbon dating. Three of the five specimens measured for bomb radiocarbon yielded valid ages of ~30–40 years from $\Delta^{14}C$ values that could be strictly calibrated to the $\Delta^{14}C$ rise period. Given that the coral record is a direct reflection of the marine environment and the carbon sources to the *B. muricatum* otolith, two of the three age estimates from growth zone counting in otoliths were low by 1 or 2 years and one was accurate within the margin of error from the coral $\Delta^{14}C$ prediction intervals. The otolith age estimates for the two younger *B. muricatum* specimens were accurate within the limited calibration of the peak $\Delta^{14}C$ period, similar to the findings for *C. undulatus*. Three of these fish were relatively large, but they were ~400–600 mm FL smaller than the largest specimens known for this species (maximum observed size 1390 mm FL; Hamilton and Choat 2012). Hence, it is possible that the potential longevity of *B. muricatum* greatly exceeds the maximum validated age of 40 years.

One possible complication for bomb radiocarbon dating of *B. muricatum* is with what appears to be a greater dietary carbon contribution to the otolith based on $\delta^{13}C$ values in the range of -6 to -8‰. Although $\Delta^{14}C$ is corrected for fractionation using this value, the $\Delta^{14}C$ reference record that is applicable for age validation may be shifted slightly from the coral $\Delta^{14}C$ reference record. This has been a regular consideration for bomb radiocarbon studies of elasmobranchs where the primary carbon source to the vertebrae is dietary (Fry 1988; Campana *et al.* 2002). In some cases, a phase lag to the bomb $\Delta^{14}C$ signal has been documented and was attributed to either a trophic level delay (sharks eating older marine organisms) or radiocarbon depleted food sources (mix of surface and deep water carbon sources; Campana *et al.* 2002). The $\delta^{13}C$ values for sharks are typically between -10 and -16‰ and indicative of metabolic carbon whether in or out of phase with the regional coral $\Delta^{14}C$ record. The otoliths of marine teleost fishes tend to have $\delta^{13}C$ values quite close to 0‰ and may range between -1 and -3‰, similar to the coral records and seemingly negligible in terms of defining a potential phase lag to the $\Delta^{14}C$ signal from such factors. However, the values measured for *B. muricatum* indicate there is a more significant contribution of metabolic carbon to the otolith than is usual. Hence, the minor offset seen as underestimated age for two of the fish may be related to a minor $\Delta^{14}C$ phase lag. The best way to truly test this hypothesis is with known age juvenile samples collected from the region during the $\Delta^{14}C$ rise period, but it is very unlikely that such specimens exist. One example of how metabolic carbon may affect temporal alignment of otolith and coral $\Delta^{14}C$ records might be exemplified by the otolith sample series from juvenile speckled hind (*Epinephelus drummondhayi*; Andrews *et al.* 2013*a*). Measured $\delta^{13}C$ levels for this species were a mean of -5.3‰, indicating a minor contribution of metabolic carbon to the otolith. Hence, the observable yet insignificant offset from the coral $\Delta^{14}C$ record from the juvenile speckled hind sample series is perhaps a manifestation of this kind of phase lag in otoliths.

A tangible source of carbon that could lead to a delayed $\Delta^{14}C$ signal for parrotfishes is the incorporation of carbon from older coral growth. The bumphead parrotfish, unlike most other parrotfishes, is corallivorous (Bellwood and Choat 1990; Bellwood *et al.* 2003; Hoey and Bellwood 2008; Bonaldo and Bellwood 2011), and it is possible that the carbonate present at some time in the past within the ingested coral would become part of the metabolic carbon sequestered by the fish, even though most is passed through the digestive system. This hypothesis, however, might not apply to the depleted $\delta^{13}C$ values, and other explanations need to be explored. Further, the two younger *B. muricatum* provided near peak bomb $\Delta^{14}C$ levels. In studies of elasmobranchs and teleost fishes where depleted radiocarbon sources were a likely factor, the peak $\Delta^{14}C$ levels were usually attenuated from coral and other more timely records (e.g. Campana *et al.* 2002; Filer and Sedberry 2008). Hence, the integration of $\delta^{13}C$ information and temporal alignment of measured otolith $\Delta^{14}C$ values with coral reference records remains somewhat unresolved, although it is likely a small factor relative to other forms of age estimate uncertainty, like the precision of growth zone counting in otoliths.

Successful measurement of ^{14}C from the core of small otoliths provides a basis for expanding the utility of bomb radiocarbon dating to other tropical reef fishes. Careful specimen preparation and use of a micromilling machine accurately removed what likely represented the first few months of growth for each of these fishes. An extracted sample mass of ~2 mg resulted in a $\Delta^{14}C$ measurement precision of ~±3‰, a significant increase from previous applications and a gateway to age validation studies of other long lived tropical reef fishes. Of interest from the GBR and other tropical regions are other parrotfishes and wrasses that have high ecological value for which there exists preliminary information on age and growth (Choat *et al.* 1996; Nardi *et al.* 2006). Additional information on age based demography is required including validation studies as described here. This is important for species of importance to fisheries. Estimates of age exist for many species, but otolith age reading is a difficult endeavour that requires validation, especially for long lived species and those whose otoliths have poorly defined annual growth zones.

Unique to bomb radiocarbon dating is the capability of providing validated age estimates from nothing more than a measured quantity within the otolith chemistry. The utility of this method as an independent chronometer was exemplified in the case of pink Hawaiian snapper (*Pristipomoides filamentosus*; Andrews *et al.* 2012). The otoliths of this species simply did not provide an annular growth zone pattern that could be clearly elucidated to allow estimation of age with any degree of consistency or reliability. Hence, bomb radiocarbon dating of *P. filamentosus* was an application blind to conventional estimates of age. Lead-radium dating is similar in this regard, but it is limited by sample mass limitations that lead to pooling of numerous fish otoliths and the end result is a mean age for a group of fish (e.g. Andrews *et al.* 2009). Bomb radiocarbon dating is unique because it can provide valid ages for individual fish and the limitations are now fewer with an increase in both methodological precision and accuracy.

Our findings also have important implications for improving the management of these large, long-lived and iconic reef fishes. Both species in this study are experiencing widespread local and regional depletions and extirpations for a variety of related reasons (Sadovy *et al.* 2003; Sadovy de Mitcheson *et al.* 2010). In particular, the habitat traits of *B. muricatum* make this species sensitive to over exploitation. Its shallow distribution, consistent and predictable schooling behaviour, and trophy inspired incidental take make this conspicuous species highly vulnerable to spearfishing and netting (Hamilton and Choat 2012; DeMartini and Smith 2014). In addition, the restriction of juvenile recruitment to high coral cover, lagoon fringing reefs increases vulnerability, especially for *B. muricatum* where nursery environments are sensitive to the effect of poor land based practices (R.J. Hamilton, pers. obs.). Furthermore, life history attributes of *B. muricatum* make it particularly sensitive to over exploitation. Longevity is likely to exceed the validated age of ~40 years and maturity is late and at a large size (7–9 years at 600 mm FL; Hamilton *et al.* 2008; Hamilton and Choat 2012). Moreover, *B. muricatum* serve a key ecological role as habitat engineers that shape the functional structure of coral reefs (Bellwood *et al.* 2003). Hence, it is imperative that key life history information, like the age validations provided by the present study, be fully considered along with repeat characterisations of size-at-age and longevity elsewhere. This kind of information can then be used to generate better input to regional fishery management plans for these species in countries and archipelagos beyond the Great Barrier Reef of Australia.

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