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# **Development and Assessment of an Ageing Criteria for the Main Hawaiian Islands Crimson Jobfish *Pristipomoides filamentosus* (‘Ōpakapaka)**

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U.S. DEPARTMENT OF COMMERCE  
National Oceanic and Atmospheric Administration  
National Marine Fisheries Service  
Pacific Islands Fisheries Science Center

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# Development and Assessment of an Ageing Criteria for the Main Hawaiian Islands Crimson Jobfish *Pristipomoides filamentosus* (‘Ōpakapaka)

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## Abstract

The economic and cultural importance of the crimson jobfish *Pristipomoides filamentosus* has long placed it at the pinnacle of the Hawaiian deepwater handline fishery. Known locally as opakapaka, the species is assessed and managed as part of a seven species bottomfish complex called the “Deep-7.” To provide length at age and somatic growth information for an age-based single-species stock assessment, we examined opakapaka otolith microstructure and age interpretation. Using standardized thin-sectioning methods for age estimation, we developed an ageing criterion based on (1) daily increment counts to confirm the location of first annulus, (2) evaluation of otolith shape between younger and older fish, and (3) comparison of annulus counts with the bomb radiocarbon age of each specimens. Between-reader age-precision estimates were at acceptable levels for deep-water species (APE, 5.5%; CV, 7.7%). We conclude that the application of this ageing criterion to thin otolith sections is a suitable method for the determination of annual age estimates for opakapaka between 1 and 44 years old. The resulting criterion will be a reference for estimating future age compositions of the fishery and evaluation of main Hawaiian Islands sex-specific growth of *P. filamentosus*.

Keywords: *Pristipomoides filamentosus*, otolith, ageing criteria, bomb radiocarbon

## Introduction

Throughout the Indo-Pacific, the crimson jobfish (*Pristipomoides filamentosus*) is a keystone commercial and recreational fisheries species (Randall 2007). However, being a deep-water snapper (100–400 m), the relative importance of this species across its distribution is closely related to the capacity of fishing effort (Newman et al. 2015). The capacity in most tropical and subtropical regions is at a much smaller scale than developed countries. Most effort is restricted to artisanal and subsistence fishing; with only a few larger efforts in developed countries with established commercial bottomfish fisheries that utilize more specialized and sophisticated gears, including Hawaii (Dalzell et al. 1996).

In Hawaii, there is a long history of exploitation of *P. filamentosus*, known locally as opakapaka, and other deep-water snappers and groupers (herein referred to as bottomfish, Haight et al. 1993). The initial harvesting of bottomfish was by the indigenous peoples of Hawaii and progressed for hundreds of years, well before the emergence of the contemporary commercial fishery in the 1950s (WPRFMC 2016). Today’s Hawaiian bottomfish fishery is not solely targeted by commercial fishers, it is composed of a complex mix of commercial, recreational, cultural, and subsistence fishermen whose fishing behaviors do not fit easily into existing legal and regulatory frameworks, thereby complicating the monitoring and management aspects of the fishery (Hospital and Beavers 2012).

Currently, the bottomfish fishery in Hawaii is assessed as a “Deep-7” bottomfish complex, which is made up of six eteline snappers (including *P. filamentosus*) and one endemic grouper, and managed as a single stock under the Western Pacific Regional Fisheries Management Council—Hawaii Fisheries Ecosystem Plan (WPRFMC 2016). The historic lack of adequate life history information is one of the reasons for the species complex approach to assessing the stock status of the Deep-7 species, especially in terms of the age-based applications to stock assessments and modeling procedures (Langseth et al. 2018; Newman et al. 2015). Following the 2005 assessment, improvements in data (maximum age of *P. filamentosus*, unreported fishery catches, and standardized catch per unit effort) were incorporated into the benchmark assessment to improve the approximation of bottomfish population dynamics (Langseth et al. 2018). However, the most recent Western Pacific Stock Assessment Review for the Deep-7 benchmark stock assessment still recommended the need for continued life-history work (age, growth, and maturity) and the inclusion of these parameters into single-species models (Martell et al. 2017).

A series of international workshops on data-poor deep-water snappers and groupers identified several areas that needed improvement for accurate length at age information for these species (Newman et al. 2015, 2016; Wakefield et al. 2016, 2017). The more recent development of standardized otolith sectioning techniques and use of oblique reflected light resulted in improvements in otolith growth zone clarity, interpretation and repeatability of annuli counts for many deepwater snappers (Newman et al. 2016). As a result, a more coordinated approach was taken to determine the life history parameters of many bottomfish in Hawaii under Pacific Islands Fisheries Science Center (PIFSC) jurisdiction, including *P. filamentosus*.

The nascent methodology outlined in Newman et al. (2016), specifically related to fish age estimation, has improved the precision and accuracy of age-based studies for other bottomfish species. Wakefield et al. (2017) examined these methodologies and reported that the ageing



precision and bias for multiple readers of *P. filamentosus* was reasonable but commensurate with reader experience. The findings also indicated that less precision was linked to younger, pre-maturation individuals because the interpretation of early growth zones (approximately 5 years or less) are much broader and more diffuse. For older individuals, the less conspicuous delineation of opaque and translucent band led to lower variation in annuli counts between readers (Wakefield et al. 2017). Wakefield et al. (2017) highlighted that a reader may need to possess a higher level of ageing experience or calibration of growth zone formation (reading criteria to interpret annuli patterns). Andrews et al. (2012) validated the upper limits of age data for *P. filamentosus*, and thus providing a solid foundation for the current development of ageing criteria in the Hawaiian Archipelago.

The objective of this study is to determine the feasibility of ageing Hawaiian *P. filamentosus* to provide accurate and precise length at age information for stock assessment purposes. First, we applied the standardized sectioning techniques to develop the most efficient method of preparing thin-sectioned otoliths. Second, we examined daily growth-zone formation to identify the deposition of the first opaque zone and aid the interpretation of first annulus. Third, we created a reference set of otoliths and evaluated the annual periodicity of growth zones using known-age (bomb radiocarbon dated) otoliths to validate the ageing criterion. Lastly, we evaluated within and between-reader precision and ageing-bias of the reference set to understand potential interpretation errors.

## Materials and Methods

### *Otolith Collection*

*P. filamentosus* were collected across the Hawaiian Archipelago (Northwestern and main Hawaiian Islands) from 1978 to 2017 from several different sources. Samples were collected aboard NOAA research vessels (1978–2017), purchased from commercial fishermen (2007–2015) using NOAA Improve a Stock Assessment funds, and donated by local recreational fishermen (2013–2017). All samples were associated with collection data that included location, date, fish length, fish weight, and macroscopic sex identification.

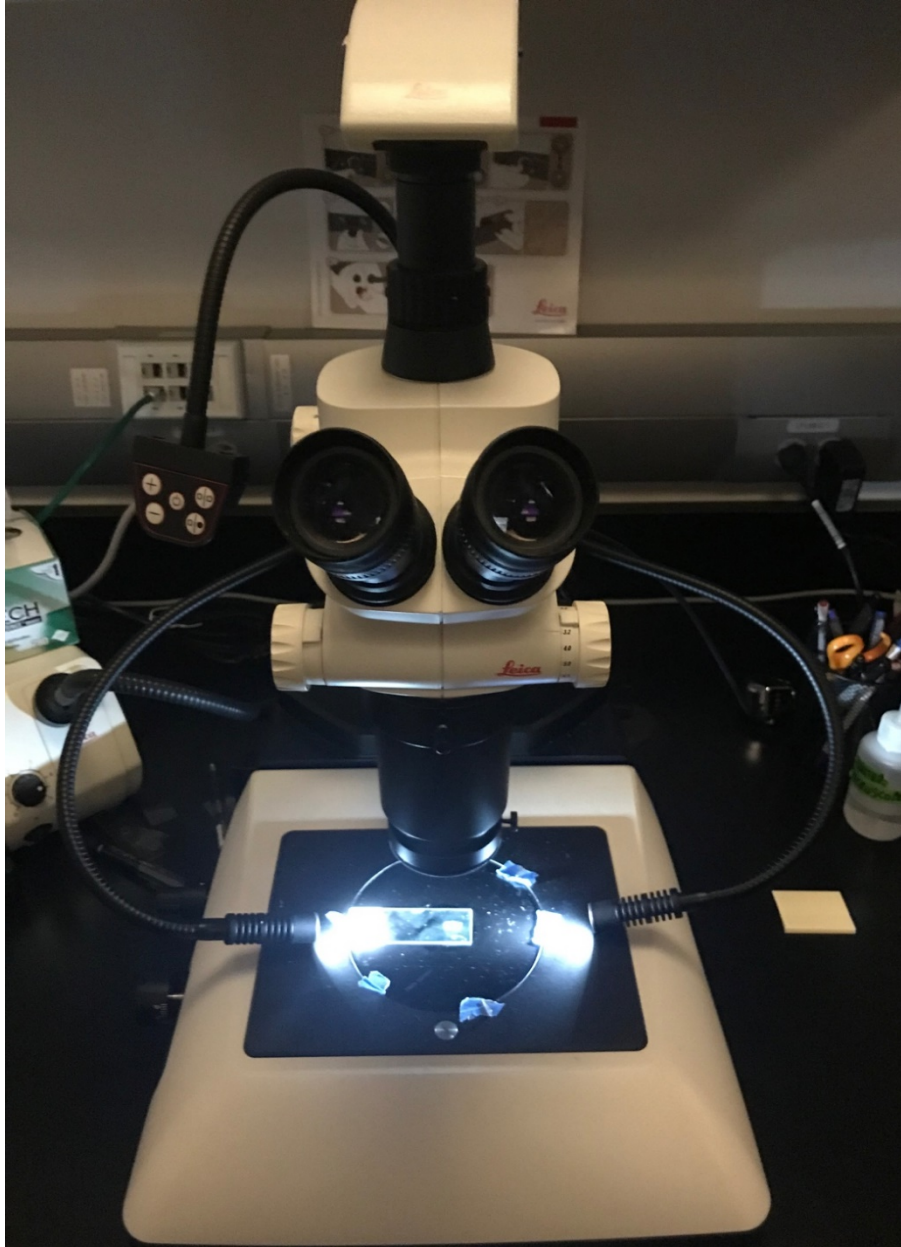
Paired sagittal otoliths were removed, cleaned, dried, and stored in open scintillation vials. After a 24-hour drying period, each otolith was evaluated for condition. Any otolith that was chipped or broken were excluded from this study. Unbroken otoliths were weighed to the nearest 0.001 g using an analytical balance.

Otoliths were selected at random for age analysis as part of the reference collection. Specimens were selected to incorporate an individual of each sex per 1 cm length bin, when possible. Individuals that lacked macroscopic sex determination were listed as unidentified sex (typically <25 cm FL).

### *Otolith Section Preparation and Age Estimation*

Otolith preparations followed standardized methods as described in Taylor and McIlwain (2010) and Newman et al. (2015 and 2016). To summarize these protocols, one otolith (either right or left, randomly chosen) was examined under a dissecting scope at 50× for condition; if suitable, the sagitta was marked to identify the primordium on the medial surface along the sulcus acusticus. The primordium was used as a reference point to ensure that the thin transverse section was aligned perpendicular through the core. Next, each individual otolith was ground using a GEMMASTA GFL8 lapping wheel, to standard thickness (0.200–250  $\mu\text{m}$ ). Then, to improve the readability of opaque bands, the thin section was swirled in a diluted 5% HCL solution for approximately 10 seconds and placed in a distilled water bath for 30 seconds. After a section was removed from the distilled water bath and dried, it was mounted onto a glass slide with crystal bond or resin and covered with a cover slip.

Each otolith section was viewed at 40–80× magnification using a Leica S8 APO stereomicroscope against a black background and two obliquely reflected light sources (Fig. 1). Transmitted light was initially used for thicker (400–650  $\mu\text{m}$ ) bomb radiocarbon sections, prior to thinning to the standard thickness (~250  $\mu\text{m}$ ) and viewed with reflected light for age estimates. “Annuli” were defined as opaque growth zones alternating with hyaline zones along the face of the transverse section (Peres and Haimovici 2004). Individual opaque growth zones were counted by the primary age reader (RN) on three separate occasions without knowledge of sample information (fish size, sex, or date of capture). The number of annuli were used as a proxy for ages in years and final age were assigned when two counts agreed.



**Figure 1. Example of Leica S8 APO stereomicroscope with sample against a black background and two obliquely reflected light sources.**

*Daily Growth and Evaluation of First Annulus*

Otolith daily growth increments (DGI) of individuals <24 cm FL were examined to identify the location of the first annulus. An Olympus BX-51 compound microscope with transmitted light source at 20–60× magnification was used to count DGIs along a various growth axis. Daily increments were counted from the primordium to the completion of each annual opaque band. Three individual counts were conducted by the primary age reader (RN) for each otolith, separated by one week, and final age was taken as the mean of three counts when counts were within 10% of the median. If samples were greater than 10% of the median than the sample was

excluded. Daily growth profiles of recently recruited *P. filamentosus* were first summarized in Uchiyama and Tagami (1984) and later by DeMartini et al. (1994).

### *Validation of Annual Periodicity*

The assumption that otolith increments were deposited annually and derived ages from thin sections indicate accurate ages was tested by using a selection of the adult otoliths (n = 20) that were evaluated for radiocarbon age in Andrews et al. (2012; Table 1). These thin sections were prepared as indicated above. Radiocarbon studies to date suggest that bomb radiocarbon can confirm the accuracy of an age and utility of an ageing method to within 2–3 years for most teleost if they were on the rise side of the reference curve (Kalish 1993; Hamel et al. 2008; Andrews et al. 2011).

**Table 1. Summary of opakapaka fractionation corrected radiocarbon (F14C) otolith data and annual age estimates (table modified from Andrews et al. 2012) Abbreviated names of NWHI reefs: FFS = French Frigate Shoals; NH = North Hampton. The Decline-age and Rise-age refer to the fit on the bomb calibration curve relative to the peak.**

| Sample number | Length (cm FL) | $\Delta^{14}\text{C}$ (‰) | Collection year | Decline-age (yr) | Rise-age (yr) | Age range (yr) |
|---------------|----------------|---------------------------|-----------------|------------------|---------------|----------------|
| FFS-3         | 66.2           | $61.6 \pm 4.0$            | 1978.6          | NA               | $15.1 \pm 1$  | 14–16          |
| FFS-4         | 63.1           | $117.7 \pm 4.9$           | 1978.6          | NA               | $13.1 \pm 1$  | 14             |
| FFS-7         | 57.6           | $158.2 \pm 4.3$           | 1978.6          | $0.3 + 3$        | $11.1 \pm 2$  | 10–11          |
| Gardner-1     | 73.0           | $96.2 \pm 5.6$            | 2007.9          | NA               | $43.1 \pm 1$  | 43–45          |
| Gardner-2     | 71.8           | $121.3 \pm 5.2$           | 2007.6          | $3.3 + 3$        | $41.9 \pm 1$  | 38–40          |
| Gardner-3     | 74.5           | $137.3 \pm 5.0$           | 2007.6          | $11.3 + 3$       | $41.3 \pm 1$  | 39–41          |
| Gardner-5     | 57.7           | $148.3 \pm 4.3$           | 1981.6          | NA               | $21.6 \pm 1$  | 18–20          |
| Maro-3        | 67.3           | $-45.5 \pm 4.8$           | 1981.1          | NA               | $27.1 \pm 2$  | 25–26          |
| Maro-5        | 74.2           | $-42.3 \pm 4.2$           | 1981.6          | NA               | $26.6 \pm 2$  | 24             |
| Necker-1      | 60.0           | $-17.9 \pm 3.6$           | 1981.1          | NA               | $22.6 \pm 1$  | 23             |
| Necker-2      | 60.4           | $135.3 \pm 4.4$           | 1981.1          | NA               | $15.1 \pm 1$  | 14–16          |
| NH-1          | 71.9           | $186.2 \pm 4.8$           | 2007.8          | NA               | $35.4 \pm 2$  | 35–37          |
| Pioneer-1     | 74.6           | $170.9 \pm 4.9$           | 2007.4          | $27.8 + 4$       | $38.1 \pm 2$  | 38–40          |
| Raita-2       | 64.9           | $160.7 \pm 5.2$           | 1980.3          | NA               | $22.2 \pm 1$  | 15–22          |

| <b>Sample number</b> | <b>Length (cm FL)</b> | <b><math>\Delta^{14}\text{C}</math> (‰)</b> | <b>Collection year</b> | <b>Decline-age (yr)</b> | <b>Rise-age (yr)</b> | <b>Age range (yr)</b> |
|----------------------|-----------------------|---|------------------------|-------------------------|----------------------|-----------------------|
| Raita-4              | 61.4                  | 170.5 $\pm$ 4.5                             | 1980.3                 | 0.5 + 3                 | 11.1 $\pm$ 2         | 11                    |
| Twin-2               | 70.6                  | 143.6 $\pm$ 5.0                             | 2008.2                 | 15.0 + 3                | 41.7 $\pm$ 1         | 16–17                 |
| M10-269              | 71.2                  | 125.8 $\pm$ 6.0                             | 2008.2                 | 23.2 $\pm$ 3            | 41.7 $\pm$ 1         | 26–27                 |
| M11-471              | 70.0                  | 121.9 $\pm$ 4.9                             | 2008.2                 | 21.8 $\pm$ 3            | 41.9 $\pm$ 1         | 16                    |
| CK10-12              | 70.8                  | 127.9 $\pm$ 3.9                             | 2008.3                 | 24.1 $\pm$ 3            | 41.7 $\pm$ 1         | 21–22                 |
| M5-219               | 73.0                  | 126.7 $\pm$ 4.5                             | 2008.3                 | 22.4 $\pm$ 3            | 41.8 $\pm$ 1         | 23–25                 |

### *Ageing Criteria Development*

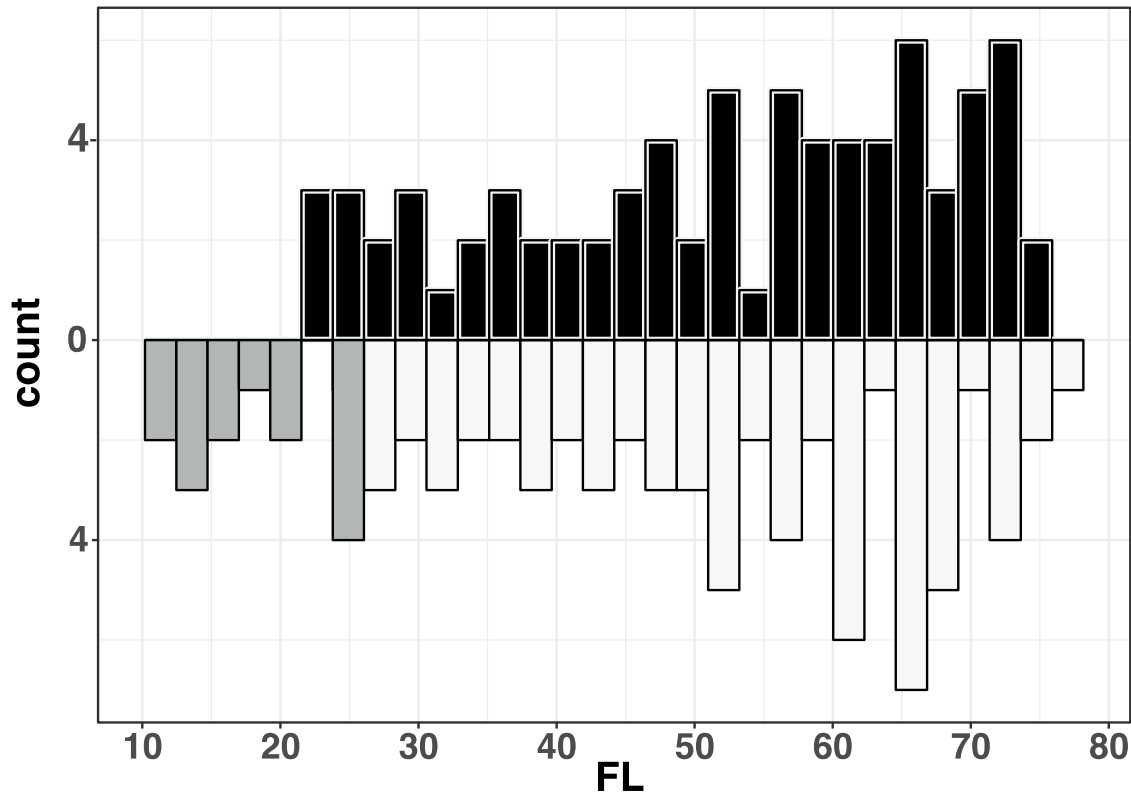
The primary reader (RN) and a second reader (ES) examined the otolith reference collection (n = 50) to evaluate ageing precision and bias and establish the ageing criterion. Prior to completion of the ageing project, both readers together created an ageing criterion (calibration of growth zone interpretation) to ensure consistency between reads. This criteria included the identification of the first annuli by using information collected during the DGI analysis, and assessment of the “transition area” from early growth zones (broad and more diffuse bands) to less conspicuous older growth (>5 annuli, wider opaque and thinner translucent bands).

After the otoliths were initially aged, the precision and bias were analyzed with traditional ageing precision metrics including: Index of Average Percent Error (IAPE; Beamish and Fournier 1981), and Coefficient of Variation (CV; Matt and Kimura 2012). Precision metrics were compared to general acceptable levels of dissimilarity  $\leq 5.5\%$  for IAPE and/or  $\leq 7.6\%$  for CV (Campana 2001; Wakefield et al. 2016). Otoliths with a large between-reader difference were re-examined and precision metrics were re-calculated. Age estimation was tested for symmetry and Bland-Altman plots were used for visual evaluation of age-bias and precision using methods described in McBride (2015).

## Results

### *Otolith Processed*

A total of 160 *P. filamentosus* otoliths collected in the Hawaiian Archipelago between 2007 and 2017 were used to develop the ageing criteria. Fish sizes ranged from 10.8 to 76.7 cm FL (Fig. 2). Macroscopic sex information, from archived collection data, was available for the majority of the fish (n = 77 female, n = 69 male); fish that were not macroscopically identified to sex were less than 25 cm FL (n = 14).

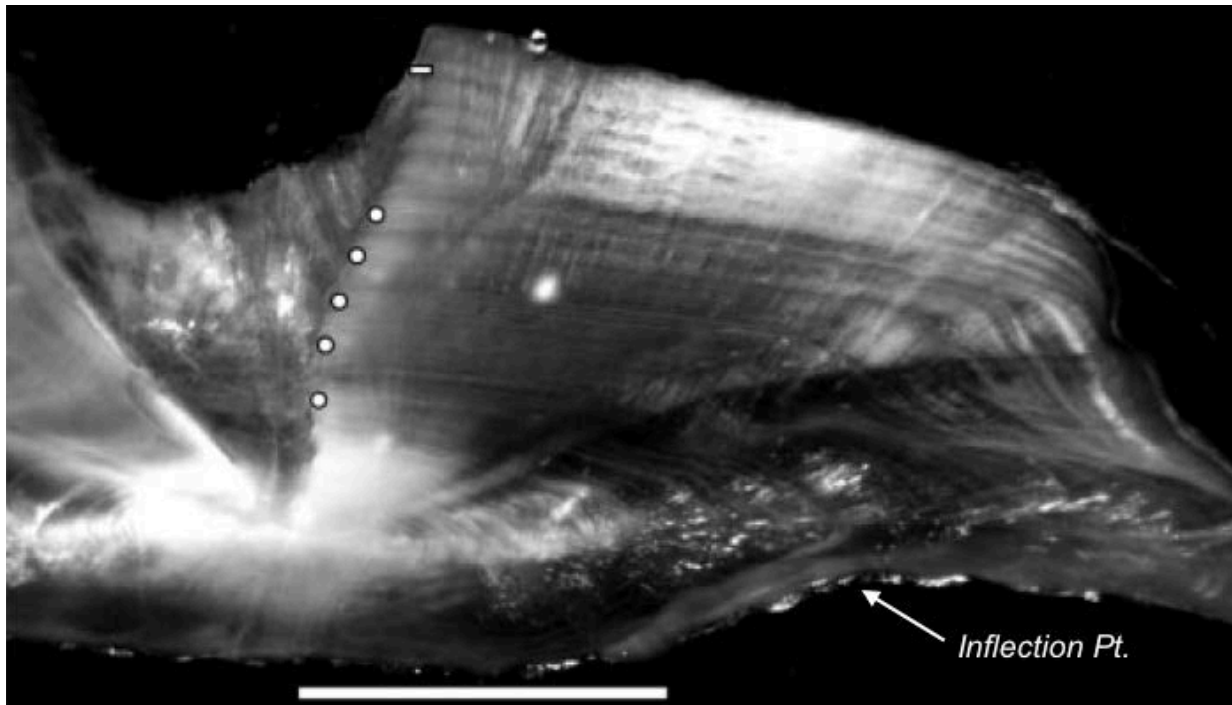


**Figure 2. Size frequency of *P. filamentosus* otolith sampled from Hawaiian Archipelago. Fish less than 22 cm FL were not macroscopically identified by sex (gray), females (black), and males (white).**

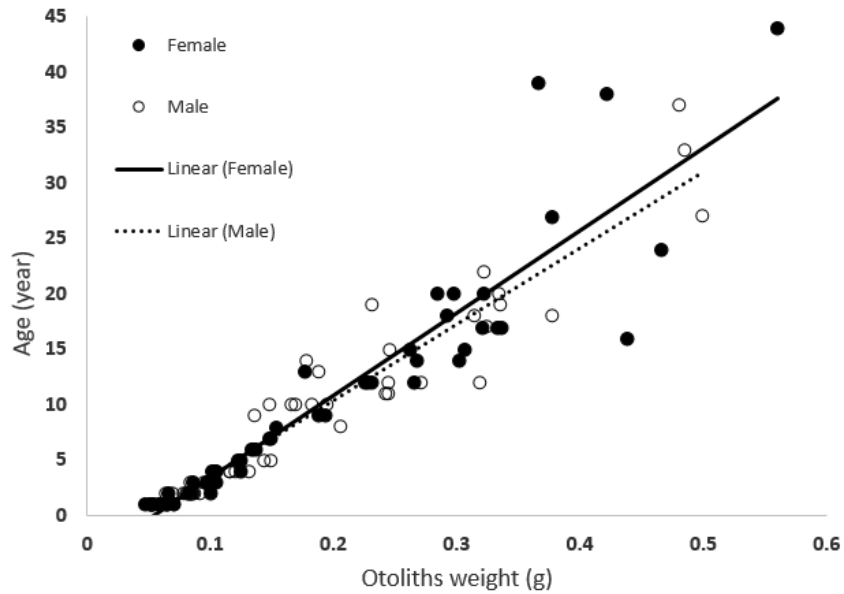
### *Otolith Sections*

The standard methodology utilized for preparing thin transverse sections maximized the clarity of the annual patterns in sagittal otoliths and indicated that *P. filamentosus* deposit clearly defined annuli under reflected light (Fig. 3). Otolith thin-sections that were fine polished to 180–250  $\mu\text{m}$  thickness clearly displayed alternating annulus pattern of opaque and translucent bands. Annuli were identified in 153 of the 160 otoliths examined (96%), the seven otoliths that lacked an annulus were all younger individuals (<1 year old, <16 cm FL) and aged using DGI (see below). Otolith weight was a strong linear predictor for age (female  $r^2 = 0.92$ , male  $r^2 = 0.93$ ; Fig. 4). The linear relationships in otolith weight between sexes began to drift after age 10 but

differences between means did not differ substantially between the sexes (ANCOVA,  $F_{2,106} = 1.67, p = 0.19$ ).



**Figure 3. Image of thin transverse section under reflected light of *P. filamentus* 44.7 cm FL with 11 opaque zones. White circles indicate individual annuli, white bar represents location of fifth opaque zone. Arrow indicates the inflection point on sagittal otolith that is associated with the first annuli. Scale bar = 1 mm.**

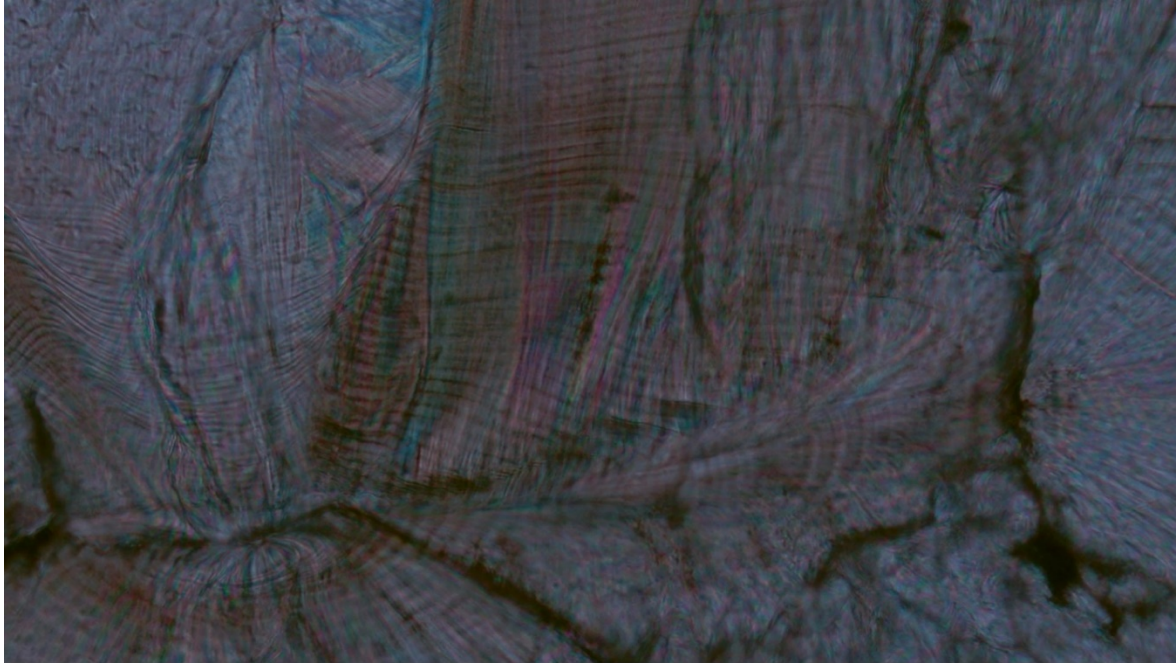


**Figure 4. Sex specific relationship between sagittal otolith weight (g) and estimated annuli for *P. filamentosus* in the Hawaiian Archipelago. Linear equation is as follows: female age =  $78.3(\text{Otolith Weight}) - 4.4$ , male age =  $68.1(\text{Otolith weight}) - 3.3$ .**

#### *First Annulus Assessment by Daily Increment Counts*

Juvenile opakapaka (10–24 cm FL) otoliths displayed clear microincrements apparent along the entire dorsal axis (Fig. 5). The counts of microincrements of 16 juvenile fish ranged from 158 to 482 days (mean 295.6 days, Table 2), with the smallest fish (10.6 cm FL) estimated at 155 days and the 465 days for the largest (24.7 cm FL). The two replicate microincrement counts were within 10% median and the CV was 3.06% for the 16 fish. Evidence of a distinct inflection point on the edge of the otolith was found on six juvenile fish greater than 22.0 cm FL (Table 2, Fig. 5). A post hoc sectioning and evaluation for annulus was completed on four juvenile fish (>22 cm FL). The paired otolith that was sectioned showed a single annulus and inflection point. A comparison of our *P. filamentosus* DGI counts did not differ significantly from previously reported DGI counts in DeMartini et al. (1994; Fig. 6) (t-test;  $p = 0.429$ ). The regression line indicated a larger intercept in this study compared to the DeMartini et al. (1994); however, there was not a significant difference in slope ( $p = 0.417$ ; Fig. 6).

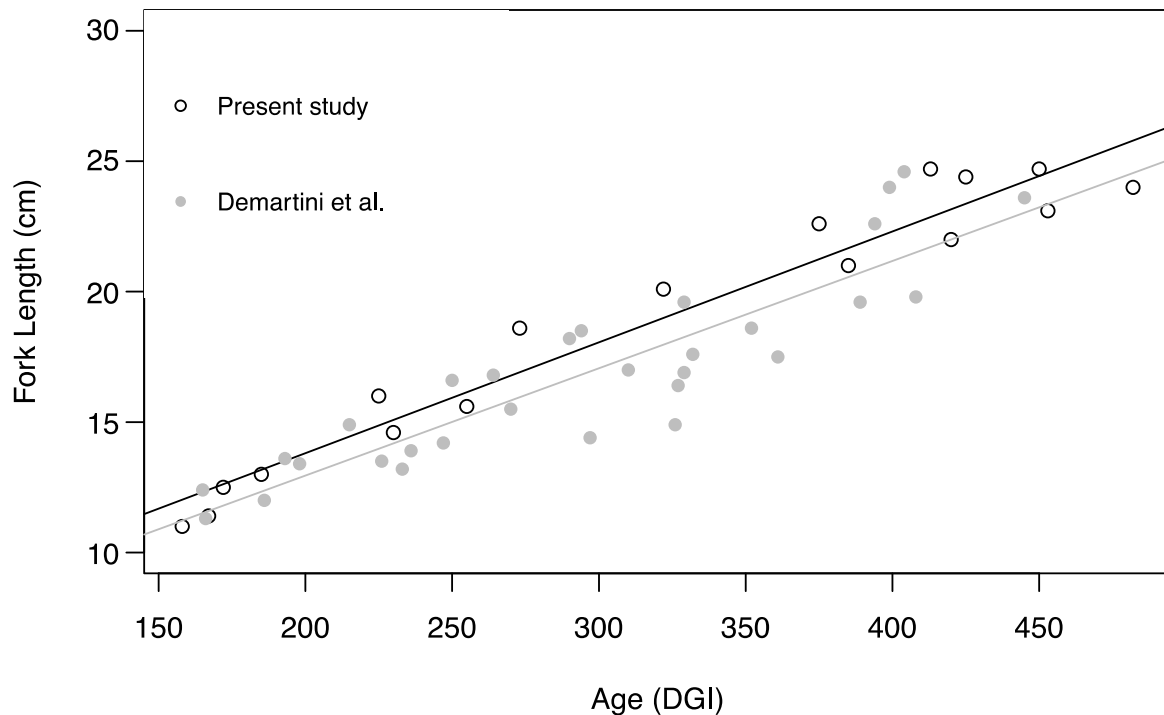




**Figure 5. View of a juvenile *P. filamentosus* (18 cm FL) daily growth increments (DGI) under transmitted light and high magnification (40 $\times$ ).**

**Table 2. Summary of three separate counts of daily growth increment (DGI). Sample number, fork length (FL cm), minimum age (days), maximum age (days), decimal age (DGI/365), and indication of completed first annulus (0 = absent, 1 = present).**

| <b>Sample #</b>   | <b>FL<br/>(cm)</b> | <b>Min. Age<br/>(DGI)</b> | <b>Max. Age<br/>(DGI)</b> | <b>Decimal<br/>Age</b> | <b>Inflection<br/>Pt.<br/>Present</b> |
|-------------------|--------------------|---------------------------|---------------------------|------------------------|---------------------------------------|
| SB9-10 PAKA       | 11                 | 153                       | 158                       | 0.42                   | 0                                     |
| SB9-15 PAKA       | 11.4               | 161                       | 167                       | 0.44                   | 0                                     |
| SB10-20 PAKA      | 12.5               | 168                       | 172                       | 0.46                   | 0                                     |
| SB9-21 PAKA       | 13                 | 162                       | 185                       | 0.44                   | 0                                     |
| SB8-5 PAKA        | 14.6               | 206                       | 230                       | 0.56                   | 0                                     |
| SB14-10 PAKA      | 15.6               | 255                       | 267                       | 0.73                   | 0                                     |
| SB4-15 PAKA       | 16                 | 210                       | 225                       | 0.58                   | 0                                     |
| SB4-17 PAKA       | 18.6               | 273                       | 275                       | 0.75                   | 0                                     |
| JA12-2-2-216 PAKA | 20.1               | 322                       | 340                       | 0.93                   | 0                                     |
| JA11-1-9-189 PAKA | 21                 | 362                       | 385                       | 0.99                   | 0                                     |
| JA16-2-8-342 PAKA | 22                 | 415                       | 420                       | 1.14                   | 1                                     |
| JA16-2-3-323 PAKA | 22.6               | 366                       | 375                       | 1.00                   | 0                                     |
| JA16-3-9-367 PAKA | 23.1               | 446                       | 453                       | 1.22                   | 1                                     |
| JA16-1-1-301 PAKA | 24                 | 468                       | 482                       | 1.28                   | 1                                     |
| SB8-9 PAKA        | 24.4               | 425                       | 438                       | 1.20                   | 1                                     |
| SB9-7 PAKA        | 24.7               | 405                       | 413                       | 1.11                   | 1                                     |
| SB8-2 PAKA        | 24.7               | 465                       | 450                       | 1.27                   | 1                                     |

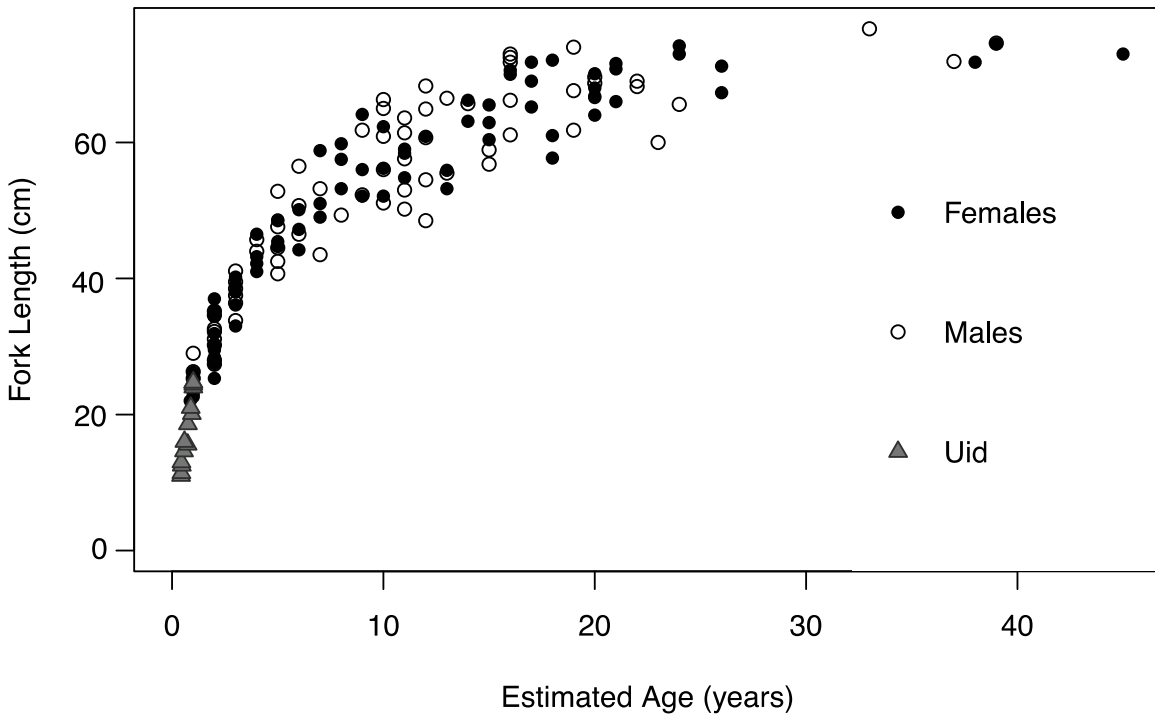


**Figure 6. Daily growth increment (DGI) counts of present study compared to DeMartini et al. (1994).**

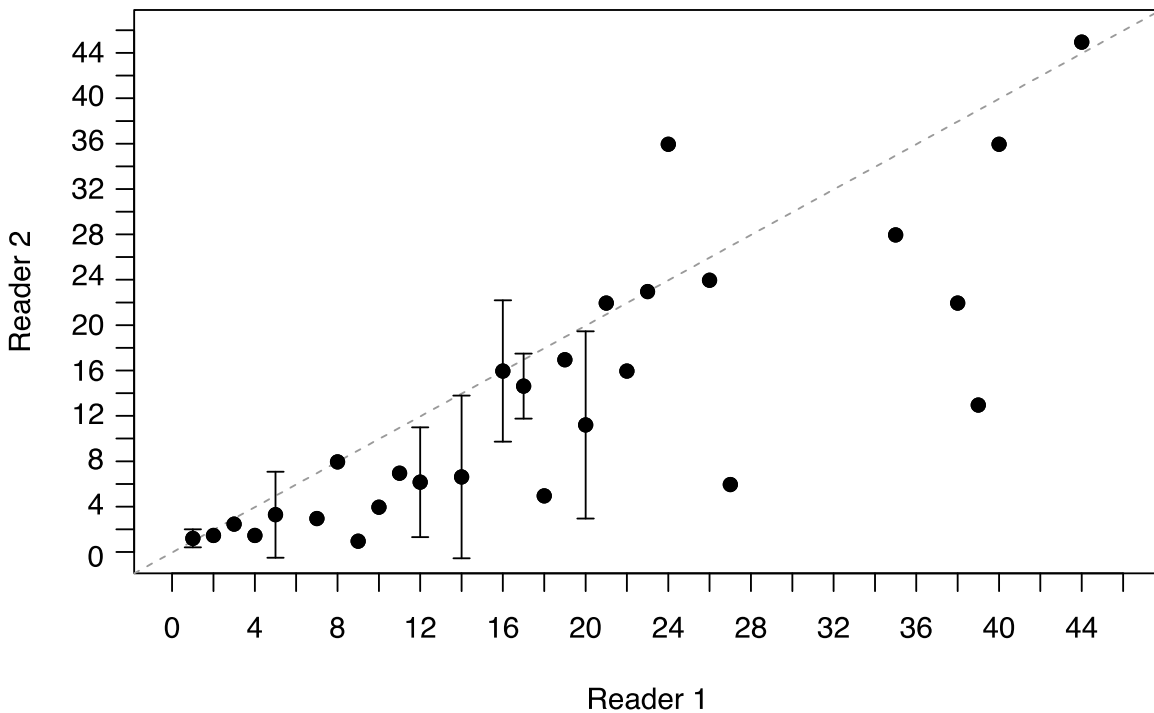
*Ageing Criteria Assessment*

Blinds reads by the primary reader indicated that interpretation and within reader repeatability of annuli counts are feasible (IAPE, 3.4%; CV, 4.8%), particularly with thinner transverse sections (~250  $\mu\text{m}$ ). The direct agreement (PA) among the initial reads of the primary reader was 53%, increasing to 77% within 1 year, and 95% within 2 years.

A wide range of annuli counts (1–45 annuli) fit well to the fish lengths indicating accurate age estimation (Fig. 7). The fish assigned 1 year of age ranged from 18.3 to 29 cm FL, and fish assigned 2 years of age ranged in size from 28.0 to 35.2 cm FL. The precision of initial read (i.e., prior to ageing criterion development) between the two readers was low and not within acceptable limits of ageing precision (IAPE, 23.6%; CV, 33.2%; Fig. 8) and direct agreement was less than 30%. Evaluation of age bias plots indicated that greatest portions of ageing error came with in the first 8 years (Fig. 8).

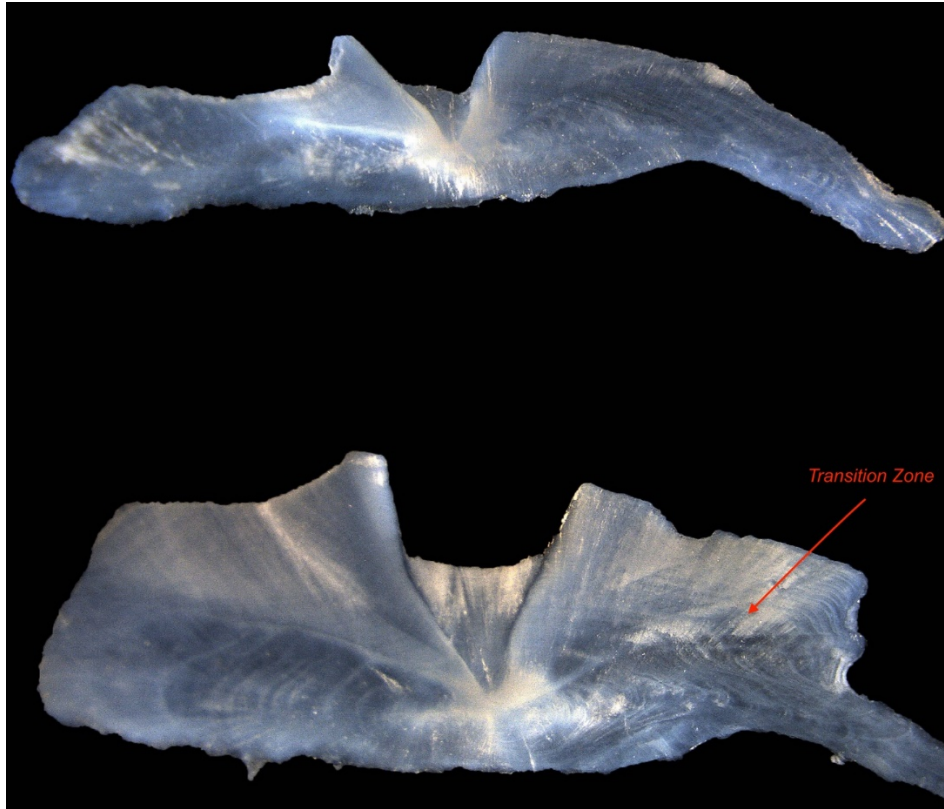


**Figure 7. Initial annuli count (prior to ageing criteria) of estimated age vs. length (FL cm).**



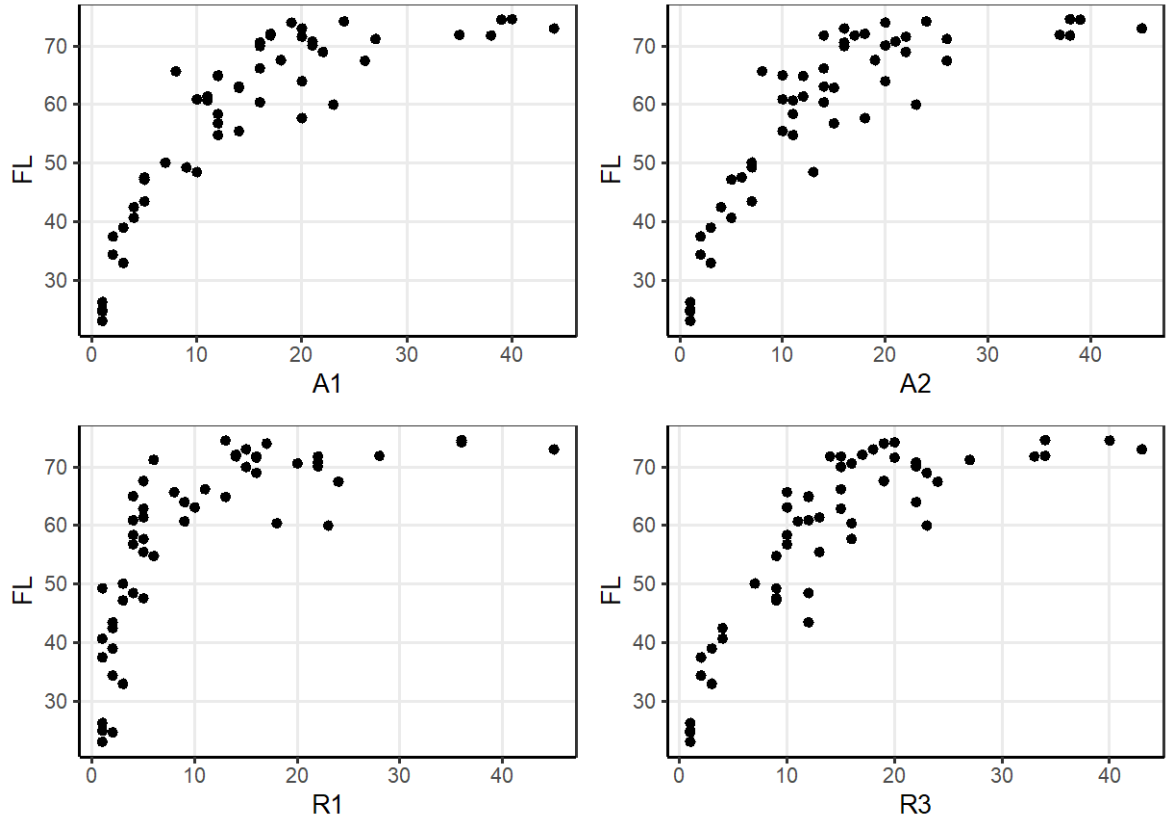
**Figure 8. Age bias plots Reader 1 (primary reader) and Reader 2 before ageing criteria was applied.**

These erroneous interpretation of early growth zones (approximately  $\leq 5$  years) was most likely due to these zones being much broader and more diffuse than those of older individuals ( $> 8$  years). The two readers examined the otoliths for inflection point corresponding with the first annulus. The formation of first annuli corresponded to sizes that ranged from 18 to 29 cm, and the transition area (i.e., narrow and less diffuse zones) of older individuals was evident in fish starting at  $> 5$  years old and FL  $> 50$  cm (Fig. 7). The ability to identifying the growth zones of older individuals ( $> 8$  years) relative to the otolith size assisted with reducing the potential to under ageing individuals (Fig. 9).

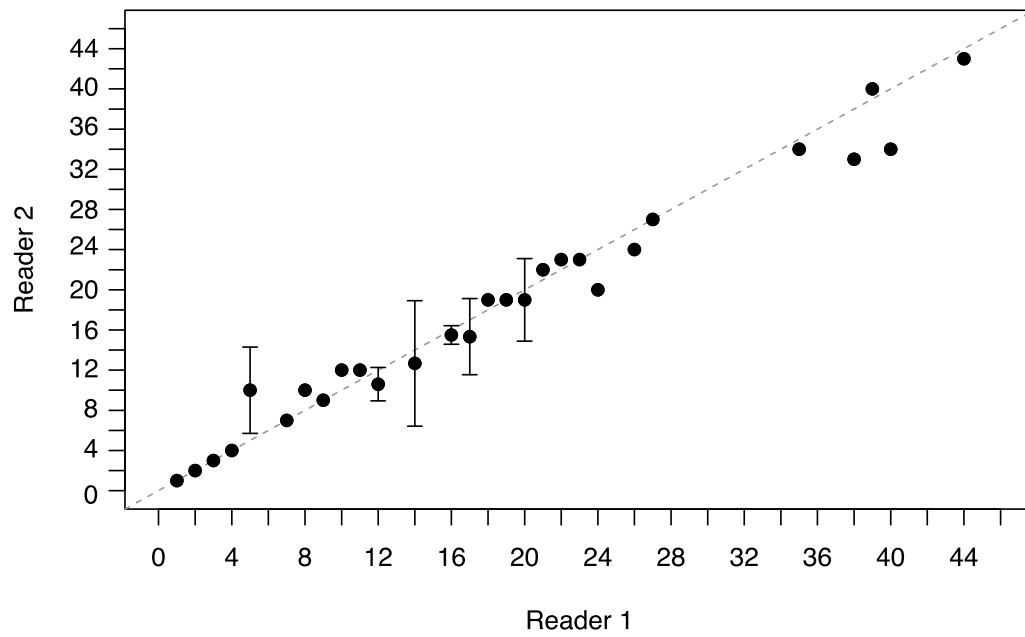


**Figure 9. Presence of transition zone on an older fish (> 5 years) and lack of transition zone on younger fish (<5 years) *P. filamentosus* relative to otolith shape and size for determining accurate ageing.**

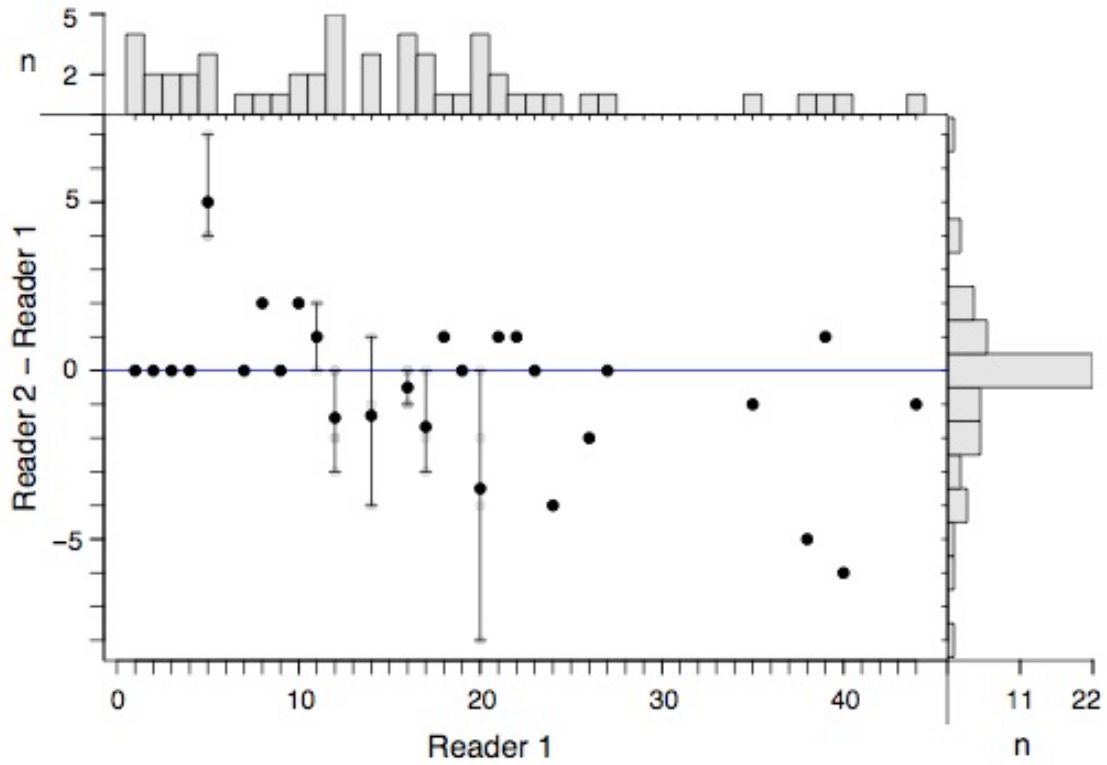
The secondary counts that incorporating the ageing criteria greatly reduced erroneous growth zone counts for individuals less than 10 years. This resulted in greater between reader precision (IAPE, 5.48%; CV, 7.76%; Fig.11). Direct agreement (PA) between readers was 53%, increasing to 72% within 1 year, and 90% within 2 years. Age bias plots suggest that there is good agreement between the age estimates of Reader 1 and Reader 2 up to a mean age of approximately 18 years, after which age estimates from Reader 2 are less than age estimates from Reader 1, with the difference between the two generally increasing with increasing mean age (Fig. 12). The incorporation of the ageing criterion also resulted in greater accuracy of age estimates. Comparison of age estimated between each reader and C-14 ages supports the effectiveness of the ageing criteria (IAPE, 2.2–5.1%; CV, 3.6–7.1%); however, there is still some evidence of under ageing by Reader 2 compared to Reader 1 (Fig. 13). The test of symmetry between readers and against the C-14 ages did not show a significant bias between age estimates (Table 3). Overall, this increase in accuracy and lack of bias resulting from the application of our ageing criteria lead to a better fit in fish length vs. assigned age compared to age estimates prior to development of the ageing criteria (Fig. 10).



**Figure 10. Size-at-age of reference otoliths for each reader before and after incorporation of ageing criteria. Reader 1 = A1 (before criteria), A2 (after criteria), Reader 2 = R1 (before criteria), R3 (after criteria).**

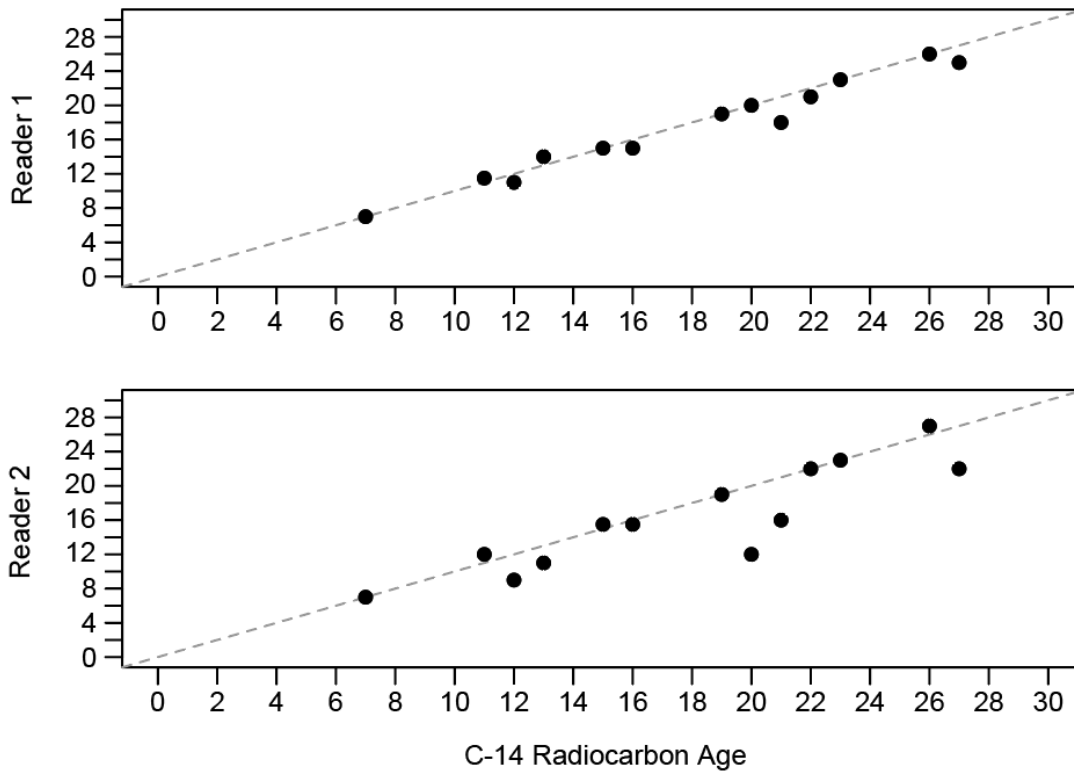


**Figure 11. Age bias plots Reader 1 (primary reader) and Reader 2 after ageing criteria was applied.**



**Figure 12. Mean (points) and range (vertical intervals) of differences between otolith annuli estimates for two readers plotted in relation to annuli counts by the first reader (more experienced reader). Top histogram is the sample size for each annuli estimates produced by first reader; right histogram is the distribution of differences between readers.**





**Figure 13. Age bias plots Reader 1 (primary reader) and Reader 2 compared to C-14 bomb radiocarbon ages.**

**Table 3. Summary of test of symmetry statistics from age agreement table (ageBias function in R software) compared between readers and C-14 bomb radiocarbon ages. The “Bowker” test as described in Hoenig et al. (1995), the “Evans-Hoenig” test as described in Evans and Hoenig (1998), and the McNemar test as described in Evans and Hoenig (1998).**

| Model               | Symmetry test | df | Chi Squared | <i>p</i> |
|---------------------|---------------|----|-------------|----------|
| Reader 1 – Reader 2 | McNemar       | 1  | 0.32        | 0.857    |
|                     | Evans Hoenig  | 6  | 3.33        | 0.766    |
|                     | Bowker        | 24 | 27.0        | 0.304    |
| Reader 1 – C-14     | McNemar       | 1  | 0.33        | 0.564    |
|                     | Evans Hoenig  | 3  | 2.48        | 0.479    |
|                     | Bowker        | 11 | 10.0        | 0.530    |
| Reader 2 – C-14     | McNemar       | 1  | 2.57        | 0.109    |
|                     | Evans Hoenig  | 3  | 7.2         | 0.408    |
|                     | Bowker        | 11 | 10          | 0.616    |

## Discussion

This study reports on the development and assessment of an ageing criterion that led to a reduction in ageing bias and acceptable between readers precision for a deep-water snapper species in the MHI. Our results indicate that the practicality of using otolith shape (i.e., location of an inflection point) were advantageous in reaffirming the validity of microincrements to the first annuli. Our high level of precision supports the efficacy of the ageing criterion, while nominal reader bias and close agreement of derived ages with true (radiocarbon) ages indicates acceptable ageing accuracy. The use of standardized thin sectioning and applying the ageing criterion to previously bomb radiocarbon dated otoliths proved to be most definitive validation of the ageing criterion for fish between 7 and 44 years old.

This study addresses several previously mentioned levels of difficulty in estimating age and examining precision of deep-water snapper species (Ralston and Miyamoto 1983; DeMartini et al. 1994; Andrews et al. 2012). Earlier work on *P. filamentosus* reported unsuccessful age estimation resulting from the use of thicker sections, and the use of an integrated method to determining ages. These methods found that greater error was associated with ages of older individuals resulted from the poor growth zone formation found in thicker sections (Ralston and Miyamoto 1983; DeMartini et al. 1994; Andrews et al. 2012). Several reviews of deep-water snapper ageing proposed that it is quite logical that the extrapolated maximum age method lacked appropriate otolith preparation (i.e., thin sectioning, obliquely reflected light, and higher magnification), or inexperienced age readers impaired age estimations in these earlier studies (Newman et al. 2015, 2016; Wakefield et al. 2016). The difficulty in interpreting growth zones in deep-water snappers is quite evident but not insurmountable and successful age estimation can be made even at lower levels of precision (Newman et al. 2016; Wakefield et al. 2016).

We applied recommendations from Newman et al. (2015) and Wakefield et al. (2016) to reduce the potential impact of ageing error in the youngest and oldest individuals when estimating ages. Our results compared DGI to similar size range as DeMartini et al. (1994), which corroborated the first annulus in younger samples relative to otolith growth. The utility of microincrements in *P. filamentosus* was similar to studies that identified changes in growth formation of the otolith (first year inflection point) and usefulness as a key characteristic of the first-year growth for snapper species in Hawaii (O'Malley et al. 2016). Additionally, the thin sectioned (~250  $\mu\text{m}$ ) protocol of Newman et al. (2015) applied to bomb radiocarbon paired otoliths from Andrews et al. (2012) resulted in accurately aged *P. filamentosus* across all years sampled (1–44 years). The thicker otolith sections used in Andrews et al. (2012) were almost double the thickness (>400  $\mu\text{m}$ ) compared to our study. This extra thickness, paired with transmitted light microscopy, most likely contributed to the previous assumption of ill-defined annual growth structure in *P. filamentosus* (Andrews et al. 2012). The high precision and lack of ageing bias between C-14 ages and reader estimates support this thinner sectioning approach, and is more consistent with other deep-water snapper species (Wakefield et al. 2010, 2015; O'Malley et al. 2016; Williams et al. 2015, 2017).

Evidence to support that annual age estimates are not insurmountable for these deepwater snappers, particularly *Pristipomoides*, has been reported in other studies and sustained in this study (Pillings et al. 2000; Fry et al. 2006; Wakefield et al. 2016; O'Malley et al. 2019). A key for accurate and precise age estimates is development of an ageing criteria. Applying the ageing

criteria proved successfully for *P. filamentosus* in this study and other deep-water teleosts (Morison et al. 2005; Hutchinson et al. 2007; O'Malley et al. 2016). However, our precision of otolith reading was close to the ceiling of acceptable levels (i.e., IAPE, 5.5%; Campana 2001), and a slight bias of under ageing was evident. While our precision is at the upper end of acceptable, lower levels of precision have been reported for deep-water snappers (IAPE, >10.0%) and the associated ageing strategy was satisfactory (Newman and Dunk 2003). Our ageing criteria reduces the potential of misleading age estimates by addressing earlier difficulties in ageing *Pristipomoides* species and incorporating nascent research.

## References

- Andrews AH, DeMartini EE, Brodziak J, Nichols RS, Humphreys RL. 2012. A long-lived life history for a tropical, deepwater snapper (*Pristipomoides filamentosus*): bomb radiocarbon and lead radium dating as extensions of daily increment analysis in otoliths. *Can. J. Fish. Aquat. Sci.* 69:1850-1869. doi:10.1139/f2012-109.
- Andrews AH, Kalish JM, Newman SJ, Johnston JM. 2011. Bomb radiocarbon dating of three important reef-fish species using Indo-Pacific D14C chronologies. *Mar. Freshw. Res.* 62:1259–1269.
- Brodziak J, Courtney D, Wagatusma L, O'Malley J, Lee HH, Walsh W, Andrews A, Humphreys R, DiNardo G. 2011. Stock assessment of the main Hawaiian Islands deep 7 bottomfish complex through 2010. U.S. Dep. Commer., NOAA Tech. Memo., NOAA-TM-NMFS-PIFSC-29. 176 p. + Appendix.
- Campana S. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Fish Biol.* 59:197-242.
- Dalzell P, Adams TJH, Polunin NVC. 1996. Coastal fisheries in the Pacific Islands. *Oceanogr. Mar. Biol. Annu. Rev.* 34:395–531
- DeMartini EE, Landgraff KC, Ralston S. 1994. A recharacterization of the age-length and growth relationships of Hawaiian snapper, *Pristipomoides filamentosus*. NOAA-TM-NMFS-SWFSC-199. Honolulu (HI).
- Evans GT, Hoenig JM. 1998. Testing and viewing symmetry in contingency tables, with application to readers of fish ages. *Biometrics* 54: 620–629.
- Fry GC, Brewer, DT, Venables WN. 2006. Vulnerability of deepwater demersal fishes to commercial fishing: Evidence from a study around a tropical volcanic seamount in Papua New Guinea. *Fish. Res.* 81:126-141.
- Hamel OW, Piner KR, Wallace JR. 2008. A robust deterministic model for bomb radiocarbon signal for use in fish age validation. *Trans. of the Am. Fish. Soc.* 137:852-859.
- Haight WR, Kobayashi DR, Kawamoto KE. 1993. Biology and management of deepwater snappers of the Hawaiian Archipelago. *Mar. Fish. Rev.* 55 (2):20–27.
- Hoenig JM, Morgan MJ, Brown CA. 1995. Analyzing differences between two age determination methods by tests of symmetry. *Can. J. Fish. Aquat. Sci.*, 52: 364–368.
- Hospital J, Beavers C. 2012. Management of the main Hawaiian Islands bottomfish fishery: fishers' attitudes, perceptions, and comments. Pacific Islands Fisheries Science Center Admin. Report. H-11-06, 46 p. + Appendices.

- Hutchinson, CE, Kastle CR, Kimura DK, Gunderson DR. 2007. Using radiometric ages to develop conventional ageing methods for shortraker rockfish (*Sebastes borealis*), p. 237-249. In Heifetz J, DiCosimo J, Gharrett AJ, Love MS, O'Connell VM, and Stanley RD (editors). Biology, Assessment, and Management of North Pacific Rockfishes. University of Alaska Sea Grant Program Report No. AK-SG-07-01. University of Alaska, Fairbanks.
- Kalish JM. 1993. Pre- and post-bomb radiocarbon in fish otoliths. *Earth Planetary Sci. Lett.* 114:549-554.
- Langseth B, Syslo J, Yau A, Kapur M, Brodziak J. 2018. Stock Assessment for the Main Hawaiian Islands Deep 7 Bottomfish Complex in 2018, with Catch Projections through 2022. NOAA Tech. Memo. NMFS-PIFSC-69, 217 p.
- Martell S, Dichmont C, Sparholt H. 2017. Western Pacific Stock Assessment Review of the 2017 Benchmark stock assessment for the main Hawaiian Islands deep7 bottomfish complex. Deep7 WPSAR Summary Report. Available at: <https://photos.fisheries.noaa.gov/directdownload.php?ti=14726262&tok=cWb5naMheUD35oMzWmPKYARR>
- Morison A, Burnett J, McCurdy W, Moksness E. 2005. Quality issues in the use of otoliths for fish age estimation. *Mar. Freshw. Res.* (5):773–82.
- Newman SJ, Dunk IJ. 2005. Age validation, growth, mortality, and additional population parameters of the goldband snapper (*Pristipomoides multidens*) off the Kimberley coast of northwestern Australia. *Fish. Bull.* 101(1):116-128.
- Newman SJ, Wakefield, CB, Williams AJ, Nicol SI, DeMartini EE, Halafih T, Kaltavara J, Humphreys RL, O'Malley J, Taylor B, Andrews A, Nichols R. 2015. International workshop on methodological evolution to improve estimates of life history parameter and fisheries management of data-poor deep-water snappers and groupers. *Mar. Pol.* 60:182-185.
- Newman SJ, Wakefield CB, Williams AJ, Nicol SI, O'Malley J, Taylor B. 2016. Review of the life history characteristics, ecology and fisheries for deep-water tropical demersal fish in the Indo-Pacific region. *Rev. Fish Biol. Fish.* 26 (3):537-562. doi:10.1007/s11160-016-9442-1.
- O'Malley JM, Taylor BM, Andrews AH. 2016. Feasibility of ageing Hawaiian Archipelago uku (*Aprion virescens*). 2016. Pacific Islands Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96818-5007. Pacific Islands Fish. Sci. Cent. Admin. Rep. H-16-06, 31 p. doi:10.7289/V5/AR-PIFSC-H-16-06.
- O'Malley JM, Wakefield CB, Oyafuso Z, Nichols RS, Taylor BM, Williams AJ, Sapatu M, Marsik M. 2019. Effects of exploitation evident in age-based demography of 2 deepwater snappers, the goldeneye jobfish (*Pristipomoides flavipinnis*) in the Samoa Archipelago and the goldflag jobfish (*P. auricilla*) in the Mariana Archipelago. *Fish. Bull.* 117:322–336 doi:10.7755/FB.117.4.5.

- Pacific Islands Fisheries Science Center. 2020. Life History Program Life History Estimates. <https://inport.nmfs.noaa.gov/inport/item/59002>.
- Pilling GM, Millner RS, Easey MW, Mees CC, Rathacharen S, Azemia R. 2000. Validation of annual growth increments in the otoliths of the lethrinid *Lethrinus mahsena* and the lutjanid *Aprion virescens* from sites in the tropical Indian Ocean, with notes on the nature of growth increments in *Pristipomoides filamentosus*. Fish. Bull. 98:600-611.
- Ralston S, Miyamoto GT. 1983. Analyzing the width of daily otolith increments to age the Hawaiian snapper, *Pristipomoides filamentosus*. Fish. Bull. 81:523-535.
- Randall JE. 2007. Reef and Shore fishes of the Hawaiian Islands. Sea Grant College Publication 543 pp.
- Taylor BM, McIlwain JL. 2010 Beyond abundance and biomass: effects of marine protected areas on the demography of highly exploited reef fish. Mar. Ecol. Prog. Ser. 411:243-258. doi:10.3354/meps08672.
- Uchiyama JH, Tagami DT. 1984. Life history, distribution, and abundance of bottomfishes in the northwestern Hawaiian Islands. In Proceedings of the Second Symposium on Resource Investigations in the Northwestern Hawaiian Islands. Edited by R.W. Grigg and K.Y. Tanoue. UNHI-SeaGrant-MR-84-01. pp. 229–247.
- Wakefield CB, Newman SJ, Boddington DK. 2013. Exceptional longevity, slow growth and late maturation infer high inherent vulnerability to exploitation for bass grouper *Polyprion americanus* (Teleostei: Polyprionidae). Aquat. Biol. 18:161–174. doi:10.3354/ab00501
- Wakefield CB, Boddington DK, Newman SJ. 2016. Rapid lateral extraction of otoliths that maintains the integrity of fish product to improve access to catches and reduce potential sampling biases. Open Fish Sci. J. 9:26-28.
- Wakefield CB, O'Malley JM, Williams AJ, Taylor B, Nichols R, Halafih T, Humphreys RL, Kaltavara J, Nicol SJ, Newman SJ. 2017 Ageing bias and precision for deep-water snappers: evaluating nascent otolith preparation methods using novel multivariate comparisons among readers and growth parameter estimates. ICES J. Mar. Sci. 74(1):193-203.
- Western Pacific Regional Fisheries Management Council [WPRFMC]. 2016. Amendment 4 to the Fishery Ecosystem Plan for the Hawaiian Archipelago. Honolulu (HI).