Pacific Islands Fisheries Science Center

Feasibility of Ageing Hawaiian Archipelago
Uku (*Aprion virescens*)

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September 2016

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Feasibility of Ageing Hawaiian Archipelago Uku (*Aprion virescens*)

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September 2016

ABTRACT

The green jobfish *Aprion virescens* (Hawai'i local name, uku) is an economically important component of Hawai'i's spearfishing, trolling, and deep handline fisheries (largest total poundage landed and third most valuable non-pelagic species). However, stock status of this large-bodied lutjanid has not been determined since 1990 and a primary reason is a lack of life history information (length-at-age, somatic growth, mortality). We examined the otoliths of this potentially long-lived fish to determine the most efficient method for thin-sectioning otoliths for age interpretation. We then developed an ageing criteria based on 1) edge analysis that confirmed the seasonality of annular mark deposition, 2) validation of the location of the first annual mark using daily ring counts and a corresponding distinct growth mark on the dorsal side of the otolith and, 3) a change in the shape of the otolith cross-section that distinguishes young fish from older fish. Bomb radiocarbon analysis and plots of otolith weight and fish length *vs*. estimated age revealed that the ageing criteria is acceptable and estimates of precision indicate that reproducible age estimates are possible. The ageing criteria (Appendix A) include annotated reference photographs of otolith thin sections to aid future age readers.

Keywords: *Aprion virescens*, otolith, age, ageing criteria

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INTRODUCTION

The green jobfish *Aprion virescens* (Hawai'ian local name, uku) is a large-bodied eteline snapper (family Lutjanidae) with an Indo-Pacific distribution from east Africa, Mauritius, and the Red Sea to Indonesia, southern Japan, the Ogasawara Islands, Australia, Micronesia, the Hawai'ian Archipelago (main Hawai'ian Islands (MHI) and Northwestern Hawai'ian Islands (NWHI)), and the Tuamotu Archipelago (Mundy, 2005).

The majority of *A. virescens* life history characteristics are poorly understood, particularly in Hawai'i, the exception being reproductive biology. Several information sources indicate that *A. virescens* spawn in Hawai'i primarily during summer months. Everson et al. (1989), using histological preparations of monthly gonad samples, found that *A. virescens* in the MHI have a protracted spawning period that lasts from May through October with peak spawning occurring in June (Fig. 1). A telemetry study in the NWHI suggests that *A. virescens* exhibit seasonal migrations that may be related to summer spawning aggregations (Meyer et al., 2007). Communications with fishermen supports the summer spawning theory because they notice that *A. virescens* become highly aggregated, and hence target them, in the late spring/early summer (R. Morioka, J. Muir, pers. comm.). This is confirmed by commercial landing data with 35% of the annual 1948-2010 catch occurred in May–July (HDAR data) (Fig. 1). Everson et al. (1989) estimated female mean size-at-50% maturity (L_{50}) at 42.5–47.5 cm fork length (FL) for MHI *A. virescens*.

Throughout their range, green jobfish are an important commercial and subsistence fishery. Caught by trolling near the surface, spearfishing, and deep handline gear, they were the largest commercial total reported poundage landed and third most valuable non-pelagic species caught in Hawai'i from 2003 to 2013 (HDAR data). Average annual landings during this period were 143,735 lbs, with an average annual value of $429,875 (Fig. 2). Possible reasons for catch exceeding that of the most valuable non-pelagic fish, *Pristipomoides filamentosus* (opakapaka), include targeting by fishermen during weather conditions that prevent bottomfishing, fishermen switching targets to *A. virescens* when the bottomfish ACL is reached, and specific targeting of summertime spawning aggregations.

Despite *A. virescens* economic importance in various Hawai'i fisheries, stock status determination has been almost nonexistent. A 1988 (Ralston and Kawamoto) size-structure analysis indicated that MHI *A. virescens* was experiencing growth-overfishing. Somerton and Kobayashi (1990) attempted an equilibrium spawning potential ratio (SPR) analysis; however, the length-frequency sample size was considered inadequate to estimate M/K. The same study produced dynamic SPR values and despite being deemed biased, it concluded that *A. virescens* was not overfished (SPR = 0.40). In 2011, *A. virescens* were included as part of a 14-species aggregated Schaefer production model analysis (Martell et. al., 2011). Although the analysis concluded that the complex was overfished and experiencing overfishing in the MHI, little information was available regarding *A. virescens* relative abundance. The reasons for this were the different biology and fishery targeting practices relative to other species in the complex.

A. virescens-specific stock assessment has not occurred since 1990. Currently, *A. virescens* are **part o**f the Bottomfish Management Unit, a complex of 14 species of Lutjanidae, Carangidae, and Serranidae managed by the Western Pacific Regional Fishery Management Council's Bottomfish and Seamount Groundfish Fishery Management Plan. *A. virescens* are not included in the contemporary 'Deep 7' multi-species deepwater snapper and grouper assessments. Various reasons account for the lack of recent *A. virescens* stock-status determination. Perhaps the most important reason is a lack of life history information. Three of the previously mentioned *A. virescens* assessments stated that the analysis was dependent on the assumption of accurate life history information (Ralston and Kawamoto, 1988) or concluded with the recommendation to collect species-specific biological and life history information (Haight et al., 1993; Martell et al., 2011). While Everson et al. (1989) provided one key life history component (L_{50}), fish age and growth information is not available for Hawai'i *A. virescens*. Age-based data can provide much of the basic information for estimating important parameters for stock assessments (e.g. length-at-age, somatic growth, longevity, mortality) (Hilborn and Walters, 1992).

Growth and length-at-age information certainly are not necessary for an accurate stock assessment however, the typically used alternative, length-based estimates of growth and assessments may not be appropriate for *A. virescens* due to its potential longevity (Newman et al., 2015; Pilling and Halls, 2003; Pilling et al., 2006). Deepwater species, including Lutjanidae, are generally considered long-lived, slow-growing fish (Andrews et al., 2012; Koslow et al., 2000; Manooch III, 1987; Williams et al., 2013). Ralston et al. (2004) classified Hawai'i bottomfish age characteristics as 20 + years old. The maximum age of central Indian Ocean *A. virescens*, based on otoliths sampled during observer trips (n = 468) in 1999, was 26 years (Pilling et al., 2000; Pilling and Mees, 2000). Thus, there is a high potential for Hawai'ian *A. virescens* to be long-lived.

Fish ages can be determined by examining calcified structures (otoliths, scales, vertebrae, and fin spines and rays) that produce annual marks. The irregular accretion of proteins to the otolith commonly corresponds to zones of faster growth during summer months and slower growth during winter months (Pannella, 1971, 1974) which appear as light and dark zones, respectively, under microscopic examination. The combination of one light and one dark zone represents one year of growth. However, in many species there is an abundance of marks, known as false annuli or checks, on the otoliths that do not correspond to annual marks. These are related instead to environmental (Neat et al., 2008) and physiological stressors (ontogenetic changes in diet [Pińeiro and Sainza, 2003], maturity [Francis and Horn, 1997], spawning [MacLellan and Fargo, 1995], and spawning migrations [Lowerre-Barbieri et al., 1994]). These marks confound the age-reading process, and interpreting these marks as annular results in biased age estimates. Hence, there is a need to validate (i.e. ensure that only annual marks are being counted) species-specific ageing criteria (Beamish and McFarlane, 1983; Choat et al., 2009). Ageing criteria can be validated by using simple and inexpensive techniques (marginal increment analysis, edge analysis, and back calculation) or ones that are complicated and expensive (tag/recapture of chemically marked fish, bomb radiocarbon and lead/radium dating). Marks on *A. virescens* otoliths from the central Indian Ocean were validated as annual marks using marginal incremental analysis (Pilling et al., 2000). However, it is a dangerous practice to assume that marks on otoliths from one location are annual based on validation that the marks were annual on

otoliths from a different location, particularly if the populations are from different oceans (Piddocke et al., 2015).

The goal of the present study is to determine the feasibility of ageing Hawai'ian Archipelago *A. virescens* to provide estimates of life history traits (length-at-age, age-at-maturity, growth, mortality) for stock assessment purposes. First, we compared different otolith preparation techniques to identify the easiest and most time-efficient method that produces the clearest potential growth increments for age determination. Second, we used daily growth zone increment analysis and published information about *A. virescens* spawning period to identify the first annual mark. Finally, we used edge analysis to validate the seasonal deposition of annual marks and bomb radiocarbon dating to evaluate ages estimated via growth zone counting. These last two steps are key components in developing the Hawai'ian *A. virescens* ageing criteria. Finally, we use different measures of ageing precision (i.e. between-reader agreement) to test the reproducibility of *A. virescens* ages. The newly developed ageing criteria (Appendix A) includes annotated reference otoliths.

METHODS

Otolith Collection

A. virescens otoliths were collected throughout the Hawai'ian Archipelago from 2007 to 2015 from a variety of different sources including 1) collections during research trips on the NOAA Ship *Oscar Elton Sette*, 2) purchases from commercial fishermen using Fishermen Disaster Release Funds (2007–2008, NWHI and MHI), Cooperative Research Funds (2009–2015, MHI), and Improve a Stock Assessment Funds (2014–2015, MHI), and 3) donations by local recreation/subsistence fishermen. Otoliths were extracted, cleaned, dried and stored in vials. Associated length, macroscopic identified sex, location, and catch date were collected when available.

Whole dried *A. virescens* sagittal otoliths were weighed to the nearest 0.0001 g using an analytical balance. The whole otoliths were then photographed and specific otolith morphometrics (whole length, core to rostrum, core to postrostrum, width and thickness) were measured using Leica V4 imaging software for later analysis to develop a morphometric-based proxy to fish ageing (Williams et al., 2015).

Otolith Preparation (Thin Sectioning)

Otoliths thin sections were prepared using two methods:
     1) Card-mount. Otoliths were centered and attached with the sulcus side face down to 2.5 × 4 cm index cards using double-stick tape. The otoliths were permanently attached to the card with Thermo Scientific™ Richard-Allan Scientific™ Cytoseal™ mounting medium. A Buehler Isomet® 5000 Linear Precision saw equipped with two diamond blades separated by a spacer was used to create a transverse thin section of the otolith. This method purposely creates thick sections (750 µm) for age interpretation so the otolith can be later ground to the desired thickness and still contain the core of the otolith. The side of the section that was closest to the core was mounted face down to a slide using Cytoseal. A subsample of the mounted otoliths was ground

using a South Bay® model 900 grinder/polisher and the thin sections were covered with a final coat of Cytoseal.

      2) Slide-mount. The otolith was affixed by thermoplastic adhesive (Crystalbond™) to a clear glass slide and ground along the longitudinal axis to the primordium using a Gemmasta® lapping machine with a 600 grit diamond lapidary wheel. The otolith was then removed and affixed to a clean slide with the flat surface down and subsequently ground until a thin transverse section (~ 400 µm) was obtained through the core.

Edge analysis

Growth increment periodicity was assessed using edge analysis. A simple edge type classification was used:
      1 = opaque zone on the edge of the otolith section;
      2 = translucent zone on the edge of the otolith section.

Identification of the First Annual Mark

Thin sections, regardless of how they were prepared, were examined using transmitted light where dark opaque zones represent narrower and presumably slower growth zones and light translucent marks represent wider and presumably faster growth zones. A Leica™ S8APO binocular stereomicroscope (magnification range 10–50×) was used to examine otoliths for annual marks. Otoliths that contained several marks that could be interpreted as the first annulus were further processed for daily increment analysis. These otoliths were ground using a diamond wheel 60-g wet sandpaper and polished using progressively finer (30, 9, 3, 0.3 µm) lapping film until daily increments were clearly visible.

Otolith daily increments were counted using an Olympus BX51compound microscope (magnification range 200–600×) along several reading axes, because one axis did not provide clear marks along the entire length of dorsal side of the otolith. Increments were counted from the core to each of the potential annual marks.

Ageing Criteria Development

Two readers examined 100 thin-sectioned otoliths (the size or the catch-date information withheld). Prior to reading, both readers came to agreement on the criteria of the start of the first annular mark based on daily increment counts. After the 100 otoliths were read, the ages were compared and precision was calculated (see below). Otoliths with large between-reader differences relative to their estimated age were re-examined and discussed until consensus was reached. These resolved otoliths and their associated problem areas were then used to refine the ageing criteria. The 100 otoliths were independently re-aged using the updated ageing criteria, including month of capture information, and precision estimates were re-calculated.

Accuracy of Ageing Criteria

Otolith weight has been used as a proxy for fish age because of their linear relationship (Lou et al., 2005; Pilling et al., 2003). This implies that otolith weight can generally be used to determine the accuracy of the ageing criteria. Otolith weight was plotted against estimated age and the coefficient of determination was calculated.

Precision

Between-reader agreement was assessed using two methods. The first method involved using the coefficient of variation (CV) expressed as the ratio of the standard deviation over the mean (Chang, 1982):

$$CV_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j}$$

where CV_j is the age precision estimate for the jth fish
X_{ij} is the ith age determination of the jth fish
X_j is the mean age estimate of the jth fish
R is the number of times each fish is aged
A mean CV is estimated by averaging the individual CV of all fish. The second method was the average percent error (APE):

$$APE_j = 100\% \times \frac{1}{R} \sum_{i=1}^{R} \frac{|x_{ij} - x_j|}{X_j}$$

Similar to CV, and index of average percent agreement (IAPE) (Beamish and Fournier, 1981), can be determined by averaging the APE across all fish.

Age-bias plots were calculated to visually assess the deviation between the two age readers from the 1:1 equivalence line (Campana et al., 1995). Typically, the x-axis is considered the known or 'better' age estimate but in this case, each reader is just as likely to be more accurate; thus, the plot is only used to visually assess if one reader is assigning higher or lower ages relative to the other.

Bomb Radiocarbon Dating

A. virescens specimens selected for bomb radiocarbon dating ranged in age from young to old. Selection was based on the otolith section ages (Table 1). Whole otolith dimensions and weight of several juvenile otoliths, coupled with observed dimensions of a clearly visible opaque region at the core, were used to refine a targeted core region within adult otolith samples. An otolith from a 37.4 cm FL juvenile measured 7.5 mm L × 3.8 mm W × 0.8-1.0 mm T (thickness) and weighed 27 mg (LY3-4-7-37). The core of this otolith was considerably larger than necessary for age determination and provided clear guidance on creating a well-centered extraction. The extraction design was well within these dimensions to reduce the possibility of extracting more recently formed material. Using a New Wave Research® (ESI–NWR Division; Fremont, CA) micromilling machine, a core extraction of 1.5 mm L × 3.0 mm W × 0.3 mm T was achieved using a 24-point line scan with a 500 µm Brasseler® (Savannah, GA) bur in 2 successive and

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overlapping scans at a depth of 150 µm for each pass. Extraction mass was 1.5–3.0 mg of $CaCO_3$, providing sufficient sample for high precision ^{14}C measurements.

The extracted otolith samples were submitted as carbonate to the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS), Woods Hole Oceanographic Institution (WHOI) in Woods Hole, Massachusetts, for small sample radiocarbon analysis using Accelerator Mass Spectrometry (AMS). Radiocarbon measurements were reported by NOSAMS as the Fraction Modern, the measured deviation of the $^{14}C/^{12}C$ ratio from a "modern" sample. This reference is defined as 95% of the radiocarbon concentration of the NBS Oxalic Acid I standard (SRM 4990B) normalized to $\delta^{13}C_{VPDB}$ (– 19‰) in 1950 AD (VPDB = Vienna Pee Dee Belemnite geological standard; Coplen, 1996). Results were normalized to – 25‰ for fractionation correction using a reported $\delta^{13}C$ value and is reported here as $\Delta^{14}C$ (Stuiver and Polach, 1977).

Validated birth years were determined by projecting the measured $\Delta^{14}C$ values to a $\Delta^{14}C$ coral and otolith reference for the Hawai'ian Archipelago (A.H. Andrews, in prep). This $\Delta^{14}C$ reference documents the decline of bomb ^{14}C across the region and provides a temporal alignment of birth year material from the *A. virescens* otoliths. A regression of the declining $\Delta^{14}C$ relationship ($\Delta^{14}C$ = -3.56*(birth yr) + 7202; R^2 = 0.969) was used to determine the birth year with a ± 4 year prediction interval to fully encompass the potential range of $\Delta^{14}C$ values. In addition, two juvenile fish were measured for ^{14}C to provide a baseline $\Delta^{14}C$ value for *A. virescens* relative to the existing $\Delta^{14}C$ decline record (Samples LA6-3-6-450 and LY3-4-7-37). Alignment of these fish with other otolith records provides an additional basis for proper calibration of core material from older fish.

RESULTS

Number of Otoliths Processed

To date, 491 *A. virescens* otoliths (308 NWHI, 183 MHI) were collected by the various sources. The Hawai'i state record for *A. virescens* is 39.5 lbs (Hawai'i Fishing News), which translates to 106.2 cm FL using Sundberg and Underkoffler (2011) length-to-weight equation. The current *A. virescens* otolith collection contains fish ranging from 20.5 to 95 cm thereby covering the majority of the known size range (Fig. 3).

The card-mount sectioning method was applied to 280 otoliths. These included the heaviest and lightest 20 otoliths and the remaining otoliths were processed in the order that they were stored. The next 50 otoliths were processed using the slide-mount method.

Otolith Thin Sectioning

Both processing methods produced thin sections with clear marks (Fig. 4). The card-mount method took longer to create thin sections for several reasons. The first was preparing the cards for otolith mounting. The second reason was the section had to be mounted to the card, then the slide and finally covered with a final coat of Cytoseal. The primary issue was the drying time of Cytoseal. In some cases it took Cytoseal > 30 days to cure thereby requiring up to 3 months for

just the mounting steps to be completed. The slide-mount method does not entail preparing and mounting otoliths to the cards and uses Crystalbond to secure the otolith to a microscope slide. Crystalbond has a low melting temperature (120° F), which means it liquefies quickly on a hotplate and cools quickly (< 30 seconds). An added advantage of thermoplastic adhesive is that the otolith can be easily manipulated on or removed from the slide simply by reheating while Cytoseal creates a permanent bond. Another problem with the card-mount method was that the proximal edges of the otolith thin sections were sometimes broken. This was due to the otolith section adhering to the double stick tape and chipping when removed from the card. The chipping occurred on the sulcus side of the thin section resulting in the removal of the final portion of all the reading axes (i.e. the most recent growth).

After initially viewing the prepared slides it was determined that the card-mount method produces thin sections that were too thick (750 µm) for age reading and required grinding, polishing and the reapplication of Cytoseal. Visual examination of numerous thin sectioned otoliths of different thickness revealed that the optimal thin section thickness for age reading (i.e. clearest annular marks) was 350 µm. Based on the extra time required to prepare otoliths, allow the mounting medium to dry, and grind and polish the thin sections the card-mount method is not recommended. The slide-mount method, where the otoliths are ground to 350 µm, is the preferred method to prepare *A. virescens* otoliths for age interpretation.

Daily Increment Counts and Identification of the 1ˢᵗ Annual Mark

Most otolith sections contained a mark inside a clear prominent mark close to the core. Either of these marks could be interpreted as the first annual mark. Most otoliths also had a distinguishing 'inflection' feature on the edge of the otolith section indicating where the otolith slightly changes shape along the dorsal growth plane. This inflection also corresponded with the clear prominent mark on the face of the thin section (Fig 4). The number of daily increments was highly dependent on the reading axis with higher counts closer to the sulcus. Daily increment counts from the core to the mark with the corresponding inflection feature ranged from 100 to 180 (Table 2, Fig. 5).

Everson et al. (1989) estimated that Hawai'ian *A. virescens* spawning season begins in May, peaks in June, and concludes in October. Otolith annual marks corresponding to Hawai'i winter growth are considered to begin in November/January (Morales-Nin and Ralston, 1990; Ralston and Williams, 1988). This indicates that there should be 1 to 6 months of translucent growth zone before the start of the formation of the opaque zone. This amount of growth corresponds to approximately 30 to 180 daily increments counts for the majority of *A. virescens* otoliths. Considering that an unknown number of daily increments is obscured inside the otolith core and that there was an average of 136 daily increment counts to the inflection feature our ageing criteria indicates that the inflection is considered the start of the first opaque zone and no marks should be counted as annuli prior to this feature.

Edge Analysis

Otolith edge type analysis was not possible for all individual *A. virescens* because of uncertainty in annual growth mark width. However, the edge type classification was conducted on 125 otoliths and these confirmed that annual marks are deposited on *A. virescens* otoliths during

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winter months. The width of the translucent zone steadily increased from May through August; soon after, the opaque zone started to appear (Fig. 6).

Assessment of *A. virescens* Ageing Criteria

Initial blind reads (no information on fish size or catch date) without development of the full ageing criteria (i.e. only identification of first annular mark was identified) suggested that otolith-based age estimates are feasible. In general, plots of otolith weight *vs.* estimated age (Fig. 7) and fish length *vs.* otolith age (Fig. 8) displayed linear and curvilinear relationships, respectively. Otolith weight accounted for 92% and 93% of the variability in age of reader 1 and reader 2, respectively. However, the plots indicated that identification of annular marks 1–4 was clearly a problem area particularly for smaller (FL = 20-55 cm, otolith weight = 0.02-0.05 g) fish. Fork length plotted against age (Fig. 8) indicated that fish estimated at 1 year of age ranged in size from 20 to 55 cm and fish estimated at age 2 years ranged in size from 24 to 55 cm.

The initial read IAPE was 6.6 and the CV was 9.4. Age bias plots indicated that neither age reader systematically under- or overestimated ages relative to the other reader (Fig. 9).

The two readers jointly examined otoliths that had poor agreement and agreed with the interpretation from the size at age plots that a large percentage of the ageing error came within the first 5 years of growth. The diffuse nature of the first annular marks combined with the lack of month of capture information also made ageing young fish accurately difficult. The ageing criteria (Appendix A) now include consensus recommendations on how to age the first 5 years.

The second set of reads utilized the updated ageing criteria and the reader had the month of capture information. These resulted in accurate age estimation (Figs. 10, 11). Otolith weight accounted for 89% and 91% of the variability in age of reader 1 and reader 2 second reads, respectively. Precision increased (IAPE = 5.5, CV = 7.8). However, age-bias plots this time indicated that reader 1 slightly, yet consistently assigned higher ages than reader 2 of fish greater than 10 years old (Fig. 12).

Bomb Radiocarbon Dating

The otoliths from 10 adult and 2 juvenile *A. virescens* were analyzed for ^{14}C from the extracted core samples and resulted in validated age estimates (Table 1). Fish ranged in length from a juvenile at 37.4 cm FL to a series of up to 80.0 cm FL. All fish were collected in the years 2007 to 2008 with counted age estimates of 1 year up to 25 years with corresponding birth years of 2007 to 1982. Measured Δ^{14}C values ranged as predicted from near low decline levels through to approaching peak levels. The progression of Δ^{14}C values relative to fish age and respective birth years increased as expected. Validated ages range from near < 4 years for the juvenile fish 37–47 cm TL to 21–25 years for the largest fish. In addition, the measured Δ^{14}C values measured for the juvenile samples were in agreement with recently collected juvenile fish that were used to establish the lower end of the Δ^{14}C decline series (A.H. Andrews, in prep). This provides validation of a maximum potential age for these fish at ~ 4 years.

DISCUSSION

This study was successful in answering the primary question of the feasibility of ageing Hawai'ian Archipelago *A. virescens*. Results indicate that it is possible to prepare *A. virescens* otoliths for growth zone counting quickly and relatively easily using the slide-mount method so the presumed growth increments are clearly visible. Edge analysis confirmed the season of annular mark deposition corresponded to the slow growth (winter) period. The location of the first annual mark was validated using daily increment counts and a corresponding distinct mark on the edge of the dorsal side of the otolith. A change in otolith shape was used to distinguish young fish from older fish. All of this information was then used to develop an ageing criteria specific for Hawai'ian *A. virescens*.

Several pieces of evidence support the conclusion that the newly developed *A. virescens* ageing criteria is accurate. First, the edge analysis indicates that one translucent and one opaque zone is deposited annually. The second is that otolith weight had a linear relationship with estimated age. This relationship, resulting from otoliths becoming disproportionally thicker with age, is common in many species (Lou et al., 2005; Pilling et al., 2003) and has been shown in several tropical Lutjanidae (Newman et al., 1996, 2000a, b). Although this relationship alone is not considered a validation method, a departure from linearity would suggest that the ageing criteria were faulty. In this study, otolith weight accounted for greater than 89% of the variability. Third, the relationship between fish length *vs*. age was curvilinear and the shape was typical of fish that conform to the von Bertalanffy growth equation. Finally, and perhaps most definitively, bomb radiocarbon dating supported the growth zone counted age estimates. The ages estimated from growth zone counting were generally in the middle of the bomb radiocarbon age ranges. These all indicate that these *A. virescens* ageing criteria provide a reliable and valid age estimation method.

The utility of bomb radiocarbon dating was expanded to the decline period to validate the age of younger fish in the Hawai'ian Islands. This is similar to work performed on speckled hind (*Epinephelus drummondhayi*) in the Gulf of Mexico (Andrews et al., 2013), but use of the decline period was more extensive in the present study. All of the adult fish were aged from near 5 years to 20–25 years with good precision (± 4 years); however, there will be further analyses using a better measure of birth year error once a new random sampling method has been established (Reimer et al., 2004, Andrews et al. *In Prep*). This monotonic decline has been documented for ^{14}C in dissolved inorganic carbon of the mixed surface layer of the north Central Pacific and consistent with the interpolated relationship (Druffel et al., 1989, 2008; Druffel and Griffin, 2008) and the addition of otolith reference material has strengthened the relationship.

The feasibility of ageing Hawai'ian *A. virescens* also includes high between-reader precision. There is no absolute benchmark for between-reader precision; however, an IAPE of 5.5, which corresponds to a CV of 7.6, is considered acceptable for production ageing laboratories (Campana, 2001). The indices of precision were slightly higher in this study. However, it is important to note that these values are primarily from temperate species and the precision of tropical species is generally lower. In addition, both age readers are confident that precision will increase with *A. virescens* ageing experience.

The diffuse nature of the annular marks of fish less than 4 years old will likely be a continuous source of ageing error as it is difficult to determine when the wide annular bands (opaque zones) end and the translucent zones start. The ageing criteria recommend that age readers maintain wide spacing until the 4th annuli, following bands from the outer edge to the sulcus. It also recommends looking for changes in the otolith shape. Following these two recommendations is the best way to avoid splitting bands too early and thus over-ageing. Providing the age reader with catch month will also aid in determining if the edge should be counted as an annular mark.

The necessity of validating ageing criteria for all species has recently been questioned. Choat et al. (2009) argued that annular increment formation in tropical marine fish species is now firmly established and it is unnecessary to validate each species prior to acceptance of life history parameters that depend on the otolith-based age information. Further, Newman and Dunk (2002) state that annual increment formation has been validated in 14 species of Indo-Pacific Lutjanidae and again, the need to validate all species is unnecessary. However, to date, only one species has been validated in the Hawai'ian Archipelago (Andrews et al., 2012) and the habitat differences between the Indo-Pacific Ocean and central Pacific Ocean surrounding the Hawai'ian Archipelago are too large to ignore. Although annual growth increments in central Indian Ocean *A. virescens* otoliths were validated using back calculations of growth and edge analysis, another validation technique, marginal increment analysis, was inconclusive (Pilling et al., 2000). Given the paucity of fish age validations in Hawai'i and the partial validation of *A. virescens* in the Indian Ocean, we used a combination of basic age validation methods (daily increment counts, edge analysis) and an advanced validation technique (bomb radiocarbon dating) to develop a Hawai'i-specific *A. virescens* ageing criteria.

CONCLUSION

Accurate age estimates are the backbone of many population dynamic parameters and age-based assessments. Subjective-based ageing criteria that did not identify the first annular mark and corresponding changes in otolith shape or establish the seasonality of opaque zone deposition will result in inaccurate ages and can lead to erroneous model parameters (Beamish and McFarlane, 1983), biased stock assessments and further, improper management schemes. Here we develop science-based objective ageing criteria for Hawai'ian *A. virescens*. The ageing criteria appear accurate and the between reader precision is high. The estimated ages based on these criteria will result in length-at-age, somatic growth, and mortality estimates that can be used for *A. virescens*-specific stock assessments.

ACKNOWLEDGEMENTS

LITERATURE CITED

Andrews, A. H., R. L. Humphreys, E. E. DeMartini, R. S. Nichols and J. Brodziak.
    2012. Comprehensive validation of a long-lived life history for a deep-water snapper (*Pristipomoides filamentosus*) using bomb radiocarbon and lead-radium dating, with daily increment data. Can. J. Fish. Aquat. Sci. 69:1-20. doi:10.1139/f2012-109.

Andrews, A. H., Barnett, B. K., Allman, R. J., Moyer, R. P. and Trowbridge, H. D.
    2013. Great longevity of speckled hind (*Epinephelus drummondhayi*), a deep-water grouper, with novel use of post-bomb radiocarbon dating in the Gulf of Mexico. Can. J. Fish. Aquat. Sci. 70, 1131-1140. dx.doi.org/10.1139/cjfas-2012-0537.

Beamish, R.J. and D.A. Fournier.
    1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. 38:982–983.

Beamish, R.J. and G.A. McFarlane.
    1983. The forgotten requirement for age validation in fisheries ecology. Trans. Am. Fish. Soc. 112:735–743.

Campana S.E., M.C. Annand and J.I. McMillan.
    1995. Graphical and statistical methods for determining the consistency of age determinations. Trans. Am. Fish. Soc. 4:131-138.

Campana, S.E.
    2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Biol. 59:197-242.

Chang, W.Y.B.
    1982. A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aquat. Sci. 39:1208–1210.

Choat, J.H., J.P Kritzer and J.L. Ackerman.
    2009. Ageing in coral reef fishes: do we need to validate the periodicity of increment formation for every species of fish for which we collect age-based demographic data? In: Green B.S., B.D. Mapstone, G. Carlos, G.A. Begg (eds). Tropical fish otoliths: information for assessment,management and ecology. Springer, New York, p 23-54.

Coplen, T.B.
    1996. New guidelines for reporting stable hydrogen, carbon, and oxygen isotope-ratio data. Geochim. Cosmochim. Acta. 60: 3359-3360.

Druffel, E.R.M. and S. Griffin.
    2008. Daily variability of dissolved inorganic radiocarbon at three sites in the surface ocean. Mar. Chem. 110:185-189.

Druffel, E.R.M., J.E. Bauer, S. Griffin, S.R. Beaupre and J. Hwang.
    2008. Dissolved inorganic radiocarbon in the North Pacific Ocean and Sargasso Sea. Deep. Sea Res. I. 55: 451–459. doi:10.1016/j.dsr.2007.12.007.

Druffel, E.R.M., P.M. Williams, K. Robertson, S. Griffin, A.J. Tull, D. Donahue, L. Toolin and T.W. Linick.
    1989. Radiocarbon in dissolved organic and inorganic carbon from the central North Pacific. Radiocarbon. 31(3): 523-532.

Everson, A.R., H.A. Williams and B.M. Ito.
    1989. Maturation and reproduction in two Hawaiian Eteline snappers, uku, *Aprion virescens*, and Onaga, *Etelis coruscans*. Fish. Bull. 87:877-888.

Francis, R. I. C. C. and P. L. Horn.
    1997. Transition zone in otoliths of orange roughy (*Hoplostethus atlanticus*) and its relationship to onset of maturity. Mar. Biol. 129, 681–687.

Haight W.R., D.R. Kobayashi and K.E. Kawamoto.
    1993. Biology and management of deepwater snappers of the Hawaiian Archipelago. In: Boehlert GW (ed.). The fisheries of Hawaii and U.S.-associated Pacific Islands. Mar. Fish. Rev. 55(2): 20-27.

Hilborn, R. and C. J. Walters.
    1992. Quantitative Fisheries Stock Assessment: Choice, Dynamics and Uncertainty. Chapman and Hall, New York. 570 p.

Lowerre-Barbieri, S.K., M.E. Chittenden and C.M. Jones.
    1994. A comparison of a validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method. Fish. Bull. 92:555-568.

Lou, D. C., B.D. Mapstone, G.R. Russ, C.R. Davies G.A. Begg.
    2005. Using otolith weight –age relationships to predict age-based metrics of coral reef fish populations at different spatial scales. Fish. Res. 71: 279 –294.

Koslow, J. A., G.W. Boehlert, J.D.M. Gordon,. R. L. Haedrich, P. Lorance, and N. Parin.
    2000. Continental slope and deep-sea fisheries: implications for a fragile ecosystem. ICES J. Mar. Sci. 57: 548–557.

MacLellan, S.E. and J. Fargo.
    1995. Validation of Age and Growth for English sole (*Parophrys vetulus*) in Hecate Strait, British Columbia. In: Secor, D.H., J.M. Dean, S.E. Campana (eds). Recent developments in fish otolith research. Univ. South Carolina Press, Columbia, SC. p. 34 1 – 355.

Manooch III, C.

1987. Age and growth of snappers and groupers. In: Polovina, J.J., editor, Tropical Snappers and Groupers: Biology and Fisheries Management, page 689. Westview Press, Boulder, CO.

Martell, S. J. D., Korman, J., Darcy, M., Christensenm, L. B., and Zeller, D.
 2011. Status and trends of the Hawaiian bottomfish stocks: 1948–2004. A report submitted under Contract No. JJ133F-06-SE-2510. Pacific Islands Fisheries Science Center, National Marine Fisheries Service, NOAA, Honolulu 96822-2396. 57 p.

Meyer, C.G., Y.P. Papastamatiou and K.N. Holland.
 2007. Seasonal, diel, and tidal movements of green jobfish (*Aprion virescens*, Lutjanidae) at remote Hawaiian atolls: implications for marine protected area design. Mar. Biol. 151:2133-2143.

Morales-Nin, B. and S. Ralston.
 1990. Age and growth of *Lutjanus kasmira* (Forsskål) in Hawaiian waters. J. Fish Biol. 36(2):191-203.

Mundy, B.C.
 2005. Checklist of the fishes of the Hawaiian Archipelago. Bishop Museum Bulletins in Zoology. Bishop Mus. Bull. Zool. 6:1-704.

Neat, F.C., P.J. Wright and R.J. Fryer.
 2008. Temperature effects on otolith pattern formation in Atlantic cod *Gadus morhua.* J. Fish Biol. 73: 2527–2541 doi:10.1111/j.1095-8649.2008.02107.

Newman, S.J. and I.J. Dunk.
 2002. Growth, age validation, mortality, and other population characteristics of the red emperor snapper, *Lutjanus sebae* (Cuvier, 1828), off the Kimberley coast of north-western Australia. Estuar. Coast. Shelf Sci. 55(1):67-80.

Newman, S.J., D. Williams and G.R. Russ.
 1996. Age validation, growth and mortality rates of the tropical snappers (Pisces: Lutjanidae) *Lutjanus adetii* (Castelnau, 1873) and *L. quinquelineatus* (Bloch, 1790) from the central Great Barrier Reef, Australia. J. Mar. Fresh. Res. 47, 575–584.

Newman, S.J., M. Cappo and D. Williams.
 2000a. Age, growth and mortality of the stripey, *Lutjanus carponotatus* (Richardson) and the brown-stripe snapper, *L. vitta* (Quoy and Gaimard) from the central Great Barrier Reef Australia. Fish. Res. 48:263-275.

Newman, S.J., M. Cappo and D. Williams.
 2000b. Age, growth, mortality rates and corresponding yield estimates using otoliths of the tropical red snappers*, Lutjanus erythropterus*, *L. malabaricus* and *L. sebae*, from the central Great Barrier Reef. Fish. Res. 48:1-14.

Newman S.J., C.B. Wakefield, A.J. Williams, J.M. O'Malley, S.J. Nicol, E.E. DeMartini, T. Halafihi, J. Kaltavara, R.L. Humphreys, B.M. Taylor, A.H. Andrews, R.S. Nichols.
2015. International workshop on methodological evolution to improve estimates of life history parameters and fisheries management of data-poor deep-water snappers and groupers. Mar. Pol. 60:182-185. doi: 10.1016/j.marpol.2015.06.020.

Pannella, G.
1971. Fish otoliths: daily growth layers and periodical patterns. Science 173:1124-1127.

Pannella, G.
1974. Otolith growth patterns: An aid in age determination in temperate and tropical fishes. In: T. B. Bagenal (editor), The ageing of fish, p. 28-39. Unwin Brothers, La., Surrey.

Piddocke, T.P., G.L. Butlera, P.A. Butchera, S.W. Purcella, D.J. Bucherd and L. Christidis.
2015. Age validation in the Lutjanidae: A review. Fish. Res. 167:48-63.

Pilling, G.M. and A.S. Halls.
2003. Age- or length-based methods of growth estimation. What drives the choice? NAGA, WorldFish Cntr. Quart. 26:4-7.

Pilling, G.M. and C.C. Mees.
2000. R7521: Implementing management guidelines arising from project R6465 – an assessment of utility. MRAG Final Technical Report.

Pilling, G.M., R.S. Millner, M.W. Easey, C.C. Mees, S. Rathacharen and R. Azemia.
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.

Pilling, G., E. Grandcourt and G. Kirkwood
2003. The utility of otolith weight as a predictor of age in the emperor *Lethrinus mahsena* and other tropical fish species. Fish. Res. 60:493-506.

Pilling, G. M, R.C. Wakeford, C.C. Mees.
2006. Comparison of length- and age-based stock assessment methods. In: D.D. Hoggarth (editor), Stock Assessment for Fishery Management: A Framework Guide to the Stock Assessment Tools of the Fisheries Management and Science Programme. FAO Fisheries Technical Papers. No. 487. Rome, FAO. 261 p.

Pińeiro, C. and M. Sainza.
2003. Age estimation, growth and maturity of the European hake (*Merluccius merluccius*) from Iberian Atlantic waters. ICES J. Mar. Sci., 60 (5):1086-1102.

Ralston, S., S. Cox, M. Labelle and C. Mees.
2004. Bottomfish stock assessment workshop – Juanuary 13-16, 2004 – final panel report. Western Pacific Regional Fishery Management Council, Honolulu, 20 p.

Ralston, S. and K.E. Kawamoto.
    1988. A biological assessment of Hawaiian bottom fish stocks, 1984-87. Southwest Fisheries Science Center Administrative Report H-88-08, 60 p.

Ralston, S. and H.A. Williams.
    1988. Numerical integration of daily growth increments: An efficient means of ageing tropical fishes for stock assessment. Fish. Bull., US. 87:l-16.

Reimer, P.J., T.A. Brown and R.W. Reimer.
    2004. Discussion: reporting and calibration of post-bomb 14C data. Radiocarbon. 46(3):1299-1304.

Somerton D.A. and D.R. Kobayashi.
    1990. A measure of overfishing and its application on Hawaiian bottomfishes. Southwest Fisheries Science Center Administrative Report H-90-10, 18 p.

Stuiver, M. and H.A. Polach.
    1977. Discussion: reporting of 14C data. Radiocarbon 19:355-363.

Sundberg, M. and K. Underkoffler.
    2011. Size composition and length-weight data for bottomfish and pelagic species sampled at the United Fishing Agency Fish Auction in Honolulu, Hawaii from October 2007 to December 2009. Pacific Islands Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Honolulu, HI 96822-2396. Pacific Islands Fish. Sci. Cent. Admin. Rep. H-11-04, 34 p.

Williams, A.J., K. Loeun, S.J. Nicol, P. Chavance, M. Ducrocq, S.J. Harley, G.M. Pilling, V. Allain, C. Mellin and C.J.A. Bradshaw.
    2013. Population biology and vulnerability to fishing of deep-water eteline snappers. J. Appl. Ichthyol., 29 (2013), pp. 395–403

Williams, A.J., S.J. Newman, C.B. Wakefield, M. Bunel, T. Halafihi, J. Kaltavara and S.J. Nicol. 2015. Evaluating the performance of otolith morphometrics in deriving age compositions and mortality rates for assessment of data-poor tropical fisheries. ICES J. Mar. Sci. first published online March 15, 2015. doi: 10.1093/icesjms/fsv042.

Table 1.--Fish, estimated age, and radiocarbon data for a selected series of *A. virescens*. Δ14C birth year range is based on an alignment of the core Δ14C value with a bomb radiocarbon decline series from a regional Δ14C reference (A.H. Andrews, in prep).

Sample code	Length (cm) FL	Collection Date	Counting age (yr)	Count birth year	Δ^{14}C birth year range	Core Δ^{14}C (‰)
KP1-1-3-103	63	5/12/07	5	2002.4	1998-2006	72.1
KP10-2-4-464	68.3	6/18/08	14	1994.5	1990-1998	91.7
KP2-4-6-140	75.6	5/29/07	18	1989.4	1985-1993	122.4
KP3-2-8-232	67.5	9/14/07	25	1982.7	1979-1987	140.4
KP4-3-5-323	80	10/19/07	23	1984.8	1981-1989	143.0
KP8-1-4-441	79.1	4/30/08	21	1987.3	1983-1991	128.7
KP9-4-1-568	74.0	5/17/08	23	1985.4	1981-1989	138.4
LA3-5-3-260	67.3	6/5/07	15	1992.4	1988-1996	110.2
LA5-3-6-414	58.4	9/19/07	5	2002.7	1999-2007	94.0
OES1-1-1-1	70.4	6/16/07	17	1990.5	1986-1994	112.2
LA6-3-6-450	46.8	11/11/07	2	2005.9	2002-2007	67.9
LY3-4-7-37	37.4	4/17/08	1	2007.3	2003-2008	57.3

Table 2.--Sample number, minimum, and maximum number of daily increment counts to the inflection on the otolith edge.Table 2. Sample number, minimum, and maximum number of daily increment counts to the inflection on the otolith edge.

Sample #	Minimum # of increments	Maximum # of increments
KP2-4-6-140	116	133
KP1-2-2-112	149	167
KP1-2-4-114	112	122
LY3-4-7-37	130	151
ROYM2-3	122	150
CTAM1-1	100	106
ROYM2-5	174	180
KP8-1-1-431	105	140
KP2-5-2-138	130	154
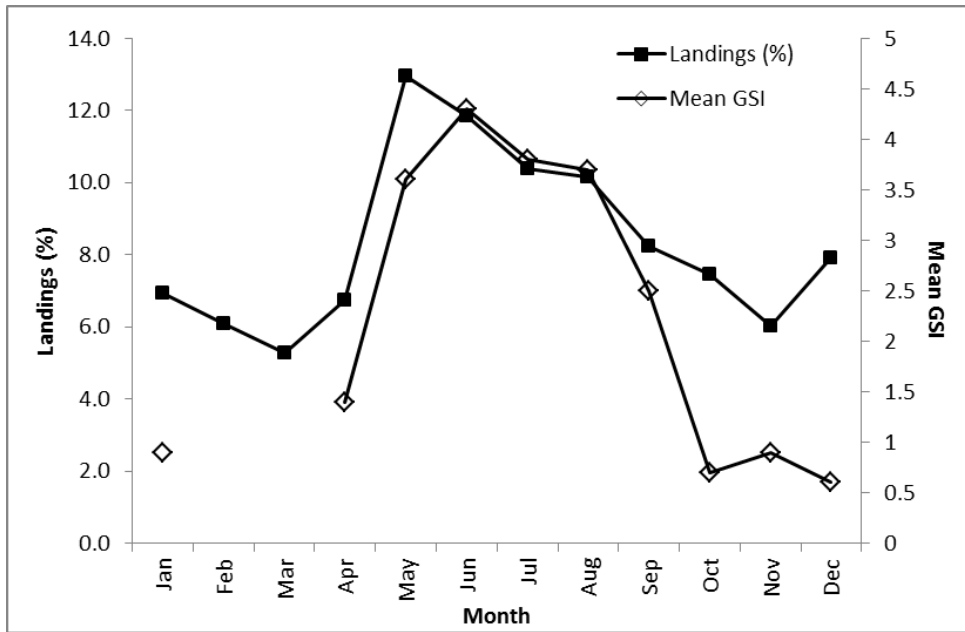
Figure 1.--Landings (monthly % of total) and monthly mean gonosomatic index (GSI) for main Hawaiian Island *A. virescens*. Catch data is reported catch to Hawaii Division of Aquatic Resources (1948–2010). GSI index only includes females, juvenile (< 500 mm FL) were excluded from analysis; modified from Everson et al. (1989).
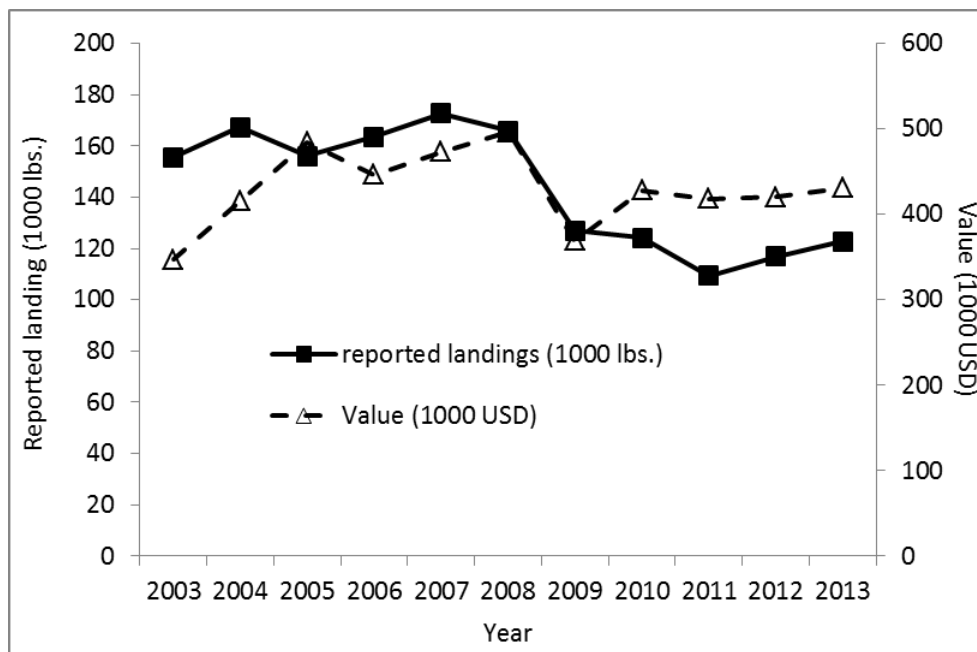
Figure 2.--Reported annual landings and annual value of *Aprion virescens* in Hawaii from 2003 to 2013. Data from Hawaii Department of Aquatic Resources.[1]

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[1] http://www.pifsc.noaa.gov/wpacfin/hi/da¹r/Pages/hi_data_3.php
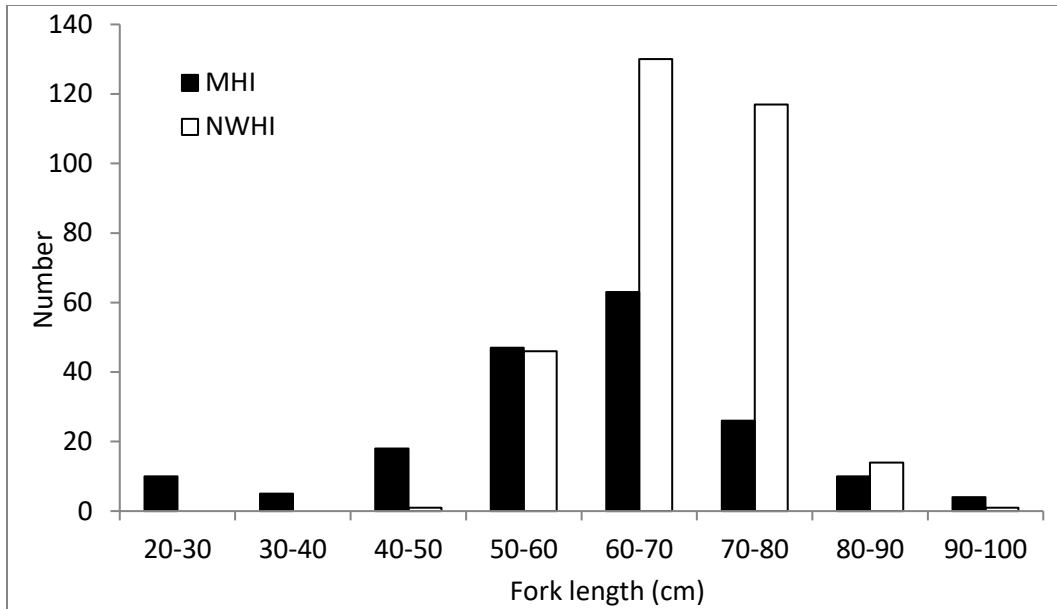
Figure 3.--Size frequency of otolith sampled *A. virescens* from the main Hawaiian Islands (MHI) and the Northwestern Hawaiian Islands (NWHI) 2007–2015.
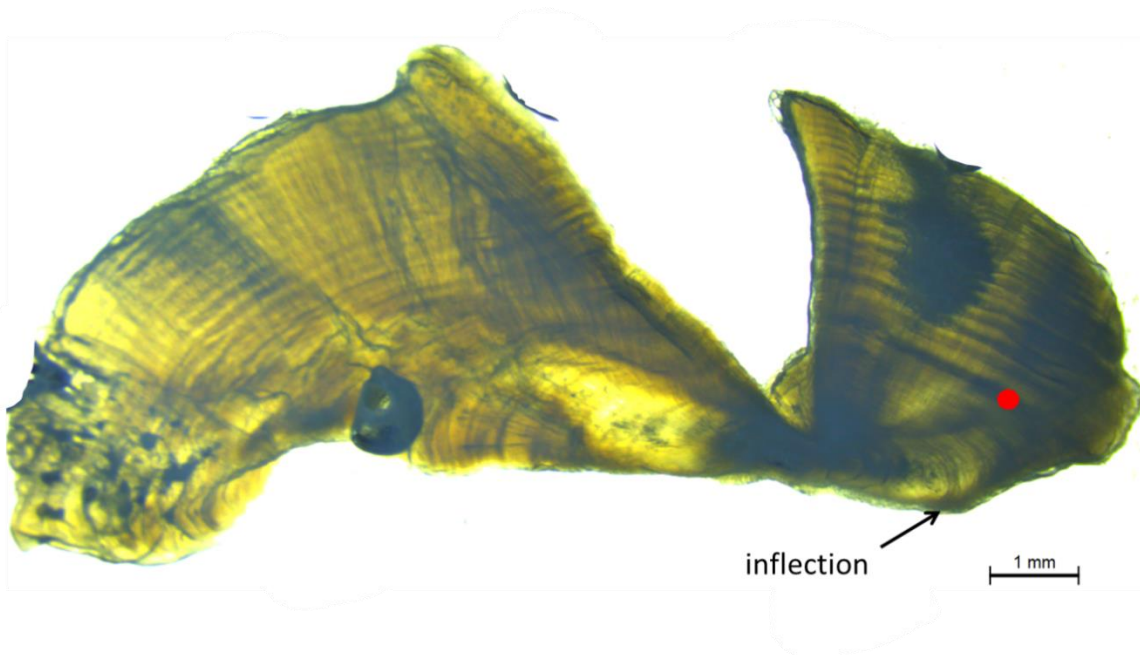
Figure 4.--*A. virescens* thin sectioned otolith, collected in June from a 70.4 cm female, displaying alternating light () and dark () zones. The inflection marks the start of the first annular mark. The second annular mark is identified with a red dot and marks where the otolith changes shape. Viewed under transmitted light.
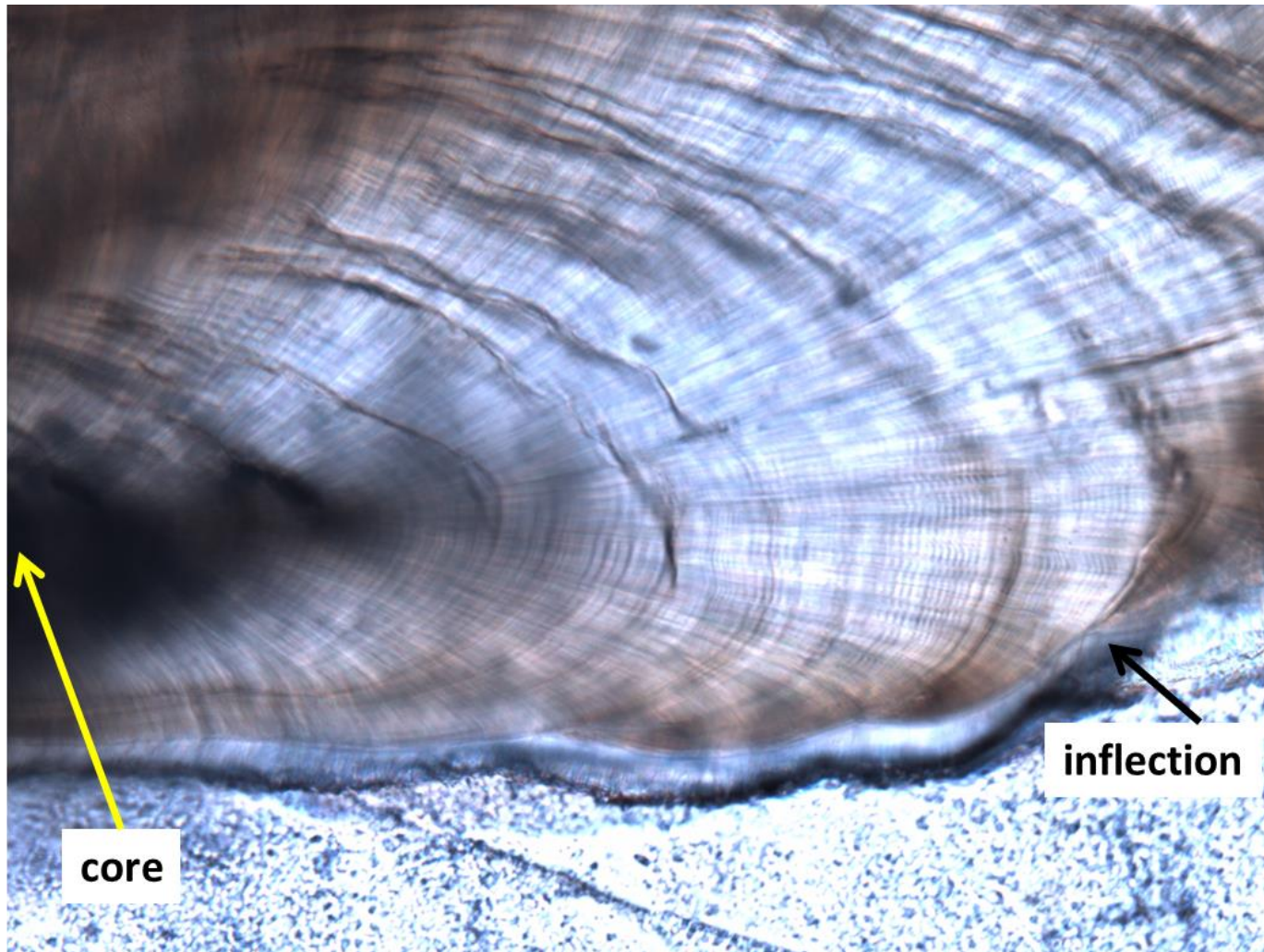
Figure 5.--High magnification (60×) view of *A. virescens* thin sectioned otolith, collected in May from a 75.6 cm male, viewed under transmitted light. Daily increment counts from core to inflection ranged from 116 to 130.
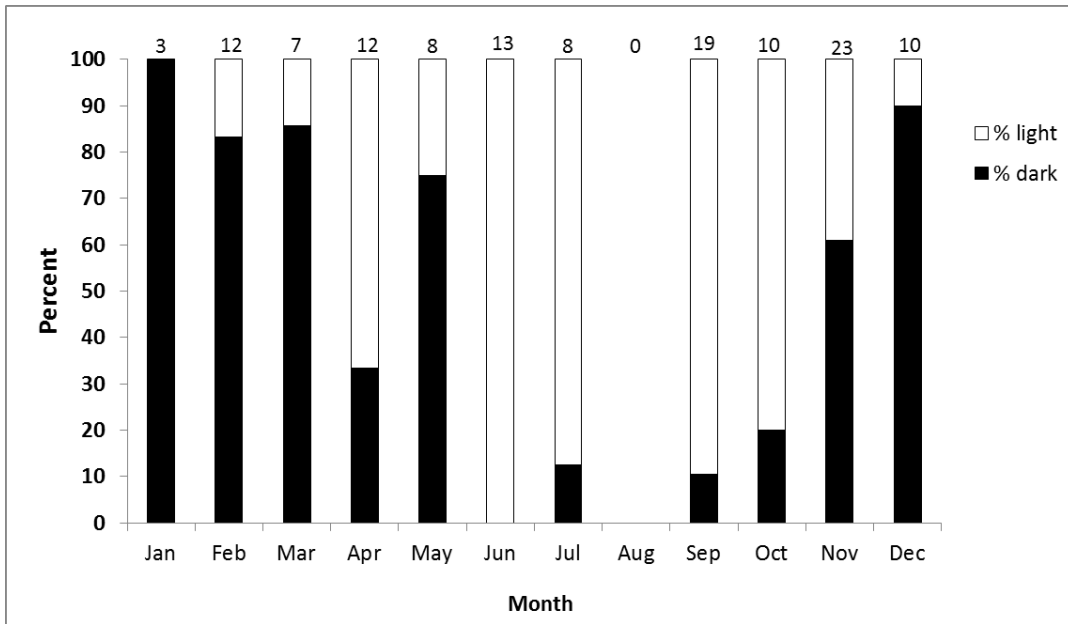
Figure 6.--Percentage of Hawaiian Island *Aprion virescens* otoliths with opaque (light) and translucent (dark) zone by month. Numbers at top of columns are the sample size.
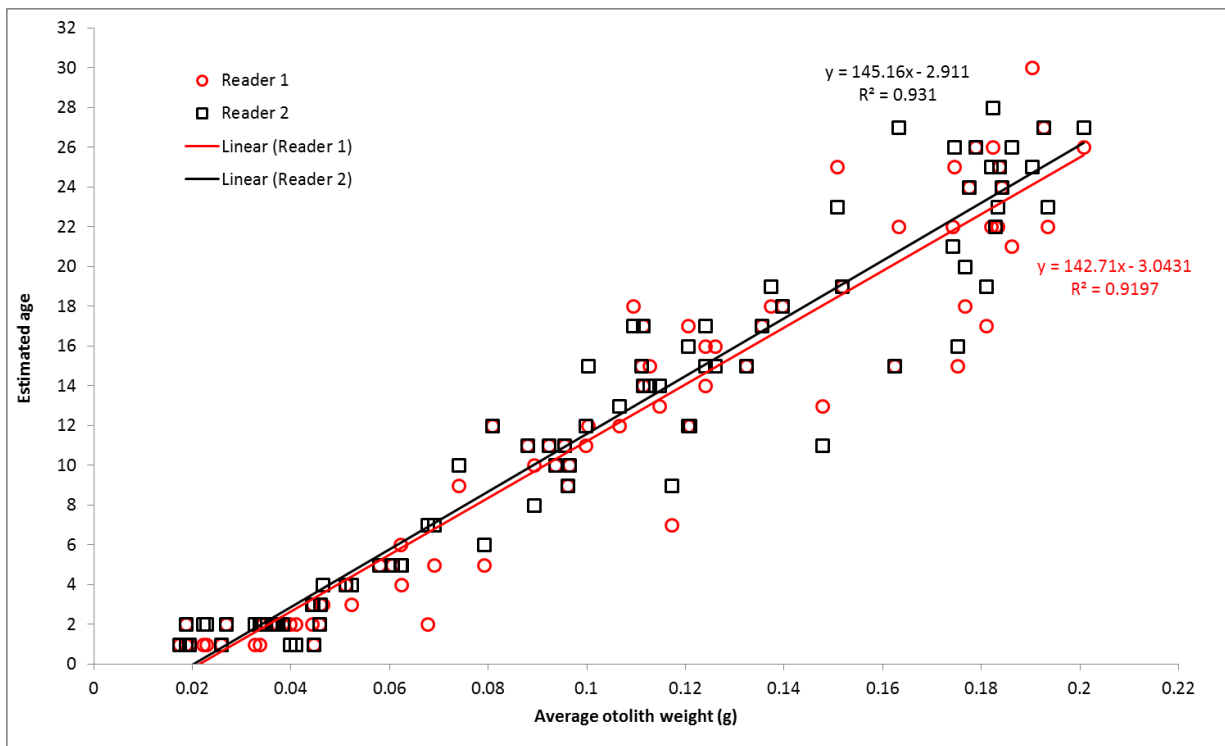

Figure 7.--*A. virescens* initial blind (no prior knowledge of month of capture) estimated age *vs*. otolith weight.
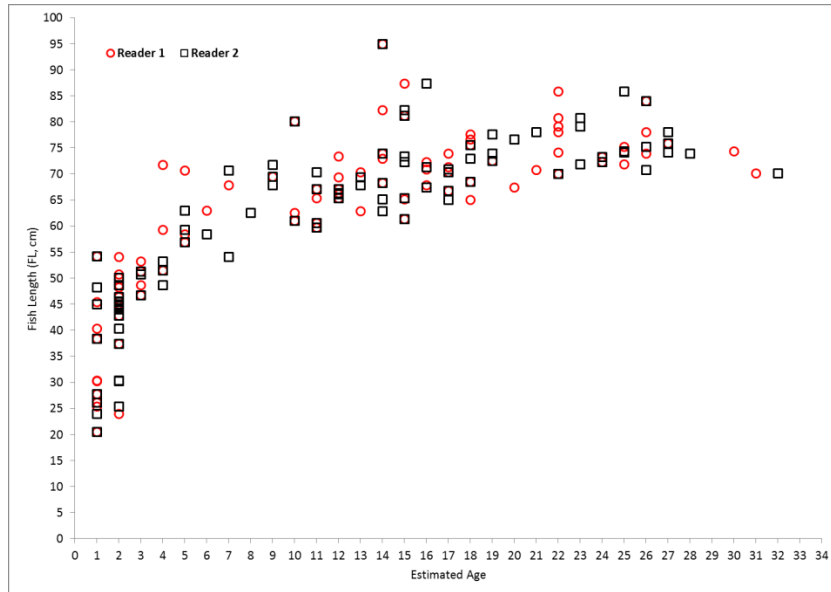
Figure 8.--*A. virescens* initial blind (no prior knowledge of month of capture) estimated age *vs.* fish length.
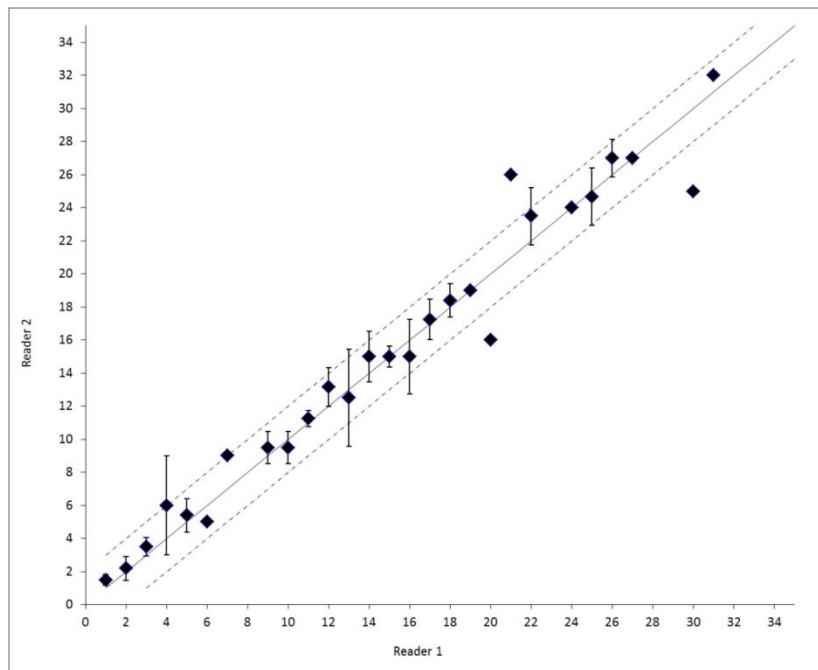

Figure 9.--Age-bias plots of Reader 1 and Reader 2 of initial blind (no prior knowledge of month of capture). Each error bar represents the 95% confidence interval about the mean age assigned by one age reader for all fish assigned a given age by a second age reader.
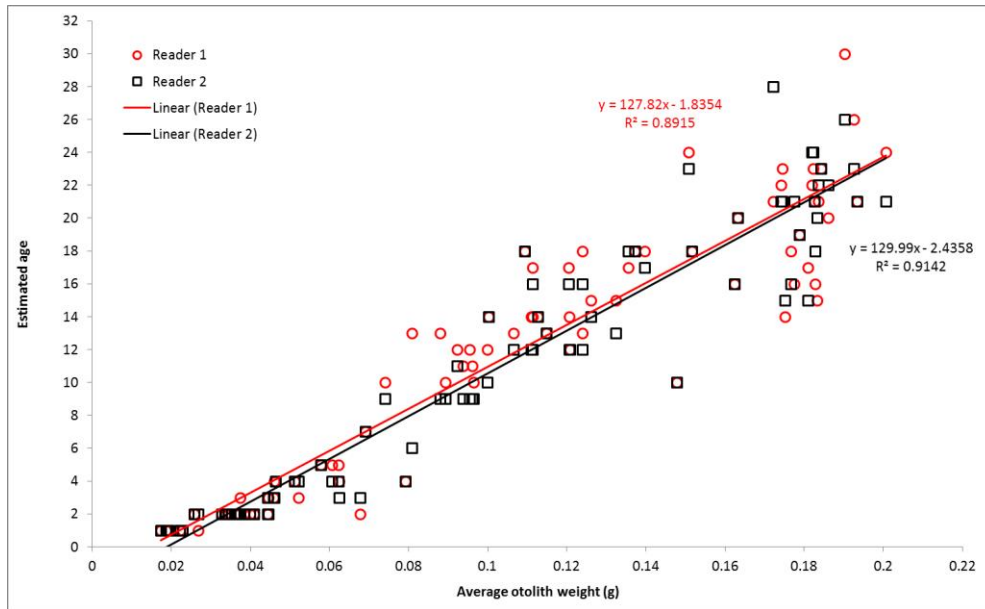
Figure 10.--*A. virescens* estimated age *vs*. otolith weight, linear fit equation and coefficient of determination. Both age readers applied the updated ageing criteria and had prior information on month of capture.
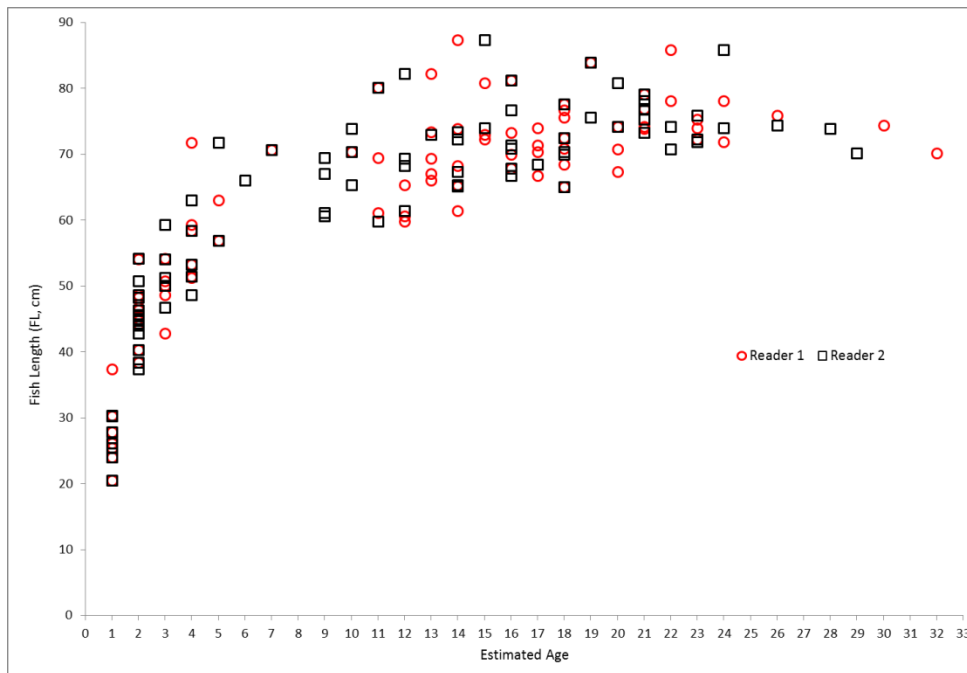

Figure 11.--*A. virescens* estimated age *vs*. fish length. Both age readers applied the updated ageing criteria and had prior information on month of capture.
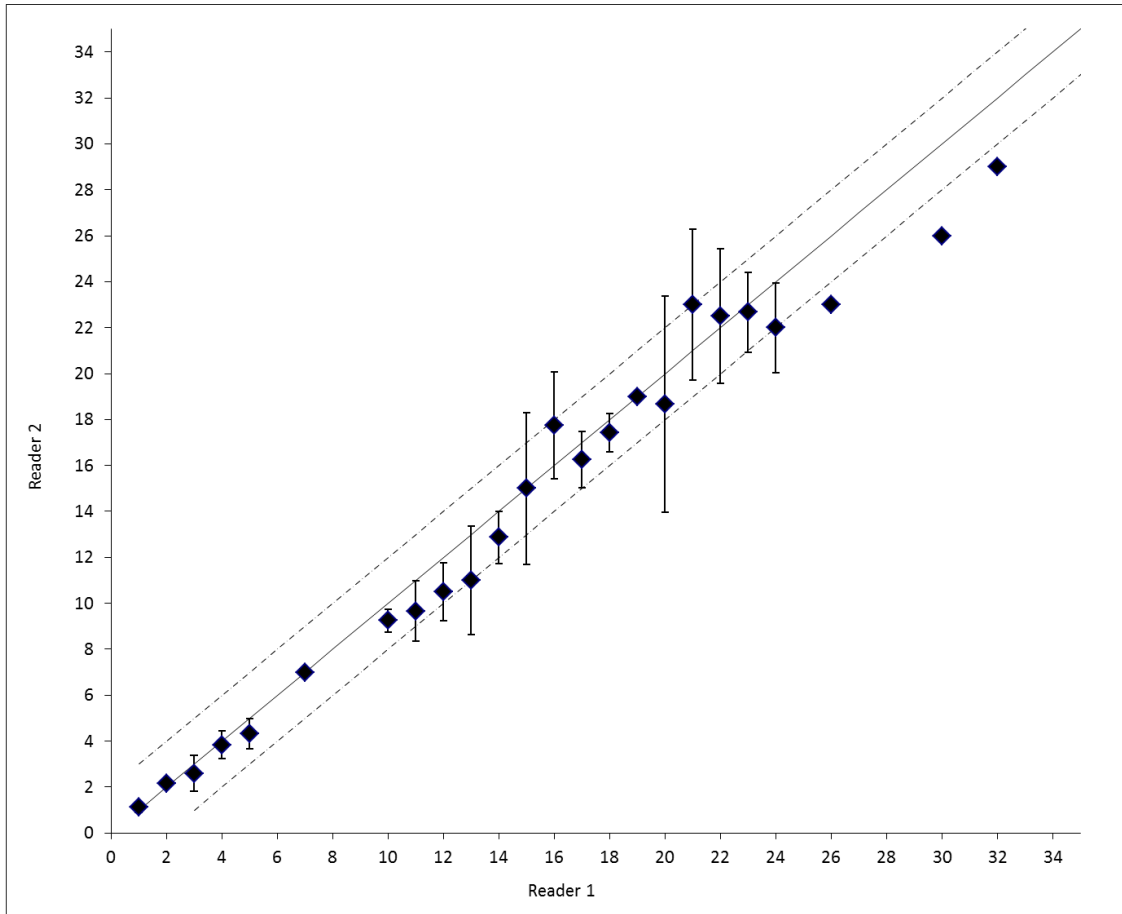
Figure 12.--Age-bias plots of Reader 1 and Reader 2. Both age readers applied the updated ageing criteria and had prior information on month of capture. Each error bar represents the 95% confidence interval about the mean age assigned by one age reader for all fish assigned a given age by a second age reader.

APPENDIX A

Aprion virescens (uku) ageing criteria

A. virescens thin sectioned otoliths have a visually apparent banding pattern however; this does not necessarily translate to easy identification of annular marks. The two problem areas are identifying the first annular mark and numerous false annular marks at younger ages (1–4). This ageing criteria focuses on providing advice in these areas.

Identification of first annual mark

The first annual mark on the thin section corresponds to a deep groove closest to the core on the otolith surface. This area on the thin section is identifiable by an 'inflection' feature on the otolith dorsal edge and annual marks 'bend' into this area. Daily increment analysis indicates that this represents the start of the first year winter growth and thus the start of the first annual mark. Many otoliths have a check inside of this mark; therefore, care must be taken not to consider any marks inside of the inflection feature as year 1. This will result in overestimating ages by much greater than 1 because the regular decreasing spacing between successive annuli will be incorrect and splitting of annuli will occur sooner than it should.

Identification of years 1-3

virescens grow quickly the first 4 years then growth abruptly slows and this is apparent in many otoliths with the first 4 annual annuli being very diffuse (i.e. they are made of numerous small marks) while the remaining annuli are distinct and appear in a clear banding pattern with equal spacing. The diffuse nature of the first 4 annuli can lure the age reader into splitting the first few years into too many annuli. While identifying the 4 four annuli it is important to keep in mind the fast growth of the first few years and keep the appropriate spacing. Recognizing this spacing pattern is integral to accurate age estimates. If the spacing between each of the successive annuli out form the core is not declining then the reader is likely misinterpreting additional marks as annuli.

Typically, each of the first few annuli is made up of several marks, some of which can be prominent, but all will originate from the same general area on the dorsal edge. Tracing a wide band from the sulcus to the section edge will ensure proper annuli identification.
The start of the slower growth typically corresponds to a change in shape of the dorsal outer edge where the otolith stops growing outward and rotates its growth axis toward the sulcal region.

The diffuse nature of the first 4 annuli makes it hard to determine if the edge should be counted in the age estimate. Therefore, this ageing criteria depends on the reader having access to the month of capture to accurately determine if the edge should be included in age estimates of young fish.

Preferred reading axis

The preferred *A. virescens* otolith reading axis for the first few years is in the middle of the dorsal side but it is important to trace each annuli to a origin on the edge. Once the otolith displays regular banding pattern (> 4 annuli) anywhere on the otolith face can be used as a reading axis. However, reading the otolith along the sulcus can result in higher estimates due to split banding.

Some otoliths have a confusing banding pattern on the dorsal side of the thin section. In these cases, the ventral side of the otolith can be used to estimate age. Also, in some cases the ventral side can also be used to verify the age estimate from the dorsal side of the thin section.
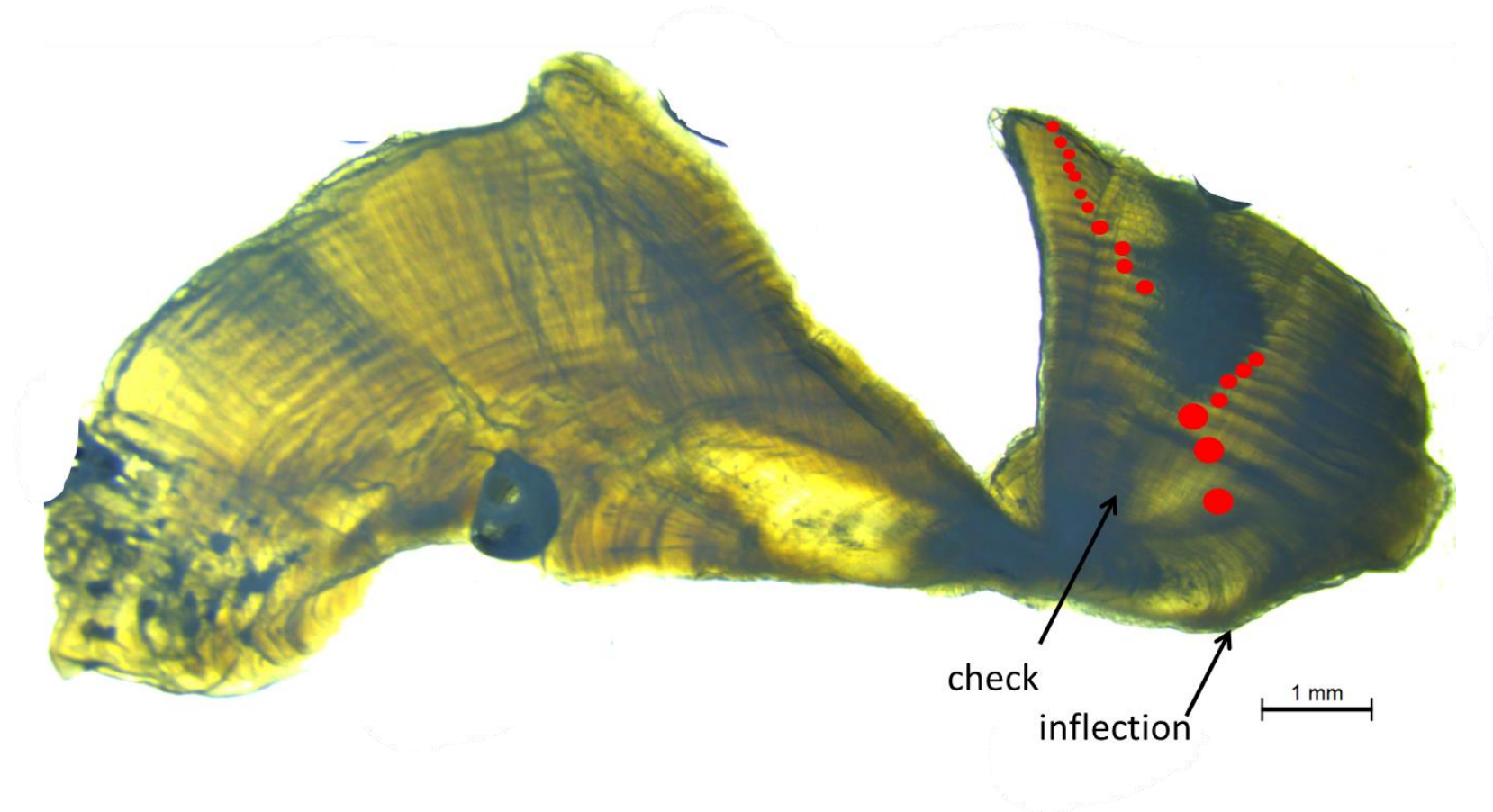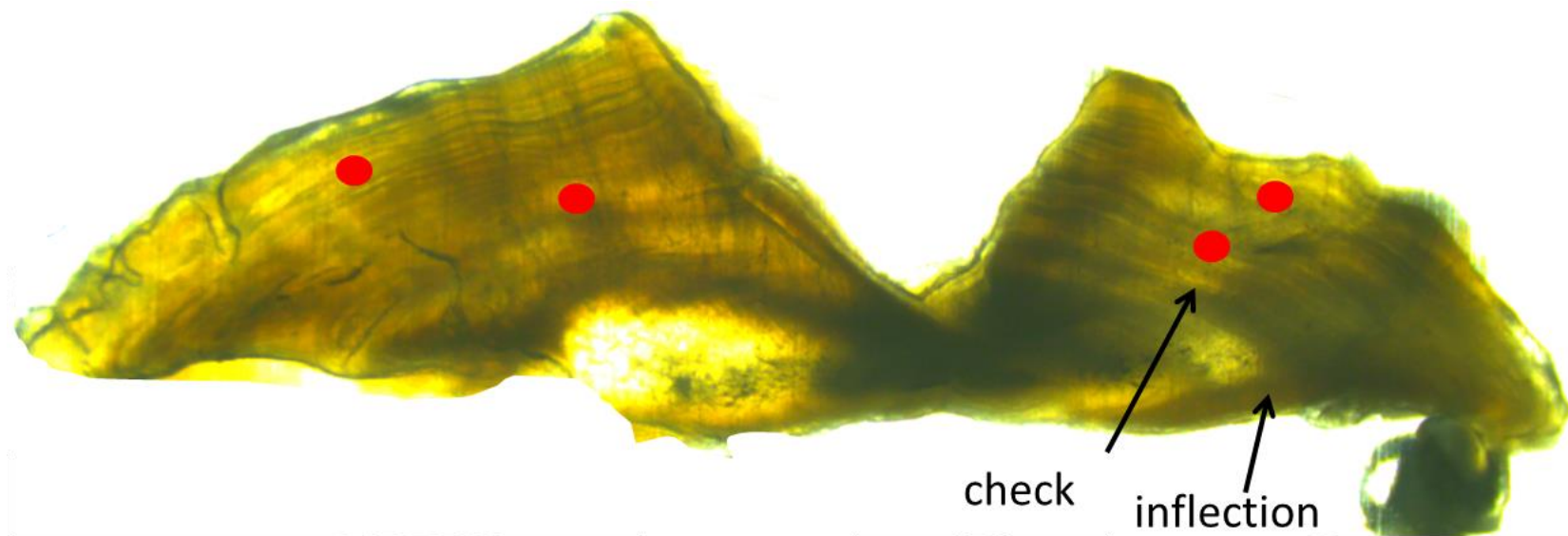
Example 1.--*A. virescens* thin sectioned otolith (OES1-1-1-1), collected in June from a 70.4-cm female, viewed under transmitted light. The start of the first annular mark (inflection) is identified along with a false first annuli (check). Red dots indicate estimated annular marks. All of the annuli have distinct marks that extend from the dorsal outer edge to the sulcus. Note the change in otolith shape after the second annuli and the abrupt change from fast growth to slower at the fourth annuli. This otolith is a good example of an easy to read otolith. Notice the first 3 years are characterized by fast growth followed by relatively slower growth start at year 4. The dorsal edge also changes after the second annular mark.
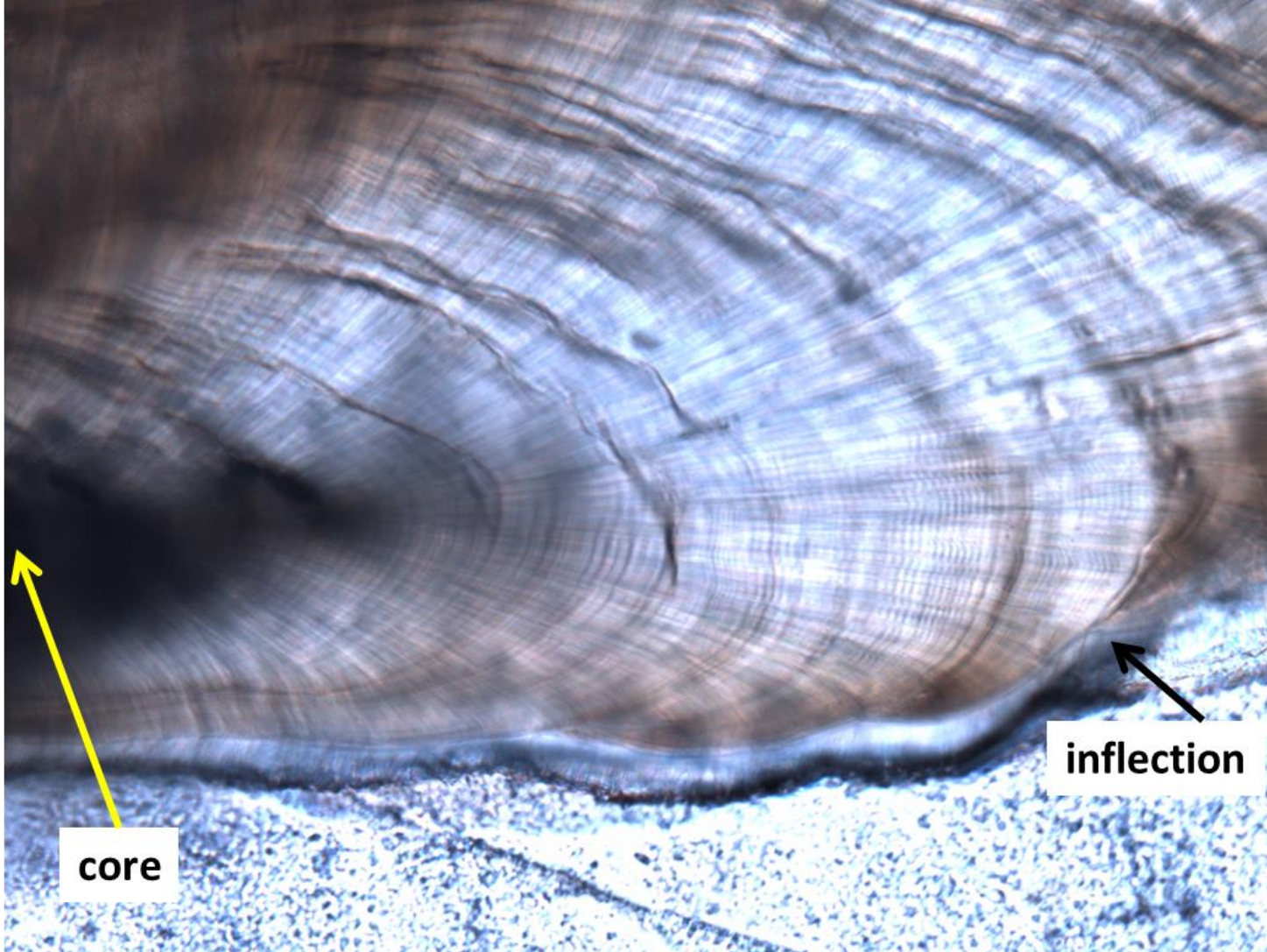
Example 2.--High magnification (60×) view of *A. virescens* thin sectioned otolith, collected in May from a 75.6-cm male, viewed under transmitted light. Daily increment counts from core to inflection ranged from 116 to 130.
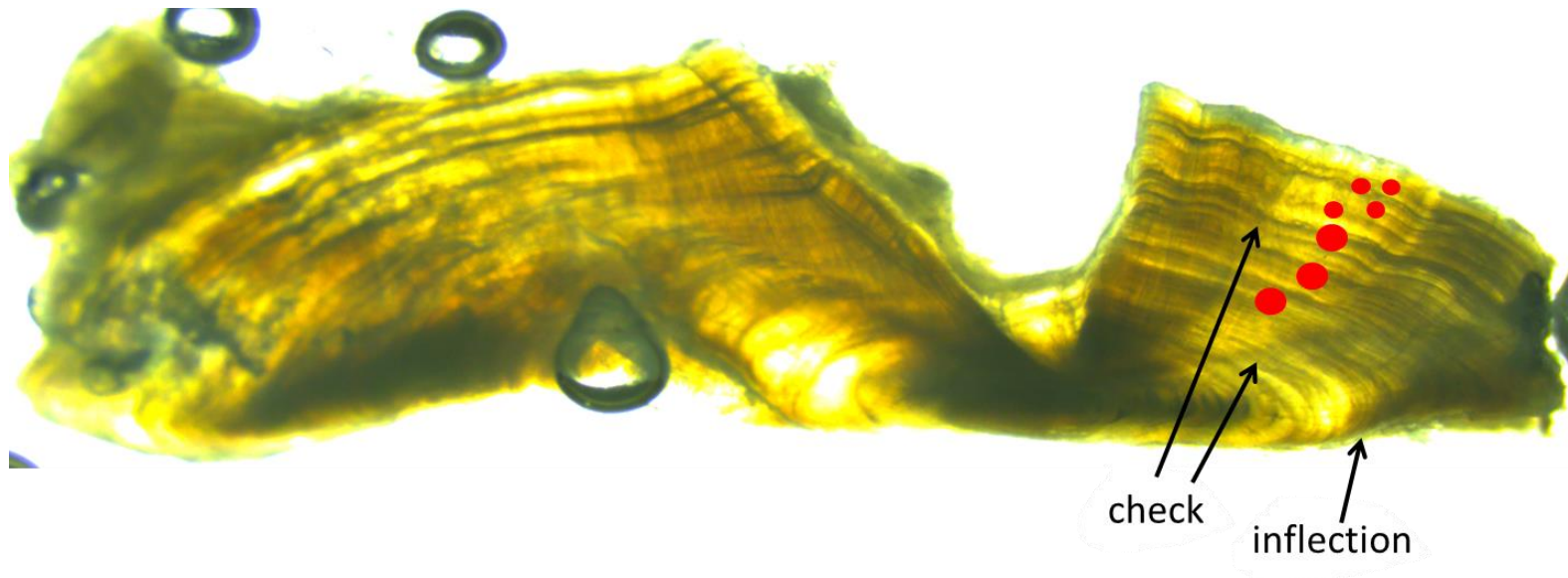
Example 3.--*A. virescens* thin sectioned otolith (KP4-2-9-248), collected in October from a 70.7-cm fish, viewed under transmitted light. The start of the first annular mark (inflection) is identified along with a false first annuli (check). Another check is apparent between 2 and 3. The spacing is not correct to call this check an annuli. Red dots indicate estimated annular marks. The dorsal edge changes shape after the second annular mark and the abrupt change from fast growth to slower growth is apparent at the fourth annuli.

check inflection

Example 4.--*A. virescens* thin sectioned otolith (LA5-3-7-403), collected in September from a 54.1-cm female estimated at 2 years of age, viewed under transmitted light. The start of the first annular mark (inflection) is identified along with a false first annuli (check). Red dots indicate estimated annular marks. This is a good example of a confusing otolith to read which is easy to over-age. The shape of the dorsal edge indicates a young fish because it has not changed shape yet.