Pacific Islands Fisheries Science Center

Preliminary Estimates of Age and Growth for the Endemic Hawai'ian Grouper (Hapu'upu'u, *Epinephelus quernus*, F. Serranidae)

Ryan S. Nichols
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December 2008

Administrative Report H-08-06

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ABSTRACT

We herein describe the general size and growth characteristics of the endemic Hawai'ian grouper or hapuʻupuʻu, *Epinephelus quernus*. All specimens were collected from commercial and research vessels by hydraulic handline; collections were made in all months and spanned the October 2005–June 2008 period. Fish weight-at-length was described using all 547 specimens collected. The best-fit relationship was $FW = 0.00002 \cdot TL^{2.997}$, where FW = total round weight of fish in kg, and TL = total length in cm ($r^2 = 0.98$). Sagittal otolith-to-fish body size and body size-at-age relationships were estimated using a random subset of 158 of the 547 specimens.

Otolith-to-fish body-size relationships were proportional, and body size was predictably related to fish age in years. Otolith length was linearly related ($r^2 = 0.81$) to fish length, and otolith weight was curvilinearly related ($r^2 = 0.70$, $p < 0.001$) to fish weight. Size-at-age was estimated using mean back-calculated body lengths-at-age, with ages estimated as the number of annuli (alternating pairs of opaque and translucent bands apparent by microscopic examination of transverse sections of sagittae). Lengths-at-age were back-calculated using the proportionality equation, $TL_t = (R_n / R_t)^b \cdot TL$, where TL_t = back-calculated total length at age t; and R_n and R_t are the distances from sagitta primordium (focus) to the n^{th} annulus and the distal edge of the cross-section, respectively. The b parameter was derived from the relation between TL and R_t ($R_t = 0.02636 \cdot TL$; $b = 1.0$; $r^2 = 0.98$). A standard, three-parameter von Bertalanffy Growth Function (VBGF) fit well ($r^2 = 0.98$) to mean back-calculated length-at-age: $L_t = 82.3\,(1 - e^{-0.157(t+1.45)})$. Back-calculated total lengths ranged from 27.5 cm at age 1 to 87.0 cm at an observed maximum age of 34 years.

The yearly periodicity of annuli was partially validated by analysis of marginal increment ratios, but additional validation, including that provided by complementary methods, is required. Although results to date are preliminary, they nonetheless indicate that the potential exists for using otolith-based age estimates to conduct an age-structured stock assessment of Hawai'ian grouper after additional specimens are analyzed to provide more precise parameter estimates and after annulus validation has been completed.

INTRODUCTION

The handline fishery for bottomfish is the last remaining major commercial extraction fishery for insular resources in the Hawai'ian Archipelago. In 2007, the total adjusted commercial landings of deep (140–330 m) reef-slope bottomfish were approximately 238 metric tons (mt) (Moffitt, pers. comm.). This total is dominated by about 15 Bottomfish Management Unit Species (BMUS) of eteline snappers, jacks, and groupers. Seven species, the so-called "Deep-7", have been protected by emergency fishing closures during the months of May through September in 2006, 2007, and until mid November in 2008 in the main Hawai'ian Islands (MHI) (WPRFMC press release, 2008). In addition to these seasonal closures, it is generally recognized that more stringent conventional management measures that include age-based approaches to stock assessment need to be implemented (WPRFMC press release, 2004). The MHI stocks of several species (onaga, an eteline snapper, and hapu'upu'u, the Hawai'ian grouper) have been identified as particularly stressed based on the previously used, non age-based criterion of a low and recently declining "Spawning Potential Ratio" (SPR; Moffitt, in prep.). Over the past decade, the catches of hapuʻupuʻu in the MHI have been variable as well as low compared to prior years, ranging from about 28,600 to 84,000 pounds (Moffitt et al., 2006; Moffitt, in prep.).

The hapuʻupuʻu *Epinephelus quernus* is endemic to the Hawai'ian Islands and Johnston Atoll. It is likely a slow-growing, long-lived and late maturing species, similar to other large-bodied groupers (Heemstra and Randall, 1993). However, little is definitively known of its age, growth or related life history attributes (Uchiyama and Tagami, 1983; Manooch, 1987). Only a preliminary description of fish size at maturity, age estimation based on subsampled daily increments found in otoliths, and DNA analysis of subtle population structure have been conducted (Everson, 1992; Williams and Lowe, 1997; Rivera et al., 2004). There is no published evidence that marks on hapuʻupuʻu otoliths (earstones) form annually and can be used to estimate age, and a relation has not been established between age estimates based on daily increments and the annuli possibly present on otoliths.

The objectives of this report are to provide (1) a general description of the growth characteristics of hapuʻupuʻu and (2) preliminary estimates of size-at-age that would be needed for an age-structured stock assessment of this species.

MATERIALS AND METHODS

Fish Collections and Measurements

All hapuʻupuʻu specimens were procured through contracted Hawaii-based commercial bottomfishing vessels during the October 2005–June 2008 period and a fisheries independent research cruise during July 2007. Sampling effort initially targeted the

Northwestern Hawai'ian Islands (NWHI) but was subsequently expanded to include the MHI (Fig. 1).

All contributing vessels used similar (hydraulic-powered) handline gear. Fish were either held on ice (aboard commercial vessels) until a NOAA biologist could obtain them dockside or processed immediately aboard the research vessel. Geographic location, date of capture, and depth of capture (in most cases) was recorded for each fish collected. All specimens were processed in a NOAA laboratory where biologists noted sex based on macroscopic criteria (Caillet et al., 1986). Body length (Total Length, TL, from tip of snout to posterior margin of caudal fin) was measured for each fish using calipers to 0.1 cm. Fish were weighed (total body weight including all viscera) using a digital bench scale to 0.01 kg. To expedite otolith extraction, heads were removed from the body using meat saws and the fish were either processed fresh or stored in freezers.

Otolith Extraction and Processing

Both left and right sagittal otoliths were extracted from fresh or frozen-thawed heads. Each pair of sagittae was cleaned with a small paint brush under a dissecting scope, rinsed with distilled water, and then stored dry in a scintillation vial. Both left and right sagittae were measured across the primordium (focus) for rostrum-postrostrum length, dorsal-ventral height, and medial-lateral thickness using digital calipers to 0.01 mm. Prior to sectioning, otoliths were weighed to 0.0001 g with an analytical balance, dried in a forced convection oven at 80º C for 24 hr, and then reweighed before further storage and processing.

Subsampled sagittae were then selected for embedding, sectioning, and age estimation. Specimens were selected across a size range to include both small and large fish from all months of collection. By convention (see Results), the left sagitta was selected. Before embedding, the nucleus and presumed primordium were marked using a pencil under a dissecting microscope. If the primordium was obscured or damaged, then the right sagitta was marked and used. Sagittae were mounted distal-side up in individual trays with clear polyester resin and sectioned transversely at 300–500 μm through the marked nucleus using a Buehler Isomet 1000 low-speed saw with two blades separated by a 0.5 mm shim. The section was then mounted onto a glass slide with thermoplastic glue and ground successively with 400, 600, and 1200 grit paper (Love et al., 1996; Choat and Axe, 1996; Pears et al., 2006).

Age Estimates

Each sectioned sagitta was examined by compound and stereozoom dissecting microscopes using transmitted light and reflected light, respectively, to ensure that the primordium was present. Each sagitta was examined for annuli using the dissecting microscope and reflected light at 20-40x on a black background; incremental opaque bands were counted as annuli. An annulus is herein defined as one opaque plus one translucent band. Ages were assigned based only on annuli that were completely formed. For example, if there were two fish—one with two completed annuli and a second fish with two completed annuli plus a developing but incomplete annulus at the margin—both fish would be placed in age-group 2. Annuli were scored by one reader at three separate sittings without reference to length or month of capture.

As a precursor to the main study, 30 sagittae were taken to the Age and Growth Lab at James Cook University, Townsville, Australia, to determine whether opaque and translucent bands were present in transverse sections and, if so, whether they might be used for estimating ages. The first author and another scientist evaluated sectioning techniques conducted by both facilities. After determining the presence of alternating opaque and translucent bands (Fig. 2) and refining sectioning techniques to expedite processing, each otolith was aged and its relative quality scored. These ages were compared for precision, and consensus agreement standards were applied (Campana, 2001).

Otolith Radius Measurement and Edge Analyses

Each sectioned sagitta was examined using Image Pro Plus 6.2 software and a stereozoom dissecting microscope equipped with a digital camera. Grey-scale images were taken, and the primordium was marked using the imaging software. The marked primordium was used as an anchor point for measuring the total radius (R_t) distance in mm. R_t is hereby defined as the distance along the counting path from the primordium to the distal-dorsal edge of the sectioned sagitta. Once established, the R_t was used as an imaginary path along which the distance between each annulus was measured. If the anchor position could not be identified or if the R_t did not overlay the counting path, then a radius was not measured for that sagitta.

Measurement of the radius for each annulus (R_n) was made to the distal edge of its translucent band. The opaque versus translucent nature of the edge of the cross-section was then noted and used as the basis for an edge analysis of the relative incidence of translucent edges present on cross-sections. A marginal increment ratio (MIR) was also measured, defined as the ratio between the width of the opaque edge and the distance separating distal edges of the nth and (n-1)th annuli. MIR was calculated for each specimen that was assigned an age estimate of 1 year or older (DeMartini et al., 2006). The MIR equation was:

$$\text{MIR} = (R_t - R_n)/(R_n - R_{n-1}),$$

where R_t = total radius of sagitta cross-section; and R_n and R_{n-1} = the distance from primordium to the distal edge of the nth and (n−1)th annuli, respectively.

Analyses of Fish Growth

Standard regression techniques were used to relate fish body length-to-weight, otolith-to-fish dimensions, and fish body length-at-age (Proc REG, Proc NLIN; SAS for Windows, vers. 9.1, SAS Inst.,Inc., Cary, NC). An unconstrained standard von Bertalanffy Growth Formula (VBGF) was fit to mean back-calculated length-at-age using Maximum Likelihood and Marquardt's Algorithm; the three-parameter VBGF model used was

$$L_t = L_\infty (1 - e^{-k(t - t_0)});$$

where L_t = mean total length (TL, in cm) at age t; L_∞ = asymptotic length; t = a specific age in yr; t_0 = hypothetical age at length zero; and k = growth coefficient.
The length-at-age of each aged individual was back-calculated using the equation

$$TL_t = (R_n / R_t)^b \cdot TL,$$

where TL_t = back-calculated total length at age t; R_n and R_t are as previously defined; and b = parameter derived from the relation of TL to R_t.
TL was related to R_t using the power equation,

$$TL = a \cdot R_t^b,$$

where a, b = fitted parameters.

RESULTS

Sampling effort included all months of the year and contributions were made by 5 commercial vessels and 1 research vessel. A grand total of 547 fish was obtained for ageing and related studies, of which approximately 95% were caught between 18–32º N lat., 153–182º W long.; only 31 specimens were caught outside the NWHI, east of 161.5º W, in the MHI (Fig. 1). Fish were obtained in all months (ranging from 16 to 74 fish per month). Median body length was about 64 cm TL (ranging from 24.1 to 110.3 cm). We evaluated all 547 fish for length-weight; the otoliths of 158 of these fish (all from the NWHI) were used for size-at-age estimation. From 10 to 27 specimens per month were used for aging; sizes of aged fish generally resembled those of the parent sample (median 63.0 cm, ranging from 24.1 to 99.0 cm TL; Figs. 3 and 4).

General Growth Characteristics

The weight-at-length relation for all hapuʻupuʻu obtained was best described by the allometric growth equation

$$FW = 0.00002 \cdot TL^{2.997},$$
$$r^2 = 0.98,$$
$$p < 0.0001,$$
$$n = 547,$$

where FW = total round weight of fish in kg and TL = total length in cm (Fig. 5).
The average 64.4 cm fish collected weighed 5.9 kg; the largest fish (110.3 cm) weighed more than 22.4 kg.

Sagittae ranged in rostrum-post rostrum length (the most precise linear metric) from about 10 to 21 mm and weighed from 0.03 to 0.7 mg. Otolith length and weight were proportional to fish length and weight, respectively. The best fit relation between otolith length and fish length was linear:

$$OL = 5.26 + 0.1563 \cdot TL,$$
$$r^2 = 0.81,$$
$$p < 0.0001,$$
$$n = 133,$$

where OL = otolith length in mm and TL = total fish length in cm.
The best fit between otolith weight and fish weight was curvilinear:

$$OW = 0.0859 \cdot FW^{0.6481},$$
$$r^2 = 0.70,$$
$$p < 0.0001,$$
$$n = 151,$$

where OW = otolith weight in mg and TL = total fish weight in kg.
Otolith cross-sections were typically less than 2 mm thick and ranged from about 0.8 to 3.7 mm along the axis from primordium to distal-dorsal edge of the section. Left and right sagittae of the same fish were indistinguishable in length (matched-pairs *t*-test: $t = 0.33$, $p = 0.74$, $n = 69$ fish). Hence, left and right sagittae could be used interchangeably when relating otolith and fish lengths.

Otolith-Fish Length Relationship

The relation between radius of the cross-sectioned otolith (R_t) and total fish length (TL) also was linear. The best fit equation was

$$R_t = 0.02636 \cdot \text{TL},$$
$$r^2 = 0.98$$
$$p < 0.0001,$$
$$n = 158,$$

where R_t = total radius in mm and TL = total length in cm (Fig. 6), and where the standard error of the coefficient was 0.00033.

The intercept term was not significant ($p = 0.90$) and was deleted from the final model fit. Prior to construction of this best-fit model, conformity to a general power curve was evaluated to test whether the relation was in fact linear. The exponent of the power curve was 1.019 (SE 0.053), which did not significantly differ from 1.0 ($p > 0.5$). The residuals of the linear fit lacked trend.

Otolith Edge Analyses

The relative frequency distribution of translucent versus opaque edges on cross-sectioned otoliths did not vary in any consistent pattern among months. However, when the edge characteristics of cross-sections were more finely examined based on MIR, there was a strong suggestion that the narrowest increments occurred in April and the widest increments occurred in June (One-way ANOVA: month effect − $F_{11,141} = 1.7$, $p = 0.08$).

Size-at-Age Relationship

For each age-group, the means of all back-calculated lengths were regressed on age using the standard three-parameter VBGF, with each age-group mean weighted by the square root of its sample size (number of fish). Length-at-age according to the best fit relation for all fish (sexes pooled) was

$$L_t = 82.3\,(1 - e^{-0.157(t+1.45)}),$$

where the standard errors of L_∞, k, and t_0 were 2.6 cm, $0.024 \cdot \text{yr}^{-1}$, and 0.62 yr, respectively.

Most growth occurred prior to age 15 yr, although fish greater than 20 yr old were not uncommon and a single fish of age 34+ yr (87 cm) was encountered (Fig. 7). The largest fish aged (99 cm TL) was an estimated 22+ yr.

DISCUSSION

Estimating Ages of Hapuʻupuʻu based on Annuli in Sagittal Otoliths

First of all, growth of the sagittal otolith of hapuʻupuʻu is proportional to fish body size, thus supporting one basic requirement when using hard parts to describe growth (Campana, 2001). Furthermore, the sagitta of hapuʻupuʻu shows clear alternation of translucent and opaque zones or bands. The formation of alternating translucent and opaque growth bands on sagittae has been demonstrated and the bands considered as annuli in age estimation for many species of groupers in the genus *Epinephelus* (e.g., Manickchand-Heileman and Phillip, 2000; Wyanski et al., 2000). Annuli were present in all cross-sectioned otoliths of hapuʻupuʻu examined in our study, and it was possible to estimate an age for each of the fish (e.g., Fig. 2). Although the relative frequency distribution of translucent edges did not vary among months, the marginal increments representing "plus" growth in hapuʻupuʻu sagittae appeared to do so, as in other *Epinephelus* spp. (Crabtree and Bullock, 1998).

Hapuʻupuʻu Growth Rate

The growth pattern of hapuʻupuʻu is very similar to those reported for other groupers of the subfamily Epinephelinae. Our estimated maximum age for hapuʻupuʻu in this study (34+ yr) is similar to those observed for other species in the genus (range 17-41 yr: Sadovy et al., 1992, Manooch and Mason, 1987; Craig, 2006; Wyanski et al., 2000; Grandcourt, 2005; Manickchand-Heileman and Phillip, 2000). Our estimated VBGF growth coefficient for hapuʻupuʻu ($k = 0.16$) is smaller than the median value of 0.18 (range 0.07 to 0.63) estimated for other *Epinephelus* spp. using similar methods (Manooch, 1987). Previous estimates of hapuʻupuʻu growth rate based on otolith daily increments derived a value almost identical to ours ($k = 0.156$ based on the Ralston and Williams [1989] method: Williams and Lowe, 1997). Although we are confident in our preliminary assessment of growth patterns and age structure, we caution that it is preliminary. Additional work is needed to address the accuracy of age estimates of older fish and the validation of annuli for this species. Our present age estimates and growth model parameters should be used with caution.

Future Research

Evaluation of additional specimens is clearly needed to better describe the potential age structure of hapuʻupuʻu for stock assessment. Our sample size of 158 is relatively small, given the magnitude of variance of the estimates. It is important to note that we in fact have nearly another 300 specimens, primarily old juveniles to old adults, available that will provide most of the additional age estimates that are needed. These additional specimens will improve the precision of our estimates of size-at-age. They will also allow exploration of possible sex differences in growth rates for this likely protogynous

(Everson, 1992) species. Our present specimen holdings are least complete for young-of-year and age 1-2 juveniles less than 35 cm TL.

Further examination of the otoliths of both very young and very old fish will also help resolve the accuracy of our age estimates. Examination of daily microincrements that are present on the otoliths of young-of-year and young juvenile specimens (Williams and Lowe, 1997) will better describe the curvature of the ascending limb of the growth curve. Radiometric analysis of the otoliths of very large, likely very old fish has the potential to calibrate the overall growth curve for realized longevity. Microincrement and radiometric analyses together could provide the validation needed for a growth curve sufficient to support age-based stock assessment.

This study has clearly increased the information available on the size and growth patterns of hapuʻupuʻu and has strongly suggested that the alternating translucent and opaque bands present on sagittal otoliths of the species are formed once yearly and can be used to estimate age and growth. However, the examination of a greater number of specimens, including complementary analyses of additional small and very large fish, is needed before an age-based stock assessment is warranted for the species.

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Table 1..--Summary of total length (TL, cm) statistics and estimated mean back-calculated lengths-at-age for 158 Hawai'ian grouper (*Epinephelus quernus*) of each age-group (sexes pooled), caught in the NWHI during 2005-2007. SD = standard deviation.

Sexes Pooled				
		TL		Back-calculated TL
Age-group	Sample size	Mean	SD	
0	3	29.8	5.0	
1	2	34.6	2.3	27.5
2	12	40.5	8.2	34.5
3	16	48.0	3.4	43.7
4	26	48.9	5.5	44.6
5	8	52.4	4.5	49.4
6	9	59.7	7.3	56.6
7	10	63.8	9.3	60.3
8	11	70.2	7.4	66.2
9	6	72.7	10.4	68.3
10	11	68.8	8.4	65.8
11	5	77.0	10.9	73.9
12	9	75.5	7.4	73.0
13	4	73.4	4.4	70.4
14	2	80.8	9.4	76.6
15	5	84.7	10.8	81.3
16	5	83.5	4.4	80.8
17				
18	2	73.2	3.7	70.0
19	3	81.1	4.3	79.4
20	1	68.2		67.3
21				
22	3	86.6	10.9	84.2
23	1	82.7		82.1
24	1	86.5		84.4
25	1	64.3		63.4
26				
27	1	88.1		85.6
28				
29				
30				
31				
32				
33				
34	1	90.1		87.0

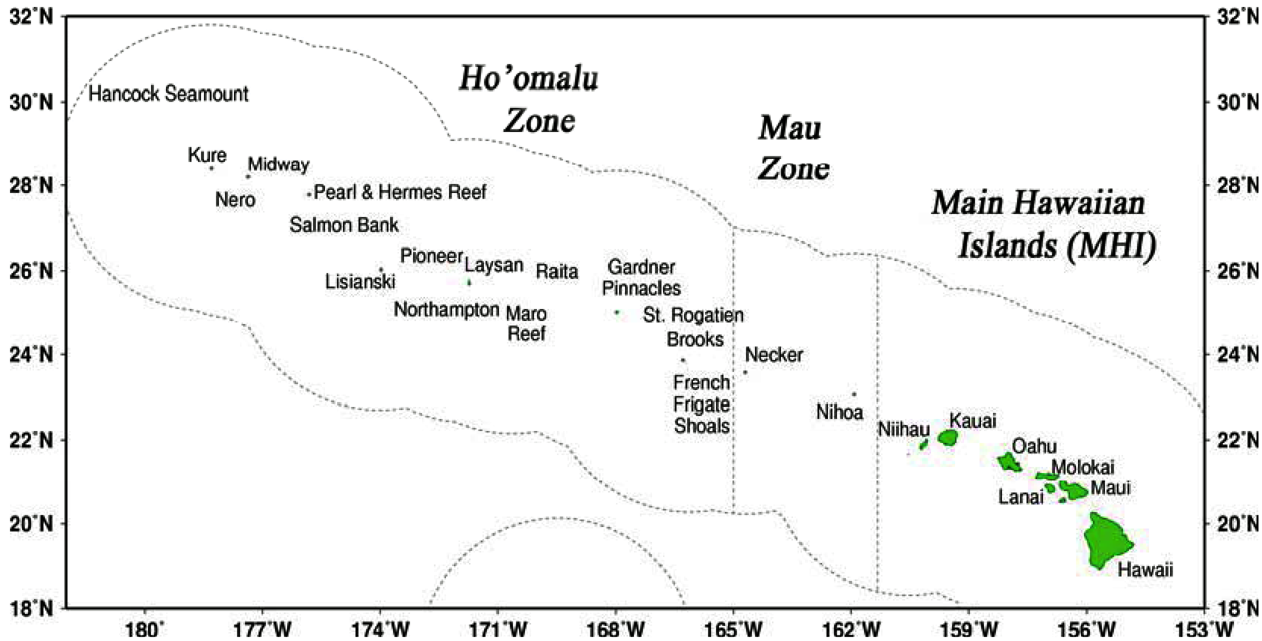

Figure 1.—Map of Hawai'ian Archipelago indicating regions of hapuʻupuʻu grouper collections. Management zones of the Hawai'ian bottomfish fishery are indicated. Only fish from the Hoʻomalu Zone were aged for this report.

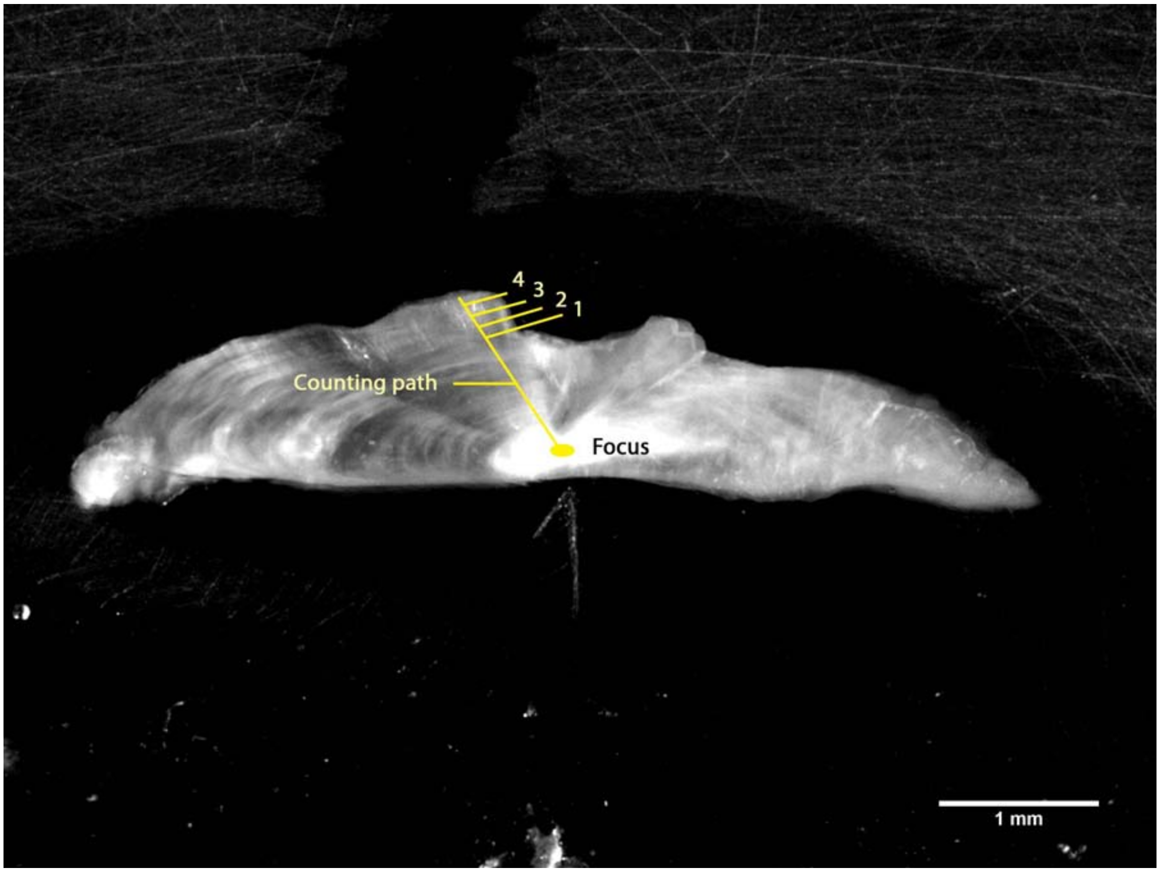

Figure 2.—Transverse section of a sagitta from a 39.1cm TL hapuʻupuʻu estimated to be in its fourth year. Labeled lines indicate completion of each of the 1st through 4th annuli. Note otolith growth distal to the 4th annulus indicating "plus" growth (age 4+).

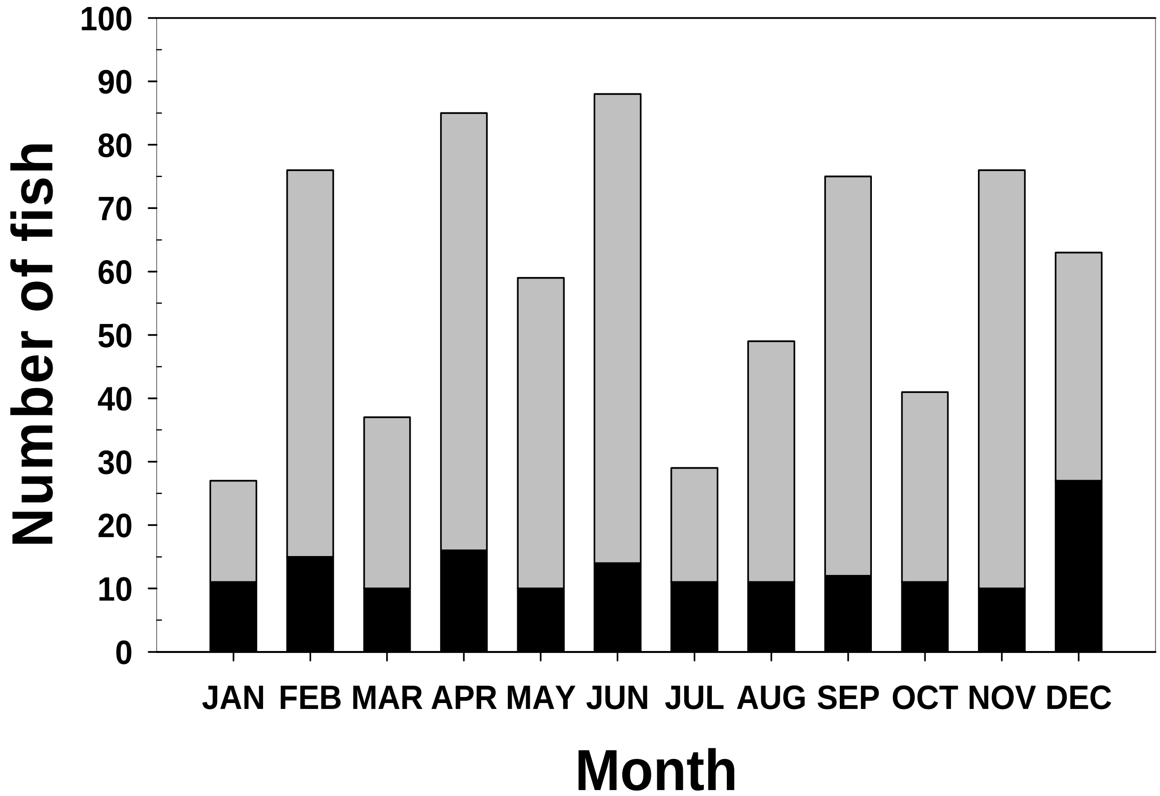

Figure 3.—Number of specimens by month of capture for hapuʻupuʻu used in study. All 547 fish collected are noted in grey; the 158 fish whose otoliths were sectioned are noted in black.

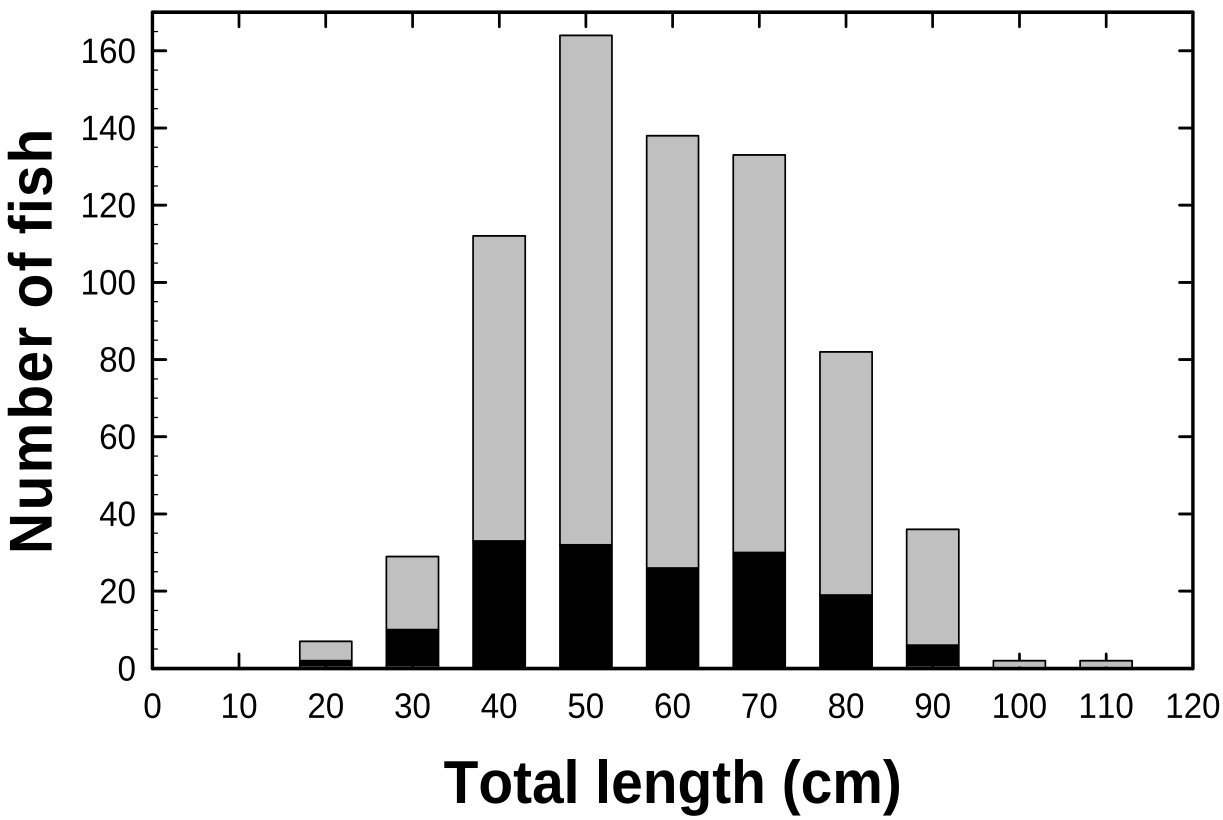

Figure 4.—Length frequency distribution of hapuʻupuʻu used in study. Total length (TL, cm) is binned by 10-cm class. As in Figure 3, collected fish are noted in grey and aged fish are noted in black.

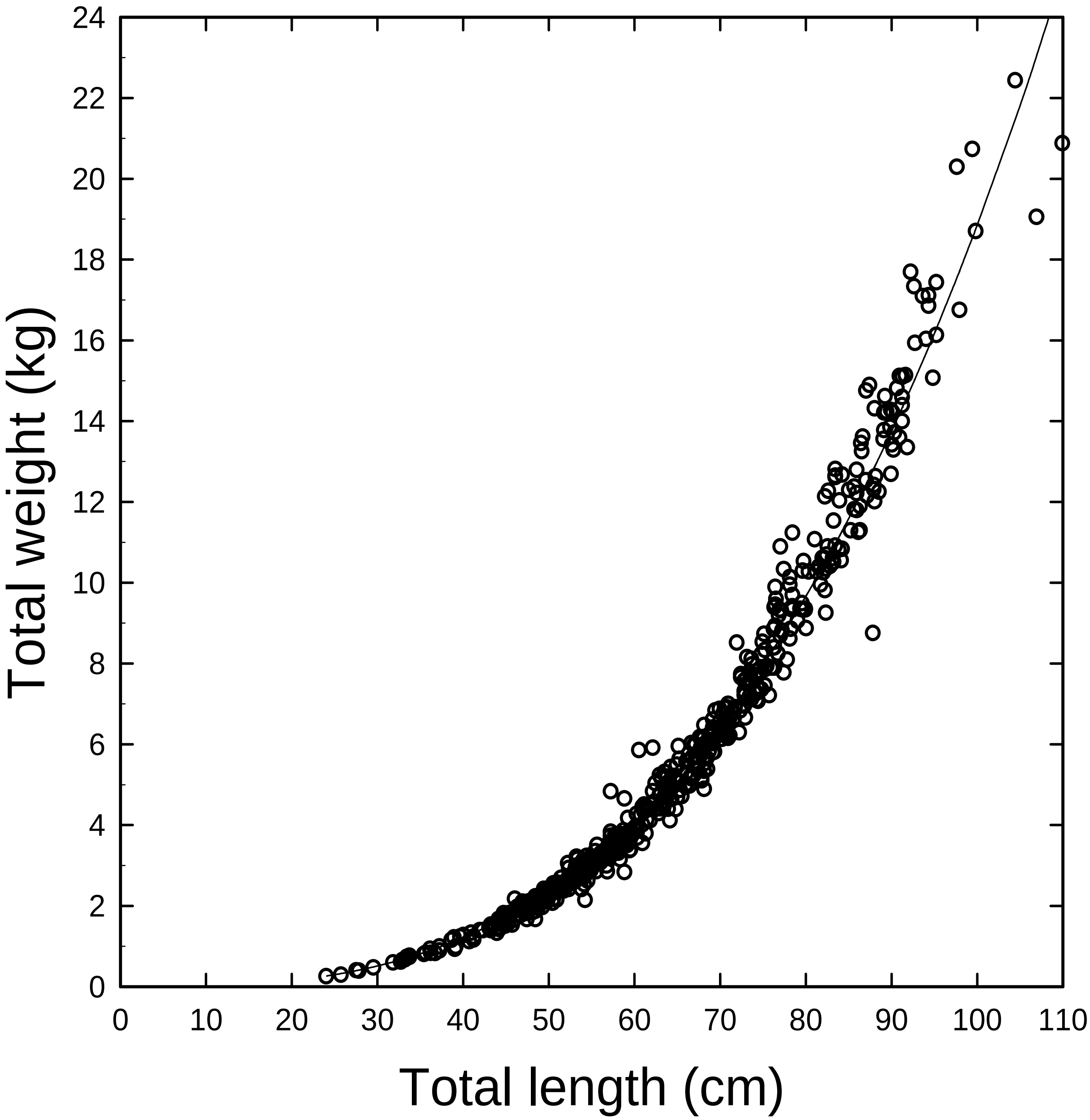

Figure 5.—Length-weight relationship for hapuʻupuʻu (n = 547 fish).

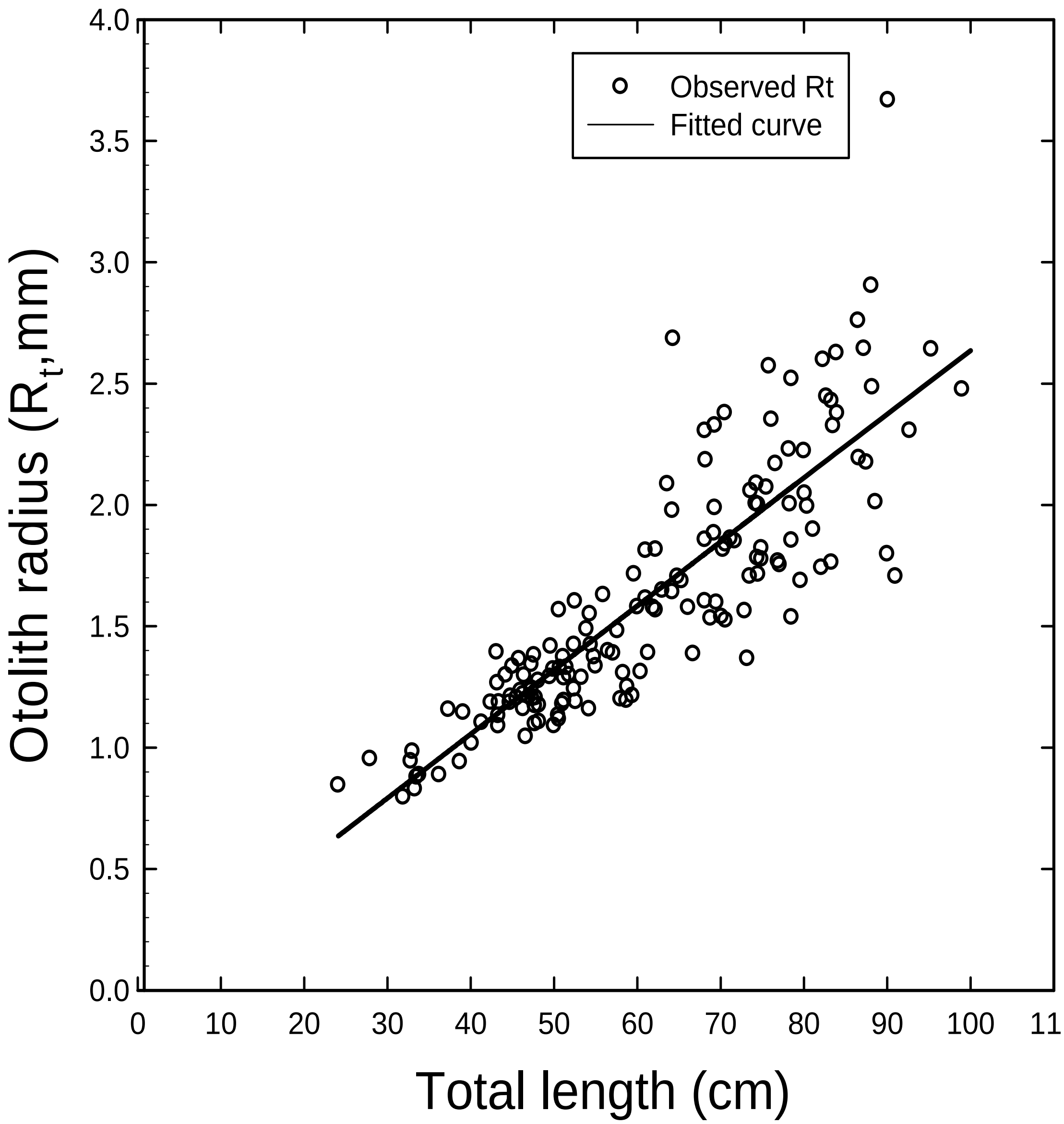

Figure 6.—Otolith radius-to-body length relationship for hapuʻupuʻu (n = 158).

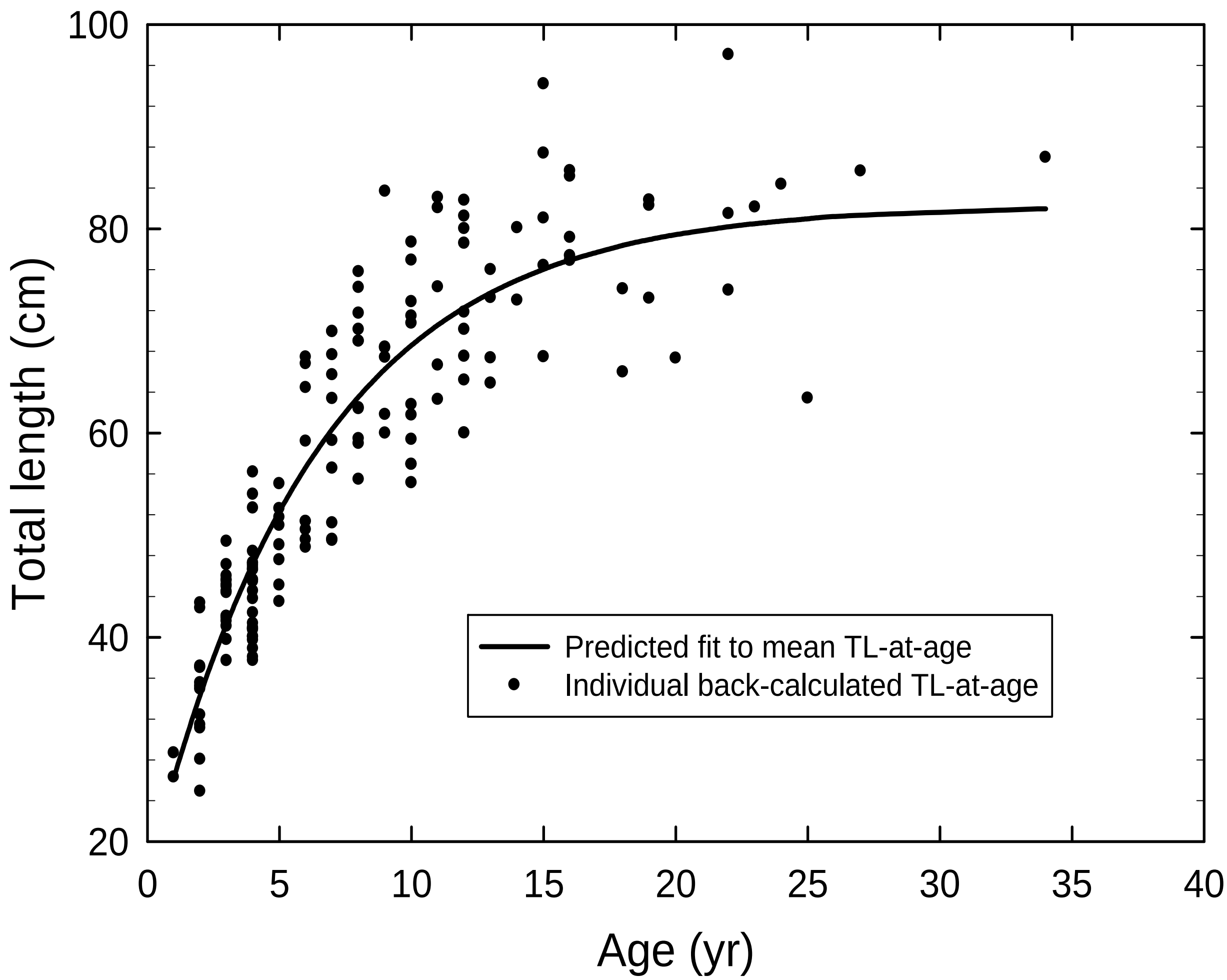

Figure 7.—Preliminary length-at-age relationship for hapuʻupuʻu based on the 158 otoliths that have been cross-sectioned and evaluated to date.