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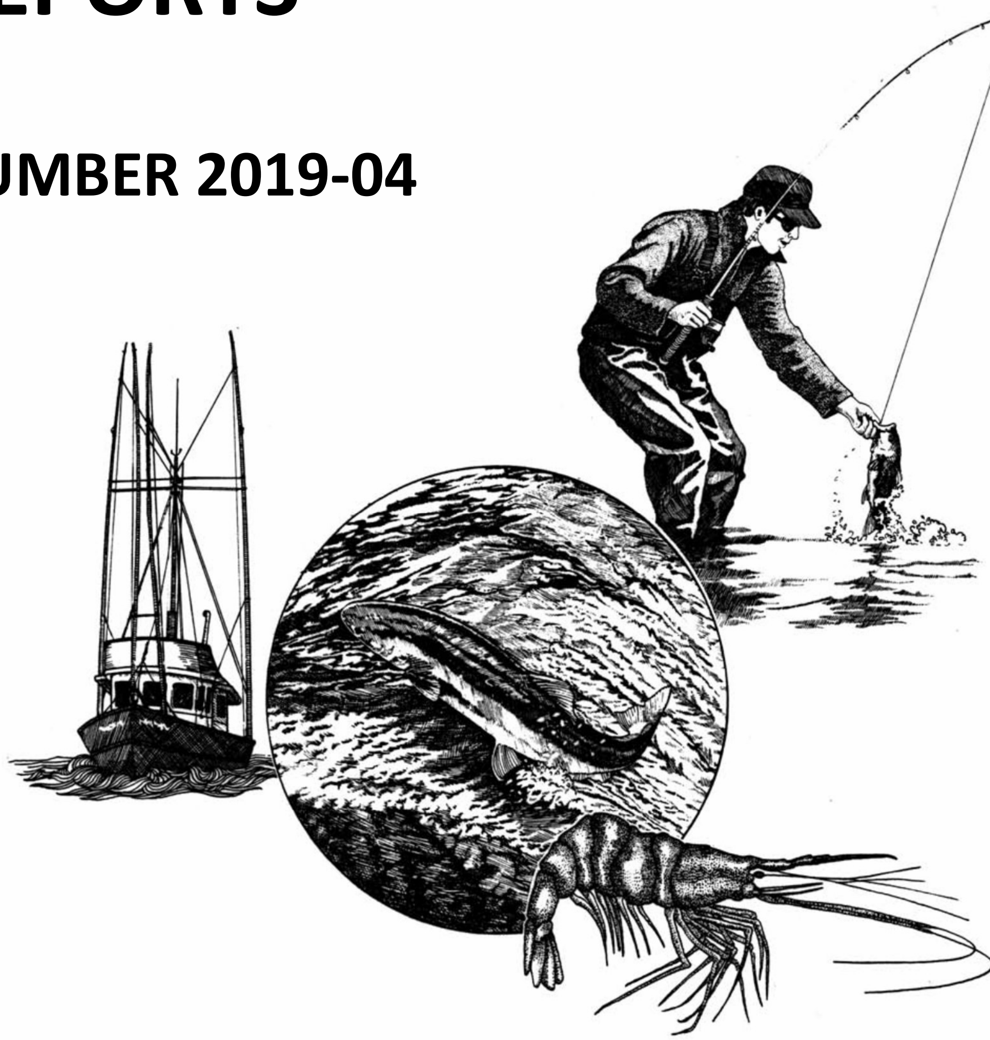

FISH DIVISION

Oregon Department of Fish and Wildlife

Age reading of Cabezon (*Scorpaenichthys marmoratus*): 1) comparison of thin-section and break-and-burn methods and 2) comparison of growth curve fits.

Age reading of Cabezon (*Scorpaenichthys marmoratus*): 1) comparison of thin-section and break-and-burn methods and 2) comparison of growth curve fits.

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Introduction

Ages are a critical component of age-structured stock assessment models (Hilborn and Walters 1992). Specifically, age information is central to the calculation of growth rate, mortality and productivity. For most species, otoliths are the primary structure used to provide ages for the stock assessment. While otoliths are relatively easy to collect and prepare for age reading, agencies often struggle to find the balance between providing enough ages for the stock assessment and ensuring the ages they provide are precise and free of bias (Campana 2001). The ease of reading otoliths (and in turn the number of ages provided to stock assessors) is dependent on how clear the annuli are on the otolith. On the West Coast of the United States, Cabezon, *Scorpaenichtys marmoratus* otoliths are some of the smallest and most difficult to interpret otoliths for the nearshore groundfish.

Historically, Cabezon ageing studies provided ages by either reading whole otoliths where ages were obtained by surface reading (O'Connell 1953, Lauth 1988) or reading otoliths that have been thin-sectioned and attached to microscope slides (Grebel 2003, Grebel and Cailliet 2010). Annuli of otoliths from larger Cabezon are difficult to interpret by surface reading, and thin-sectioning has been considered the preferred method for ageing. Thin-sectioning otoliths does have its drawbacks, however. The process of thin-sectioning and sanding otolith sections is labor intensive, resulting in fewer structures being read and fewer ages being provided to stock assessors. Additionally, improper processing of otolith sections can skew ages and can be confounded by species-specific pattern challenges.

Previous ageing work on Cabezon by the Oregon Department of Fish and Wildlife was completed using the thin-section method because of the small otolith size and a perceived increase in pattern clarity. Recently however, the ODFW marine age reading team elected to try to decrease the amount of time spent on sample preparation while maintaining pattern clarity. A few common methods of otolith preparation were tested, but ultimately the best clarity came from soaking the otoliths in a 50% ethanol solution for at least a week and applying the break-and-burn method. Thus, one of the goals of this study was to 1) determine how much bias there was between break-and-burn and thin sectioning and 2) assess bias and precision between current and previous ODFW age readers.

The growth function obtained from fitting a nonlinear model to a set of age and length data is one of the most essential pieces of information for an age-structured population model (Hilborn and Walters 1992). Recent work suggests time-varying growth- and age-based selectivity makes estimating growth rates for stocks more complex than previously thought (Francis 2016). Thus, the need to examine the effects of both geographic and fisheries based dynamics on growth rates and their associated growth functions is important. Especially given that we often define stock boundaries based on political boundaries rather than analyses of stock dynamics.

In addition to examining how methodological differences in age and growth affect bias and precision of age estimates, we also wanted to examine how these differences ultimately impact parameter estimates obtained when fitting growth functions. Further, previous growth function parameter estimates in Oregon were generated solely from the recreational fishery and with a temporally restricted dataset. Therefore, we reanalyzed the data, examining the effect of port and fishery on the larger dataset while accounting for differences between readers. Finally, in the most recent Cabezon stock assessment, Cope and Key (2009) note a significant difference between Oregon and California male growth function parameters. Therefore, we reassessed parameter estimates for male Cabezon in Oregon to determine whether increasing the size of our dataset affected the growth function parameter estimates.

In recent assessments (e.g. kelp greenling), the lack of young and small fish has been shown to have a profound impact on the ability of the model to establish the scale of the stock (Berger et al. 2015). Therefore, we also tested the effect of adding size data for young and small fish by: 1) assessing different techniques for anchoring the growth function at or near the origin and 2) testing how these different anchors affect the estimated growth function parameters.

Methods

Sample Collection

Adult fish from both the non-live fish commercial fishery and the recreational fishery were sexed and measured to the nearest cm or mm. Otoliths were then collected, cleaned of tissue, and stored dry in trays for later analysis. Most fish caught for commercial purposes are intended for the live fish market and therefore cannot have their otoliths extracted. Therefore, samples were primarily obtained from the recreational fishery. A total of 2,661 adult structures were collected (2,296 from the recreational fishery and 365 from the commercial fishery) from 1999-2018.

To anchor the growth function, lengths were included via pelagic juvenile fishes (transitioning from the pelagic larval life stage to the benthic adult life stage) collected through a collaborative project run by the ODFW Marine Reserves program and Oregon State University. This project is part of long-term monitoring to quantify abundance and diversity of newly settled fishes recruiting into nearshore habitats in two of the five of Oregon's marine reserves and their associated comparison areas (Redfish Rocks Marine Reserve, Humbug Mountain, Otter Rocks Marine Reserve, and Cape Foulweather). Newly settled juvenile fishes are collected using Standard Monitoring Units for the Recruitment of Fishes (SMURFs; Ammann 2004). The SMURF is a plastic mesh unit, which resembles settlement habitat, attached five feet below the surface from a mooring line deployed in 15-20 m of water in hard bottom habitat. For the duration of the recruitment season (April through September), four replicate SMURFs are sampled biweekly at each location. These collections have occurred from 2011-2018 and are ongoing. The standard length (in mm) is recorded for all fishes collected before they are preserved. The juvenile Cabezon sizes included in this study represent all Cabezon collected from SMURFs between 2011-2018, totaling 2152 samples. OSU graduate students are studying the daily age and growth of SMURF fishes using otolith microstructure analysis; results are forthcoming.

Otolith Preparation

Thin-Sectioning

While working under a fume hood, small plastic molds were filled half-way with the appropriate ratio of TAP Clear-Lite Casting Resin and MEKP liquid catalyst. Once the resin became slightly hard and tacky, otoliths were placed in the molds, and covered with a new mixture of resin. Molds were left to cure under the fume hood until the resin fully hardened, at least 24 hrs. To remove the cross sections from the otoliths, individual resin blocks were tightly clamped into the chuck attachment of a Buehler Isomet 1000 hi-speed saw, with the dorsal-ventral axis of the otolith lined up perpendicular to the blade. To capture as many years as possible, the saw blade was aligned to the right of the otolith nucleus and the first cut of the section made using a 0.5 mm diamond wafering saw blade (7.5" diameter). A second cut was made 0.9 mm beyond the first. The resulting thin-section was dried and glued to a frosted micro-slide using Cytoseal adhesive. Once the glue dried, the thin-section was sanded down to ~0.2 mm thickness using a Buehler sanding wheel, water, and 2000 grit sand paper. For viewing, thin sections were brushed with mineral oil to enhance growth zones and viewed with reflected light under a dissecting microscope.

It is worth noting this method differs from Grebel (2003) where a low speed saw was used. Also, at ODFW the 2008 recreational thin sections were prepared by outside company. ODFW's previous age reader noted that the end products developed by this company "did not turn out to be quite as phenomenal as hoped".

Break-and-Burn

We considered several techniques for preparing Cabezon otoliths for ageing (Table 1), but ultimately the best clarity came from soaking them in ethanol and applying the break-and-burn method. Since ODFW stores their ageing structures dry, otoliths were transferred from storage trays to 0.5 ml vials containing a 50% ethanol solution. The otoliths were then soaked in ethanol for a week and rechecked for clarity. Smaller otoliths tended to hydrate faster than larger ones so some required longer than one week. Once ready, whole otoliths were viewed under a dissecting microscope, a reference line drawn transversely through the nucleus, and the otolith cut in half with a scalpel. One-half of the otolith was then briefly held over an alcohol flame until lightly browned and the growth zones enhanced. To view the burned half, it was placed upright in a small amount of clay, brushed with mineral oil, and viewed under a microscope with reflected light (Matta and Kimura 2012).

Method Comparison

We selected 181 otoliths from ODFW's repository of Cabezon otoliths that 1) had prepared thin-sections, 2) recorded ages from ODFW's former age reader and 3) a second otolith was complete and intact for use in generating a break-and-burn age. Sixty-four female and 117 male samples were available for comparison (Figure 1).

Due to the relatively small sample size, and early evidence of low precision in age estimates, multiple methods were employed to test for bias and precision. Age bias plots were generated using the methods described by Campana et al. (1995) and further examined using a Bland-

Altman plot proposed by McBride (2015) and Giarvina (2015) but modified to generate a best fit line with a generalized additive model rather than a generalized linear model. Calculation of symmetry was analyzed using the methods of McNemar (1947), Evans-Hoenig (1998), and Bowker (1948). Precision of the different age readers and methods were compared using the formulas outlined by Beamish and Fournier (1981) and Chang (1982). Finally, the methods of Punt et al. (2008) were used to calculate ageing bias and ageing imprecision in such a way that an age-reading error matrix could be generated. In these analyses we considered the break-and-burn age to be the true age as we only had one reader for this dataset and tested for evidence of bias in the thin-section ages. Analyses were conducted using R version 3.5.1 "Feather Spray", and either the "FSA" package version 0.8.21 or the "nwfscageingerror" package version 1.0.

Growth Function Comparison

Growth functions were fit to multiple subsets of the data to examine the relative effects of different variables on the parameter estimates of the function. Only von Bertalanffy growth functions were considered in this study. Growth was modeled as:

$$L_t = L_\infty\left(1 - e^{K(t-t_0)}\right)$$

Where L_t is the total length (cm) at age t, L_∞ is the hypothetical maximum length, K is the growth rate per year, and t_0 is the age of the fish at a size of 0 cm.

We first analyzed the differences in the parameter estimate for the structures aged for methodological comparison study (Figure 1). Due to the small sample sizes (males n=117 and females n=64) each sex was fit individually using the von Bertalanffy growth function in the "FSA" package version 0.8.21 in combination with the minpack.lm package 1.2-1 interface for the nonlinear least-squares algorithm. Model fits were compared by calculating the coefficient of variation using the cvTools package version 0.3.2 and calculating the R^2 of the model fit following Ogle et al. (2017).

We then developed hierarchical models to assess the effect of reader, fishery, sex, and port location on the growth function fits. Port location is defined as north or south of Cape Blanco. Functions were fit using the nlme package version 3.1-137 by modeling the growth function as:

$$L_{t_i} = L_{\infty_{hj}}\left(1 - e^{K_{hj}(t-t_{0_{hj}})}\right) + \varepsilon_{hj_i}$$

Where ε are the within-population random errors and *h* and *j* are the indices for the first and second levels of the population. All possible model formulations were considered and compared using the Bayesian Information Criterion (BIC). Unfortunately, both readers did not read the commercial fishery data so we were required to examine the fits of two different model structures. Our first model only considered the recreational fishery data and included both sex and port as fixed effects and reader as a random effect (n=2225, Figure 1). Our second model only considered data from the reader who read both the commercial and recreational fishery samples and included both sex and port as fixed effects and fishery as a random effect (n=1707,

Figure 1). For any best-fit models which contained a random effect, the standard errors of the fixed effects were estimated using the deltamethod function in the mms package version 3.0.11.

We then tested different methods of anchoring the growth function to determine how these differences affected the overall fit of the model. Data were fit to all of the available ages regardless of reader, port, or fishery with an interaction with sex included (n=2661, Figure 2). Four model fits were generated. First, we fit the growth function to the production ages without fixing t_0 to a specific parameter or incorporating presumed age zero fish. Second, we forced the model to fit $t_0=0$. Next, we used the fish captured in the SMURFs by OSU and ODFW's Marine Reserve program to provide lengths for fish that were presumed to be ~ age 0. As these fish were not sexed, the dataset was duplicated and each copy denoted as male or female. These data were then incorporated into the production ages to assess the relative fit of the growth function. As the SMURF fish were unaged, we tested two different model fits 1) where we presumed the SMURF fish were age 0, and 2) where we presumed the fish were age 0.5.

To assess how our different growth curves affected the estimated age at maturity for Oregon we used the length at 50% maturity reported in Hannah et al (2009), 42.05 cm, and calculated the corresponding age of this fish by rearranging the growth function as

$$t = \left(\frac{\log(1 - \frac{L_t}{L_\infty})}{-K} \right) + t_0$$

We back calculated age at 50% maturity using the growth functions from Cope & Key (2009), each of the best-fit hierarchical models and the fits from each of the different anchoring methods.

Results & Discussion

Method Comparison

Cabezon sagittal otoliths are small, opaque structures measuring approximately 5 mm in length (Figure 3). The first year is not always easy to distinguish from surrounding growth checks, but it frequently occurs between approximately 1.2 and 1.5 mm. The second year is more prominent and is typically seen at about 1.9 mm (Figure 3). Splitting of the annuli during years 1-3 also occurs, making it easy to overestimate the age of young fish.

Soaking the otoliths in ethanol helps make the annuli of whole otoliths more prominent, but this method cannot be used alone since it tends to underestimate the age of large fish (Grebel 2003). Burning otolith halves over an alcohol flame is often considered one of the quickest ways to process large numbers of otoliths in a short amount of time. In this study it was shown to produce otoliths with the best clarity when compared to other methods (Table 1). An additional advantage to using a burned otolith half is that the structure is three-dimensional and it can be manipulated to follow annuli from the proximal side to the burned face, information that is not available when working with a flat object such as a thin-section. The best method of otolith preparation for production ageing of Cabezon appears to be a combination of soaking the structure in 50% ethanol then burning one half or more for ageing.

While preparation of an otolith for break-and-burn ageing is quick compared to producing a thin-section, many of the species-specific pattern challenges are still the same. As noted by Grebel (2003), the early years have several checks due to rapid growth, and the late years of older fish tend to have vague annuli. Unlike other species where counts can be compared between the ventral and dorsal axis, the annuli along the dorsal axis of Cabezon are not easily visible, especially as the fish ages and growth slows. Several examples of Cabezon otolith patterns are available in Appendix A of this document.

Improperly prepared thin-sections contribute additional challenges. Otolith sections cut too thick remove faint annuli; if cut too thin, checks are almost as prominent as annuli (an added challenge to the Cabezon's regularly spaced check marks). Sections sanded at an angle become gradually thinner, washed out, and disappear altogether.

The average coefficient of variation and percent error were both very high between readers and methods while the average percent agreement between methods was very low (Table 2). The average amount of agreement was higher when the method or reader were similar between the two datasets; however, the amount of agreement was only 31 or 34%, respectively (Table 2). Although no standard cut off values for the average coefficient of variation have been proposed, Campana (2001) suggested that values <5% were relatively precise estimates and at values >5% ages are suspect. Our average coefficient of variation for all three datasets was 17.42%, strongly suggesting our age reading estimates are imprecise.

Comparison of the age bias plots show that there is clear evidence of age bias between all of the different reader and method combinations (Figure 4 & 5, Table 3). All three tests of symmetry (McNemar, Evans-Hoenig, and Bowker) indicate that the different method/reader combinations are not symmetrical around the 1:1 axis; in other words there is strong evidence of age reading bias (Table 4). McBride (2015) notes that especially in situations where precision estimates are poor, tests of symmetry of data struggle to identify whether or not datasets are biased or not.

AIC values did not differ between the 16 models where the error and bias terms were compared between age readers and otolith preparation methods (Table 5). This indicated that there was equal support for no-bias or error in the data as there was for any of the linear or curvilinear models of error. To ensure these results were correct, the model was run using each age reader as the "correct age" and there was no effect on the AIC values. Punt et al. (2008) note that their model is sensitive to sample size, and ultimately this may be an artifact of our relatively small number of samples. Further, methods to determine and identify age reader bias have been shown to be highly influenced by the precision of the dataset, which also may be driving the inability of the model to coalesce on a best fit. Parameter estimates suggest a large disagreement in ages from the thin-section method and those from the break-and-burn method (Figure 6, Table 6).

However, there is some consistency in the relative proportion of individuals in the observed and expected populations (Figure 7).

Growth Function Comparison

For the method comparison structures, the von Bertalanffy growth parameters did not differ much for the females or males regardless of the reader or the technique (Table 7, Figure 8) except for in the case of break-and-burn males, where the algorithm had to be given many iterations to converge on a best fit. In general, the female had a better R^2 value than the males despite the much smaller sample size (n=64 female versus n=117 male). These results suggest that although otolith preparation methods result in significant variability in the generated ages, both techniques are capable of being fit to growth functions.

Our best-fit hierarchical model for the recreational data included sex as a fixed effect and reader as a random effect (Table 8 and 9, Figure 9). A model also including port as a fixed effect only differed by 7.5 BIC units suggesting there may be some effect of capture location. Further, a model with only sex (no random effect of reader) differed from the best-fit model by 599 BIC units, strongly suggesting that there is a strong effect of reader on the data.

Our best-fit hierarchical model for the fishery model only included sex as a fixed effect (Table 8 and 10, Figure 10). Unlike the recreational only model, the ΔBIC for the other models was quite high, suggesting a good fit of the sex-only model. It is worth re-iterating the fishery model only included reads from one reader, further strengthening the arguments and concerns of the variability in the ages generated by different readers.

A potential concern with these fishery analyses is that the commercial fishery data were only obtained from the non-live fish fishery. Inherent in the differences between the dead fish and live fish fishery is a selectivity for smaller “dinner plate-sized” individuals. Thus, the live-fish fishery is likely selecting not only smaller fish but also fish that grow more slowly. Therefore, during the stock assessment process when back calculating ages using the length at age key for the live fish fishery, we suggest a sensitivity analysis using the lower confidence bound of our best-fit model as the length at age-key for this fishery. Unlike the commercial fisheries, in the recreational fishery it is likely the sizes and ages are representative of Oregon’s Cabezon population. During bottomfish charters with fisheries observers, 91% of all Cabezon caught were retained. Of the 9% that were released, 86% of those were released because they were below the legal limit. In other words only 1.25% of the Cabezon caught were released due to potential high grading.

Growth function parameters for the production ages from Oregon (combining samples read using both break and burn and thin-sectioning methods and samples from both fisheries) in this study differed quite a bit from those previously published for both Oregon and California (Table 11 and 12). In the previous assessment, Cope and Key (2009) noted the disparity between the growth function parameters between Oregon and California. While the difference in t_0 values provide strong evidence of difficulties with the early ages of fish, the overall similarity of the L_∞ values does suggest that the larger maximal size in Oregon is real.

The parameter estimates generated by anchoring the growth curve by forcing $t_0=0$ or including fish from the SMURFs as age 0 or 0.5 drastically altered the parameter estimates and overall shape of the best-fit line (Table 11, Figure 11 and 12). Due to the increase in the sample size of our dataset we were unable to use our BIC approach to determine a best fit model. Therefore, we examined the residuals of the models to assess which model overall had a better fit to the data (Figure 13). Overall we see that the residuals from not including the SMURF fish and not forcing $t_0=0$ had the best overall fit. This is not surprising considering an inherent quality of the von Bertalanffy growth function is that a better fit is achieved the function is not anchored at zero. Although including the SMURF fish makes biological sense, the goodness of fit is reduced when forcing $t_0=0$.

However, when comparing the three other anchoring methods, the residuals associated with the assumption that SMURF fish are best represented as age 0 fish appears to have the best residuals. This is not surprising as the average length of the SMURF fish was 3.9 cm, and Grebel & Cailliet (2010) report fish in the 15-20 cm range as age zero. Ultimately, this suggests that in their first year of growth Cabezon could grow extremely quickly; more information about these early growth rates will be obtained via the OSU-led otolith analyses of the SMURF fish. Our back calculation of the age at 50% maturity suggests that the differences in the best-fit growth functions for the females has a large effect on the age at 50% maturity, resulting in a difference in age of ~1.5 years (Table 13).

Conclusions

In this study we find that ethanol-soaked otoliths read using the break-and-burn method provide a dramatic increase in the number of structures that can be aged each day. However, our work also demonstrates that there is a large amount of age reading bias and overall lack of precision between otolith preparation methods and readers. The large difference between the ages generated using either thin-sectioning or break-and-burn is a concern because ages from 2005-2008 were read using thin-sectioning and all other years were read using break-and-burn. This work highlights the difficulties of ageing Cabezon and strongly argues for the need to conduct age validation studies for future stock assessments (Campana 2001).

Grebel (2003) also read vertebrae of Cabezon and found that annuli were much clearer in this structure than others. This may suggest that ageing Cabezon using vertebrae and spines in future assessments may be a more accurate method. Future work should be done to compare ages obtained from otoliths, vertebrae and spines. Should other structures not be a viable ageing structure, in Oregon it is possible that small Cabezon captured in the SMURFs could be tagged with oxytetracycline. As Cabezon have a relatively small home range, during the marine reserves subsequent hook and line sampling, Cabezon could be examined for the presence of oxytetracycline. Tagged individuals could then be retained and their otoliths read to determine their age. This project assumes that Cabezon entering the SMURFs are young-of-the-year.

This work also highlights the need for aging studies that extend to juvenile Cabezon, as there is a marked paucity of data points on the growth function below 40 cm (around 2 years old). The inclusion of the SMURF fish as age=0 represents a novel data input to the Cabezon stock assessment, but these data cannot explain the patterns of growth over the entire first two years. The growth rates during these early stages are likely quite fast, variable, and particularly sensitive to environmental conditions. An increased understanding of the factors that modulate juvenile growth will improve estimates of the steepness of the growth function, with significant impacts on the stock assessment.

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Table 1. Comparison of methods used to determine ages from Cabezon otoliths. Several techniques have been tried by the ODFW to improve the readability of Cabezon otoliths, with the additional goal of keeping the preparation time of structures to a minimum.

Method	Pros	Cons
Surface of otolith viewed under tap water	No preparation	Underestimates age. Surfaces not always clear. Double banding early years difficult to interpret.
Soak in 50% ethanol (1 week)	Easier to see nucleus in many structures. Increased clarity of pattern.	Time to transfer from dry storage to vial. Labels needed for vials. Needs a week or more of soak time.
Break with scalpel and flame burn	Can tip otolith and follow annuli from burned face to proximal side. Quick to cut and burn. Good clarity.	Depending on surface topography, can be difficult to locate the nucleus to cut through. Doesn't always break through center. Doesn't always burn well. Easy to over burn.
Break with scalpel and oven bake	Can tip otolith and follow annuli from burned face to proximal side. Quick to cut and can bake many at once.	Can be difficult to find the nucleus to cut through. Doesn't always break through center. Very faint patterns- some readable, others less so.
Stain of cut otolith half (aniline blue, WS)	Can see some etching from acetic acid.	Need to cut otolith in half, label containers, stain and rinse otoliths. Aniline blue does not adhere well to otolith.
Stain of cut otolith block half (aniline blue, WS)	Can see some etching from acetic acid.	Labor intensive. Otoliths need to be set in resin blocks, labeled, cut in half with low speed saw, and then stained. Aniline blue does not adhere well to otolith.
Thin sectioning	Faint annuli visible	Labor intensive. Otoliths need to be set in resin, labeled, sectioned, glued to slides, and sanded to desired thickness. Improper cutting or sanding can impact ageing. No longer have the information a 3-D structure provides.

Table 2. Generalized precision estimates for all method and reader combinations (A) and by number of years of age discrepancy for each method reader combination (B). Values in table B are percentage of samples by discrepancy.

A.

Comparison Model	Average Coefficient of Variation	Average Percent Error	Percent Agreement
Break & Burn (LK), Thin Section (JT), Thin Section (LK)	17.42	12.87	7.24
Break & Burn (LK), Thin Section (JT)	21.11	12.22	14.93
Break & Burn (LK), Thin Section (LK)	14.82	10.48	31.11
Thin Section (JT), Thin Section (LK)	10.46	7.40	33.89

B.

Comparison	Age Discrepancy (years)									
	-6	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**
Break & Burn (LK) –Thin Section (LK)	1.1	1.1	1.7	6.2	19.6	33.5	31.3	4.5	1.1	0.0
Break & Burn (LK) –Thin Section (JT)	1.7	2.2	7.3	13.4	25.7	31.8	12.3	4.5	0.6	0.6
Thin Section (LK)–Thin Section (JT)	0.0	0.6	1.7	4.5	13.4	31.8	33.5	10.6	3.9	0.0

Table 3. Parameter estimates for each method/reader combination to examine the potential for reader's bias using the 'FSA' package. p-values <0.05 denotes a significant difference between the non-reference age from the reference age.

Break & Burn (LK) – Thin Section (LK)

Age	n	Min age	Max age	Mean age	Standard Error	t-score	p-value	Lower Confidence Interval	Upper Confidence Interval
2.00	5.00	3.00	8.00	5.20	0.86	3.72	0.08	2.81	7.59
3.00	28.00	3.00	6.00	4.57	0.20	8.04	0.00	4.17	4.97
4.00	25.00	4.00	7.00	5.16	0.18	6.46	0.00	4.79	5.53
5.00	37.00	4.00	10.00	5.84	0.18	4.77	0.00	5.48	6.19
6.00	39.00	5.00	10.00	7.00	0.18	5.56	0.00	6.64	7.36
7.00	21.00	6.00	9.00	7.38	0.16	2.36	0.09	7.04	7.72
8.00	10.00	7.00	10.00	8.30	0.33	0.90	0.79	7.54	9.06
9.00	8.00	7.00	11.00	8.75	0.49	-0.51	0.79	7.59	9.91
10.00	2.00	15.00	16.00	15.50	NA	NA	NA	NA	NA
11.00	1.00	14.00	14.00	14.00	NA	NA	NA	NA	NA
12.00	1.00	13.00	13.00	13.00	NA	NA	NA	NA	NA
14.00	2.00	14.00	16.00	15.00	NA	NA	NA	NA	NA

Break & Burn (LK) – Thin Section (JT)

Age	n	Min age	Max age	Mean age	Standard Error	t-score	p-value	Lower Confidence Interval	Upper Confidence Interval
2.00	5.00	4.00	6.00	4.60	0.40	6.50	0.01	3.49	5.71
3.00	28.00	3.00	7.00	5.07	0.18	11.20	0.00	4.69	5.45
4.00	25.00	4.00	10.00	5.68	0.31	5.43	0.00	5.04	6.32
5.00	37.00	4.00	9.00	6.32	0.18	7.28	0.00	5.96	6.69
6.00	39.00	4.00	11.00	7.69	0.27	6.32	0.00	7.15	8.23
7.00	21.00	6.00	10.00	8.05	0.26	3.99	0.00	7.50	8.60
8.00	10.00	8.00	12.00	9.70	0.45	3.79	0.01	8.69	10.71
9.00	8.00	6.00	13.00	9.88	0.77	1.14	0.29	8.06	11.69
10.00	2.00	15.00	16.00	15.50	NA	NA	NA	NA	NA
11.00	1.00	17.00	17.00	17.00	NA	NA	NA	NA	NA
12.00	1.00	16.00	16.00	16.00	NA	NA	NA	NA	NA
14.00	2.00	15.00	16.00	15.50	NA	NA	NA	NA	NA

Thin Section (LK) – Thin Section (JT)

Age	n	Min age	Max age	Mean age	Standard Error	t-score	p-value	Lower Confidence Interval	Upper Confidence Interval
3.00	7.00	4.00	5.00	4.29	0.18	6.97	0.00	3.83	4.74
4.00	13.00	3.00	6.00	4.62	0.21	2.89	0.07	4.15	5.08
5.00	40.00	4.00	8.00	5.58	0.16	3.51	0.01	5.24	5.91
6.00	41.00	4.00	11.00	6.44	0.23	1.94	0.18	5.98	6.90
7.00	36.00	5.00	11.00	7.67	0.21	3.22	0.02	7.25	8.09
8.00	23.00	6.00	12.00	8.78	0.30	2.60	0.07	8.16	9.41
9.00	6.00	8.00	12.00	9.67	0.56	1.20	0.57	8.23	11.10
10.00	6.00	8.00	11.00	10.00	0.45	0.00	1.00	8.85	11.15
11.00	1.00	13.00	13.00	13.00	NA	NA	NA	NA	NA
13.00	1.00	16.00	16.00	16.00	NA	NA	NA	NA	NA
14.00	2.00	15.00	17.00	16.00	NA	NA	NA	NA	NA
15.00	1.00	16.00	16.00	16.00	NA	NA	NA	NA	NA
16.00	2.00	15.00	16.00	15.50	NA	NA	NA	NA	NA

Table 4. Symmetry analyses for each method/reader combination using the McNemer, Evans-Hoenig or Bowker tests. p-values < 0.05 denote instances where the two datasets are not symmetrical. In other words evidence of bias in the age reading.

Break & Burn (LK) – Thin Section (LK)

Symmetry Test	Degrees of Freedom	Chi Square	p-value
McNemar	1	86.25203	1.58E-20
Evans-Hoenig	6	87.19714	1.16E-16
Bowker	30	94.04444	1.58E-08

Break & Burn (LK) – Thin Section (JT)

Symmetry Test	Degrees of Freedom	Chi Square	p-value
McNemar	1	119.5478	7.95E-28
Evans-Hoenig	6	121.1836	9.19E-24
Bowker	37	124.4667	2.15E-11

Thin Section (LK) – Thin Section (JT)

Symmetry Test	Degrees of Freedom	Chi Square	p-value
McNemar	1	37.72269	8.16E-10
Evans-Hoenig	5	40.32258	1.29E-07
Bowker	28	53.28442	0.002719

Table 5. Models tested to compare the different error and bias combinations as well as the best minus age and plus age to use. Minus age is the minimum where age-composition is estimated. Plus-age is the maximum age the age composition is estimated.

JT Thin Section		LK Thin Section		LK Break and Burn				
Error	Bias	Error	Bias	Error	Bias	Minus Age	Plus Age	AIC
Linear	Linear	Linear	Linear	Linear	Linear	4	4	1986.8
Curvilinear SD	Linear	Linear	Linear	Linear	Linear	4	4	1986.8
Curvilinear Bias	Linear	Linear	Linear	Linear	Linear	4	4	1986.8
No Error	Linear	Linear	Linear	Linear	Linear	4	4	1986.8
Linear	Linear	Curvilinear SD	Linear	Linear	Linear	4	4	1986.8
Linear	Linear	Curvilinear Bias	Linear	Linear	Linear	4	4	1986.8
Linear	Linear	No Error	Linear	Linear	Linear	4	4	1986.8
Linear	Linear	Linear	Linear	Curvilinear SD	Linear	4	4	1986.8
Linear	Linear	Linear	Linear	Curvilinear Bias	Linear	4	4	1986.8
Linear	Linear	Linear	Linear	No Error	Linear	4	4	1986.8
Linear	Curvilinear	Linear	Linear	Linear	Linear	4	4	1986.8
Linear	No Bias	Linear	Linear	Linear	Linear	4	4	1986.8
Linear	Linear	Linear	Curvilinear	Linear	Linear	4	4	1986.8
Linear	Linear	Linear	No Bias	Linear	Linear	4	4	1986.8
Linear	Linear	Linear	Linear	Linear	Curvilinear	4	4	1986.8
Linear	Linear	Linear	Linear	Linear	No Bias	4	4	1986.8
Linear	Linear	Linear	Linear	Linear	Linear	6	4	1986.8
Linear	**Linear**	**Linear**	**Linear**	**Linear**	**Linear**	**4**	**8**	**1920.2**
Linear	Linear	Linear	Linear	Linear	Linear	4	10	1928.7
Linear	Linear	Linear	Linear	Linear	Linear	4	12	1930.9
Linear	Linear	Linear	Linear	Linear	Linear	4	14	1936.0
Linear	Linear	Linear	Linear	Linear	Linear	4	16	1945.8

Table 6. Parameter estimates for each method/reader combination to examine the potential for reader's bias using the 'nwfscageingerror' package.

	JT Thin Section			LK Thin Section			LK Break and Burn		
True Age	CV	SD	Expected Age	CV	SD	Expected Age	CV	SD	Expected Age
0	0.187	0.187	1.092	0.125	0.125	1.092	0.179	0.179	0.5
1	0.187	0.187	2.138	0.125	0.125	2.138	0.179	0.179	1.5
2	0.187	0.375	3.209	0.125	0.251	3.209	0.179	0.359	2.5
3	0.187	0.562	4.304	0.125	0.376	4.304	0.179	0.538	3.5
4	0.187	0.749	5.426	0.125	0.501	5.426	0.179	0.717	4.5
5	0.187	0.936	6.574	0.125	0.627	6.574	0.179	0.897	5.5
6	0.187	1.124	7.749	0.125	0.752	7.749	0.179	1.076	6.5
7	0.187	1.311	8.951	0.125	0.878	8.951	0.179	1.255	7.5
8	0.187	1.498	10.182	0.125	1.003	10.182	0.179	1.435	8.5
9	0.187	1.685	11.442	0.125	1.128	11.442	0.179	1.614	9.5
10	0.187	1.873	12.731	0.125	1.254	12.731	0.179	1.793	10.5
11	0.187	2.060	14.050	0.125	1.379	14.050	0.179	1.972	11.5
12	0.187	2.247	15.401	0.125	1.504	15.401	0.179	2.149	12.5
13	0.187	2.434	16.783	0.125	1.630	16.783	0.179	2.324	13.5
14	0.187	2.622	18.197	0.125	1.755	18.197	0.178	2.492	14.5
15	0.187	2.809	19.645	0.125	1.881	19.645	0.176	2.645	15.5
16	0.187	2.996	21.127	0.125	2.006	21.127	0.173	2.762	16.5
17	0.187	3.184	22.643	0.125	2.131	22.643	0.164	2.789	17.5
18	0.187	3.371	24.195	0.125	2.257	24.195	0.145	2.607	18.5
19	0.187	3.558	25.784	0.125	2.382	25.784	0.101	1.924	19.5
20	0.187	3.745	27.409	0.125	2.507	27.409	0.003	0.057	20.5

Table 7. von Bertalanffy growth function parameter estimates for and goodness of fit metrics for the method comparison study.

Reader	Method	Sex	L_∞: Estimate (CI)	**K**: Estimate (CI)	t_0: Estimate (CI)	CV	R^2
JT	TS	M	59.4 (55.4 - 66.8)	0.22 (0.12 - 0.37)	-1.87 (-5.32 - 0.22)	42.92	0.58
JT	TS	F	71.1 (64.9 - 84.6)	0.16 (0.08 - 0.26)	-2.10 (-5.72 - -0.09)	46.43	0.75
LK	BB	M	205.9 (93.7 - 450.1)	0.01 (0.00 - 0.03)	-26.07 (-39.32 - -15.51)	44.4	0.25
LK	BB	F	71.2 (64.58 - 83.88)	0.19 (0.10 - 0.31)	-2.34 (-5.38 - -0.66)	48.29	0.73
LK	TS	M	60.4 (54.6 - 82.0)	0.19 (0.05 - 0.42)	-3.04 (-10.5 - 0.36)	43.4	0.39
LK	TS	F	73.8 (64.9 - 105.3)	0.14 (0.05 - 0.28)	-3.09 (-8.79 - -0.54)	47.35	0.64

Table 8. Hierarchical model selection for the recreational model and the fishery model. Port denotes if the fish was landed north or south of Cape Blanco. RE denotes the variable was modeled as a random effect. BIC is the Bayesian information criterion.

Recreational Model		Fishery Model	
Model Structure	Δ BIC	Model Structure	Δ BIC
No Interaction	921	No Interaction	340
Reader(RE)	469	Fishery(RE)	348
Sex	599	Sex	0
Sex+Reader(RE)	0	Sex+Fishery(RE)	22
Port	913	Port	354
Port+Reader(RE)	447	Port+Fishery(RE)	345
Sex*Port	602	Sex*Port	36
Sex*Port+Reader(RE)	7.5	Sex*Port+Fishery(RE)	31

Table 9. Model parameter estimates for the recreational fishery model. Standard error estimates for the fixed effects were estimated using the deltamethod to account for the use of a random effect.

Random Effects

Variable	Intercepts JT	Intercepts LK	Std. Dev
L_∞	-0.37	0.37	3.70 e-01
K	-2.61 e-09	2.61 e09	3.07 e-06
t_0	0.90	-0.90	9.08 e-01
Residuals	NA	NA	3.90

Fixed Effects

Variable (Sex)	Estimate (95% CI)	Std. Error
L_∞ (Female)	69.57 (66.82 - 72.31)	1.40
L_∞ (Male)	58.00 (52.28 - 63.72)	0.70
K (Female)	0.20 (0.16 - 0.23)	0.02
K (Male)	0.29 (0.19 - 0.39)	0.02
t_0 (Female)	-2.01 (-3.44 - -0.57)	0.73
t_0 (Male)	-1.45 (-3.84 - 0.93)	0.72

Table 10. von Bertalanffy growth function parameter estimates for the fishery comparison study.

Variable (Sex)	Estimate (95% CI)	Std. Error
L_∞ (Female)	71.34 (67.95 - 76.53)	1.92
L_∞ (Male)	57.85 (56.54 - 59.52)	0.68
K (Female)	0.19 (0.14 - 0.23)	0.02
K (Male)	0.32 (0.25 - 0.38)	0.03
t_0 (Female)	-3.14 (-4.29 - -2.29)	0.46
t_0 (Male)	-2.12 (-3.02 - -1.43)	0.36

Table 11. Parameter estimates of the von Bertalanffy growth function testing the effect of not anchoring the growth curve, forcing the growth curve through T_0=0, and anchoring using the SMURF fishes assuming an age of 0.5 or 0 years.

	No SMURF		**No SMURF, t0=0**		**SMURF Age=0.5**		**SMURF Age=0**	
Variable (Sex)	Estimate (95% CI)	Std. Error	Estimate (95% CI)	Std. Error	Estimate (95% CI)	Std. Error	Estimate (95% CI)	Std. Error
L_∞ (Female)	73.14 (68.83 - 80.26)	2.69	58.87 (58.27 - 59.48)	0.38	57.97 (57.61 - 58.33)	0.19	59.93 (58.85 - 59.64)	0.22
L_∞ (Male)	60.48 (57.96 - 64.63)	1.47	52.80 (52.37 - 53.23)	0.27	52.23 (51.98 - 52.59)	0.14	52.98 (52.71 - 53.26)	0.13
K (Female)	0.12 (0.09 - 0.16)	0.02	0.55 (0.52 - 0.57)	0.01	0.67 (0.65 - 0.69)	0.01	0.51 (0.50 - 0.53)	0.01
K (Male)	0.16 (0.11 - 0.21)	0.02	0.69 (0.66 - 0.72)	0.01	0.86 (0.83 - 0.89)	0.01	0.65 (0.63 - 0.67)	0.01
t_0 (Female)	-5.8 (-7.66 - -4.43)	0.79	NA	NA	0.40 (0.39 - 0.40)	0	-0.13 (-0.14 - -0.13)	0
t_0 (Male)	-5.90 (-8.27 - -4.24)	0.91	NA	NA	0.41 (0.40 - 0.41)	0	-0.12 (-0.12 - -0.11)	0
	CV: 4.58		**CV: 4.95**		**CV: 4.58**		**CV: 4.57**	

Table 12. Growth function parameter estimates for other studies of Cabezon growth.

Study	Location	Sex	L_∞	K	t_0
O'Connel 1953*	California	Male	53.80	0.46	-0.23
		Female	67.83	0.23	-1.40
Lauth 1987	Puget Sound	Male	69.03	0.24	-1.23
		Female	47.09	0.35	0.64
Grebel & Cailliet 2010	California	Male	44.07	0.35	-1.49
		Female	64.72	0.17	-1.74
Cope & Key 2009	California	Male	41.78	0.50	-0.72
		Female	58.97	0.21	-1.28
	Oregon	Male	59.05	0.25	-1.22
		Female	68.83	0.20	-1.19

* O'Connel 1953 estimates obtained from Grebel & Cailliet 2010

Table 13. Back calculated age at 50% maturity for Oregon based on different von Bertalanffy growth functions assuming a length at 50% maturity of 42.05 cm (Hannah et al. 2009).

Study or Model	Model Parameters	A_{50} (yrs)
Cope & Key 2009	L_∞= 68.83, K=0.2, t_0=-1.19	3.87
Recreational Fishery Model	L_∞= 69.57, K=0.2, t_0=-2.01	2.63
Fishery Comparison Model	L_∞= 71.34, K=0.19, t_0=-3.14	1.55
No SMURF Model	L_∞= 73.14, K=0.12, t_0=-5.8	1.33
No SMURF, t_0=0	L_∞= 58.87, K=0.55, t_0=0	2.28
SMURF Age=0.5	L_∞= 57.97, K=0.67, t_0=0.4	2.32
SMURF Age=0	L_∞= 59.93, K=0.51, t_0=-0.13	2.24

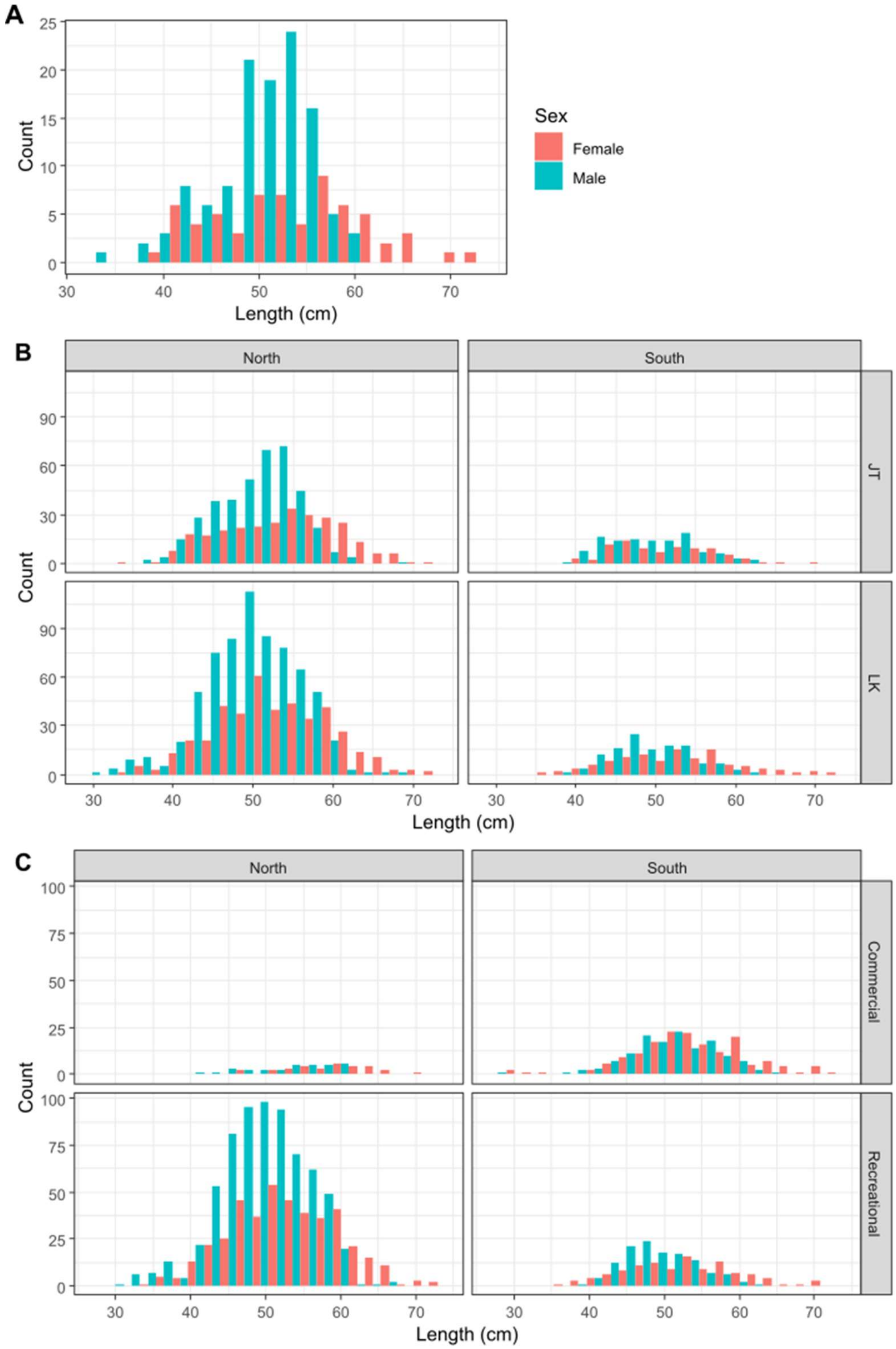


Figure 1. Size distribution of male and female Cabezon samples used to compare thin sectioning and break-and-burn methods (A) for the recreational fishery by port and reader (B) and for the fishery by region and fishery (C). Port is defined as samples landed north or south of Cape Blanco. Fishery (C) only include reads by one reader using the break-and-burn method.

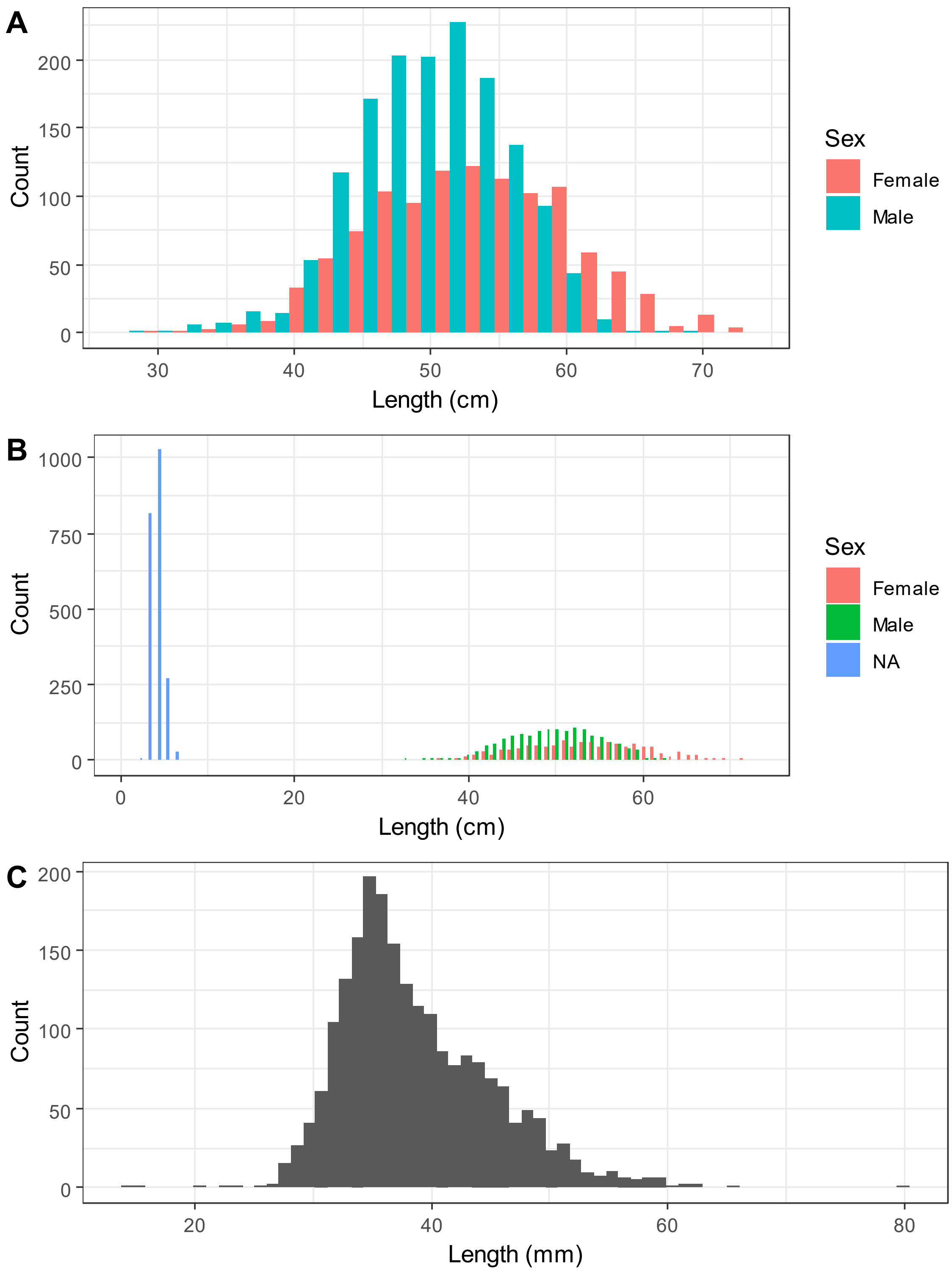


Figure 2. Distribution of size of fish used to test the effectiveness of anchoring the growth curve using the SMURF data. A) distribution of raw production ages, b) raw production ages plus the SMURF fish, c) distribution of SMURF fish. Due to their small size, the sex of the small SMURF fish was not determined.

A)

B)

B)

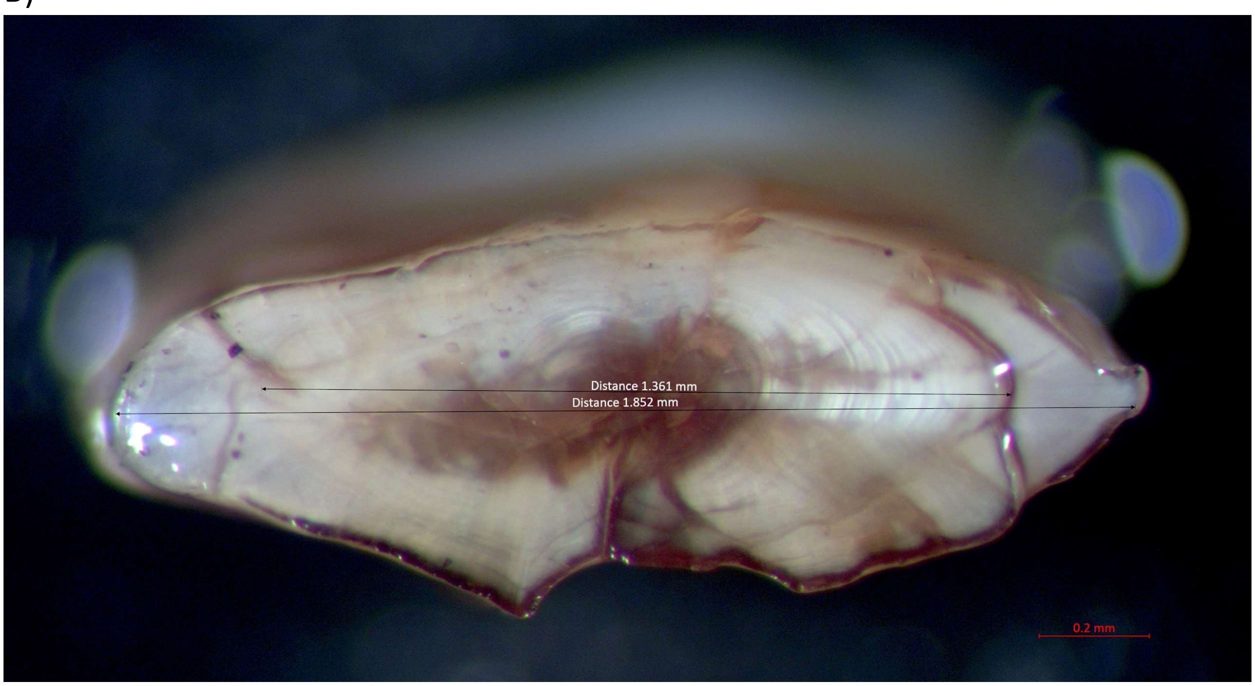


Figure 3. Pair of Cabezon sagittal otoliths measuring approximately 4mm (A) and Cabezon otolith aged as 2 year old (B). 340 mm male collected 10 July 2018. Newport, OR. First year placed at 1.36 mm and second year at 1.85 mm. Sagittal otoliths are the largest of the three pairs in Cabezon.

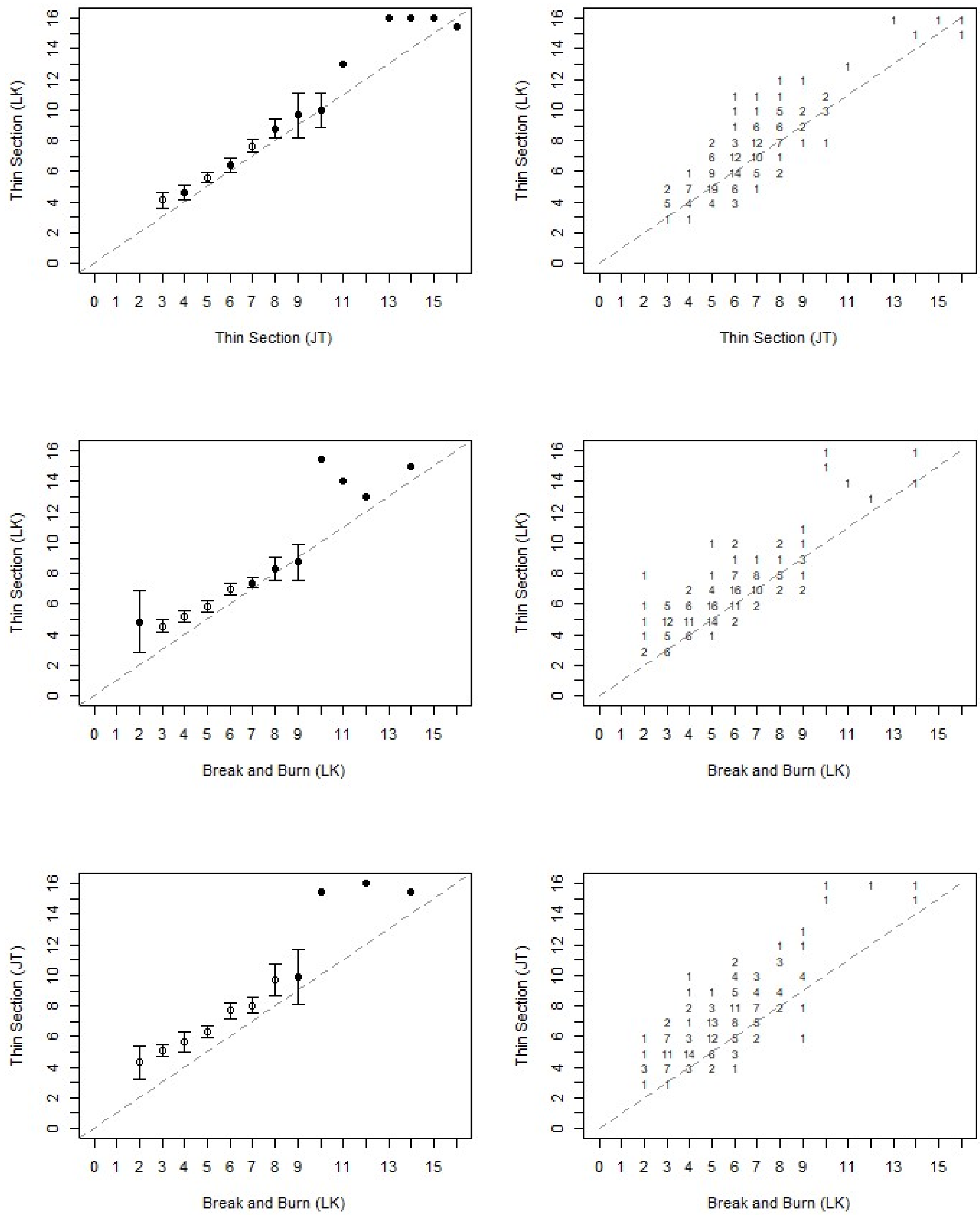


Figure 4. Age bias plots for each of the method reader comparisons and their associated age frequency tables. Circles denote the mean age and the lines are the 95% confidence interval. Open circles denote situations where the non-reference age is significantly different from the reference age and closed circles situations where the non-reference age is not significantly different from the reference age. Numbers in the age frequency table denote the number of samples with the corresponding ages. Dashed lines denote the 1 to 1 line.

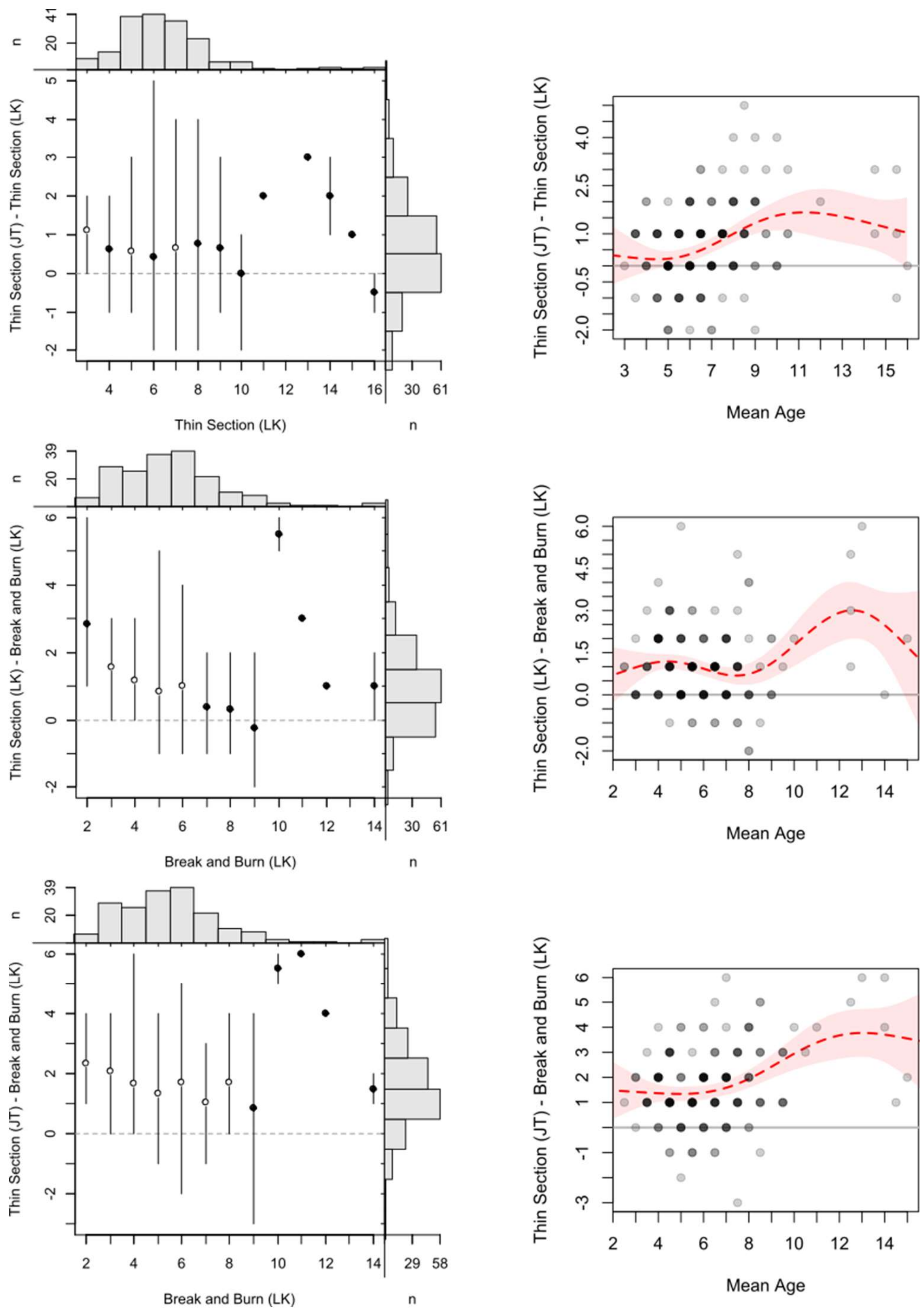


Figure 5. Differences in age estimates between readers and methods (left side) and modified Bland-Altman plot fit with generalized additive models depicting average discrepancy for each age (right side). On the right side: Circles denote the mean age and the lines are the 95% confidence interval. Open circles denote situations where the nonreference age is significantly different from the reference age and closed circles situations where the nonreference age is not significantly different from the reference age. Histograms on the side are the distribution of the reference and nonreference ages. On the left side darkness of the dots denotes how many samples are represented by the dot. The red line is the best fit smoother line fit using the mgcv package.

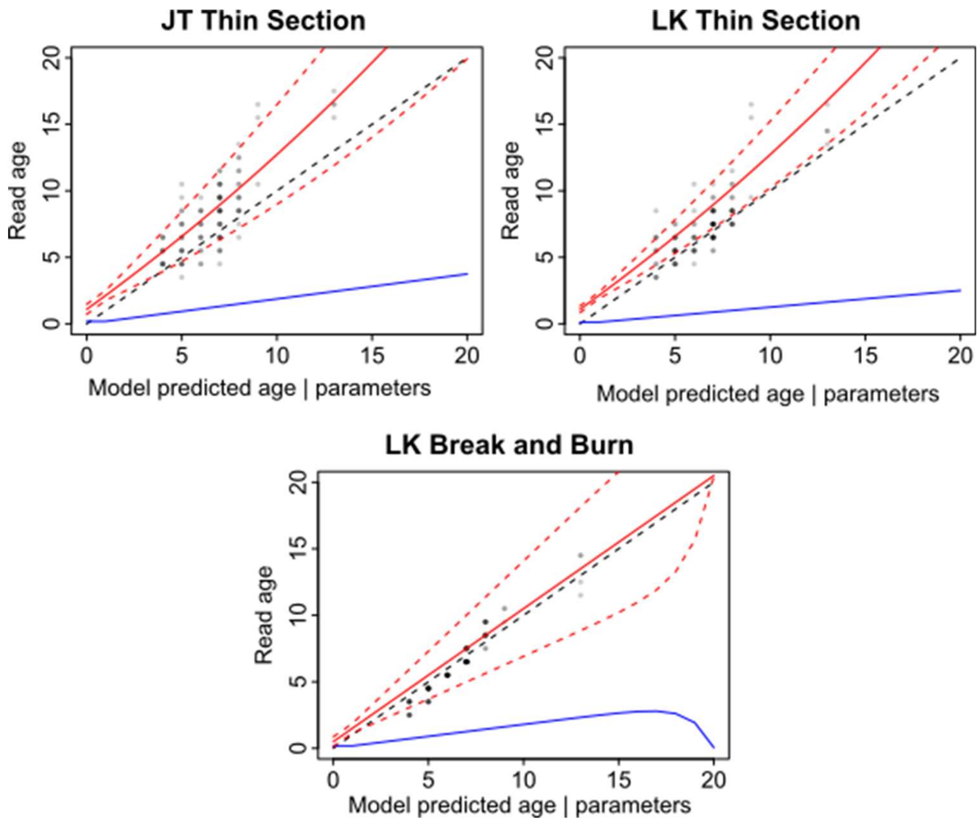


Figure 6. True vs observed reading data. Blue line denotes the standard deviation of the read, the red solid line the expected read given the data and the red dotted lines the 95% confidence interval. Black dots denote the data used in the model and the darker the dot denotes more data.

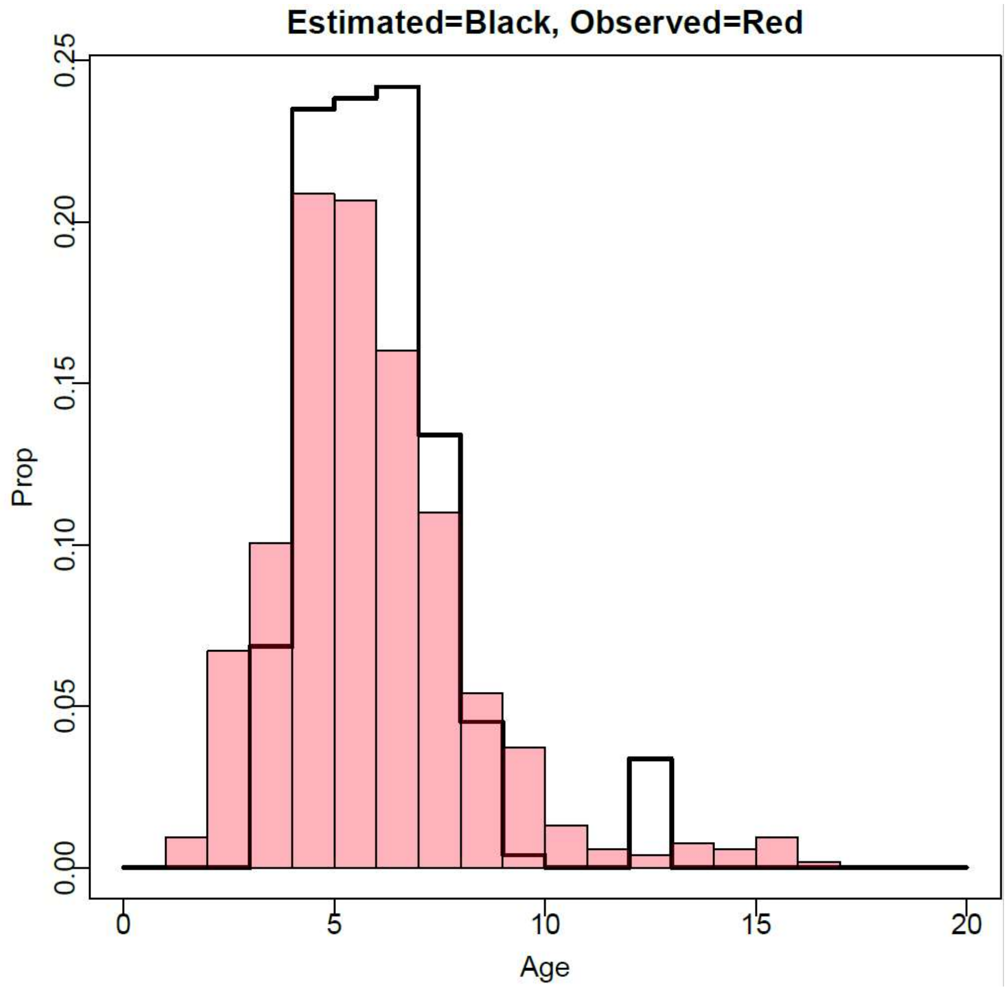


Figure 7. Estimated vs observed distribution of proportions of individuals for each age.

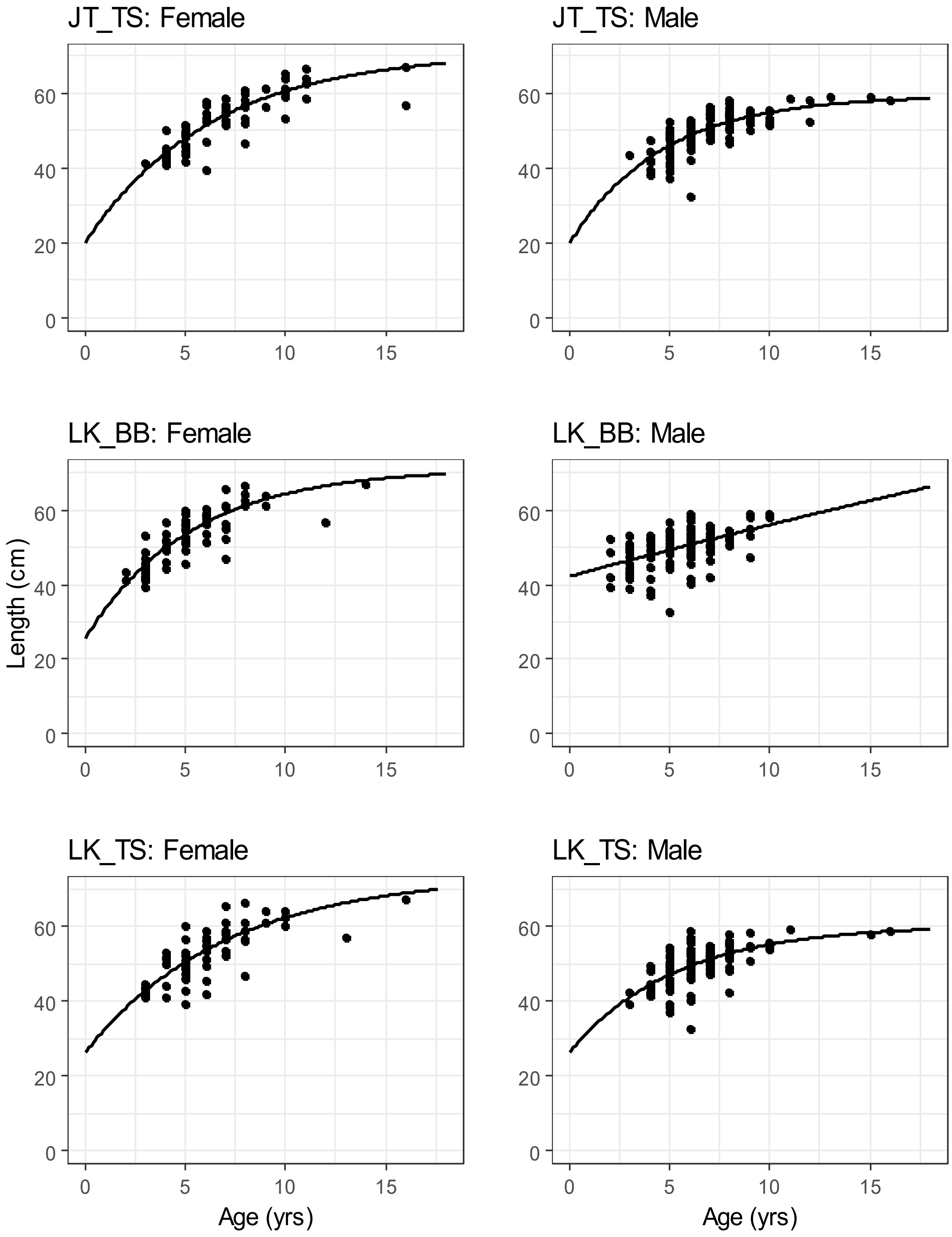


Figure 8. Age and growth fits for the von Bertalanffy growth function for the Oregon methodological comparison data sets (Table 7). TS: Thin-section, BB: Break-and-Burn. LK & JT denote the different age readers. To achieve a best-fit for the break-and-burn males, the model had to be given 10,000 iterations.

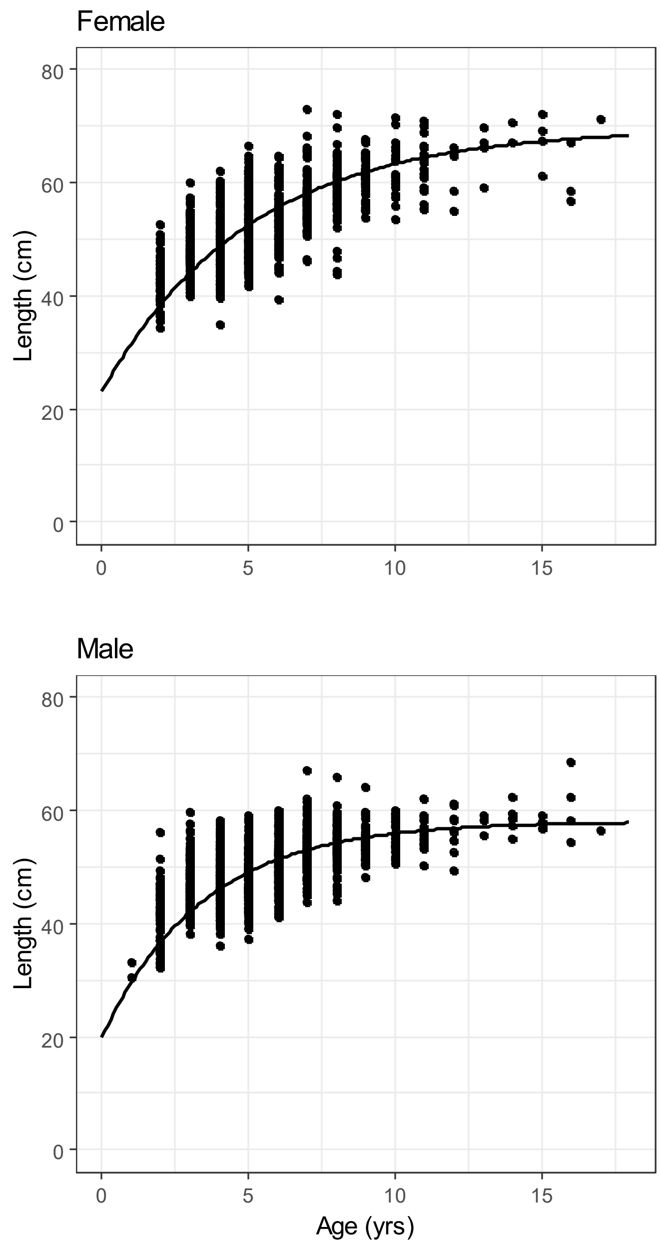


Figure 9. Age and growth fits for the von Bertalanffy growth function for the comparison of recreational data. The best fit model included a random effect of reader (Table 9)

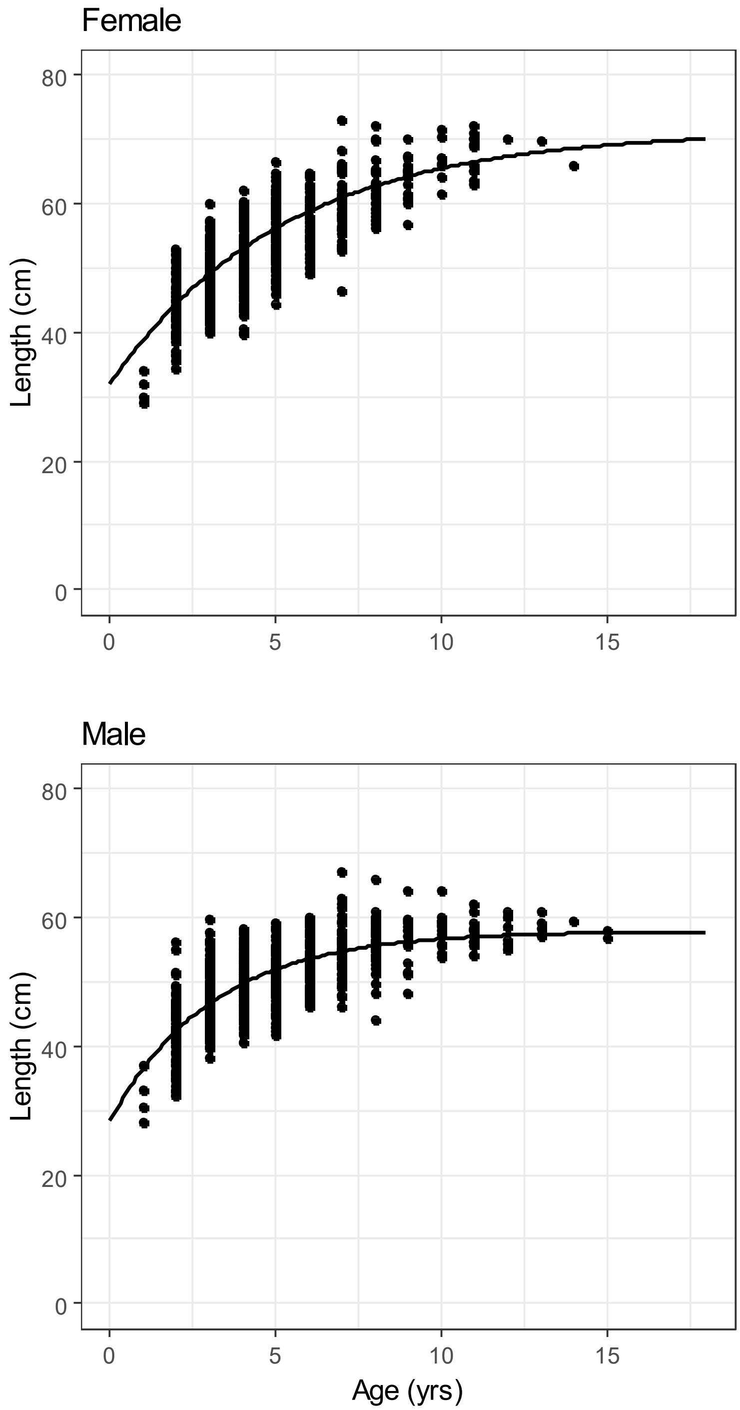


Figure 10. Age and growth fits for the von Bertalanffy growth function for the comparison of fishery data (Table 10).

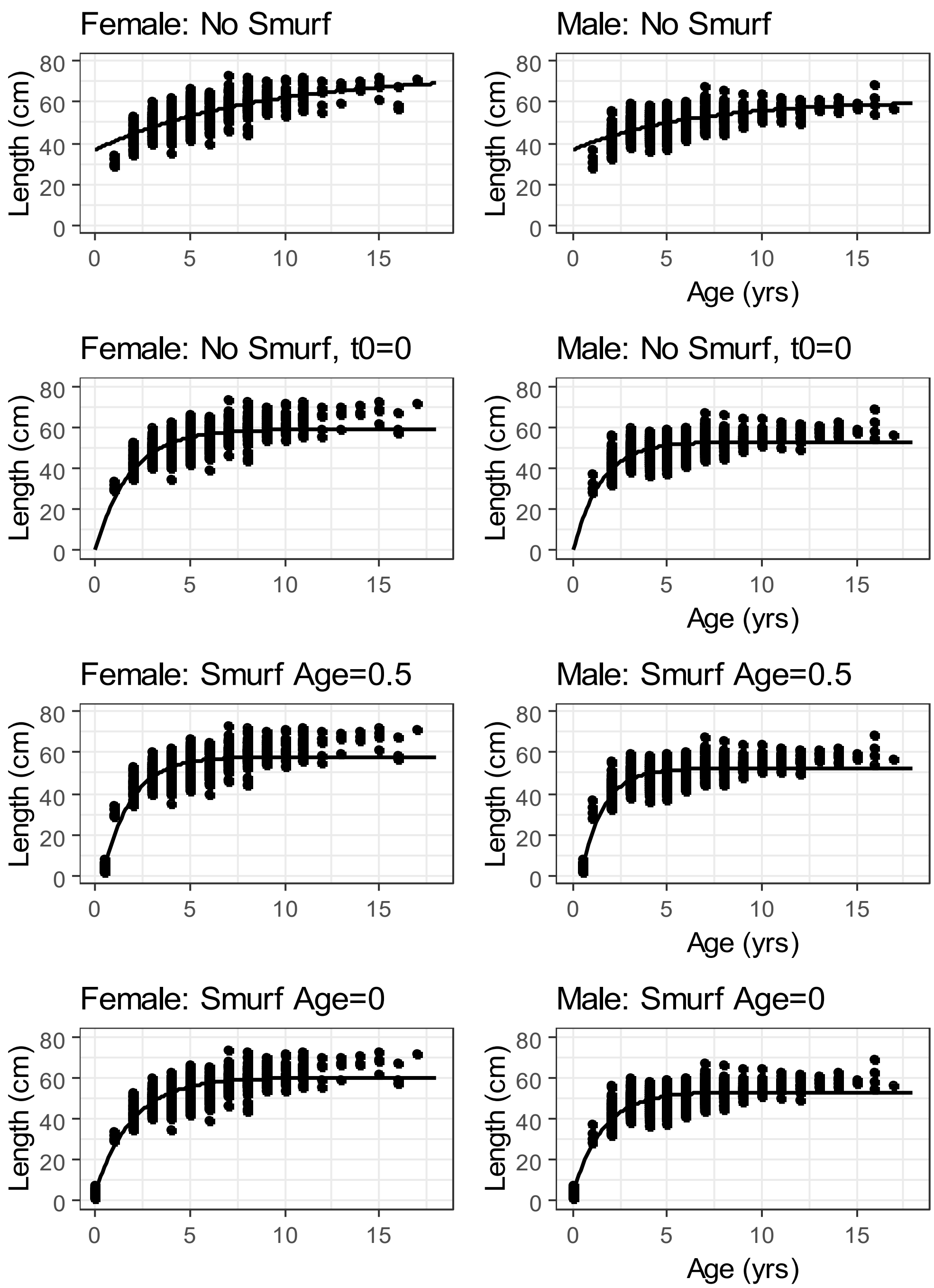


Figure 11. Age and growth fits for the von Bertalanffy growth comparing the anchoring of the growth curve by forcing T_0=0 and incorporating fish caught in the SMURF assuming they are either age 0.5 or 0 yrs (Table 11).

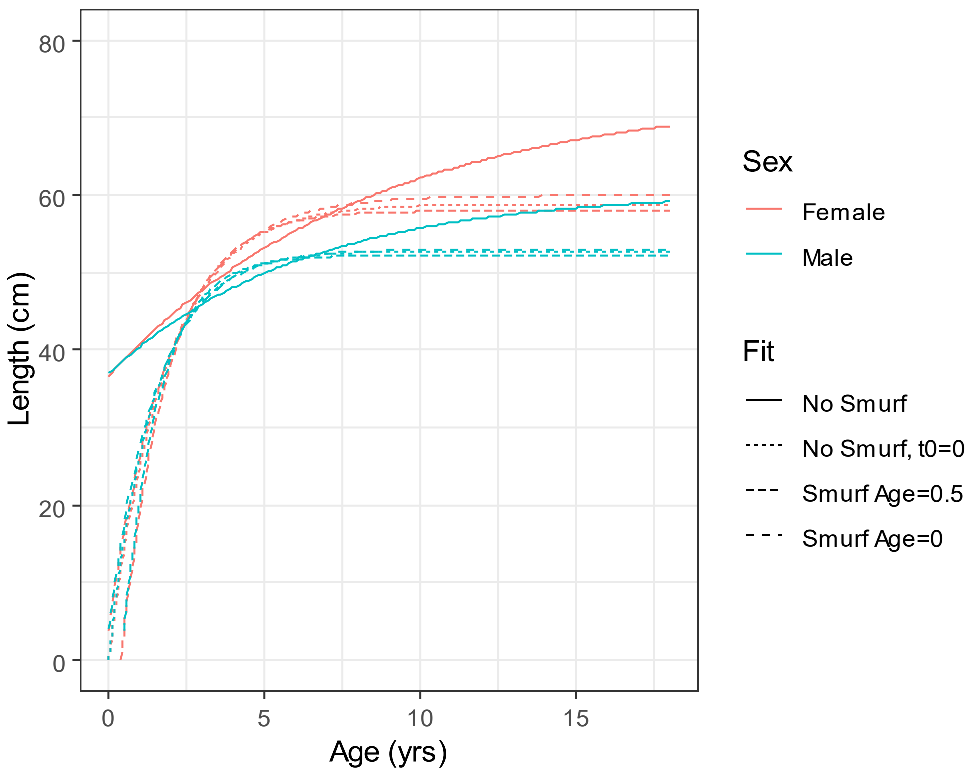


Figure 12. Age and growth fits for the von Bertalanffy growth comparing the anchoring of the growth curve by forcing T_0=0 and incorporating fish caught in the SMURFs assuming they are either age 0.5 or 0 yrs overlain on top of each other (Table 11).

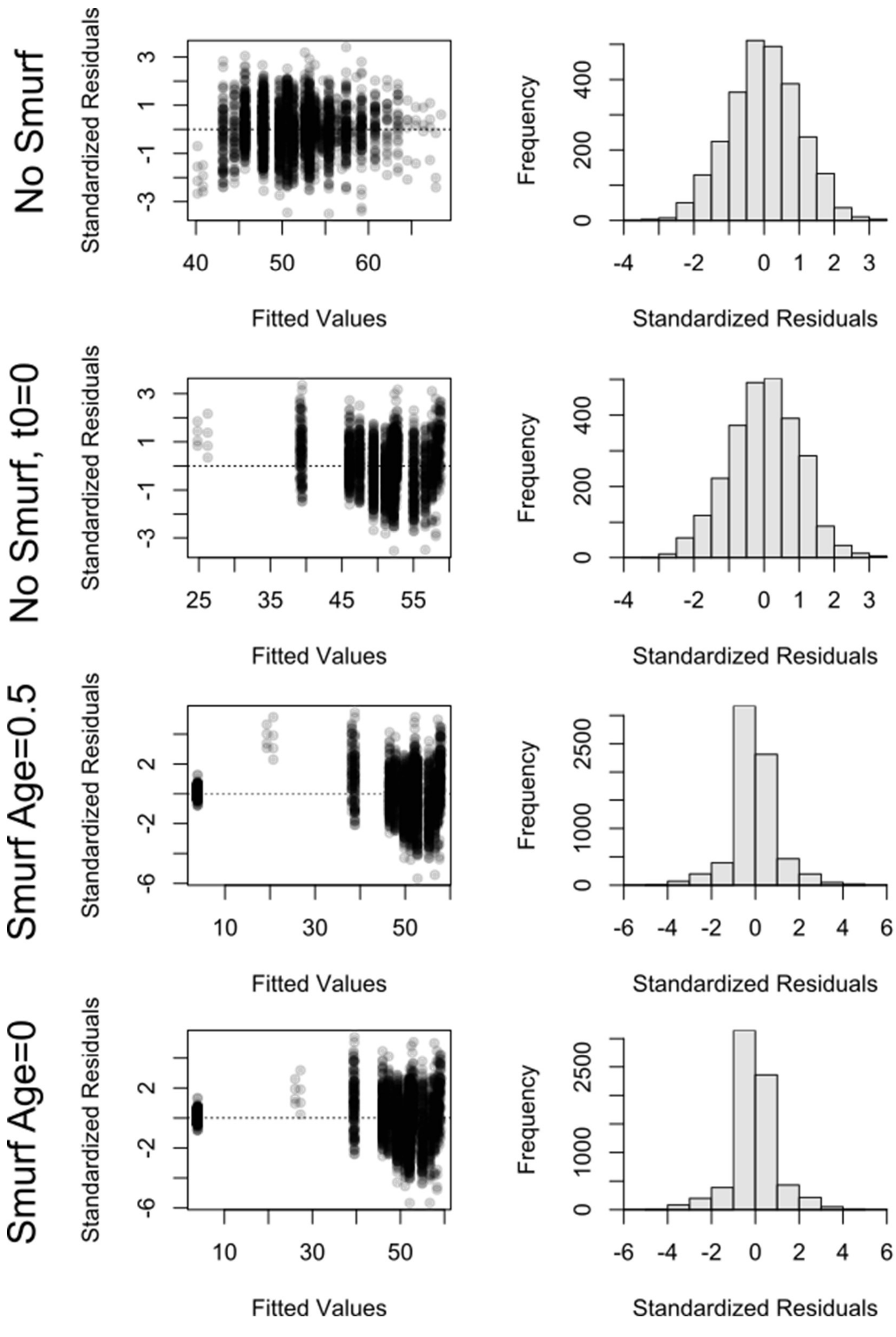
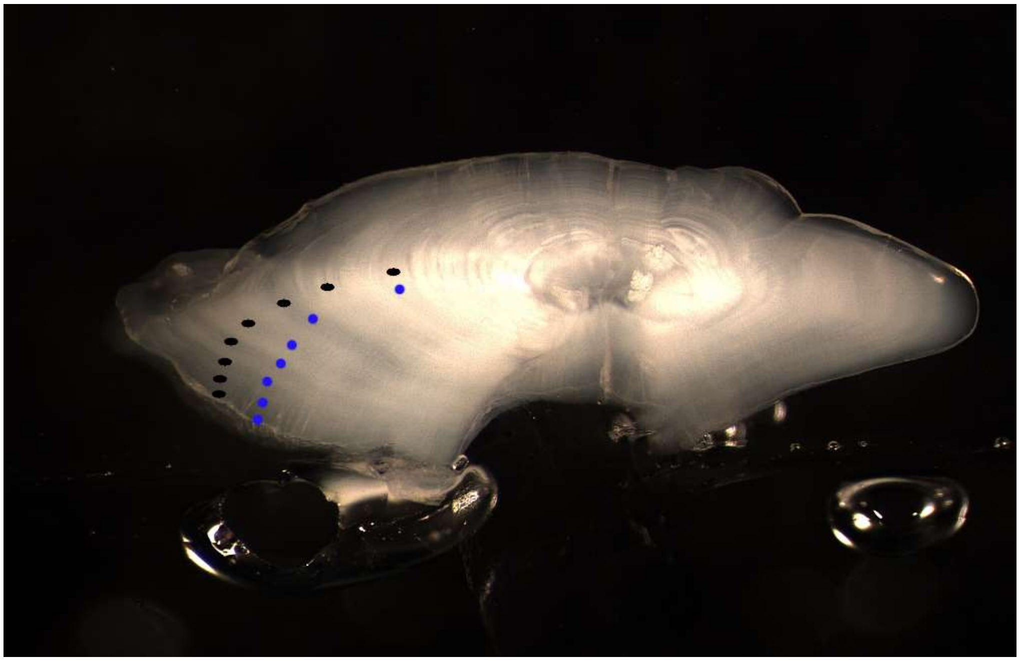


Figure 13. Residuals from fitting the von Bertalanffy growth functions comparing the anchoring of the growth curve by forcing T_0=0 and incorporating fish caught in the SMURFs assuming they are either age 0.5 or 0 yrs overlain on top of each other (Table 11).

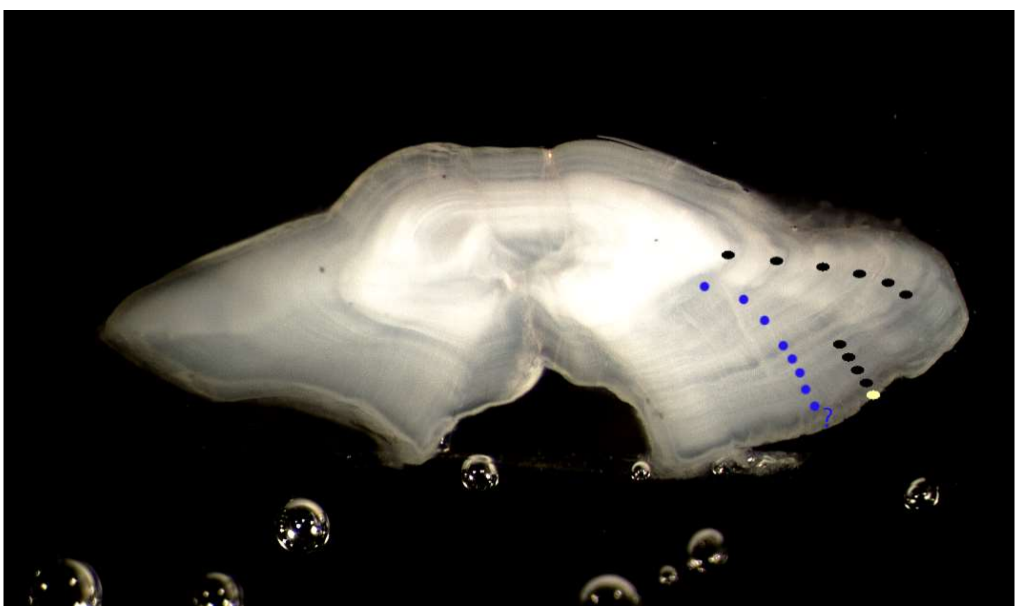
Appendix A. Example images of Cabezon otoliths

Thin Sections

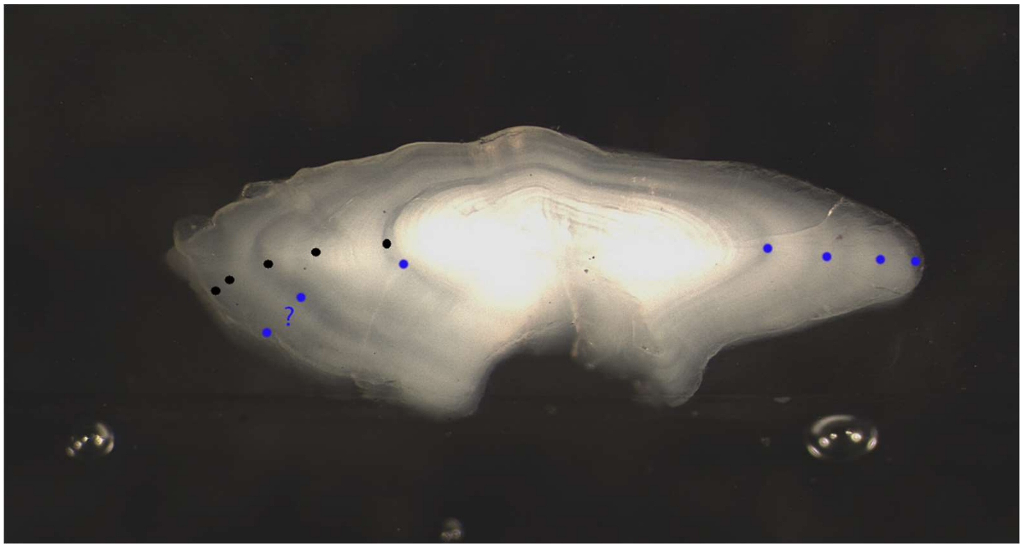
JT ages in black. LK ages in blue



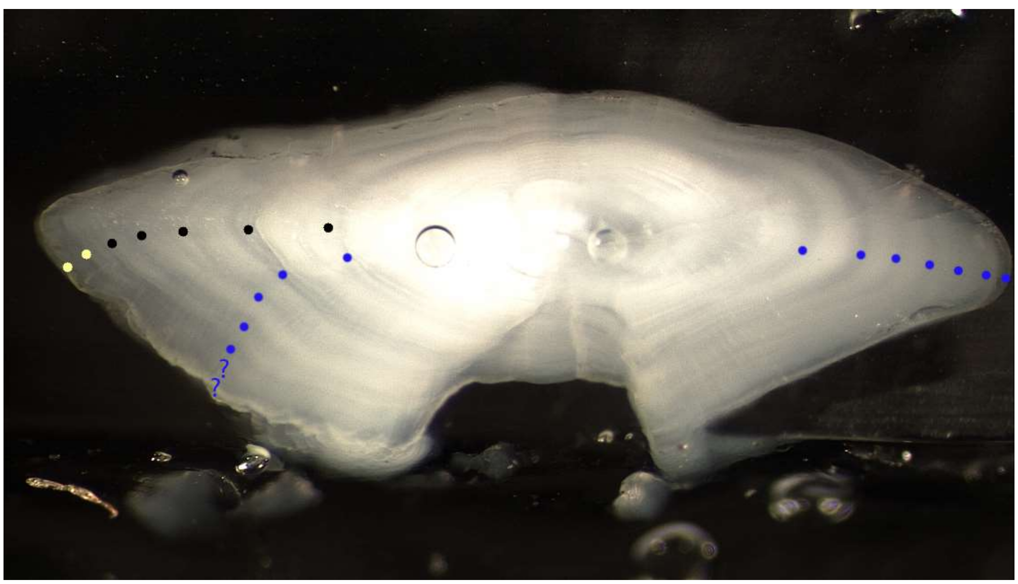
JT=8 yr, LK=7 yr. Is there something between the first two blue dots or is it doubling?



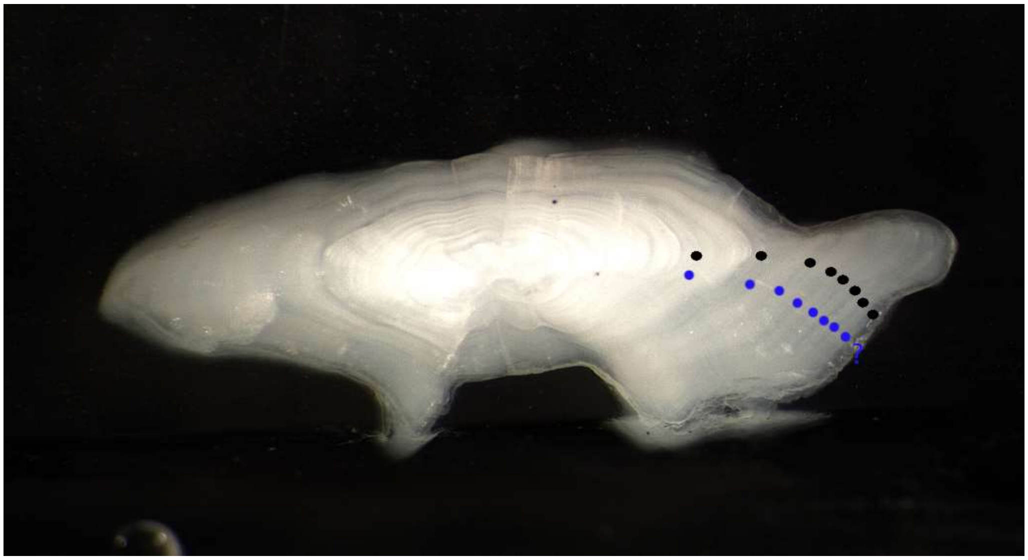
JT= 10-11 yr. LK= 9 yr Not sure about late years.



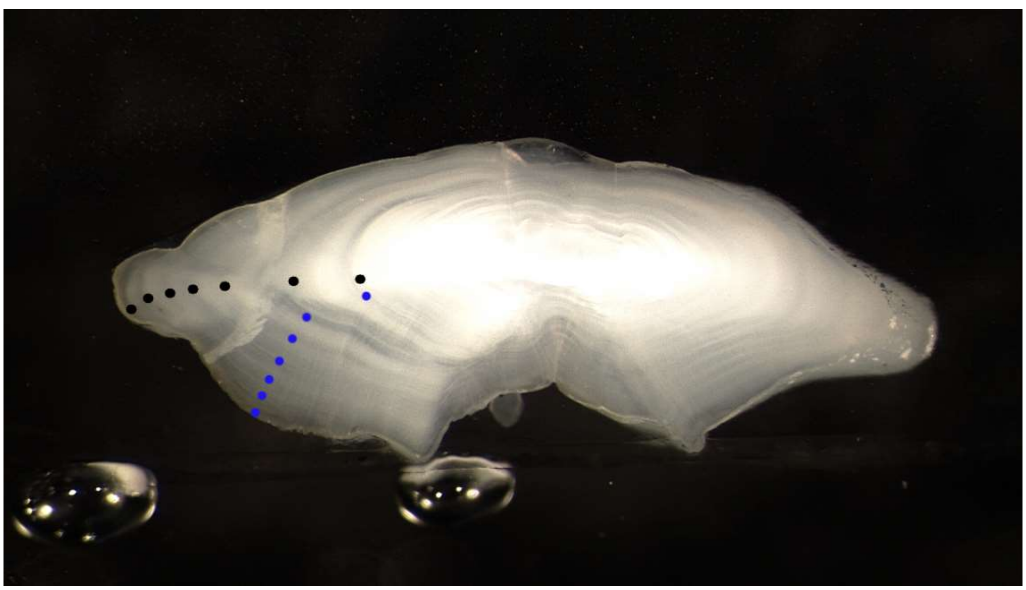
JT= 5 yr. LK= 3-4 yr.



JT= 7 yr. LK= 6-7 yr, not sure about the edge.



JT=8 yr. LK= 8 yr. Not sure about placement of years 3 & 4.



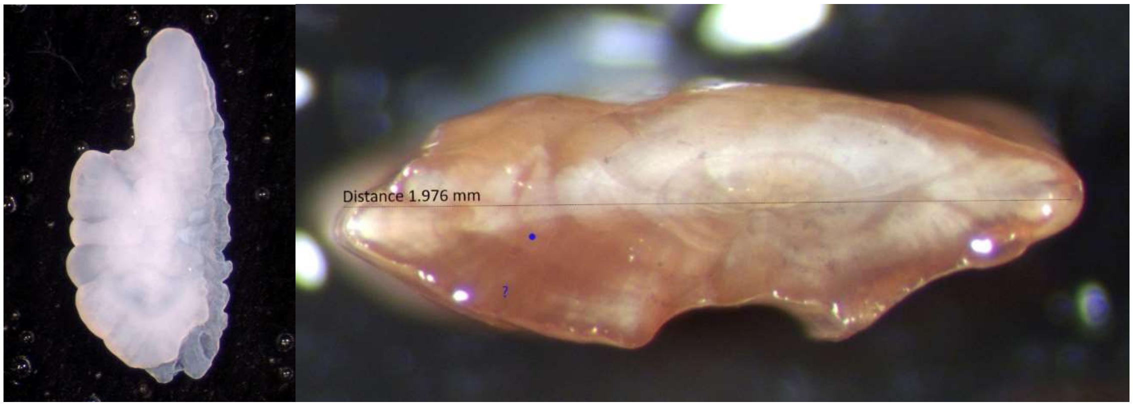
JT= 7 yr, LK=7 yr. Not sure about 4th year placement.

Break-and-Burn

First year is typically seen between 1.2 and 1.5 mm and the second year near 1.9 mm. Measurements are approximate.

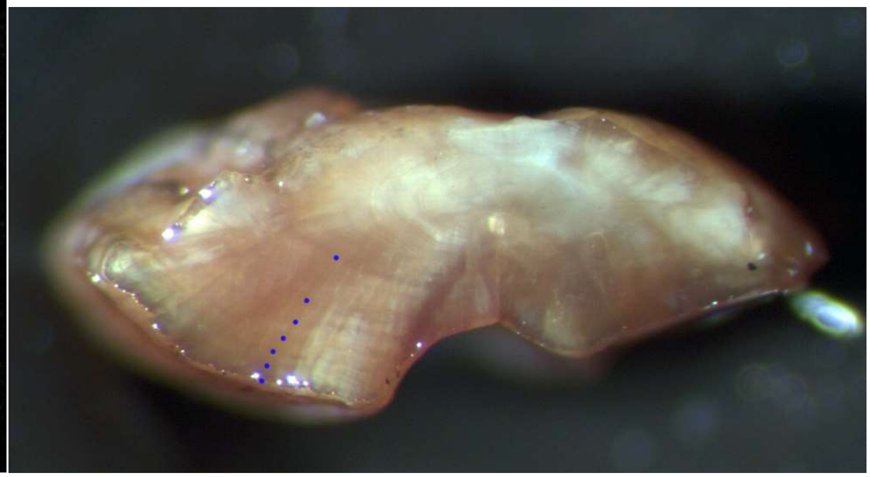
Aged as 1-year-old. Depoe Bay, OR male. 300 mm, collected 11 August 2001. (Sample GE01.0022 #3).

Aged as 2-year-old. Garibaldi, OR male. 366 mm, collected 6 August 2001. (Sample GE01.0006 #18).

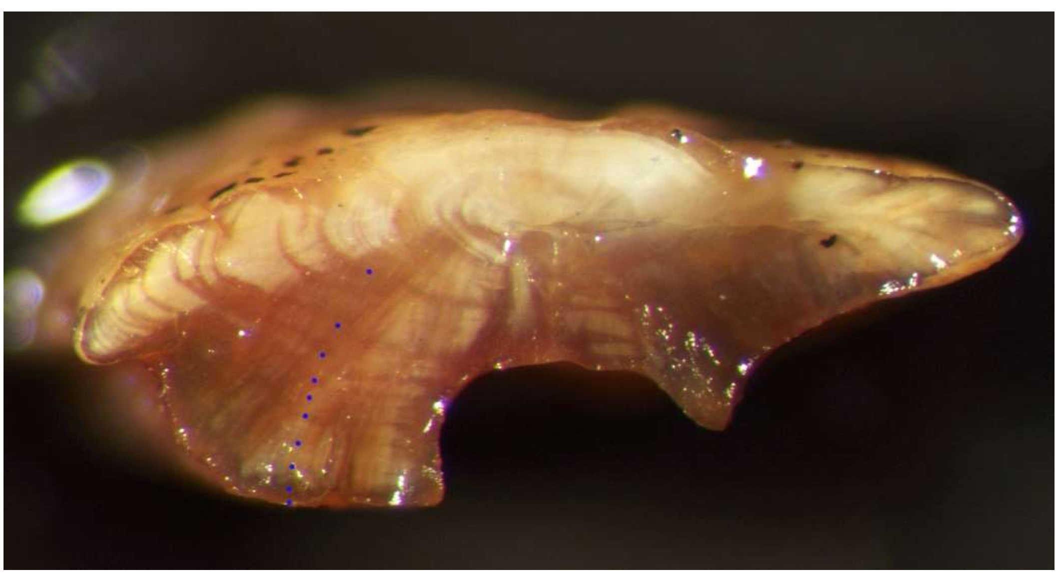


Aged as 2-year-old. Garibaldi, OR female. 360 mm, collected 6 August 2001. Does not fit typical measuring criteria (Sample GE01.0006 #19).

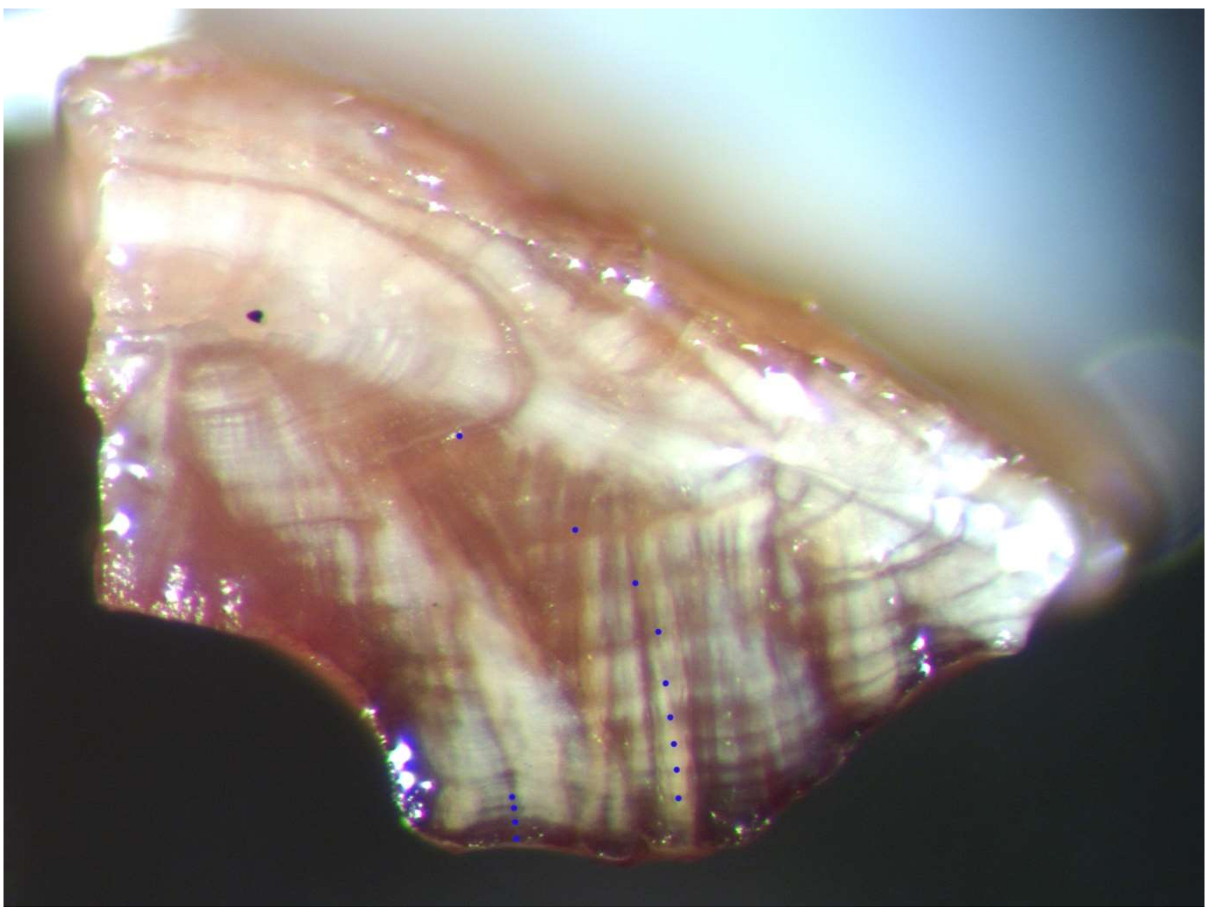
Aged as 7-year-old. Garibaldi, OR female. 500 mm, collected 25 August 2013 (RC13.1016 #16).



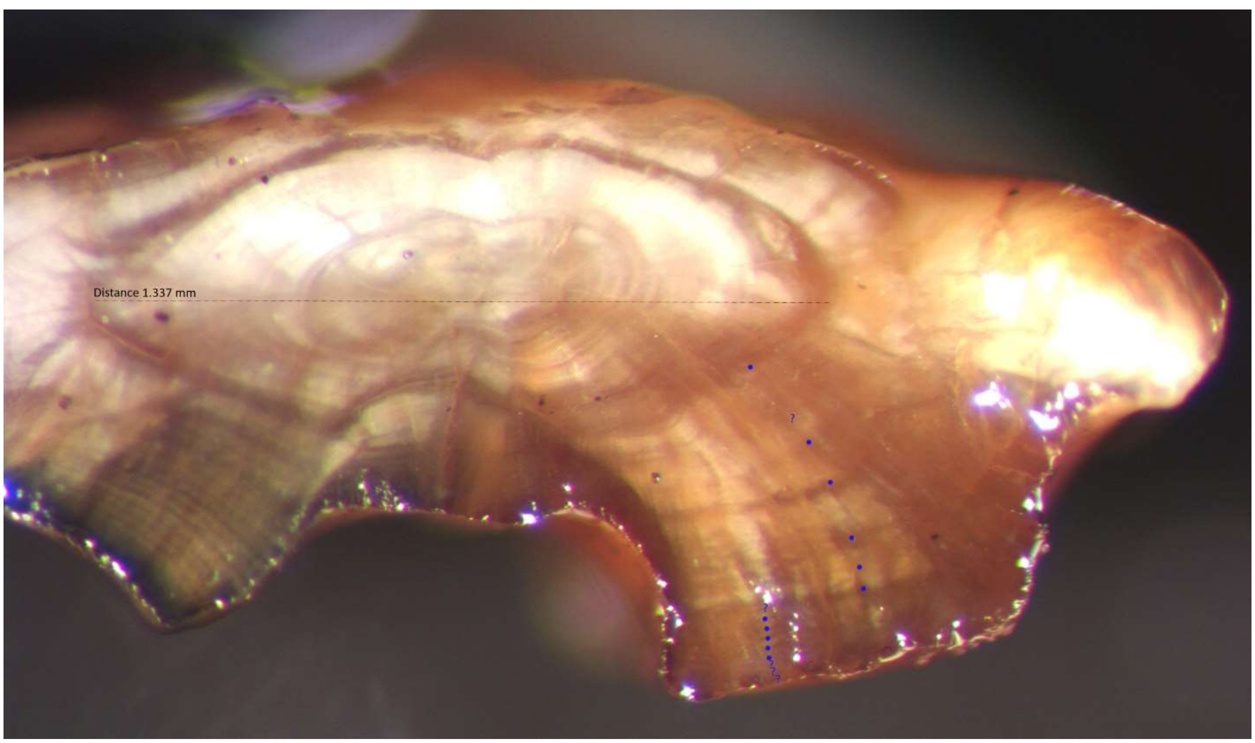
Aged as 7-year-old. Depoe Bay, OR female. 540 mm, collected 30 May 2009 (RC09.1005 #45).



9-12-year-old. Depoe Bay, OR male, 590 mm, collected 4 August 2013. (RC13.1017 #15)



Aged as 13-year-old. Brookings, OR female, 700 mm, collected 30 July 2009. (RC09.3086 #3).



Aged as 15-year-old. Depoe Bay, OR male, 570 mm, collected 28 May 2011. (RC11.1004 #76).

Whole otolith pairs

Sagittal otolith pair of 43cm female Cabezon collected 29 August 2017. Port Orford, OR. Commercial fishery.

Sagittal otolith pair of 51cm female Cabezon collected 28 August 2017. Port Orford, OR. Commercial fishery.

Sagittal otolith pair of 41cm male Cabezon collected 6 November 2017. Port Orford, OR. Commercial fishery.

Sagittal otolith pair of 57cm male Cabezon collected 29 August 2017. Port Orford, OR. Commercial fishery.

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