

1 **Age validation of four rockfishes (genera *Sebastes* and *Sebastolobus*) with bomb-**
2 **produced radiocarbon**

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22 **Abstract**

23 In rockfish (Family Scorpaenidae) age determination is difficult and the annual nature of
24 otolith growth zones must be independently validated. We applied routine age determination
25 to four species of Gulf of Alaska rockfish: two shallower water species, harlequin rockfish
26 (*Sebastes variegatus*) and redstripe rockfish (*S. proriger*), and two deepwater species,
27 shortspine thornyhead (*Sebastolobus alascanus*) and shortraker rockfish (*S. borealis*). The
28 estimated ages (counts of presumed annual growth zones in the otoliths) were then evaluated
29 with bomb-produced radiocarbon (^{14}C) and Bayesian modeling with Markov Chain Monte
30 Carlo simulations. This study successfully demonstrated the level of accuracy in estimated
31 ages of redstripe rockfish (a 35% probability of under-ageing, and about a 5% probability of
32 over-ageing) and harlequin rockfish (a 100% probability that they were under-aged by about 3
33 or 4 years). Measured $\Delta^{14}\text{C}$ in shortspine thornyhead and shortraker rockfish otoliths was
34 lower and increased later than expected. Hence, incorrect age determination could not be
35 evaluated. This is likely caused by dissimilar environmental and biological availability of ^{14}C
36 between these two species and the Pacific halibut (*Hippoglossus stenolepis*) reference
37 chronology, or under-ageing of these two species.

38

39 **Additional keywords**

40 Rockfish, Otolith, Bomb-produced radiocarbon, Age accuracy, Bayesian modeling, Markov
41 Chain Monte Carlo simulations, Scorpaenidae, age determination.

42

43

44 **Running head**

45 Rockfish age validation with bomb-produced radiocarbon

46

47 **Lay summary**

48 Determining the age of rockfish is difficult. Their otoliths (ear stones) have growth zones,
49 which are difficult to count. Hence, we used bomb-produced radiocarbon to confirm the fish
50 age independently. In redstripe rockfish there was a small probability of under-ageing, in
51 harlequin rockfish there was a large probability that they were under-aged, and in shortspine
52 thornyhead and shorttraker rockfish the bomb-produced radiocarbon was probably not an
53 effective tool.

54 **Introduction**

55 Rockfish (Scorpaenidae) are a valuable component of Alaska groundfish fisheries. The ex-
56 vessel value of rockfish harvested in 2017 was about \$29 million (Fissel *et al.* 2019). The
57 biological reference points used to determine harvest specifications for optimal management
58 of a fishery stock depend on accurate fish age estimation (Parker *et al.* 2000; Tribuzio *et al.*
59 2017). A classic example of under-ageing is demonstrated with Pacific ocean perch (*Sebastes*
60 *alutus*) when it was determined that interpretations of growth zones viewed on otolith's
61 surface under-estimated the ages relative to their cross sections (Beamish 1979). In this
62 example of Pacific ocean perch, the otolith cross-section ages provided a reduced estimate of
63 natural mortality compared to that from otolith surfaces (Beamish and McFarlane 1983).

64

65 Fish age estimation relies on consistent methods of otolith preparation and interpretation of
66 the otolith's annual growth zones. Two common methods of otolith preparation are the break
67 and burn (Goetz *et al.* 2012a) and thin sectioning (Hutchinson *et al.* 2007). The interpretation
68 of growth zones requires the application of a set of rules, or age determination criteria, in a
69 consistent fashion (Matta and Kimura 2012). This is often difficult, and age estimates in long-
70 lived species of fish suffer from low accuracy and precision (Campana 2001; Pearson and
71 Gunderson 2003; Kimura and Anderl 2005; Hutchinson *et al.* 2007). Ideally, age
72 determination criteria should be based on otoliths from fish of known age. However, such
73 samples are rarely available, so validation of age determination methods via independent
74 methods is required (Campana 2001; Kimura *et al.* 2006). Typically, ages are estimated by
75 counting posited annual growth zones, and then a variety of age validation methods can be
76 applied to confirm that the estimated ages are accurate (Campana 2001, Kimura *et al.* 2006).

77 If the estimated ages are deemed inaccurate by the validation method, the age determination
78 criteria can be revised to correct a bias from the true age (Kastelle *et al.* 2017).

79

80 This study focuses on age determination in harlequin rockfish (*S. variegatus*), redstripe
81 rockfish (*S. proriger*), shortspine thornyhead (*Sebastolobus alascanus*), and shorttraker
82 rockfish (*S. borealis*). These species are managed by the North Pacific Fishery Management
83 Council with limited information on life history traits such as life span, proportion mature at
84 age, size at age, mortality rates, and population age structure. Since vital rates and life history
85 parameters for assessment models depend on reliable age data, it is necessary to validate age
86 determination criteria and quantify age determination precision and bias, if it exists.

87

88 Previous research on age determination and age validation in redstripe rockfish, harlequin
89 rockfish, shorttraker rockfish, and shortspine thornyhead is limited. For redstripe and harlequin
90 rockfish, two species which occupy shallower water (Love *et al.* 2002; Rooper 2008) than
91 most congener species, age validation information is not available. Otoliths in these two
92 species display growth patterns that are similar to northern rockfish, a species that is routinely
93 aged for stock assessment with criteria validated using bomb-produced radiocarbon (Kastelle
94 *et al.* 2016). Shorttraker rockfish and shortspine thornyhead are found in deeper waters of the
95 Gulf of Alaska (Love *et al.* 2002; Rooper 2008). Shorttraker rockfish age determination has
96 been attempted with resolved methods common to other Scorpaenids, but interpretation of
97 growth zones is problematic with low precision between age readers (Hutchinson *et al.* 2007).
98 They are thought to have a lifespan as high as 150 years (Munk 2001) which can exacerbate

99 age reading difficulty. Radiometric age validation, using the ratio of $^{210}\text{Pb}/^{226}\text{Ra}$ in otoliths,
100 confirms that they are long-lived (Kastelle *et al.* 2000; Hutchinson *et al.* 2007). Unfortunately,
101 confidence intervals of radiometric age estimates become large in fish over about 60 years,
102 and it does not provide information on individual fish. Age determination of shortspine
103 thornyhead, also using common resolved methods, is similarly difficult with a maximum
104 observed age of about 100 years. These methods and criteria have also been validated with the
105 radiometric method (Butler *et al.* 1995; Kline 1996; Kastelle *et al.* 2000). In both of these
106 species, the interpretation of otolith growth zones is known to be problematic because of their
107 compact and faint nature (Kline 1996; Hutchinson *et al.* 2007). The point here is, for all four
108 species, resolved and established age determination methods and criteria exist, but needs to be
109 validated. This is based on the foundation of previous age reading in other Scorpaenids and
110 the confirming age validation research.

111

112

113 In this study, we apply what is often considered the “gold standard” method of age validation,
114 bomb-produced radiocarbon (^{14}C) (Kalish 1995; Campana 2001; Kimura *et al.* 2006). This
115 method relies on above-ground testing of atomic bombs conducted during the Cold War era,
116 which caused a large increase of ^{14}C in the marine environment from the late 1950s to about
117 1970 (Nydal 1993; Kumamoto *et al.* 2013). This increase is recorded in marine carbonates,
118 including fish otoliths, formed during that era, providing a time reference. The amount of ^{14}C
119 (measured as $\Delta^{14}\text{C}$) in otolith material deposited during the first year of a fish’s life (the birth
120 year, calculated from the catch date minus the estimated age of the fish) can be compared to
121 an established reference $\Delta^{14}\text{C}$ chronology (Piner and Wischniowski 2004; Wischniowski *et al.*

122 2015). So in a simple evaluation, if $\Delta^{14}\text{C}$ values of test specimens (test specimens are those
123 whose ages are under investigation) are synchronous with the reference chronology, then the
124 ages estimated from counting growth zones is deemed accurate. In a more complex
125 evaluation, the synchrony, or lack thereof, can be used to estimate the probability of ageing
126 error (Kastelle *et al.* 2016). This age validation method has successfully been applied to a
127 number of North Pacific species (for example: Piner and Wischniowski 2004; Keer *et al.*
128 2005; Kastelle *et al.* 2008; Andrews *et al.* 2011; Wischniowski *et al.* 2015; Kastelle *et al.*
129 2016).

130

131 Age determination of redstripe rockfish, harlequin rockfish, shortraker rockfish, and
132 shortspine thornyhead is difficult. The methods and criteria used here for age determination
133 are largely based on those used in other *Sebastes* species, and on variations of these methods.
134 To facilitate the use of this age data in stock assessments, there is a need for new and better
135 age validation. Therefore, our first goal was to use bomb-produced ^{14}C to validate ages
136 estimated by otolith growth zone counts in all four species. In our second goal, we wished to
137 evaluate the probability of ageing error when these age determination methods and criteria are
138 applied to these four species.

139

140 **Materials and methods**

141 *Specimen collection*

142 Shortspine thornyhead, shortraker rockfish, redstripe rockfish, and harlequin rockfish otoliths
143 were collected in the Gulf of Alaska from 1977 to 2015 during National Marine Fisheries

144 Service's (NMFS) Alaska Fisheries Science Center (AFSC) scientific bottom trawl surveys
145 and by NMFS fishery observers aboard commercial vessels (Fig. 1). Specimens were selected
146 as explained for each species below, and was based in part on estimated birth years (using the
147 age determination methods described in the following sections) such that they were hatched
148 during the era of increasing ^{14}C (Table S1¹).

149

150 *Age determination and specimen selection-redstripe and harlequin rockfish*

151 Ages of redstripe and harlequin rockfish were estimated using the break-and-burn method
152 (Goetz *et al.* 2012a) as part of routine age determinations by the AFSC Age and Growth
153 Program to support fishery stock assessments. Interpreting the otolith's innermost and
154 outermost growth zones were typically the most difficult components of the age determination
155 for these species. The innermost are difficult due to break-and-burn irregularities and
156 occurrence of indistinct or possibly non-annual growth zones. The outermost are difficult due
157 to the compact nature of growth zones deposited in older adults.

158

159 Otolith selection for age validation was based on an initial sample of 446 redstripe rockfish
160 and 563 harlequin rockfish that had been aged twice as part of routine quality control
161 precision testing (Kimura and Anderl 2005). One of these age readings was done by an
162 "expert age reader" who had the most experience in applying the break-and-burn method and
163 standard age determination criteria, the other was done by a second experienced "reader"
164 (Fig. 2). Of this initial sample, we selected all specimens with estimated birth years (birth year

¹ Supplementary data are available for this article through the journal at <http://...>

165 = collection year – the expert age reader’s age estimate) prior to 1980 (redstripe rockfish, n =
166 215) and prior to 1983 (harlequin rockfish, n = 108) to ensure the era of marine radiocarbon
167 increase would be represented. Next, when more than two specimens had the same estimated
168 birth year, only two were randomly chosen for analysis. This process yielded 41 redstripe
169 rockfish and 40 harlequin rockfish for bomb radiocarbon analysis (Table S1A and S1B). For
170 these chosen specimens, ages were independently estimated again by up to four different
171 readers to provide up to six age estimates per specimen. The expert age reader’s estimates of
172 age were used as the validation ages to be tested.

173

174 *Age determination and specimen selection-shortspine thornyhead*

175 Shortspine thornyhead otoliths have historically been collected during AFSC scientific bottom
176 trawl surveys, but they do not currently undergo routine age determination; therefore,
177 previously aged specimens were not available. Otoliths from fish collected from 1996 to 2007
178 with lengths ≥ 24 cm were selected for age reading (n = 66). Longer specimens were used at
179 the onset because we only needed older specimens which had birth years posited to be during
180 the era of increasing bomb-produced ^{14}C .

181

182 Shortspine thornyhead otoliths were prepared and read using the thin-section method, similar
183 to that established in Butler *et al.* (1995), Kline (1996), McCurdy *et al.* (2002), Hutchinson *et*
184 *al.* (2007), and Goetz *et al.* (2012b). Otoliths were embedded in clear polyester resin and cut
185 transversely through the core to produce thin sections 0.3-0.4 mm thick that were then
186 mounted on glass slides. Thin sections were coated with mineral oil and viewed under a

187 dissecting microscope with reflected light and a black background (Fig. 2). The interpretation
188 of growth zones and application of age reading criteria were difficult, especially for
189 seemingly older specimens. There were often faint growth zones amongst those posited to
190 form annually, as well as growth zones that did not conform to consistently spaced laminar
191 patterns. A set of juvenile shortspine thornyhead otoliths were surfaced aged and then broken
192 transversely through the nucleus to help determine measurements of the first three years for
193 each specimen.

194

195 Two different age readers (an expert and a second experienced reader) independently
196 estimated the ages, and the average age was used to estimate the birth year for ^{14}C analysis.
197 The average age was used because the shortspine thornyhead stock assessments do not use
198 age-structured population dynamics models, are managed in a species complex (Echave and
199 Hulson, 2018), and because of the difficulty in interpreting otolith growth zones, even though
200 they were aged by established methods and criteria. Hence, the average age was used here as
201 the best way to make a starting point for age validation. Shortspine thornyhead specimens
202 with posited birth years prior to 1979 were separated into two categories, those with ages ≥ 30
203 years and those with ages ≤ 29 years (Table S1C). Nine specimens were randomly chosen
204 from the older category and 20 specimens were randomly chosen from the younger category
205 such that there were three or fewer specimens within any given birth year. The two categories
206 were used to help incorporate as wide an age range as possible.

207

208 *Age determination and specimen selection-shortraker rockfish*

209 The shortraker rockfish selection process had minor deviations from that of the other three
210 species. Shortraker rockfish otoliths were prepared for age determination by the same
211 established otolith thin-section preparation methods and growth zone interpretations described
212 for shortspine thornyhead (McCurdy *et al.* 2002; Hutchinson *et al.* 2007; Goetz *et al.* 2012b)
213 (Fig. 2). Much like shortspine thornyhead, applying age reading criteria is difficult. To
214 generate an adequate number of candidates, specimens collected between the years 1996 to
215 2006, and aged independently by two age readers (an expert and a seconder experienced
216 reader) (n = 699) were available. From those, specimens with estimated average birth years
217 between 1952 and 1985 were considered. Similar to shortspine thornyhead, the average age of
218 shortraker rockfish was considered the best way to make a starting point for age validation.
219 Eighteen of the specimens were chosen because they were subjectively clear and the two age
220 estimates differed by 5 years or less. Nine more specimens were chosen because there was a
221 large difference between age estimates (7 to 20 years), and subjectively these otoliths were
222 not clear and were difficult to interpret (Table S1D). There was no regard for the number of
223 specimens with the same estimated birth year.

224

225 *Inter-reader precision and bias*

226 All multiple readings per specimen were used to calculate 95% confidence intervals for
227 estimated ages and birth years. In the cases of harlequin and redstripe rockfish up to six
228 readings were used. In the cases of shortspine thornyhead and shortraker rockfish, just two
229 readings were made and hence used to calculate the confidence intervals. Only paired inter-
230 reader readings (expert and second experienced reader) were used for precision statistics.
231 Precision was evaluated by percent agreement, average percent error (APE; Beamish and

232 Fournier 1981), and the coefficient of variation (CV; Chang 1982). Inter-reader bias (relative
233 bias) was evaluated graphically using age bias plots (Campana *et al.* 1995). From the six
234 readings of harlequin and redstripe rockfish otoliths, only the initial two age estimates (those
235 of the expert and a second reader) were used to calculate precision statistics and construct age
236 bias plots. In the instances of shortspine thornyhead and shorttraker rockfish just one set of
237 paired readings were made.

238

239 *Sample preparation and mass spectrometry*

240 Age reading is a destructive process; therefore, only one remaining otolith from each fish was
241 available for ^{14}C examination. Cores from these otoliths, representing the first 2 years of life,
242 were extracted to provide material for ^{14}C analysis. A 2-year core was necessary to meet the
243 sample mass requirements of mass spectrometry. As a guide, target core sizes were
244 determined on a per species basis by measuring the size and mass of otoliths from 2- and 3-
245 year-old juvenile specimens. To extract each core, growth zones outside of the first two
246 translucent zones were removed (wet-sanded) with a Buehler MetaServ 250TM grinder-
247 polisher (Lake Bluff, IL, USA). The inner 2 or 3 translucent growth zones typically became
248 more visible as outer material was removed and also served as a guide in this coring process.
249 See Kestelle *et al.* (2016) or Kestelle *et al.* (2008) for more information on coring methods.
250 The cores were cleaned ultrasonically in distilled and deionized water, dried, weighed, placed
251 in acid-washed glass vials, and shipped to the National Ocean Sciences Accelerator Mass
252 Spectrometry Facility (NOSAMS) at the Woods Hole Oceanographic Institution, Woods
253 Hole, MA, USA, where they were analyzed for ^{14}C and ^{13}C . The results are reported as $\Delta^{14}\text{C}$
254 in ‰ (Stuiver and Polach 1977) which represents the

255 in an international standard and the sample. Values of otolith $\Delta^{14}\text{C}$ were normalized to 1950,
256 corrected for isotopic fractionation with $\delta^{13}\text{C}$, and normalized to a $\delta^{13}\text{C}_{\text{VPDB}}$ value of -25‰
257 (Woods Hole Oceanographic Institution 2018).

258

259 *Age validation*

260 To perform the age validation, the increase (pulse function) of the $\Delta^{14}\text{C}$ in the test validation
261 rockfish otolith cores, as a function of birth year, was compared to a Gulf of Alaska Pacific
262 halibut (*Hippoglossus stenolepis*) reference $\Delta^{14}\text{C}$ chronology (Piner and Wischniowski 2004).
263 The Pacific halibut $\Delta^{14}\text{C}$ reference chronology is based on juvenile fish whose age is
264 considered known, or without any error. We used a coupled-function model (product of
265 Gaussian and exponential models) (Hamel *et al.* 2008; Kestelle *et al.* 2016):

$$\hat{y}_x = \lambda + ke^{\left[(\mu \cdot r) + \frac{(\sigma^2 \cdot r^2)}{2}\right]} \times e^{(-r \cdot x)} \Phi(\mu + \sigma^2 \cdot r, \sigma, x) + \sigma_e^2,$$

266 where: \hat{y}_x = estimated $\Delta^{14}\text{C}$ and x = birth year. The model parameters are λ = average pre-
267 bomb $\Delta^{14}\text{C}$ value (lower predicted asymptote), k = the total predicted increase of $\Delta^{14}\text{C}$ to
268 reach the upper asymptote, μ = mean or peak year of radiocarbon Gaussian pulse curve
269 (which is the birth year corresponding to the midpoint, 50%, of the $\Delta^{14}\text{C}$ increase), σ =
270 standard deviation of the Gaussian pulse curve, r = exponential decay rate (per year) of the
271 post-peak decline, and σ_e^2 = the error variance. The symbol Φ represents the cumulative
272 normal function. The difference between the predicted μ of the reference chronology (R) and
273 that of the test validation sample (S), $\mu_R - \mu_S$, is a measure of dissimilarity in the year of 50%
274 increase of the two curves, and hence age determination bias (Hamel *et al.* 2008; Kestelle *et*

275 *al.* 2016). This means that if the validation sample birth years are estimated correctly (birth
276 year = collection year – estimated age) the value of $\mu_R - \mu_S = 0$. For the purposes of this
277 model, a midpoint of otolith deposition for every sample must be used. Hence, the birth year
278 of each test validation specimen was adjusted by +1 year to account for the 2-year core, and
279 the Pacific halibut birth years were adjusted by + 0.5 years to account for using whole otoliths
280 from 1-year-old juveniles (Kastelle *et al.* 2016). Bayesian methods were used to fit the models
281 using Markov Chain Monte Carlo (MCMC) simulation (2,000,000 samples, burn-in =
282 1,000,000, thinned at 1,000) and the converged posterior sample, $n = 1,000$, was used to
283 compute the probability of ageing bias. As presented in Kastelle *et al.* (2016) and summarized
284 here, the MCMC probability density of $\mu_R - \mu_S$ is a measure of age determination bias. If the
285 probability density is centered on zero, then the estimated ages of the specimens in the test
286 validation sample can be considered accurate. An indication of bias in the estimated ages can
287 be assessed by calculating the tail probability greater or less than zero; that is, $Prob[(\mu_R -$
288 $\mu_S)] > 0$.

289

290 **Results**

291 *Age determination and inter-reader precision*

292 Ages estimated by six independent readers for harlequin rockfish test validation specimens
293 spanned 14 to 87 years. The expert's age estimates spanned 14 to 66 years, resulting in
294 predicted birth years from 1949 to 1982 (Table S1A). Ages estimated by six independent
295 readers for redstripe rockfish spanned 7 to 38 years. The expert's age estimates spanned 7 to
296 36 years, resulting in predicted birth years from 1945 to 1979 (Table S1B). Age estimation of

297 harlequin rockfish was more difficult than that of redstripe rockfish. This is demonstrated in
298 percent agreement of 47.6% and 51.35%, respectively; differences in the other inter-reader
299 precision statistics (Table S2); and by typical standard deviations of average ages from the six
300 readers (Table S1A and S1B). This difficulty was also noted subjectively by the expert age
301 reader and may be partially related to the fact that age estimates for chosen harlequin rockfish
302 specimens were older on average (32.8 years, n = 40) than for chosen redstripe rockfish (22.7
303 years, n = 41). For redstripe rockfish, in specimens greater than about 20 years old, the
304 reader's age estimates were biased low compared to the expert's age estimates (Fig. 3A). For
305 harlequin rockfish, there was a bias between the expert's and reader's age estimates; the
306 reader's age estimates were more often higher than the expert's (Fig. 3B).

307

308 The shortraker rockfish and shortspine thornyhead specimens were only read two times, and
309 the ages for each specimen were then averaged. In shortspine thornyhead specimens (by
310 design all specimens were ≥ 24 cm) the age estimates ranged from 12 to 49 years. The
311 specimens' average age estimates ranged from 13 to 46 years (Table S1C). The individual
312 shortraker rockfish age estimates ranged from 11 to 59 years, and the specimens' averaged
313 age estimates ranged from 11 to 53 years (Table S1D). The 95% confidence intervals around
314 averaged age estimates for both shortspine thornyhead and shortraker rockfish were large,
315 over 10 years when the two age estimates differed greatly. Hence, the confidence intervals are
316 not as informative compared to the other two species. The inter-reader percent agreement for
317 shortspine thornyhead and shortraker rockfish was 21.21% and 5.58%, respectively. The
318 relative age determination difficulty is further reflected in other inter-reader precision
319 statistics (Table S2). For shortspine thornyhead, agreement between reader and expert was

320 generally good until about age 20 year. Beyond the age of 20 years there was more variability
321 between the two sets of ages, and a bias did exist (Fig. 3C). Low precision in shortspine
322 thornyhead may have been partially a result of preferentially choosing otoliths from fish that
323 were ≥ 24 cm, resulting in overall older age estimates (Table S1C). For shortraker rockfish,
324 there was a large variability in the reader's ages with respect to the expert's ages throughout
325 the full range of ages. This variability in shortraker rockfish ages was larger compared to the
326 other species, but there was no discernable bias between the two readers (Fig. 3D). The age
327 readers indicated that age estimation of these two species was generally difficult, and
328 confidence in age estimates was relatively low.

329

330 $\Delta^{14}C$ analysis and age validation

331 Statistical inference of ageing bias is based on the properties of the model. We evaluated
332 MCMC simulation performance by examining the posterior sample. Here the Bayesian model
333 and MCMC simulation were computationally efficient, yielding 1,000 samples with which to
334 compute summary statistics and develop a framework to assess ageing bias. Initial testing of
335 the MCMC simulation showed burn-in was achieved after 10,000 samples, and between-
336 sample autocorrelation of estimated parameters was non-significant after a log of 10 sample
337 parameter sets. We nevertheless discarded the first half of the 2 million samples and thinned
338 at a rate of 1,000. These results are shown in Figure S1, which provides trace, autocorrelation
339 and posterior density plots. The simulation traversed the parameter space efficiently which is
340 indicated by smooth unimodal posterior density, low autocorrelation, and a large effective
341 sample size.

342

343 Trends in the redstripe rockfish $\Delta^{14}\text{C}$ values (chronologies) were largely similar to the Pacific
344 halibut reference chronology. Generally, in redstripe rockfish and the reference chronology,
345 $\Delta^{14}\text{C}$ began to rise above pre-bomb levels shortly after the onset of above-ground nuclear
346 testing in the late 1950s to early 1960s, after which $\Delta^{14}\text{C}$ increased more rapidly in the middle
347 1960s. The estimated model parameters of chronologies in redstripe rockfish and Pacific
348 halibut reference were nearly identical (Table 1, Fig. 4A); $\Delta^{14}\text{C}$ in redstripe rockfish reached
349 an asymptotic maximum around 1970, similar to the halibut reference, with a total increase of
350 182.8‰. Here, the parameter of primary interest is μ , 50% of the $\Delta^{14}\text{C}$ rise; redstripe rockfish
351 had a μ_S of 1963.9, and the Pacific halibut reference chronology's μ_R was 1963.1. In redstripe
352 rockfish, the posterior distribution of $\mu_R - \mu_S$ was centered on about -0.7 years, with about an
353 85% probability of being less than zero, indicating a mostly negative bias (under-ageing) of
354 redstripe rockfish ages (Fig. 4A). However, there was only about a 35% probability of under-
355 ageing by 1 year, about a 5% probability of under-ageing by 2 or more years, and less than a
356 5% probability of over-ageing by 1 or more years.

357

358 Trends in the harlequin rockfish $\Delta^{14}\text{C}$ values (chronologies) were only somewhat similar to
359 the Pacific halibut reference chronology, and indicated some notable age determination bias.
360 The estimated model parameters and general shapes of chronologies in harlequin rockfish and
361 Pacific halibut reference were similar (Table 1, Fig 4B). Harlequin rockfish $\Delta^{14}\text{C}$ values had
362 nearly the same pre-bomb values, reached about the same maximum (having a total increase
363 of 250.8 compared to Pacific halibut's of 185.2), but exhibited a post-peak decline after 1972.
364 However, harlequin rockfish $\Delta^{14}\text{C}$ values were right-shifted compared to Pacific halibut,

365 representing a delay in rise of several years with a μ_S at 1967.2. The $\mu_R - \mu_S$ between the
366 harlequin and the Pacific halibut reference chronologies was centered on about -3.8 years, and
367 indicated a 100% probability of a negative bias, or that under-ageing by about 3 or 4 years
368 was most probable. However, there was only about a 5% probability of an under-ageing bias
369 greater than 5 years.

370

371 The coupled function was not fit to the measured $\Delta^{14}\text{C}$ values in shortspine thornyhead or
372 shortraker rockfish otoliths. Neither of these two test species displayed a trend similar to the
373 Pacific halibut reference chronology; hence, the models (parameter estimation) did not
374 converge (Figs. 4C and 4D). Their $\Delta^{14}\text{C}$ values were all scattered low and to the right of the
375 Pacific halibut reference chronology. The Bayesian model and MCMC simulations did not
376 converge to estimate parameter sets. Therefore, we were unable to derive an estimate of
377 ageing bias from the $\Delta^{14}\text{C}$ values in either shortspine thornyhead or shortraker rockfish. In
378 both of these species, there appeared to be about a 10-year delay in the start of the bomb-
379 produced increase (Figs. 4C and 4D). The shortspine thornyhead $\Delta^{14}\text{C}$ values ranged from
380 about -150‰ to 0‰, and the pre-bomb values were clustered around 130‰. The shortraker
381 rockfish $\Delta^{14}\text{C}$ values ranged from about -175‰ to 60‰, with pre-bomb $\Delta^{14}\text{C}$ values clustered
382 around -150‰. Both species did not appear to have well-defined upper asymptotes. Without a
383 fit model, little can be concluded about the accuracy of the estimated ages. All of the nine
384 difficult to age shortraker rockfish specimens had $\Delta^{14}\text{C}$ values in the extreme upper or lower
385 range; that is, in the range of the expected upper and lower asymptotes. Therefore, because
386 the $\Delta^{14}\text{C}$ values were not in an informative range, it was not even possible to subjectively
387 evaluate the accuracy of estimated ages.

388

389 **Discussion**

390 Our age validation of redstripe rockfish was successful. The estimated ages appeared to be
391 close to accurate, with the most probable age determination bias being only about 0.7 years
392 less than true age (Fig. 4A). Hence, only minor revision, if any, in the age determination
393 methods should be made. An age validation and estimated ageing bias for redstripe rockfish
394 did not exist previously. Therefore, this study is the first to independently confirm age
395 estimates of redstripe rockfish, with a maximum validated age of 36 years. Maximum
396 estimated ages of 46 years and 55 years have previously been reported for this species from
397 the Gulf of Alaska (Alaska Fisheries Science Center 2017) and British Columbia (Munk
398 2001) waters, respectively. While it is generally not appropriate to extrapolate beyond those in
399 the study, these previous studies used age determination methodology similar to ours and
400 therefore it could reasonably be assumed that their reported maximum ages are also accurate.

401

402 Our results, relative to the first goal, indicate that current age determination practices for
403 harlequin rockfish do not yield accurate estimates of age. Our second goal of estimating the
404 probability of age reading bias was attained; it was highly probable (about 95%) that an
405 under-ageing bias of about 3 or 4 years exists in the harlequin rockfish samples (Fig. 4B).
406 Therefore, our results can be used to revise and improve otolith preparation methods and age
407 determination criteria for harlequin rockfish. The maximum age reported previously for
408 harlequin rockfish is 76 years (Alaska Fisheries Science Center 2017), and the maximum age
409 estimated by the expert reader in this study was 75 years. Therefore, given the probable

410 negative bias, this represents a new maximum age estimate, which is vital information for
411 estimating natural mortality rates and other vital rates. There are two harlequin rockfish
412 samples that appear as outliers (under-aged), with deposition years of 1972 and 1974 (Fig.
413 4B). In our processing of these samples they appeared normal for this species, and hence,
414 probably represent real variation. If these two samples were left out of the analysis, the
415 estimated bias would be less.

416

417 Rockfish age determination is generally difficult, and there are several possible explanations
418 for the observed small negative bias in redstripe rockfish and larger bias in harlequin rockfish
419 age estimates (McCurdy *et al.* 2002; Goetz *et al.* 2012a). The earliest 1 to 3 annuli in rockfish
420 otoliths are often the most difficult to interpret, especially if the otolith was not cut directly
421 through its core during preparation. Compact annuli near the otolith's edge can also be
422 challenging to interpret. Diffuse or faint growth patterns can occur within an otolith on even
423 the clearest reading axis due to the degree of burning applied and to fading over time after
424 preparation (McCurdy *et al.* 2002). In the future, great care should be made to interpret fine
425 and compact growth zones in these areas. These issues may be especially true for harlequin
426 rockfish, which were generally more difficult to age as indicated by lower inter-reader
427 precision. Once refinements to the age determination methods are made, further independent
428 confirmation of the ages should be done, especially the first year's growth zone interpretation
429 (Stewart and McKillup 2002; Guido *et al.* 2004). The best precision is achieved by careful
430 preparation of the otolith and calibration of ageing criteria between readers especially with
431 regard to interpretation of early growth zones. Goetz *et al.* (2012b) gives some suggestions on
432 cutting through the core and burning Scorpaenid otoliths. After revised methods and new

433 criteria are developed, previously aged specimens can be re-aged and comparisons to the first
434 ages made.

435

436 Our conclusions on redstripe rockfish and harlequin rockfish rely on the main assumption for
437 this type of age validation study, that of an environmental and biological similarity between
438 the test validation and reference species. This means that in the absence of ageing error, the
439 timing and magnitude of the ^{14}C increase should be similar in both the reference chronology
440 and validation specimens. The importance of this assumption has been demonstrated in
441 previous bomb-produced ^{14}C age validation studies by Kalish 1995, Campana and Jones
442 (1998), Haltuch *et al.* (2013), Helser *et al.* (2014), and Wischniowski *et al.* (2015). The
443 similarities of the redstripe rockfish and Pacific halibut pulse curves in the parameters we
444 estimated, not only μ , is notable (Fig. 4A, Table 1). The observed pre-bomb values of $\Delta^{14}\text{C}$ in
445 harlequin rockfish were nearly the same as in Pacific halibut, rising to a similar maximum and
446 then decreasing; the Pacific halibut pulse curve does not show a decrease (Fig. 4B, Table 1).
447 Our definition of bias uses μ ; however, consideration of σ , which defines the slope of the
448 pulse curve, is important. If the validation and reference curves had estimates of σ which were
449 different, this could be an indication of environmental and biological differences in the rate of
450 ^{14}C uptake. This was not the case in our validation and reference curves (Figs. 4A, 4B, and
451 Table 1). Pacific halibut are well known to inhabit nearshore areas as juveniles, migrating
452 deeper as they reach maturity (Norcross *et al.* 1996; Norcross *et al.* 1999; Abookire, *et al.*
453 2001). Redstripe and harlequin rockfish are less researched than halibut, but are also thought
454 to inhabit nearshore areas as juveniles, the time period corresponding to the ^{14}C measured in
455 the otolith cores (Gunderson and Sample 1980; Love *et al.* 2002). Further, the samples of

456 these three species were all collected from same oceanic basin, the Gulf of Alaska. Therefore,
457 they all would be expected to encounter generally similar concentrations of bomb-produced
458 $\Delta^{14}\text{C}$ prior to ontogenetic migrations to deeper water. Small regional differences in conditions
459 such as nearshore water column mixing or less continental freshwater input could conceivably
460 cause a post-peak decline, as noted for the harlequin rockfish. However, the similarities
461 between Pacific halibut and harlequin rockfish in other parameters (σ , k , and λ) suggest that
462 the main assumption was met in our comparisons. In situations where the assumption of
463 environmental and biological similarities between a correctly aged test validation species and
464 reference species is clearly not met, the differences between the pulse curves are greater
465 (Campana and Jones 1998; Haltuch *et al.* 2013; Wischniowski *et al.* 2015).

466

467 Our results for shortspine thornyhead and shortraker rockfish are inconclusive. The $\Delta^{14}\text{C}$
468 measured in otolith cores from both of these species did not display the form of an expected
469 pulse curve; the values were scattered below and to the right of the reference chronology.
470 Therefore, the coupled-function model did not describe the $\Delta^{14}\text{C}$ values, and we did not
471 attempt to fit this model to these data. Consequently, the estimated fish ages could not be
472 validated and the probability density of $\mu_R - \mu_S$ was not estimated. The shortraker rockfish had
473 a special sample selection protocol, a set with clear ages and a set of difficult-to-age samples.
474 In the nine difficult-to-age samples, two of the samples had $\Delta^{14}\text{C}$ values slightly below, but
475 near the expected upper asymptote. The remaining seven samples had some of the lowest
476 resulting $\Delta^{14}\text{C}$ values, about 50 ppm below the lower asymptote of the Pacific halibut
477 reference chronology. Our hope was that when these nine $\Delta^{14}\text{C}$ values were plotted versus the
478 average age and compared to the values of the reference chronology (in the era of increasing

479 $\Delta^{14}\text{C}$) an indication of the correct age would be clear. Unfortunately, this was not the case. In
480 our study the estimated maximum ages of shortspine thornyhead and shortraker rockfish were
481 49 and 59 years old, respectively, from specimens chosen to coincide with the era of
482 increasing bomb-produced $\Delta^{14}\text{C}$, not the maximum age available. Maximum ages reported
483 elsewhere are up to 89 and 157 years, respectively, for shortspine thornyhead and shortraker
484 rockfish (Munk 2001; Alaska Fisheries Science Center 2017). Using radiometric age
485 validation, ages older than observed in this study were confirmed as generally accurate for
486 both species (Kline 1996; Kestelle *et al.* 2000; Hutchinson *et al.* 2007).

487

488 There are two possible explanations for the low and delayed $\Delta^{14}\text{C}$ in shortspine thornyhead
489 and shortraker rockfish. First, in comparing these two species to Pacific halibut, the
490 assumption that they are biologically and environmentally similar may not hold true.
491 Shortraker rockfish and shortspine thornyhead are both known to often inhabit waters deeper
492 than 400 m during their benthic juvenile stages (Jacobson and Vetter 1996; Orlov 2001).
493 Juvenile Pacific halibut usually become benthic at depths less than 120 m (Norcross *et al.*
494 1996; International Pacific Halibut Commission 1998; Norcross *et al.* 1999; Abookire *et al.*
495 2001). This distinction in depths occupied by these rockfish and Pacific halibut during their
496 early life histories may violate the main assumption of environmental and biological
497 similarity due to depth-related differences in oceanic mixing of ^{14}C . Following the period of
498 atomic bomb testing, ocean surface water largely received bomb-produced ^{14}C through
499 exchange at the air-sea interface. Below the mixed surface layer the input rate of ^{14}C is
500 reduced due to a lengthened mixing process from the surface, and by the influence of deep
501 ^{14}C -depleted water (Nydal 1993; Kumamoto *et al.* 2013). Thus, in shortspine thornyhead and

502 shortraker rockfish the low initial level and delayed increase of $\Delta^{14}\text{C}$ may be explained by the
503 differences in depths occupied by the test validation and reference specimens. In other species
504 that are influenced by deeper water from below the mixed layer, a delay in the $\Delta^{14}\text{C}$ pulse
505 curve has also been seen (Haltuch *et al.* 2013). Second, it is possible that both of these species
506 were under-aged, but these results are not useful as an indicator of this. Previous radiometric
507 age validations indicate that shortraker rockfish and shortspine thornyhead can both reach old
508 ages, and in some cases under-ageing can occur (Kline 1996; Kastle *et al.* 2000; Hutchinson
509 *et al.* 2007). The possibility of under-ageing cannot be ignored given the difficulties of age
510 determination in these two species. The age determination problems described previously in
511 this paper, regarding the earliest years and the growth zones on the otolith's edge, are
512 pertinent to the question of under-ageing of shortspine thornyhead and shortraker rockfish.
513 Also, shortspine thornyhead otoliths occasionally have faint growth zones amongst those
514 posited to form annually. This was especially true in otolith regions representing fish growth
515 prior to maturity, which is common in rockfish species (Goetz *et al.* 2012a), but also occurred
516 in otolith regions representing adult life history, which is less common amongst other rockfish
517 species. The interpretation of these faint growth zones in shortspine thornyhead is a source of
518 poor accuracy and low precision because otolith readers must make subjective decisions as to
519 their annual nature. These areas could be a source of under-ageing if the growth zones are
520 more compact and fine than previously thought. This makes shortspine thornyhead unique
521 compared to other rockfish species aged at the AFSC. Both shortspine thornyhead and
522 shortraker rockfish were aged by otolith thin sectioning, instead of the break-and-burn
523 method, due to these age reading difficulties. Importantly, these two explanations, the unmet
524 assumption and under-ageing, are completely confounded and cannot be separated.

525

526 This validation study successfully demonstrated the level of accuracy in estimated ages of
527 redstripe and harlequin rockfish. This was useful because it indicates that future revisions are
528 necessary in applying age determination criteria to harlequin rockfish. The interpretation of
529 the first two or three annuli and of the seasonal growth on the otolith's edge are the most
530 likely areas for revisions, especially for harlequin rockfish. The results here will help in
531 utilizing age data in stock assessments of these two species. Results for shortspine thornyhead
532 and shortraker rockfish were inconclusive, indicating that the Pacific halibut reference was
533 not biologically or environmentally appropriate for an age validation of these species or that
534 under-ageing occurred. The dramatic difference in outcomes between these four species
535 highlights the importance of using the correct known-age reference chronology. Future
536 sampling of $\Delta^{14}\text{C}$ in shortspine thornyhead and shortraker rockfish otoliths to estimate an
537 upper asymptote, using specimens with estimated birth years in the range of 1980 to the
538 2000s, could help to separate these two possibilities.

539

540 This study is unique in the fact that two different habitat preferences are represented by four
541 species. Further, our study was unique in that the sample size for each species was far larger
542 than most other single-species age validation studies, lending more confidence in our results.
543 Also, using the MCMC probability densities to estimate age determination bias is unique
544 among many previous age validation studies.

545

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553 Reference to trade names does not imply endorsement by the National Marine Fisheries
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555

556 **Conflicts of Interest**

557 The authors declare no conflicts of interest; in our knowledge, conflicts of interest do not
558 exist.

559

560 **Animal ethics**

561 The biological samples used in this study were collected during authorized National Marine
562 Fisheries Service scientific bottom trawl surveys or during commercial fishing operations.

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765

766 Table 1. Estimated parameters for the coupled-function model using three data sets: Pacific
 767 halibut (*Hippoglossus stenolepis*) reference chronology, harlequin rockfish (*Sebastes*
 768 *variegatus*), and redstripe rockfish (*Sebastes proriger*).

Model parameter	Model attribute	Pacific halibut reference, n = 36			Harlequin rockfish, n = 40		
		Median	95% credibility interval		Median	95% credibility interval	
λ (‰)	pre-bomb $\Delta^{14}\text{C}$	-106.4	-117.5	-96.3	-99.7	-160.0	-54.4
k (‰)	Absolute $\Delta^{14}\text{C}$ rise	185.2	168.8	201.9	240.5	139.4	400.5
μ (year)	Year of 50% rise	1963.1	1962.5	1963.8	1967.2	1965.5	1968.5
σ	Pulse curve SD	2.61	1.76	3.52	3.314	0.641	8.668
r (per year)	Decay rate	0.004	-0.015	0.025	0.0569	0.0038	0.1226
σ_e^2	Error variance	343.7	190.2	510.1	1936.9	1092.3	2886.7

Model parameter	Model attribute	Redstripe rockfish, n = 41		
		Median	95% credibility interval	
λ (‰)	pre-bomb $\Delta^{14}\text{C}$	-87.8	-102.4	-73.2
k (‰)	Absolute $\Delta^{14}\text{C}$	157.7	139.9	174.8
μ (year)	Year of 50% rise	1963.9	1963.1	1964.6
σ	Pulse curve SD	2.356	1.247	3.399
r (per year)	Decay rate	0 ^a	-0.101	0.034
σ_e^2	Error variance	375.8	221.7	565.0

769 ^aNot different from 0, therefore not used in parametrization.

770 **Figure Captions**

771 Figure 1. Map of Gulf of Alaska collection locations for harlequin rockfish (*Sebastes*
772 *variegatus*), redstripe rockfish (*Sebastes proriger*), shortspine thornyhead (*Sebastolobus*
773 *alascanus*), and shortraker rockfish (*Sebastes borealis*).

774

775 Figure 2. Images of whole otoliths and break-and-burned preparations for (A) harlequin
776 rockfish (*Sebastes variegatus*) and (B) redstripe rockfish (*Sebastes proriger*). Images of
777 whole otoliths and thin section preparations for (C) shortspine thornyhead (*Sebastolobus*
778 *alascanus*) and (D) shortraker rockfish (*Sebastes borealis*).

779

780 Figure 3. Age bias plots (reader vs. expert) for candidate samples (grey circles) and samples
781 randomly chosen for ^{14}C analysis (red stars): (A) redstripe rockfish (*Sebastes proriger*), (B)
782 harlequin rockfish (*Sebastes variegatus*), (C) shortspine thornyhead (*Sebastolobus alascanus*),
783 and (D) shortraker rockfish (*Sebastes borealis*).

784

785 Figure 4. Validation specimen $\Delta^{14}\text{C}$ pulse curves (chronologies), dashed line and points
786 compared to the Pacific halibut (*Hippoglossus stenolepis*) $\Delta^{14}\text{C}$ reference pulse curve
787 (chronology), solid line, and the resulting MCMC probability density of ageing bias, $\mu_R - \mu_S$:
788 (A) redstripe rockfish (*Sebastes proriger*), and (B) harlequin rockfish (*Sebastes variegatus*).

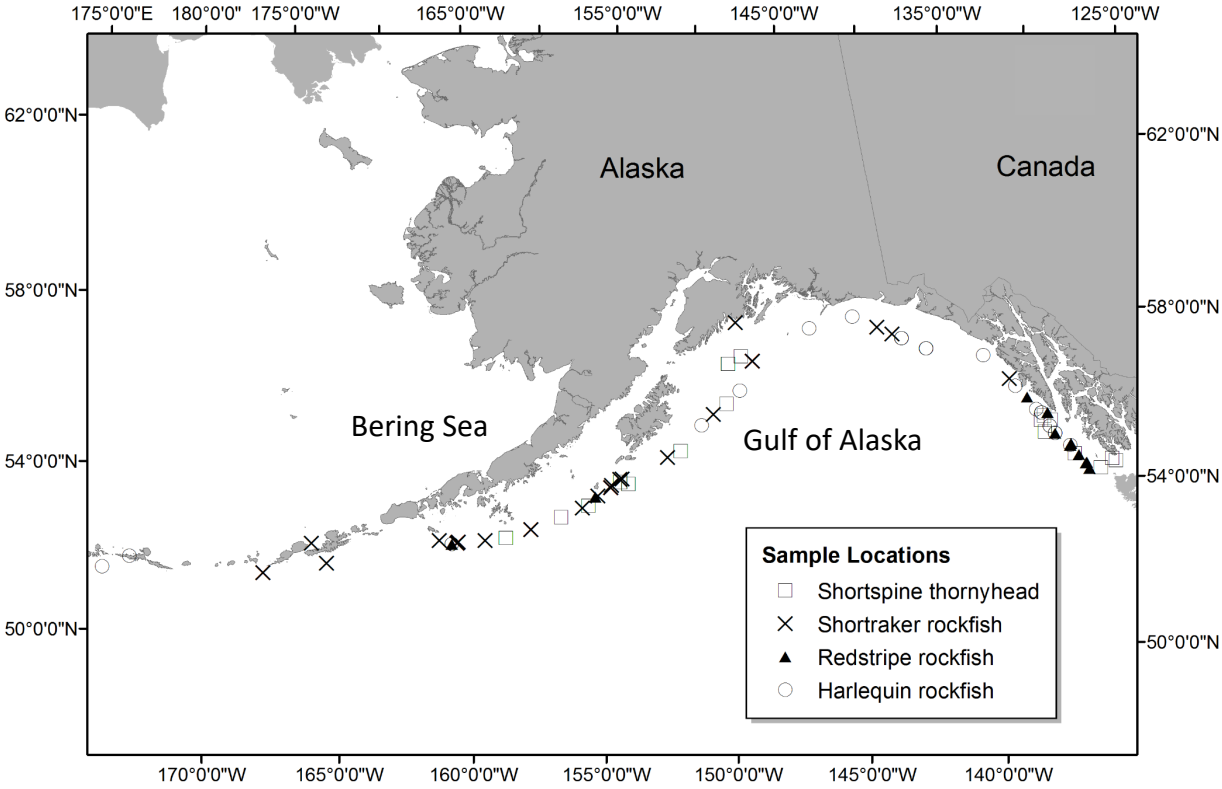
789 Validation specimen $\Delta^{14}\text{C}$ data points compared to the Pacific halibut $\Delta^{14}\text{C}$ reference

790 chronology: (C) Shortspine thornyhead (*Sebastolobus alascanus*) (D) Shortraker rockfish
791 (*Sebastes borealis*). Error bars are 95% confidence intervals.

792

793 Figure 1.

794



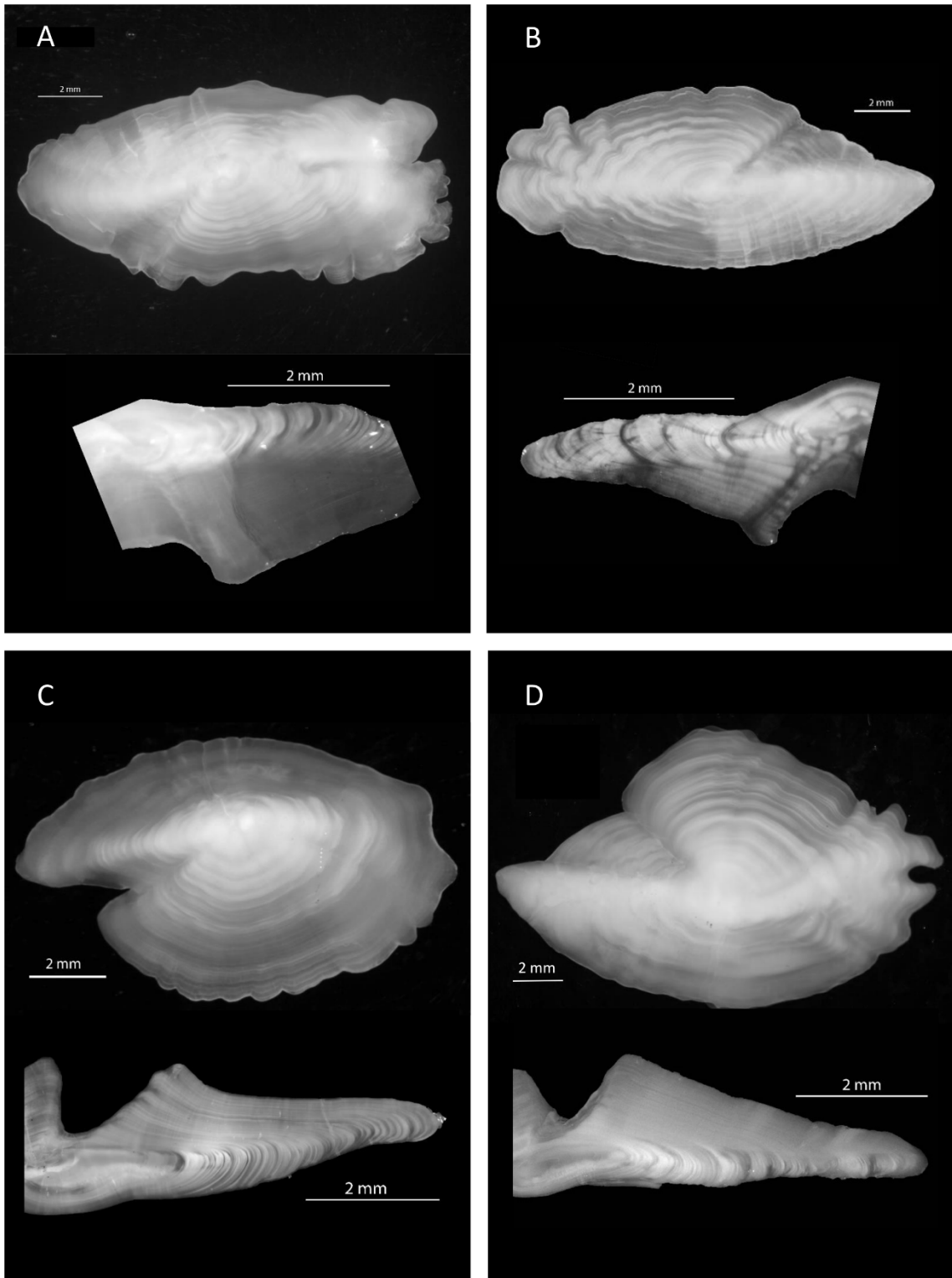
795

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798 Figure 2.

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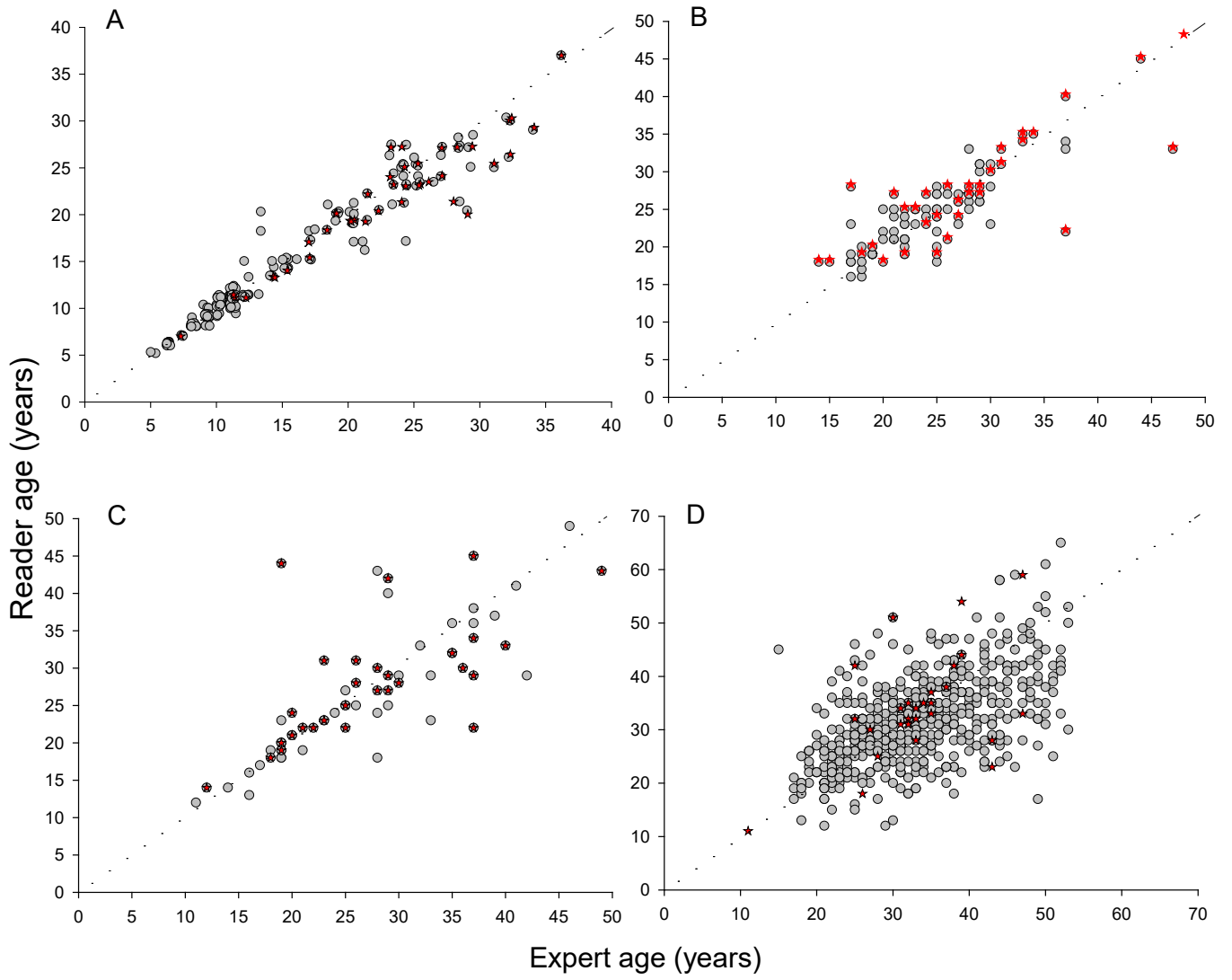


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801

802 Figure 3.

803

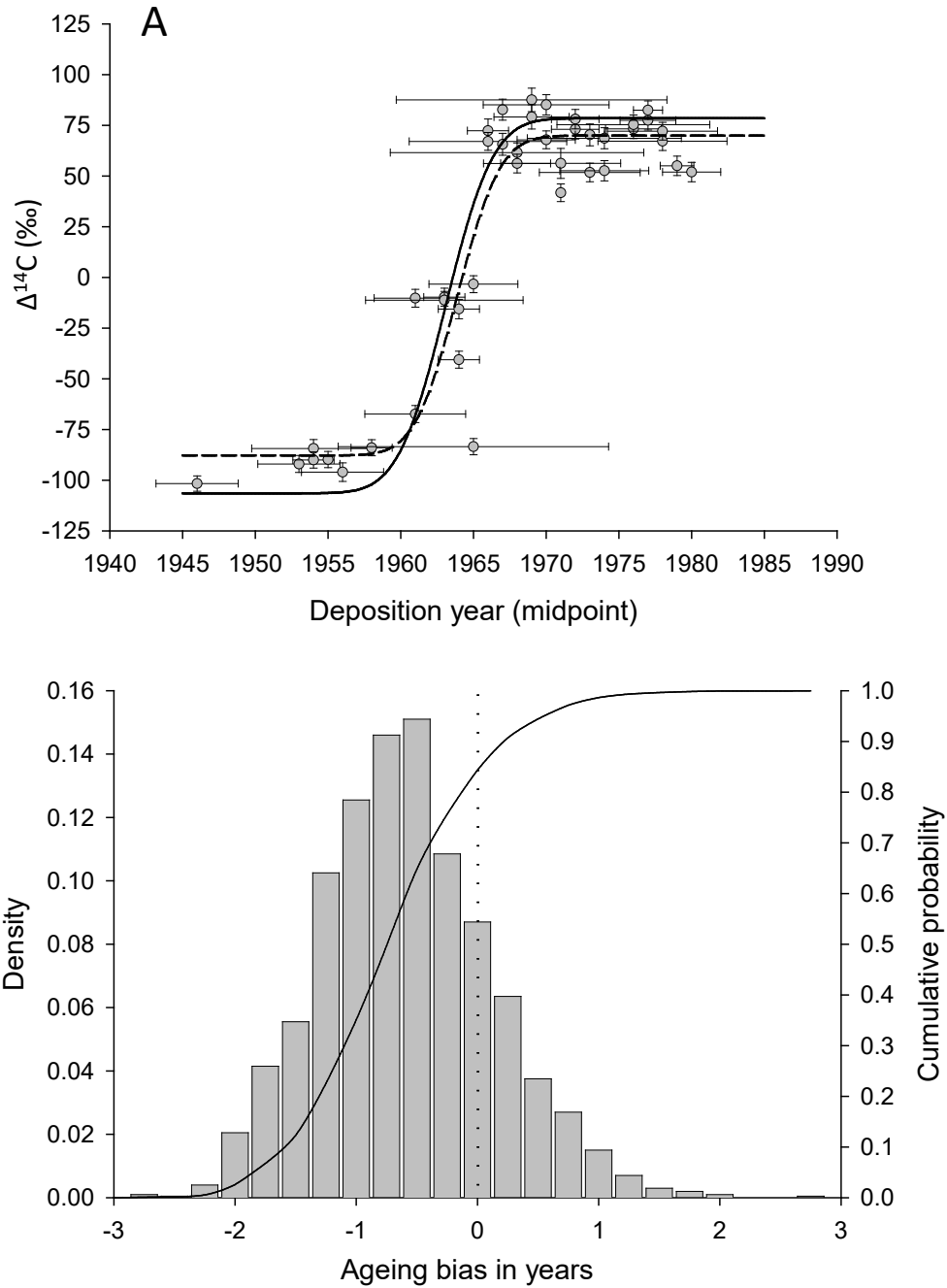


804

805

806 Figure 4

807

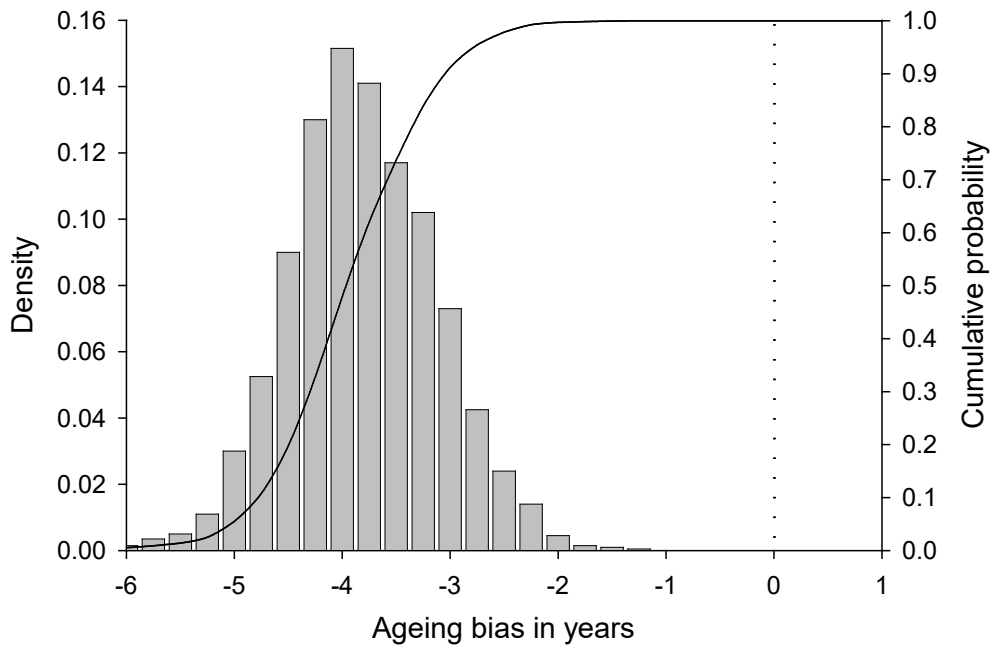
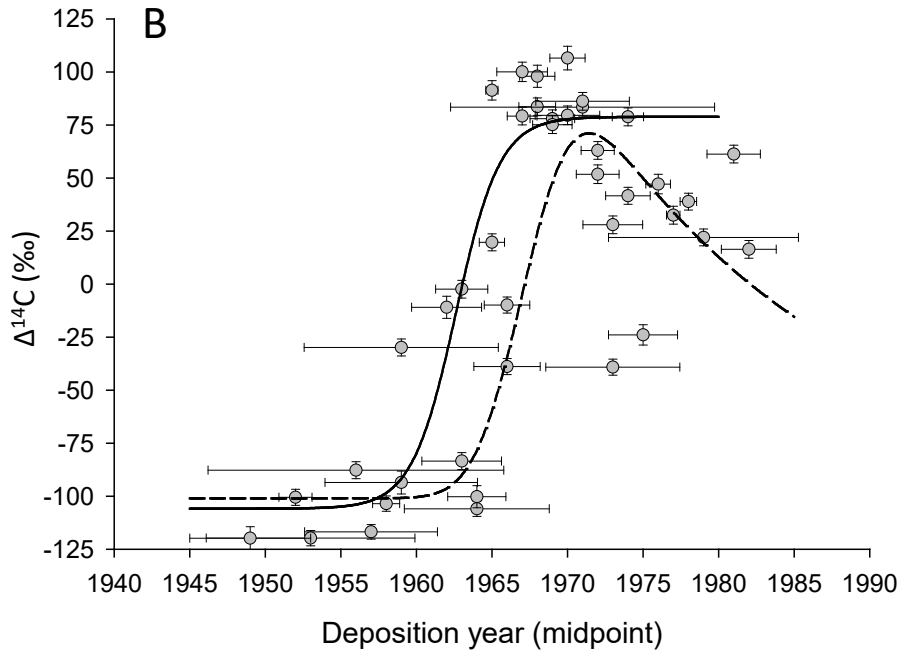


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809

810 Figure 4

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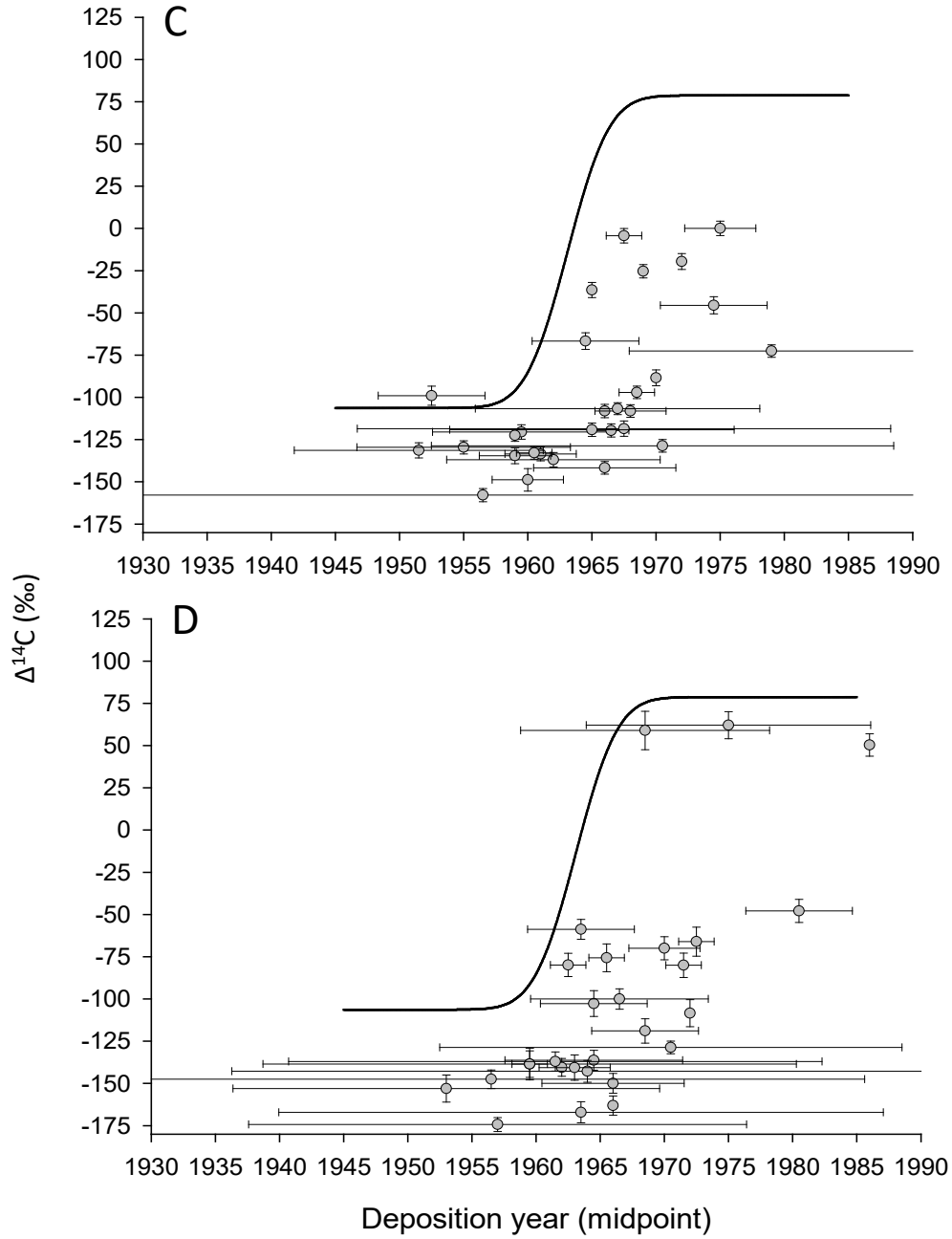


812

813

814 Figure 4

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816