

1 Evidence for systemic age underestimation in shark and
2 ray ageing studies

3 Systemic age underestimation of cartilaginous fishes

4 Effects of systemic age underestimation in cartilagnious fishes

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9 Running title: Age underestimation in sharks and rays

10 Abstract

11 Numerous studies have now demonstrated that the most common method of ageing sharks and rays, counting
12 growth zones on calcified structures, can underestimate true age. I reviewed bomb carbon dating (n=15) and
13 fluorochrome chemical marking (n=44) age validation studies to investigate the frequency and magnitude
14 of this phenomenon. Age was likely to have been underestimated in nine of 29 genera and 30% of the 53
15 populations studied, including 50% of those validated using bomb carbon dating. Length and age were
16 strongly significant predictors of occurrence, with age typically underestimated in larger and older individuals.
17 These characteristics suggest age underestimation is likely a systemic issue associated with the current
18 methods and structures used for ageing. Where detected using bomb carbon dating, growth zones were
19 reliable up to 88% of asymptotic length (L_{∞}) and 41% of maximum age (A_{Max}). The maximum magnitude
20 of age underestimation, Δ_{Max} , ranged from five to 34 years, averaging 18 years across species. Current
21 perceptions of shark and ray life histories are informed to a large extent by growth studies that assume
22 calcified ageing structures are valid throughout life. The widespread age underestimation documented here
23 shows this assumption is frequently violated, with potentially important consequences for conservation and
24 management. In addition to leading to an underestimation of longevity, the apparent loss of population age
25 structure associated with it may unexpectedly bias growth and mortality parameters. Awareness of these
26 biases is essential given shark and ray population assessments often rely exclusively on life history parameters
27 derived from ageing studies.

28 Keywords: age validation; bomb carbon dating; chondrichthyes; fluorochrome marking; life history; longevity

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42 Introduction

43 The credibility of fish age estimates derived from counts of concentric growth zones on calcified structures relies
44 on validating the temporal periodicity of formation over the lifespan of the species (Beamish and Mcfarlane
45 1983; Campana 2001). This basic tenet underlies a substantial amount of modern fisheries science including
46 any stock assessment that relies on ages derived from these methods, ranging from simple equilibrium methods
47 such as catch curves or per-recruit analyses to integrated population dynamics models (Maunder and Punt
48 2013). Age is also used in many qualitative risk-based methods (Patrick *et al.* 2010; Hobday *et al.* 2011)
49 and measures of intrinsic vulnerability (Dulvy *et al.* 2004; Cortés 2016), ultimately informing management
50 strategies for sustainable exploitation and conservation.

51 In chondrichthyan fishes (hereafter “sharks and rays”), where ageing methodology developed at a much
52 slower pace than teleosts, the first concerted efforts to determine age (Prince and Pulos 1983; Cailliet *et al.*
53 1986) coincided with a renewed focus on age validation across the wider discipline (Beamish and Mcfarlane
54 1983). The many reviews on shark and ray ageing have repeatedly emphasized the importance of validation
55 to practitioners (Cailliet *et al.* 1986, 2006; Cailliet 1990, 2015; Cailliet and Goldman 2004; Goldman *et al.*
56 2012). Accordingly, validation has become an integral and expected part of most studies, particularly when
57 using the most common method of counting growth zones on thin sections of vertebrae or spines.

58 Within the past two decades global ageing of shark and ray populations has increased rapidly, in parallel
59 with attempts by governments to implement policies of fisheries management and biodiversity conservation
60 to meet international commitments (Fischer *et al.* 2012). Operationalising these policies invariably requires
61 the assessment of fishing impacts on populations. This has major implications for shark and ray species, of
62 which an estimated one quarter are threatened by overfishing (Dulvy *et al.* 2014). In practice, it has meant
63 more regular stock assessments on target species, and risk assessments on non-target components of the catch
64 including bycatch and threatened species. With greater numbers and diversity of sharks and rays being aged
65 to support these assessments, corresponding studies have successfully validated part or all of the lifespan in a
66 growing number of species (Cailliet 2015).

67 Yet the increasing attempts at validating growth zones have not always yielded successful or expected results.
68 One issue that has become increasingly apparent is that of age underestimation, a phenomenon that appears
69 to result from growth zones ceasing to form or becoming unresolvable or unreliable beyond a certain size or
70 age. The first detailed account of age underestimation was in New Zealand porbeagle sharks (*Lamna nasus*,
71 Lamnidae) (Francis *et al.* 2007). Using bomb radiocarbon dating, levels of $\Delta^{14}\text{C}$ radio isotopes were found to
72 be 'phase-shifted' relative to reference chronologies. This implied that some sharks were more than twice as
73 old as originally thought and that growth zones were only reliable in individuals up to 20 years old. Similar
74 phase-shifts, which have also been termed 'missing time', have now been reported in several other species
75 studied using bomb carbon dating, including for populations where well-established and even previously
76 validated ageing protocols existed (Andrews *et al.* 2011; Passerotti *et al.* 2014).

77 Age underestimation is not only limited to bomb carbon dating and direct evidence has also been found using
78 other age validation techniques such as chemical marking. This includes cases where recaptured individuals
79 failed to deposit expected annual growth increments after several years at liberty (Chin *et al.* 2013; Harry *et*
80 *al.* 2013). Further circumstantial evidence that growth zone counts underestimate true longevity is apparent
81 from some long-term tagging studies, most strikingly in the school shark (*Galeorhinus galeus*, Triakidae)
82 where tag recaptures of up to 42 years at liberty are more than double the highest age based on growth zone
83 counts (Moulton *et al.* 1992; Francis *et al.* 2007).

84 The full extent of age underestimation in shark and ray ageing studies is unknown, and its implications
85 have not been investigated. McPhie and Campana (2009) suggest that improper age assignment due to age
86 underestimation in skates could have potentially severe management implications. Chin *et al.* (2013) state
87 that the underestimation of longevity would impact demographic analyses. Noting the increasing prevalence
88 in long-lived species, Natanson *et al.* (2014) questioned the value of vertebral ageing. Despite these concerns,
89 the *a priori* assumption that annual growth zones form throughout life persists (Cailliet and Goldman 2004;
90 Francis *et al.* 2007; Passerotti *et al.* 2014). In light of the mounting evidence for age underestimation there is
91 a need to re-examine this assumption.

92 To investigate this issue I reviewed two common approaches for age validation; chemical marking and bomb

93 carbon dating. Fluorescent dyes such as oxytetracycline (OTC) and calcein have successfully been used to
94 mark the calcified structures of tagged and recaptured or captive sharks and rays since the 1970s (Holden
95 and Vince 1973). The technique involves injecting a fluorescent marker into the muscle or body cavity of
96 the fish that is then rapidly incorporated into calcified structures providing a permanent record of when
97 the individual was caught (Izzo *et al.* 2007). The use of bomb carbon dating is more recent, but has been
98 applied to sharks and rays since the 2000s (Campana *et al.* 2002). This method involves comparing $\Delta^{14}\text{C}$
99 radio isotope levels in growth zones of unknown age with known-age reference chronologies (Kalish 1993).
100 The rapid increase in $\Delta^{14}\text{C}$ in marine dissolved inorganic carbon that occurred globally as a result of nuclear
101 testing during the late 1950s is preserved as a distinct signature in calcified tissues of organisms alive during
102 this period, allowing age estimates to be corroborated. These methods were chosen as they are generally
103 considered to be the most robust methods for validating periodicity of growth zone deposition in sharks and
104 rays (Goldman *et al.* 2012) and marine organisms more generally (Campana 2001). While the two techniques
105 differ considerably in their applications and are subject to a range of assumptions (Campana 1999), they are
106 nonetheless the most likely to detect age underestimation.

107 In reviewing evidence for age underestimation I (1) quantify its frequency of detection, (2) quantify its
108 magnitude and the lengths and ages at which it manifests, and (3) assess how pervasive it is likely to be in
109 shark and ray ageing studies. Using the New Zealand porbeagle shark as an example, I formulate hypotheses
110 about the potential impacts and management implications. I conclude by discussing options for addressing
111 the issue and the challenges of shark and ray age validation more generally.

112 **Materials and methods**

113 To determine the frequency of age underestimation, I conducted a comprehensive review of all bomb carbon
114 dating and chemical marking studies. I primarily restricted my search to peer-reviewed primary literature,
115 although some influential technical reports or books were also included. Prior to 2006 a series of literature
116 reviews on age determination in sharks and rays provided an exhaustive list of validation studies (Cailliet *et*
117 *al.* 1986, 2006; Cailliet 1990; Cailliet and Goldman 2004). Studies from 2006 onward were obtained through a

118 search of online databases. Long-term studies that resulted in multiple publications were grouped e.g. Smith
119 (1984), Kusher *et al.* (1992), and Smith *et al.* (2003).

120 Evidence of age underestimation was clear and definitive in some cases, but subjective in others. Based
121 on the strength of evidence, age underestimation was classified as either ‘likely’ or ‘possible’. The latter
122 category included cases where evidence was ambiguous, circumstantial in nature, or was retrospectively
123 invoked as an explanation. For example, interpretation of $\Delta^{14}\text{C}$ signatures used for bomb carbon dating
124 can be confounded by multiple factors. As diet is the source of carbon in shark and ray vertebrae, $\Delta^{14}\text{C}$
125 signatures are expected to be phase-shifted relative to reference chronologies because they feed upon prey
126 with a ^{14}C content different to the surrounding water (Campana *et al.* 2002). Depth-related attenuation,
127 interspecific variability, regional differences, ontogeny, and incorrect interpretation of growth zones may
128 further affect $\Delta^{14}\text{C}$ signatures (Campana 1999; Goldman *et al.* 2012; Andrews and Kerr 2015), making it
129 necessary to formulate hypotheses about the potential cause of any observed phase-shift.

130 Where age validation studies were accompanied by a full age and growth study, I also attempted to quantify
131 the magnitude of age underestimation at the population level. Information was collected on the range of
132 ages validated, the apparent longevity of the species (based on the highest growth zone counts), and the true
133 longevity of the species (based on age validation). These data were used to classify the lifespan into three
134 components; validated ages, uncertain ages, and underestimated ages (Table 1). The apparent proportion of
135 the lifespan that growth increments were reliable for was calculated based on these classifications, assuming
136 that the periodicity of growth zone deposition was the same for all ages (unless otherwise specified). Where
137 only lengths of validated individuals were provided, the inverse of the corresponding growth function was
138 used to determine the approximate range of ages validated.

139 Most bomb carbon dating studies reported the details of all individuals studied. This allowed the lengths
140 and ages in which age underestimation manifested to be investigated. In the subset of populations where it
141 was detected, data on the length, sex, apparent age, and true age (Table 1) were collated for each individual
142 studied. The mean and maximum difference between true and apparent age, Δ_{Mean} and Δ_{Max} respectively,
143 were determined for each population. Age was divided against the oldest individual reported (A_{Max}), and

length divided against asymptotic length (L_{∞}) or, if unavailable, the largest individual reported, to provide two dimensionless indices of relative age and length. These indices were used to statistically investigate the incidence of age underestimation as a function of length and age. Incidence of age underestimation was modeled separately as a function of relative length and age using Generalized Linear Models of the form

$$Y_i \sim \text{Bernoulli}(p_i)$$

148

$$p_i = \text{logit}^{-1}(\beta_1 + \beta_2 \cdot x_i)$$

where the probability, Y_i , that the age of sample i was underestimated, was approximated by a Bernoulli random variable, x_i is relative length or relative age, β_1 and β_2 are parameters estimated using maximum likelihood, and $\text{logit}^{-1}(X)$ is the logistic function $\exp(X)/(\exp(X)+1)$. Data were insufficient to include other explanatory variables (e.g. species, sex) in the model structure. All analyses and graphics were undertaken using R (R Core Team 2016).

Results and Discussion

Between 1973 and 2016 there were 58 unique studies including 44 that used chemical marking and 15 that used bomb carbon dating (a single study by Andrews *et al.* (2011) used both methods). In total 69 populations of chondrichthyans were studied of which 16, all chemically marked, were excluded from further analysis because they either failed to validate any regular temporal periodicity in growth zones, were purely methodological, or did not provide any details of the lengths or ages validated. The final data analysed included 20 populations validated using bomb carbon dating and 33 using chemical marking (see Supplementary Material for complete reference list and data).

The majority (91%) of studies were conducted on sharks including 25 species from 17 genera and four orders, with ground sharks (Carcharhiniformes) and mackerel sharks (Lamniformes) studied most frequently. Studies on rays and skates consisted of five species from five genera and two orders. No studies were done on chimaeras. Vertebrae were the primary ageing structure validated, making up 89% of studies, although a

166 small number of studies on dogfish (Squaliformes) and horn sharks (Heterodontiformes) used spines. Most
167 studies (85%) counted growth zones in thin-sections of vertebrae or spines, with the remainder using whole or
168 half structures for ageing.

169 **Frequency of detection**

170 Across both validation methods there were 21 populations identified where age underestimation was likely or
171 possible (Table 2). Age underestimation was detected most frequently with bomb carbon dating where it was
172 inferred from phase-shifted $\Delta^{14}\text{C}$ signatures (Fig. 1). The first phase-shift attributed to age underestimation
173 was documented in school sharks by Kalish and Johnston (2001), however the phenomenon did not gain
174 widespread exposure until its discovery in New Zealand porbeagle sharks (Francis *et al.* 2007). To date,
175 phase-shifts in 10 populations have been directly attributed to age underestimation. Documented cases
176 involved eight species from seven genera (Table 2). In general, the large temporal shift in $\Delta^{14}\text{C}$ signature (\geq
177 five years) led authors to conclude that age must have been underestimated, as phase-shifts of this magnitude
178 are inconsistent with other possible explanations (Goldman *et al.* 2012).

179 There were also several populations where unexplained, shorter, phase-shifts were observed. These were
180 distinct from phase-shifts attributable to the metabolically-derived carbon present in ageing structures, which
181 generally results in radiocarbon signatures lagging reference chronologies by a few years (Campana *et al.*
182 2002). Ontogenetic dietary and depth changes were invoked to explain the $\Delta^{14}\text{C}$ values in a northwest
183 Atlantic tiger shark (*Galeocerdo cuvier*, Carcharhinidae) that were phase-shifted relative to other individuals
184 studied (Kneebone *et al.* 2008). The authors noted parallels with a study on northeast Pacific white sharks
185 (*Carcharodon carcharias*, Lamnidae) (Kerr *et al.* 2006), however the phase-shift in that study was later
186 attributed to age underestimation (Andrews and Kerr 2015). Methodological error was thought to explain
187 phase-shifts in some samples of spiny dogfish (*Squalus* spp., Squalidae) (Campana *et al.* 2006) and shortfin
188 mako (*Isurus oxyrinchus*, Lamnidae) (Ardizzone *et al.* 2006) — the latter species is also further complicated
189 by its potentially variable growth zone deposition (Natanson *et al.* 2006; Kinney *et al.* 2016). Although
190 not considered in these cases, underestimation of age by a few years could conceivably be a causal factor.

191 Excluding these studies where evidence was ambiguous, age underestimation still likely occurred in a minimum
192 of 50% of bomb carbon dating studies (Table 2).

193 Fewer chemically marked populations exhibited signs of age underestimation; it occurred in eight populations,
194 including six considered to be likely and two possible (Fig. 2, Table 2). Likely cases involved six species
195 from four genera. They included several instances where chemical marks were visible on the distal margin of
196 vertebrae of individual specimens following recapture after several years, indicating that no growth zones
197 had been formed subsequently (Pierce and Bennett 2009; Chin *et al.* 2013; Harry *et al.* 2013; Frazier *et al.*
198 2014). Walker *et al.* (2001) also provided population-level evidence of age underestimation in school sharks, a
199 finding consistent with the bomb carbon dating by Kalish and Johnston (2001). Using analysis of variance,
200 the mean number of annual growth zones was found to be significantly less than one in length classes >1000
201 mm, with sharks > 1400 mm producing, on average, one growth zone every four years.

202 Age underestimation was possible in a further two chemically marked populations. Natanson (1993) hy-
203 pothesized that growth zone deposition may cease in reproductively active females based on a captive study
204 of little skates (*Raja erinacea*, Rajidae). Davenport and Stevens (1988) aged Australian blacktip sharks
205 (*Carcharhinus tilstoni*, Carcharhinidae) to 12 years old, using chemical marking and marginal increment
206 techniques for validation. A subsequent recapture after 18 years (estimated to have been at least two when
207 tagged) suggests substantially greater longevity (Harry *et al.* 2013). Similar long-term recaptures of school
208 shark and bonnethead sharks (*Sphyrna tiburo*, Sphyrnidae) have also been reported (Francis *et al.* 2007;
209 Frazier *et al.* 2014). The overall level of detection in chemical validation studies was lower than that of bomb
210 carbon dating. Nonetheless it was likely to have occurred in at least 18% of populations.

211 The limitations on both methods for detecting age underestimation, and also the difficulty of comparing
212 detection levels between them, are highlighted in Figures 1 and 2. Bomb carbon dating was typically done of
213 a small number of the oldest and largest individuals sampled and the method validates the entire lifespan
214 of those individuals (Fig. 1). As age underestimation is generally assumed to occur in older and larger
215 individuals, it follows that this method is more likely to detect it. Except in a few cases, chemical marking
216 typically only validated a small subset of the total lifespan of the population and did not include older

217 individuals, which are presumably both less abundant and less amenable to tag and recapture due to their
218 larger size (Fig. 2). As such, age underestimation may not be expected to be detected in as many chemical
219 marking studies.

220 At least two other factors may confound or limit the ability to detect age underestimation using these methods.
221 The failure of fluorochrome dyes to actually mark the vertebrae of some individuals was noted in numerous
222 chemical marking studies. For example, McFarlane and Beamish (1987) reported that OTC marks failed to
223 form in 34% of Pacific spiny dogfish (*Squalus suckleyi*, Squalidae) spines, and that marking success rates
224 varied between seasons. Walker *et al.* (2001) attributed this type of failure to either a lack of vertebral
225 mineralization due to low somatic growth rates or methodological error such as a failure to properly inject
226 chemicals into the body cavity of the fish. Since age underestimation is also typically attributed to the slowing
227 or cessation of growth, these outcomes may be confounded. If slow growth does indeed limit or stop the
228 incorporation of chemical markers in calcified structures it implies that they would no longer be useful as a
229 validation tool. The ability to detect a small underestimation of age using bomb carbon dating is also unclear
230 since it would presumably be difficult to distinguish from phase-shifts related to the metabolically-derived
231 carbon present in shark and ray ageing structures.

232 **Magnitude and manifestation**

233 The mean magnitude of age underestimation, Δ_{Mean} , detected using bomb carbon dating ranged from
234 five years in thorny skates (*Leucoraja ocellata*, Rajidae) (McPhie and Campana 2009), to 22 years in New
235 Zealand porbeagle sharks (Francis *et al.* 2007), averaging 13 years across all studies (Table 2). The greatest
236 individual disparity between true and apparent age, Δ_{Max} , was also in a New Zealand porbeagle shark
237 that was underaged by 34 years. However, this population appeared to be exceptional and the next largest
238 disparity was in a northwest Atlantic white shark underaged by 21 years (Hamady *et al.* 2014). Δ_{Max}
239 averaged 18 years across all studies. For chemically marked populations the estimation of Δ_{Mean} and Δ_{Max}
240 was limited by the short duration of studies and small sample sizes; both ranged from 2 to 3 years.

241 Among the 10 bomb carbon dating studies where age underestimation was likely, 61 individual samples
242 were pooled for analysis. With the exception of three outliers (two northeast Pacific white sharks and one
243 school shark), there were consistent patterns in the relative lengths and ages that it occurred in (Fig. 3a).
244 Relative length, in particular, was a strong and highly significant predictor of occurrence (Likelihood ratio test:
245 $\chi^2=62.33$, $d.f.=1$, $P<0.01$). At lengths above 88% of L_∞ more than half of all individuals were underaged
246 (Fig. 3b, Table 3). Relative age was also a highly significant predictor of occurrence (Likelihood ratio test:
247 $\chi^2=58.39$, $d.f.=1$, $P<0.01$). At ages above 41% of A_{Max} more than half of individuals were underaged (Fig.
248 3c, Table 3). This implies that, on average, ageing structures provided a valid record of age for less than half
249 of the maximum longevity of the species.

250 Using the definitions in Table 1, the extent of age underestimation in bomb carbon dating studies is even
251 more pronounced, since any ages above the age that it is first detected are effectively uncertain. Based on
252 these definitions, growth zones were a reliable and valid indicator of true age for approximately a third of
253 total lifespan (median = 34%) (Fig. 1, Table 2).

254 **How pervasive is age underestimation?**

255 Determining just how pervasive age underestimation is in sharks and rays is difficult since chemical marking
256 seems inherently less likely to detect it than bomb carbon dating. It is also possible that both methods may
257 be unable to detect it or decouple it from other processes in certain situations. Shark and ray age validation
258 studies in general were characterized by very low sample sizes – 57% of studies were based on fewer than 10
259 samples, and 17% were based on only a single individual (Fig. 1, Fig. 2). The relatively high frequency of
260 detection in spite of these small sample sizes suggests it must be relatively common.

261 Age underestimation occurred in at least nine different genera (Table 2), indicating it is a widespread
262 phenomenon. Although predominantly detected in two orders, the Lamniformes and Carcharhiniformes,
263 these were also by far the most intensively studied (see Supplementary Material). Age underestimation does
264 not seem to be restricted to particular life history types, and has been reported in some relatively small

265 and short-lived species such as blue-spotted mask rays (*Neotrygon kuhlii*, Dasyatidae) (Pierce and Bennett
266 2009) as well as large and long-lived species such as dusky sharks (*Carcharhinus obscurus*, Carcharhinidae)
267 (Natanson *et al.* 2014).

268 Although common and widespread, it does not seem to be ubiquitous, and was absent from some well-studied
269 species. Age underestimation has not been reported in gummy sharks (*Mustelus antarcticus*, Triakidae) a
270 species studied concurrently with school sharks over many decades in southern Australian waters (Walker
271 *et al.* 2001). Likewise, there is no evidence for it in the well-studied leopard shark (*Triakis semifasciata*,
272 Triakidae) (although Kusher *et al.* (1992) noted that OTC uptake was insufficient for age validation in 65%
273 of recaptured sharks). There is also evidence of intraspecific variability in its occurrence; it was absent in
274 northwest Atlantic porbeagle sharks (Campana *et al.* 2002) but present in New Zealand porbeagles (Francis
275 *et al.* 2007) (differences in sampling and exploitation history (Cassoff *et al.* 2007) should be noted here,
276 however).

277 Two interrelated and mutually inclusive hypotheses have been proposed to explain age underestimation in
278 shark and ray ageing studies:

279 1) it results from growth zones becoming vanishingly small and unresolvable on the margin of ageing
280 structures as individuals get larger and older, and as growth decreases (Francis *et al.* 2007; Chin *et al.*
281 2013; Hamady *et al.* 2014), or

282 2) it results from a temporary or permanent cessation of growth and growth zone formation (Natanson *et*
283 *al.* 2014) potentially occurring at any life stage, including in response to a reallocation of resources to
284 reproduction (Natanson 1993; Harry *et al.* 2013) or from “feast and famine” feeding behavior (Andrews
285 and Kerr 2015).

286 Current evidence does not clearly favor either hypothesis. On the one hand, age determination in many
287 sharks and rays is inherently difficult, and progress in ageing slow due in large part to the limitations of
288 available ageing structures (Cailliet and Goldman 2004; Goldman *et al.* 2012). Intraspecifically, counts and
289 readability of growth zones have been shown to vary depending on the method of preparation (Irvine *et al.*

290 2006), reader experience (Officer *et al.* 1996), type of structure used (Bubley *et al.* 2012), and region of the
291 vertebral column sampled (Natanson and Skomal 2015). The hypothesis that growth zones simply become
292 unresolvable is appealing since it implies that, with the right method of preparation, the true age may still
293 be attainable. However, it also risks overlooking the extensive research done with the aim of enhancing the
294 readability of ageing structures. This includes histology (Natanson 1993), radiography (Aasen 1963), scanning
295 x-ray fluorescence microscopy (Raoult *et al.* 2016), micro-computed tomography (Geraghty *et al.* 2012) and
296 a multitude of staining and sectioning techniques (Cailliet *et al.* 1983; Goldman *et al.* 2012). While these
297 techniques have sometimes revealed underageing (Francis *et al.* 2007; Maurer 2009), none has yet indicated
298 that counts may be underestimated by the large magnitudes documented here.

299 On the other hand, the hypothesis that age underestimation is due to growth zones ceasing to form also has
300 foundation. Otolith mineralization differs from most other calcified structures found in vertebrates, taking
301 place within an acellular medium known as endolymph with deposition of new material occurring daily
302 (Morales-Nin 2000; Payan *et al.* 2004). Francis *et al.* (2007) note that “vertebrae form part of the axial
303 skeleton of a shark so, in theory, if the shark’s somatic growth ceases, then deposition of material on the
304 outer margin of the vertebrae should also cease”. Elasmobranch vertebral centra, the structures used most
305 commonly for age determination, are a form of densely calcified ‘areolar’ cartilage found uniquely within
306 the vertebrae (Dean and Summers 2006). Unlike bone, mineral content in shark and ray cartilage has been
307 shown to vary within individuals, intraspecifically, and interspecifically (Porter *et al.* 2007). Indeed, the
308 vertebral centra of sharks and rays are so variable that they were initially thought to be useful for taxonomic
309 classification (Ridewood 1921). In addition to this, the physiological and mechanical factors that mediate
310 mineralization of vertebrae are still largely unknown (Dean and Summers 2006; Porter *et al.* 2007), despite
311 long being recognized as centrally important to ageing (Cailliet *et al.* 1986). So while counts of growth zones
312 obtained from shark and ray ageing structures are essentially treated the same as those from otoliths, the
313 underlying processes that generate them have not been demonstrated to be the same, and may well differ.

314 Evidence from bomb carbon dating, where age underestimation manifested almost entirely in larger and
315 older individuals, is consistent with the suggestion that growth zone periodicity changes or ceases later in life,

316 potentially after the onset of sexual maturity (Casey and Natanson 1992; Natanson *et al.* 2016). While no
317 conclusive link with maturation has been demonstrated, this would provide a physiological mechanism to
318 explain age underestimation. Growth zone formation has also recently been shown to shift from biannual
319 to annual after maturity in shortfin mako vertebrae (Kinney *et al.* 2016). Many sharks and rays have
320 reproductive cycles that last two or more years, so if the timing and frequency of growth zones are linked to
321 reproductive events, associated changes in the frequency of growth zone formation could be difficult to detect,
322 and may only gradually become evident after several full reproductive cycles.

323 At present little can be definitively said about the mechanisms underlying age underestimation. In summary
324 though, it appears to be common and widespread and manifests in similar ways across a range of species. On
325 the balance of this evidence, it seems likely to be a systemic issue associated with the current methods and
326 structures used for ageing sharks and rays. While it may not occur in all species, it will probably continue to
327 be detected as validation studies increase and expand across taxa, particularly if ageing methods remain
328 unchanged.

329 **Implications**

330 The findings of this study are relevant to the numerous species that have been and continue to be aged
331 by counting growth zones on calcified structures. Importantly, this study confirms that it is no longer
332 sufficient to assume growth zones are deposited regularly on ageing structures throughout life, reinforcing
333 the need for ongoing validation of all age classes. Yet while numerous studies have now documented age
334 underestimation, there has still been no directed investigation of its effects and only limited discussion of
335 its possible implications. As a starting point I consider the potential effects in relation to two fundamental
336 population processes, growth and mortality.

337 **Growth**

338 One of the most clearly apparent effects of underestimating age relates to modelling growth. Since ages from

339 growth zone counts are an unknown function of true age, it follows that any growth parameters estimated
340 from these data are likely to be biased. Francis *et al.* (2007), the only authors to so far correct for age
341 underestimation, used a modified Von Bertalanffy growth function to adjust growth zone counts to reflect
342 true ages. Natanson *et al.* (2016) on the other hand, opted to remove all ages deemed unreliable (> 14 years)
343 and used only back-calculated data when re-fitting growth curves to account for age underestimation in
344 common thresher sharks (*Alopias vulpinus*, Alopiidae). In both these particular cases the resultant growth
345 curves were similar to those fit to the original data, suggesting that any bias was minimal. Indeed, this may
346 be expected since age was underestimated in individuals that had completed most of their growth already.

347 Simulating data from the models in Francis *et al.* (2007) suggests that age underestimation could still have
348 a pronounced impact, especially when sample sizes are small. Through the underestimation of age, there
349 is an apparent ‘loss’ of older individuals from length-at-age data. This effectively truncates the asymptote
350 of the growth curve, presumably making it more difficult to obtain an unbiased estimate of L_{∞} (Fig. 4a).
351 L_{∞} is strongly, negatively correlated with the growth coefficient, k , in many commonly used models for
352 describing fish growth. As such, a positively biased (larger) L_{∞} associated with this apparent absence of
353 older individuals would lead to a negatively biased (lower) k , somewhat analogous to the bias generated by
354 length-selective fishing gears such as gillnets (Walker *et al.* 1998; Thorson and Simpfendorfer 2009). This
355 phenomenon was seen in northwest Atlantic dusky sharks; using validated data points corrected for age
356 underestimation led to an increase in the estimated growth coefficient of 6-17% and a decrease in L_{∞} of 8-9%
357 (Natanson *et al.* 2014). Growth coefficients, k , estimated from vertebral ageing of school sharks, where ages
358 are known to be substantially underestimated, were 24-26% lower than estimated from two long-term tagging
359 studies (Moulton *et al.* 1992). Thus, a paradox of underestimating age is that the bias introduced may lead
360 to an underestimate of the growth coefficient, not an overestimate, as would be intuitively expected.

361 Francis *et al.* (2007) showed that simple corrections can account for some of the bias associated with age
362 underestimation. However, under even slightly more complex assumptions it is clear that additional biases
363 would be more difficult to correct for. For example, assuming that the growth coefficient (i.e. k) varies among
364 individuals in a population and that age underestimation is predominantly a function of length, it would be

365 expected to disproportionately impact faster growing individuals (Fig. 4b). At the very least it is likely to
366 further add to the challenge of estimating robust growth parameters in sharks and rays by compounding
367 biases caused by length selective fishing, low sample sizes, and growth model uncertainty.

368 Mortality

369 The more directly obvious impact of age underestimation is on longevity. The frequency and magnitude of
370 age underestimation documented here indicates that many sharks and rays are likely to live much longer
371 than currently thought. This is important since longevity is frequently used to make inferences about natural
372 mortality, M , a parameter that is highly influential in population models and that nearly always has to be
373 pre-specified for information-limited taxa such as sharks and rays (Kenchington 2014). Reduced longevity is
374 also symptomatic of a more general ‘loss of age structure’ caused by age underestimation, which presents a
375 potentially more serious issue than biased growth parameters. When using age structured population models
376 that are fit to these data such as catch curves (Simpfendorfer 1999; Robbins *et al.* 2006), this apparent loss
377 of population age structure could be inadvertently attributed to or indistinguishable from fishing mortality
378 (Fig. 4c).

379 Understanding the impacts of these biases on populations, which are influenced by complex and non-linear
380 dynamics, is not straightforward. Shorter-lived species are generally more productive (i.e. have a higher
381 recruitment). The much greater longevity found for many species may indicate they have a lower natural
382 mortality and in turn lower productivity and resilience to fishing than currently thought. In contrast, in a
383 simple demographic analysis assuming Hoenig mortality for New Zealand porbeagles, use of the true longevity
384 of 65 years results in a much greater net reproductive rate, R_0 , than if the apparent longevity of 38 years is
385 used. This translates to a population doubling time of ~9 years compared with ~22 years (Fig. 4d), implying
386 much greater population productivity. In sand tiger sharks (*Carcharias taurus*, Odontaspidae) it was noted
387 that the upward revision of lifespan to 40 years would lead to at least five times more reproductive years
388 than previously thought, again implying increased productivity (Passerotti *et al.* 2014).

390 These examples, while simplistic, highlight the potential ways that age underestimation may have skewed
391 understanding of shark and ray population dynamics. With only a handful of long-term, empirical observations
392 of wild shark and ray populations (e.g. Feldheim *et al.* (2014)), current perceptions of population dynamics,
393 including key areas such as fisheries productivity reference points and extinction risk, are strongly shaped by
394 comparative life history studies (Cortés 2000; Frisk *et al.* 2001, 2005; Pardo *et al.* 2016) and meta-analyses
395 (García *et al.* 2008; Hutchings *et al.* 2012; Zhou *et al.* 2012) that draw heavily upon age and growth studies.

396 Such are the data limitations for many populations that assessments are often based solely on life history
397 information (Cortés 2002, 2016; Brooks *et al.* 2009). This means that the above issues have the potential
398 to extend well beyond the specific examples above. For example, age-at-maturity, another key determinant
399 of population productivity (Smith *et al.* 1998; Pardo *et al.* 2016), is frequently obtained from the inverse
400 of a growth curve at length-at-maturity, meaning it too would be susceptible to biased growth parameters.
401 Sidestepping the use of biased parameters altogether is also difficult. While it may be possible to use
402 assessment techniques that avoid specifying, say, longevity (Xiao 2002; Skalski *et al.* 2008), it is generally not
403 possible to avoid M altogether. Furthermore, almost all life history invariant techniques that are used to
404 pre-specify M require the use of length-at-age, growth parameters, or longevity (Kenchington 2014; Then *et*
405 *al.* 2014). These dependencies mean there is considerable potential for ageing error to propagate through
406 population assessments.

407 Through these types of biases, age underestimation may have important flow-on effects for fisheries management
408 and conservation. With age-structured population models increasingly being used to support management
409 of commercially exploited stocks (Cortés *et al.* 2012), model misspecification due to ageing error has the
410 potential to affect scientific advice such as acceptable catch limits. The impacts that these types of errors
411 can have are well documented in teleosts (Yule *et al.* 2008; Melvin and Campana 2010). For example,
412 underestimation of longevity in orange roughy (*Hoplostethus atlanticus*, Trachichthyidae) (Smith *et al.* 1995)
413 and Pacific ocean perch (*Sebastes alutus*, Sebastidae) (Beamish 1979) led to overly optimistic estimates of
414 stock productivity, in both cases contributing to serious, long-term ecological and socio-economic impacts on
415 these resources.

416 Given that sharks and rays are often not the target of fishing activities, management strategies are typically
417 less responsive. They are often centered around the identification and protection of high-risk species or
418 mitigation of high-risk activities using measures such as spatial and temporal closures and species catch
419 restrictions. Because of this, the ultimate impacts of age underestimation from a conservation or fisheries
420 management perspective may well be more insidious, occurring through the misclassification of high-risk
421 species, leading to inefficient prioritization of research, monitoring, and management measures. If age
422 underestimation is indeed as widespread and common as indicated from this study, the impacts could also
423 be substantial from a wider scientific perspective, affecting the many disciplines that also use baseline life
424 history data.

425 The implications of age underestimation are also by no means restricted to sharks and rays, and this study
426 serves as a reminder of the potential impacts on fish more generally. While the processes governing otolith
427 mineralization are well understood, many species, particularly long-lived and deep-water, are nonetheless
428 difficult to age, which can lead to underestimation of true age. Similar phase-shifts to those reviewed here
429 have been documented in bocaccio rockfish (*Sebastes paucispinis*, Sebastidae) (Andrews *et al.* 2005), for
430 example. The use of whole rather than sectioned otoliths has also frequently been shown to underestimate
431 age (Bennett *et al.* 1982; Dwyer *et al.* 2016). For example, the use of whole otoliths likely resulted in a biased
432 growth curve for southern bluefin tuna (*Thunnus maccoyii*, Scombridae) (Gunn *et al.* 2008). In addition
433 to this, a range of structures other than otoliths are routinely used to age fish, many of which have been
434 shown to underestimate age including scales (Secor *et al.* 1995), fin-rays (Rien and Beamesderfer 1994), and
435 vertebrae (Gunn *et al.* 2008). In most of these instances, the impacts of ageing error are presumably isolated
436 to specific populations. Nonetheless, for groups of species that are difficult to or unable to be aged using
437 otoliths, the potential exists for more widespread biases to manifest.

438 **Confronting the challenges of shark and ray ageing**

439 The outcomes of this study highlight the ongoing difficulties of ageing sharks and rays using calcified
440 structures, particularly the validation of growth zones in older individuals. At present it is unclear whether

441 the underestimation of ages is a major problem, or perhaps a simple bias that, for some applications, could
442 be corrected with adjustments like those made to otolith back-calculations (Campana 1990) or worn spines
443 (Taylor *et al.* 2013). Although this study is the first time data have been synthesized and quantified on age
444 underestimation, the issue is far from new (Kalish and Johnston 2001). But despite repeated invalidation of
445 ageing structures in older individuals of a range of species, it has not yet led to widespread changes to ageing
446 practices or growth modeling.

447 Understanding the extent of the problem and ultimately addressing it will require, in the first instance, more
448 data. While the number of age validation studies has increased in recent years, the studies reviewed here
449 cover fewer than 5% of described species (Fig. 1 and Fig. 2). As noted by Cailliet (2015) and emphasized by
450 Figure 2, even fewer of these species have been validated convincingly. Bomb carbon dating appears to be
451 the most effective tool currently available for age validation of sharks and rays; it is the most likely to give a
452 true indication of longevity and detect whether age has been underestimated. The high cost of such studies,
453 the increasing difficulty of obtaining archived samples born prior to the rise in $\Delta^{14}\text{C}$, and the unsuitability
454 for shorter-lived species mean it is not a 'silver bullet'.

455 Fluorochrome chemical marking is clearly an effective tool in some situations, however this study raises
456 questions about its usefulness for validating older age-classes and why it seemingly fails in many cases. The
457 ongoing use of chemical markers in laboratory-based studies to quantify mineralization rates may help resolve
458 some of these questions (Officer *et al.* 1997), particularly if they can be extended to examine the influence
459 of factors such as length, age, and maturity stage. Extending the inferences from these studies to wild
460 populations is always problematic, and therefore long-term tag-recapture studies targeting both juveniles and
461 adults are the only way to convincingly validate age and the efficacy of chemical marking in older individuals.
462 As this study shows, the costs and logistical difficulties of conducting either type of study have so far made
463 them feasible for a very small number of stocks globally, and this seems unlikely to change in the foreseeable
464 future.

465 As the enormity and complexity of the conservation challenges facing sharks and rays globally becomes
466 increasingly clear (Dulvy *et al.* 2014, 2017), there may be a need to confront the wider issue, that calcified

467 structures may be insufficient to meet the ageing needs of many species. While there are many excellent
468 examples that illustrate that sharks and rays can be aged successfully using calcified structures, after more
469 than 50 years of ageing, one of the most consistent feature of such structures is their inconsistency (Goldman
470 *et al.* 2012; Cailliet 2015). With no foreseeable end to the difficulties associated with traditional ageing
471 structures, it may be time to look for solutions that complement existing methods and can assist in building
472 the deeper understanding of growth and mortality needed for effective conservation and management.

473 One such solution could include a shift in focus toward the use of alternative data sources, for example
474 tag-recapture and length-based data for estimating growth. Although commonly used, there has been a
475 tendency to view these data as less credible compared to those derived from calcified ageing structures which,
476 until now, have been seen as the more reliable source of information. Indeed, many of the studies reviewed
477 here also included tag-recapture components that were often discussed in less detail and rarely considered the
478 ‘preferred’ growth model (Davenport and Stevens 1988; McAuley *et al.* 2006; Kneebone *et al.* 2008; Andrews
479 *et al.* 2011). Comparison of growth parameters derived from populations studied simultaneously using both
480 methods may also be one of the only ways to empirically determine whether some of the expected biases
481 proposed here really exist.

482 In recent years, tagging programs for sharks and rays have increasingly been initiated for various non-fishery
483 related applications (e.g. acoustic telemetry, ecological study, human-safety) and by non-fisheries institutions
484 (e.g. universities, ENGOs). Such programs can potentially be of considerable value in supplementing the
485 limited resources of fisheries agencies for obtaining long-term data on growth. The use of tagging and
486 length-based methods is also consistent with the shift towards non-lethal alternatives to sampling that are
487 now increasingly being advocated (Hammerschlag and Sulikowski 2011). Opportunities for collecting and
488 making tag-recapture data publicly available for age and growth estimation purposes should be embraced
489 where possible.

490 Conclusions

491 *The prevalence and impact of inaccurate age determinations on the accuracy of population dynamics*
492 *studies cannot be overstated* (Campana 2001)

493 The study of shark and ray life history has evolved in much the same way as in teleost fish. It is, in large
494 part, built upon the ageing of individuals from which parameters such as growth, age at maturity, longevity,
495 and mortality are estimated or indirectly inferred. The simple assumption underpinning this process is
496 that counts of growth zones do indeed provide a valid record of age throughout life. The comprehensive
497 review of bomb carbon dating and chemical marking age validation studies undertaken here shows that this
498 assumption is frequently violated, highlighting a systemic problem associated with the most common method
499 and structures used for ageing. Given the inherent difficulties in studying wild populations, age and growth
500 studies form the basis of much of the current understanding of shark and ray population dynamics. So while
501 age underestimation, in isolation, may be regarded as a relatively unimportant or minor issue, its impacts
502 could still be far reaching and warrant closer attention. The ongoing potential for it to compound the existing,
503 significant challenges in obtaining accurate estimates of growth, longevity, and mortality for sharks and rays
504 should not be overlooked.

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