1 **Using genetic inference to re-evaluate the minimum longevity of the lemon shark,**
2 ***Negaprion brevirostris***

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28   Accurate life history parameters are critical for effective fisheries management, in particular,

29   knowing the maximum life span of a species. Here we use a combination of mark-recapture and

30   genetic sampling to extend the minimum longevity of an elasmobranch species. Using this

31   approach, life span estimate of the lemon shark, *Negaprion brevirostris*, was increased

32   conservatively from 20.2 years to 37 years. This increase in longevity means higher vulnerability

33   and a longer recovery time from exploitation.

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38   Accurately modeling population dynamics is impossible without an understanding of how fast

39   individuals are naturally removed from the population, and how many offspring they produce

40   over a lifetime, both of which are influenced by life span (Hoenig, 1983). Life spans of long-

41   lived fishes are often underestimated, which has implications for calculating natural mortality

42   rates and intrinsic rebound potentials and can lead to mismanagement of stocks (Cailliet and

43   Andrews, 2008). If a species is longer-lived than originally modelled, for example, life-time

44   fecundity is underestimated, but instantaneous rates of natural mortality are overestimated.

45   Across shark species, population productivity is negatively correlated with maximum

46   reproductive age (Smith *et al*., 1998), therefore an increase in the known maximum age within a

47   population suggests the stock is actually more vulnerable to overexploitation.

48   Several methods for validating age have been developed and applied to sharks and batoids

49   (elasmobranchs; Campana, 2001). Traditional age determination techniques involve analyzing

50   growth rings in vertebral centra, dorsal fin spines and caudal thorns (Brown & Gruber, 1988;

51   Cailleit *et al*., 2006). However, considerable variability exists in calcification patterns, and thus

52   the utility of aging techniques, within and among taxonomic groups of elasmobranchs (Goldman,

53   2005). The precision (verification) and accuracy (validation) of aging techniques should be

54   assessed in all studies (see review by Cailliet *et al*., 2006) using determinate methods such as

55   mark-recapture of known-age or chemically tagged fish or dating using lead:radium or bomb

56   radiocarbon, or through indeterminate methods such as marginal increment analysis (Beamish &

57   McFarlane, 1983; Brown & Gruber, 1988; Campana, 2001). Age estimation of older sharks

58   using band pairs is also less accurate due to a decrease in somatic growth, leading to a decrease

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59 in the rate of deposition of vertebral material (Francis *et al*., 2007; Andrews *et al*., 2011; Hamady

60 *et al*., 2014). Many original life span estimates have been re-evaluated using bomb radiocarbon

61 methods and have often been revised upwards substantially (Campana, 2001; Cailliet &

62 Andrews, 2008).

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64 The lemon shark *Negaprion brevirostris* (Poey, 1868) is a large coastal shark that is found on

65 both sides of the Atlantic Ocean and the Eastern Pacific, where it is exploited by recreational and

66 commercial fisheries (Compagno 1984; Carlson *et al*., 2012; Shiffman & Hammerschlag, 2014).

67 Brown & Gruber (1988) used vertebral centra to age 110 *N. brevirostris* in Florida, U.S.A. and

68 Bimini, Bahamas. Annual band deposition on the vertebrae was validated through mark-

69 recapture of chemically-tagged individuals. Of the 110 animals aged, the oldest estimated was

70 20.2 years for a 226 cm pre-caudal length (L_{PC}) male. The study suffered, as many do, from low

71 sample sizes and hence the growth curve did not asymptote. Hoenig & Gruber (1990) estimated

72 that the theoretical maximum age for lemon sharks was 26 years since the largest specimens

73 from the Brown & Gruber (1988) study were considerably smaller than the maximum reported

74 size. It was hypothesized that the maximum longevity for lemon sharks had been largely

75 underestimated and could be extended using previously documented age at maturity (Brown &

76 Gruber, 1988), and an extensive genetic pedigree of offspring within the nurseries (Feldheim *et*

77 *al*., 2014).

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78 Adult female *N. brevirostris* were aged in one of two ways: parental genotype reconstruction or

79 parental genotype reconstruction combined with tag-recapture. Parental genotype reconstruction

80 involved exhaustively sampling 0-3 year old *N. brevirostris* at Bimini (25°44′N, 79°18′W),

81 Bahamas, every year since 1993 (Feldheim *et al*., 2014). Sampling occurred each June using

82 monofilament gillnets (180 m length, 2 m height) set perpendicular from shore. All captured *N.*

83 *brevirostris* were measured (cm) for pre-caudal length (L_{PC}), fork length (L_F) and total length

84 (L_T), sex determined, and tagged with a passive integrated transponder tag (PIT, Destron Fearing,

85 South St. Paul, MN, USA). See Gruber *et al*., 2001, for a full description of methods. A small

86 piece of fin was removed and stored in 20% DMSO solution for genetic analysis. Individuals

87 were released alive after a brief holding period of no more than 7 days (Feldheim *et al*., 2014).

88 DNA from all *N. brevirostris* were genotyped at 11 polymorphic microsatellite markers followed

89 by sibship and parental genotype reconstruction (for detailed methods see Feldheim *et al*.,

90 2002*a*; Wang, 2004; DiBattista *et al*., 2008; Feldheim *et al*., 2014). Genetic profiling of *N.*

91 *brevirostris* from 20 consecutive cohorts (1993-2012), showed that certain females faithfully

92 gave birth at this site for nearly two decades (Feldheim *et al*., 2014). This enabled estimation of

93 minimum age of these individuals, with the conservative assumption being made that their first

94 genetically confirmed litter in Bimini was their first litter. Brown & Gruber (1988) estimated age

95 of maturity of 12.7 years with first parturition at 14 years, which was concordant with direct

96 observations of philopatric females of known age first returning to Bimini for parturition

97 (Feldheim *et al*., 2014). This study, therefore, conservatively estimated the age of 19 females that

98 gave birth multiple times at Bimini by assuming that they were age 14 years the first year we

99 sampled any of their offspring, and died the last year we sampled any of their offspring.

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101   Tag recapture was also used to directly estimate age of one tagged female. Gravid females are

102   annually targeted for capture in late March and April, as they enter the Bimini lagoon as part of

103   an ongoing study of natal philopatry. Individuals were either captured by closely following the

104   shark from a skiff in shallow water (< 2 m depth), then placing a dip-net on its snout to incite it

105   to bite the mesh and become tangled, or encircling the shark with a large seine net deployed from

106   two small skiffs. Individuals were fitted with National Marine Fisheries Service (NMFS) M-type

107   dart tags (Kohler *et al*., 1998) and tagged with electronic PIT tags unless they were already

108   tagged.

109   Parental genotype reconstruction (Feldheim *et al*., 2014) revealed that at least 19 female *N.*

110   *brevirostris* giving birth at Bimini were older than Brown & Gruber's (1988) oldest individual

111   (Table I), including B-female 12, estimated to be at least 37 years old when last detected.

112   A mature female *N. brevirostris* with fresh mating wounds was captured using the netting

113   method on May 20th, 2014. A Vemco ® V16-6H tag was surgically implanted into the body

114   cavity (see Kessel *et al*., 2014 for method).The shark had a broken NMFS dart tag and an intact

115   PIT tag, (ID A4D11). Records indicate that her first capture was 17 years earlier on April 18th,

116   1997, at a site less than 8 km away (Figure 1), with L_{PC} (196 cm) and L_T (252 cm), and a bulging

117   abdomen with movement of pups indicating this shark was pregnant at the time. The second

118   capture of this shark was on April 28th, 2005, and again, she was suspected to be pregnant.

119   According to the sibship pedigree (Feldheim *et al*., 2014), a total of 65 of her pups have been

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120 sampled on a biennial basis between June 1997 and June 2013 (Table II). Brown & Gruber

121 (1988), estimated age of maturity of 12.7 years and first parturition at 14 years, which is

122 concordant with Feldheim *et al.* (2014). Based on this, A4D11 was at least 31 years old when

123 captured in 2014, and 32 years old when she was detected on multiple Vemco ® acoustic

124 receivers in Bimini waters in April, 2015 (Guttridge, T. L. pers. observation). This shark had a

125 pre-caudal length measurement of 203 cm in 2014; however, 23 cm smaller than the 20.2 year

126 old male sampled by Brown & Gruber (1988).

127 This study provides the first example of using genetic pedigree reconstruction and mark-

128 recapture to estimate the age of a chondrichthyan fish. It revises the minimum longevity of *N.*

129 *brevirostris* by nearly a factor of two from 20.2 years to 37 years, however, actual life span could

130 be longer. For example, genetic sampling of *N. brevirostris* in Bimini began in 1990; 'B-Female

131 12' (the oldest shark) was first detected in Bimini in 1989 with one offspring (sampled as a 1-

132 year old in 1990), so could have pupped in the years prior to sampling efforts. Further, our age

133 estimates are based on a minimum age at first parturition of 14 years. Of the six known-aged

134 sharks in Feldheim *et al.*'s (2014) genetic pedigree, the range of ages of first parturition in

135 Bimini was 14-17 years, although we cannot be sure they only gave birth in the Bimini nurseries.

136 Accurate age and life span information are an important component of any demographic

137 modeling, as maximum age and age at maturity are often used to estimate instantaneous rates of

138 natural mortality (Simpfendorfer *et al.*, 2005), as well as, intrinsic rebound potentials and

139 population doubling times (Simpfendorfer 2005, Smith *et al.*, 1998). This study was limited to

140 aging only female *N. brevirostris*. However, previous longevity studies have shown maximum

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141 ages of females are greater than those of males in numerous shark species (Carlson & Parsons,

142 1997; Driggers *et al*., 2004). In addition, it is pertinent to note that for population modelling,

143 female life history parameters are considered more relevant as total lifetime fecundity and

144 mortality determine population numbers (Bishop *et al*., 2006). Intrinsic rebound potential is a

145 demographic parameter that gives a population's productivity after removal of a proportion of

146 the population, and can be useful for understanding the status of exploited populations (Au *et al*.,

147 2015). Rebound potential is easily calculated for a given total mortality, needing only age at

148 maturity and natural mortality for a given species (Au *et al*., 2015). Then *et al* (2014) concluded

149 that from over 200 direct estimates of natural mortality rates, using a maximum-age based

150 estimator performs the best among all estimators evaluated. Previous intrinsic rebound

151 calculations have incorporated the longevity estimate of 25 years for *N. brevirostris* (Smith *et al*.,

152 1998), or Hoenig's (1983) formula for natural mortality rates (which also uses the maximum age

153 of 25 years). With this updated minimum longevity, the estimated natural mortality rate

154 ($M=4.899*tmax^{-0.916}$) for *N. brevirostris* has decreased from $0.257yr^{-1}$ to $0.179yr^{-1}$ (Then *et al*.,

155 2014). This increase in life span could mean an increase in the time it takes an exploited

156 population to rebound back to stable numbers.

157 Although there is evidence of gene flow amongst *N. brevirostris* Northern Hemisphere

158 populations (Ashe *et al*., 2015) and telemetry data indicating connectivity between the USA and

159 the Bahamas (unpublished data), the genes related to longevity have not yet been identified so

160 we cannot be certain that the longevity found in this study applies throughout their entire

161 distribution. This study may prove difficult to replicate in other species but highlights the

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162 advantage and importance of long-term, standardized tagging projects, and the collection and

163 analysis of genetic samples for life-history parameter research.

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