- 1 Inferring the Demographic History of Japanese Eel (*Anguilla japonica*) from Genomic Data:
- 2 Insights for Conservation and Fisheries Management
- 4 Leanne Faulks¹, Prashant Kaushik¹, Shoji Taniguchi¹, Masashi Sekino², Reiichiro
- 5 Nakamichi², Yuki Yamamoto², Hiroka Fujimori¹, Chiaki Okamoto¹, Sakie Kodama¹, Ayu
- 6 Daryani¹, Angel Faye Manwong¹, Ishmerai Galang¹, Noritaka Mochioka³, Kiyo Araki⁴,
- 7 Motoo Suzuki⁵, Yoshitsugu Kaji⁶, Takumi Ichiki⁷, Tetsuya Matsunaga⁸, Hiroshi Hakoyama¹*
- 9 ¹ Nagano University, Ueda, Japan
- ² National Research Institute of Fisheries Science, Fisheries Research and Education Agency,
- 11 Yokohama, Japan

8

- ³ Bioresource Sciences, Faculty of Agriculture, Kyushu University, Fukuoka, Japan
- ⁴ Kumamoto Prefectural Fisheries Research Center, Kamiamakusa, Japan
- ⁵ Shizuoka Prefectural Research Institute of Fishery Hamanako Branch, Hamamatsu, Japan
- 15 ⁶ Wakayama Prefectural Museum of Natural History, Kainan, Japan
- ⁷ Kagoshima Fisheries Technology Development Center
- 17 ⁸ Miyazaki Prefectural Inland Fishery Promotion Center
- * Correspondence: hiroshi-hakoyama@nagano.ac.jp
- 20 Abstract

19

- 21 1. Assessing the status or population size of species is a key task for wildlife conservation
- and the sustainable management of harvested species. In particular, assessing historical
- changes in population size provides an evolutionary perspective on current population
- 24 dynamics and can help distinguish between anthropogenic and natural causes for population
- 25 decline.

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

2. Japanese eel (Anguilla japonica) is an endangered yet commercially important catadromous fish species. Here we assess the demographic history of Japanese eel using the pairwise and multiple sequentially Markovian coalescent methods. 3. The analyses indicate a reduction in effective population size (N_e) from 38 000 to 10 000 individuals between 4 and 1 Ma, followed by an increase to 80 000 individuals, between 1 Ma and 22–30 kya. Approximately 22–30 kya there is evidence for a reduction in Ne to approximately 60 000 individuals. These events are likely due to changes in environmental conditions, such as sea level and oceanic currents, especially around the last glacial maximum (19–33 kya). 4. The results of this study suggest that Japanese eel has experienced at least two population bottlenecks, interspersed by a period of population growth. This pattern of demographic history may make Japanese eel sensitive to current and future population declines. Conservation management of Japanese eel should focus on practical ways to prevent further population decline and the loss of genetic diversity that is essential for the species to adapt to changing environmental conditions such as climate change. Keywords climate change, last glacial maximum, pairwise and multiple sequentially Markovian coalescent, whole genome sequence Introduction

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

75

Assessing the status or population size of species is a key task for wildlife conservation and the sustainable management of harvested species. However, one-time static estimates of population size provide a limited amount of information; assessing changes in population size over time yields greater insight into population dynamics. For example, understanding historical changes in population size places current population decline in an evolutionary context and can help distinguish between anthropogenic and natural causes for population decline (Dong et al., 2021). In addition, changes in population size have predictable effects on genetic variation. Bottlenecks, drastic reductions in population size over a short period of time, result in the loss of genetic variation and if the original population size was small or the population growth rate is low, recovery of genetic variation is slow (Nei et al., 1975). In such populations, sustained low levels of genetic diversity can reduce population fitness and limit the ability to adapt to environmental changes (Reed & Frankham 2003). Understanding this 'genetic background' of a species that is experiencing current population decline can indicate the susceptibility of that species to further genetic deterioration or even extinction (Abascal et al., 2016). Therefore, assessing changes in population size over historical timescales, termed demographic history, is applicable to contemporary conservation and management. The demographic history of species can be inferred by estimating the effective population size (N_e) ; the size of an ideal population that experiences genetic drift at the same rate as the observed population) of the species at various points in time. One approach is to use the pairwise and multiple sequentially Markovian coalescent (PSMC and MSMC) models to estimate N_e from whole-genome sequences derived from just a single individual (Li & Durbin 2011). This approach has even been successful for ancient DNA samples, including extinct species. Studies of the demographic history of extinct species observe two main patterns: fluctuations in population size, e.g., passenger pigeon (Hung et al., 2014), scimitar-horned

77

78

79

80

81

82

83

84

85

86

87

88

89

90

91

92

93

94

95

96

97

98

99

100

oryx (Humble et al., 2020), and woolly rhinoceros (Lord et al., 2020); and gradual population reduction over time, e.g., Steller's sea cow (Sharko et al., 2021) and two New Zealand passerines (Dussex et al., 2019). Similar patterns of demographic history have also been observed in some studies of threatened species, e.g. fluctuations: Iberian lynx (Abascal et al., 2016), Sumatran rhinoceros (Mays Jr et al., 2018), greater bamboo lemur (Hawkins et al., 2018), and red pandas (Hu et al., 2020); gradual reduction: brown hyena (Westbury et al., 2018); and long term low population: vaquita (Morin et al., 2021), African wild dog (Campana et al., 2016), and pygmy hog (Liu et al., 2021). Assessment of the demographic history of these threatened species is anticipated to contribute to their conservation management. However, these patterns of demographic history are not evident in all at risk species. For example, species that have experienced recent population declines, such as the commercially exploited fish species European eel (Nikolic et al., 2020), Atlantic cod and haddock (Tørresen et al., 2018), and yellowfin tuna (Barth et al., 2017), all show evidence of historical population size increase leading up until the last glacial maximum (LGM, 19–33 kya; Clark et al., 2009), after which they experienced population bottlenecks. Such bottleneck events may be considered as random catastrophes which can contribute to a species risk of extinction (Lande 1993). In these cases, it may be difficult for population size to recover given the combination of an LGM bottleneck and contemporary human-induced harvesting pressure. These examples highlight the importance of considering the demographic history of species in order to more fully understand current population dynamics and develop management strategies that are comprehensive and contribute to the long-term sustainability of species. One commercially important species that has experienced recent declines in census population size attributed to fishing pressure, climate change, pollution, and habitat

destruction (Tsukamoto et al., 2009), and is now listed as endangered by the IUCN, is Japanese eel (Anguilla japonica) (Pike et al., 2020; Tsukamoto et al., 2009). Japanese eel is a catadromous migratory species distributed across the rivers and estuaries of the northwestern Pacific, including Japan, China, Korea, Philippines, and Taiwan (Pike et al., 2020). After 5 to 15 years living in rivers and estuaries, yellow eels transform into silver eels and migrate to the spawning area near the West Mariana Ridge in the Pacific Ocean (Yokouchi et al., 2009). Following spawning the adults die and larvae drift on the North Equatorial and Kuroshio Currents where they metamorphose into glass eels and then arrive in waves along the east coast of Asia (Gong et al., 2019). Due to the complex life history and commercial importance of Japanese eel, there has been considerable research into its population genetic structure and population dynamics. Most studies on the population genetic structure of Japanese eel suggest that the species is panmictic (Gong et al., 2019, 2014; Han et al., 2010; Ishikawa et al., 2001; Minegishi et al., 2012; Sang et al., 1994). However, some studies have argued for the existence of low- and high-latitude groups (Chan et al., 1997; Tseng et al., 2006, 2009) and one recent study found evidence for the genetic differentiation of one population from the Kuma River, southern Japan (Igarashi et al., 2018). It is possible that these anomalous results are due to temporal fluctuations in genetic structure in an otherwise panmictic population, as also observed in European eel (Dannewitz et al., 2005; Pujolar et al., 2009). To date, studies of the demographic history of Japanese eel have found evidence for population size expansions 110-350 kya and 9-25 kya (Tseng et al., 2012), and a decline in population size 3.5–8 kya (Tseng et al., 2003). However, these inferences are limited in scope due to the use of few molecular markers (6–8 microsatellites, mtDNA D-loop sequences) and sampling from just a single location (1 river in Taiwan), so further analyses are warranted.

101

102

103

104

105

106

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123

126

127

128

129

130

131

132

133

134

135

136

137

138

139

140

141

142

143

144

145

146

147

148

Determining N_e has been made easier and more accurate by the abundance of genomic data available, as well as the recent development of a variety of data analysis approaches (Fuentes-Pardo & Ruzzante, 2017; Spence et al., 2018). Investigating long-term historical changes in N_e can provide an evolutionary context within which to assess the nature of current population dynamics. This approach also improves our understanding of the response of species to past and future environmental change (Gattepaille et al., 2016). The overall aim of the current study was to assess the historical demography of Japanese eel. Inferences of past changes in N_e were made by utilising whole genome re-sequencing data of Japanese eel from two sources: the previously published data of Igarashi et al., 2018 and new data described here. The results were compared with those from previous studies on Japanese eel using 'traditional' markers (Tseng et al., 2012, 2003), as well as with similar analyses in other Anguilla species (Barth et al., 2020; Nikolic et al., 2020), to provide fisheries management agencies with a broader perspective of the current status of Japanese eel populations. Materials and Methods Inference of population demographic history Past changes in Ne were estimated using the pairwise and multiple sequentially Markovian coalescent (PSMC and MSMC) methods (Li & Durbin, 2011; Schiffels & Durbin, 2014). These approaches examine an individual's whole genome sequence and estimate the time to the most recent common ancestor of windows along the genome based on the density of

heterozygous sites within those regions (Li & Durbin, 2011; Schiffels & Durbin, 2014).

150

151

152

153

154

155

156

157

158

159

160

161

162

163

164

165

166

167

168

169

170

171

172

173

The model is then used to estimate ancestral population sizes. For these analyses, a previously published dataset of 84 whole genome re-sequenced Japanese eels (Igarashi et al., 2018) was obtained from the National Centre for Biotechnology Information (NCBI) database under BioProject PRJDB5707. Duplicate reads were removed from the dataset and mean read depth assessed for each sample. As genome coverage is known to influence the accuracy of P/MSMC, due to the potential for false negative calling of heterozygotes in low coverage data (PSMC manual), only samples with a mean read depth greater than 18 (Nadachowska-Brzyska et al., 2015) were considered for the analyses. This resulted in just one glass eel from Sagami River, Kanagawa, Japan (sample ID: DRR091902). Thus, to obtain additional data with higher mean read depths, whole genome re-sequencing was performed on ten adult eel samples collected from Kuma River, Kumamoto Prefecture, Japan in 2018 (May–September). This data is available upon reasonable request to the authors. The ten specimens from Kuma River were kept at -20°C until DNA extraction. Genomic DNA was extracted from a small piece of muscle tissue by using a Maxwell RSC Blood DNA Kit with a Maxwell RSC Instrument (Promega). A shotgun DNA library was constructed using a TruSeq Nano DNA High Throughput Library Prep Kit (Illumina), and the resulting library was subjected to pair-end sequencing with a Novaseq 6000 sequencer (Illumina) by Macrogen Japan. Trimmomatic v0.39 (Bolger et al., 2014) was used for adapter trimming and initial filtering (SLIDINGWINDOW, threshold phred-scale quality score of 25 within a window size of 4 bases; LEADING and TRAILING, 20; MINLEN, 50). The resulting pair-end reads were mapped onto a reference genome sequence of Japanese eel (Nakamura et al., 2017) using BWA v0.7.17 (Li 2013) with default parameters (the bwa-mem algorithm). Samtools v1.7 (Li et al., 2009) was used for additional filtering. The following were removed: reads with a mapping quality less than 10 (the subprogram, view); reads with

175

176

177

178

179

180

181

182

183

184

185

186

187

188

189

190

191

192

193

194

195

196

197

198

an XA or SA tag (alternative hits and chimeric reads, respectively; standard grep command); unmapped reads (fixmate); and PCR duplicates (markdup). Reads from the 11 samples were aligned to the draft reference assembly of Japanese eel (Nakamura et al., 2017) using BWA (Li 2013) and the consensus sequence for each was called using the mpileup command in beftools (Danecek et al., 2021). Minimum base and mapping quality thresholds were set to 20. The total number of single nucleotide polymorphisms (SNPs) across the genome was estimated by veftools (minQ 20, minDP 15, maxDP 120, max-missing 0.9) (Danecek et al., 2011). For the PSMC, variants were called using vcfutils.pl, setting the minimum and maximum read depths to one third and twice the mean depth of each sample, respectively. Then the PSMC package was used to convert files from vcf to psmc format and the analysis was performed with the following settings: iteration length 30, T_{max} 15, initial θ/ρ 5, and atomic time interval 64 (4+25*2+4+6). For the MSMC, the data was un-phased, so analyses were performed separately for each individual. Variant and input files were created for each individual using the MSMC tools scripts bamCaller.py and generatemulithetsep.py. The MSMC method requires separate input files for each chromosome, or in this case scaffold. Scaffolds that were longer than N75 (115 160 basepairs) were selected, resulting in 1719 input files per individual. The MSMC was then performed with default settings. Parameter Estimation and Plotting To be scaled into time in years, the PSMC and MSMC output requires estimates of the species generation time and genome-wide mutation rate. A review of life history studies of Japanese eel indicated that silver eels (eels that are ready to undergo the spawning migration)

200

201

202

203

204

205

206

207

208

209

210

211

212

213

214

215

216

217

218

219

220

221

222

223

range in age from 4 to 17 years, with a mean of 8 years for females (Han et al., 2009; Kotake et al 2007, 2005; Yokouchi et al., 2009). The spawning migration is estimated to take 6 months (Chang et al., 2016; Han et al., 2009), thus a generation time of 8.5 years was used to convert the PSMC and MSMC output to real time. The genome-wide mutation rate of Japanese eel was calculated by comparison with the European eel, Anguilla anguilla. The reference genome of European eel was obtained from the NCBI database under BioProject PRJNA561979. Each chromosome (1–19 and the mitochondrial genome) was aligned to the Japanese eel draft assembly using LASTZ (Harris, 2007). The number of matches and mismatches between the sequences of the two species were counted and then summed across all chromosomes. The mutation rate (per nucleotide per year) was then calculated as $\mu = \text{(number of mismatches/total length)/}2t$, where t is the divergence time between Japanese and European eel. The divergence time was estimated to be 13.6 million years (Santini et al., 2013). Thus, $\mu = 8.7 \times 10^{-9}$. The mutation rate per generation was then calculated as $\mu \times 8.5$, resulting in a mutation rate of 7.4×10^{-8} per nucleotide per generation. The results for the PSMC and MSMC analyses were scaled to real time using the values described above. Changes in N_e over time, for each individual and for the mean of all 11 individuals, were plotted by using R 4.1.0 (R Core Team, 2021). Results Following the alignment of the 11 samples to the Japanese eel draft genome, 2 373 329 SNPs were identified. The 10 re-sequenced individuals had mean read depths of 42–59. The PSMC results indicated that the Ne of Japanese eel was stable at around 8 000 (7 700–8 400) individuals 1 Ma and then from approximately 500 kya N_e started to increase (Figure 1a,

b). This population size increase continued until it peaked at approximately 88 000 (68–102

225

226

227

228

229

230

231

232

233

234

235

236

237

238

239

240

241

242

243

244

245

246

247

248

000) individuals around 30–40 kya. There was then a sudden reduction in N_e to approximately 63 000 (46–80 000) individuals around 30 kya. A population increase to approximately 74 000 (54-94 000) individuals occurred around 17 kya. The MSMC results indicated an initial reduction in N_e from 38 000 (32–60 000) to 10 000 (10–13 000) individuals between 1 and 4 Ma (Figure 1a, c). This was followed by a steady increase in N_e to approximately 81 000 (64-105 000) individuals around 22-32 kya. There was then a sudden reduction in N_e to approximately 56 000 (45–77 000) individuals around 22 kya. A population increase to 97 000 (60–178 000) individuals occurred around 14 kya. The PSMC and MSMC inferences were very similar, although MSMC was able to infer Ne further back in time (5 Ma vs. 2 Ma). In addition, the inferences based on one individual from previously published data (Figure 1 b, c, yellow lines) were similar to those based on the data from 10 re-sequenced individuals obtained in this study (Figure 1 b and c, red and blue lines, respectively). Discussion Demographic history of Japanese eel This study provided valuable insights into the demographic history and N_e of Japanese eel, a species of conservation and fisheries management concern. In summary, the results of this study provide the following general picture of the demographic history of Japanese eel: population reduction between 1 and 4 Ma, population growth between 30 and 800 kya, sudden population reduction around 25 kya (during the LGM), followed by population growth around 15 kya (post-LGM). Interestingly, the findings of this study were similar to those of a previous study that used mtDNA sequences (Tseng et al., 2012), suggesting that

250

251

252

253

254

255

256

257

258

259

260

261

262

263

264

265

266

267

268

269

270

271

272

273

'traditional' methods remain relevant and informative, especially in cases where the availability of funds, equipment, expertise, or high-quality DNA may prevent the use of NGS techniques. In addition, our analyses demonstrate the potential for publicly available genome data to contribute to conservation and fisheries management. The long-term view of population dynamics presented here helps provide some perspective on current population declines. The MSMC inferred a reduction in the N_e of Japanese eel between 1 and 4 Ma, during the Pliocene. During this time, the formation of the Isthmus of Panama, together with the closure of the Indonesian seaway, caused a general shift in global oceanic circulation and a drop in sea surface temperatures that led to glaciation in the northern hemisphere (Cane & Molnar, 2001; Gallagher et al., 2015). It is also postulated that at this time the Kuroshio current did not reach the Japan/East China Seas, and that the Tsushima current inflow was weak and periodical until about 1.7 Ma (Gallagher et al., 2015). These factors may have restricted the larval drift and/or migration of Japanese eels, leading to declines in N_e . Both the PSMC and MSMC inferences indicated that the N_e of Japanese eel started to increase around 0.5-1 Ma. Notable changes to the oceanic environment in the western Pacific occurred during this period. Firstly, global sea levels were relatively high and large areas of south-eastern China and the Ryukyu Islands (southern Japan) were inundated, influencing the circulation of ocean currents (Kimura, 2000). Secondly, stronger glacial/inter-glacial cycles intensified the Kuroshio Current and the North Pacific Gyre (Gallagher et al., 2015). Together these conditions may have facilitated a population size expansion in Japanese eel, which continued until approximately 30 kya. The reduction in Ne that occurred around 30–22 kya is most likely due to changes in sea level, ocean current patterns, and sea surface

temperatures associated with the LGM (19–33 kya). For example, during the LGM a land bridge between Taiwan and the Ryukyu Islands deflected the Kuroshio Current eastwards (Ujiié & Ujiié, 1999), thereby preventing the drift of Japanese eel larvae northwards to the rivers and estuaries of the Japanese coastline. Because the PSMC and MSMC methods perform less well for inferences of demographic history within the last 20 ky (Mather et al., 2020), we interpret population size changes after the LGM with caution. However, the results of this study (population growth 14–17 kya), a previous study of Japanese eel (population expansion 9–25 kya; Tseng et al., 2012), and studies of other species (three tropical Anguilla species: Barth et al., 2020), as well as other northwest Pacific fishes (Lu et al., 2020; Xu et al., 2019), all provide evidence for post-LGM population growth, suggesting that oceanic conditions were generally suitable for population expansion at that time. In the case of Japanese eel, perhaps abrupt sea level rise at the end of the LGM (14.5 kya; Clark et al., 2009) and the inundation of the Taiwan-Ryukyu land bridge 10 kya (Ujiié & Ujiié, 1999), which restored the Kuroshio Current to its present-day path, provided an opportunity for geographic population expansion and population size increase. We recommend further studies to clarify changes in N_e of Japanese eel within the last 20 ky, and to estimate the current N_e .

Implications for conservation and fisheries management

274

275

276

277

278

279

280

281

282

283

284

285

286

287

288

289

290

291

292

293

294

295

296

297

298

The results of this study suggest that, on an evolutionary time scale, Japanese eel may have experienced two population bottlenecks interspersed by a period of population growth. One previous study also detected evidence of a population bottleneck 3–8 kya (Tseng et al., 2003), a time period not covered by the PSMC or MSMC analyses. Finally, comparison to analyses of population demographic history in other *Anguilla* species suggest that Japanese eel has

300

301

302

303

304

305

306

307

308

309

310

311

312

313

314

315

316

317

318

319

320

321

322

323

always had a relatively lower N_e compared to its congeners (Barth et al., 2020; Nikolic et al., 2020). Together, these findings suggest that Japanese eel has a demographic history of population size fluctuations and has experienced population bottleneck events. The genetic consequences of this demographic history may include reduced levels of genetic diversity and loss of adaptive potential that may make Japanese eel sensitive to current and future population declines. The results of this study demonstrate how climate induced changes in the pattern and intensity of ocean currents, particularly the Kuroshio and Tsushima currents, and consequent shifts in the availability of suitable habitat, led to changes in the historical N_e of Japanese eel. It is likely that these factors will also be important for the natural and long-term maintenance of N_e in the future. However, we acknowledge that it is difficult to predict the influence future climate change will have on Japanese eel population size because of the interaction between the effects of changes in ocean currents and sea surface temperatures, and the uncertainty surrounding the degree to which other anthropogenic activities like habitat degradation and harvesting will continue to contribute to population decline. In conclusion, this study found that Japanese eel has experienced at least two population bottlenecks throughout its evolutionary history, a genetic background that warrants caution. Conservation and fisheries management of Japanese eel should focus on practical ways to prevent further population decline and the loss of genetic diversity that is essential for the species to adapt to changing environmental conditions such as climate change. Finally, we wish to highlight the valuable perspective that can be gained by assessing historical population demography and hope that this and similar studies continue to contribute to conservation and fisheries management.

325

326

327

328

329

330

331

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

347

Acknowledgements This work was supported by the Research and assessment program for fisheries resources, the Fisheries Agency of Japan. We thank Dr. Mayumi Kobayashi for helpful comments. We have no conflict of interest to declare. References Abascal, F., Corvelo, A., Cruz, F., Villanueva-Cañas, J. L., Vlasova, A., Marcet-Houben, M. et al. (2016). Extreme genomic erosion after recurrent demographic bottlenecks in the highly endangered Iberian lynx. Genome Biology, 17(1), 1–19. https://dx.doi.org/10.1186/s13059-016-1090-1 Barth, J. M., Damerau, M., Matschiner, M., Jentoft, S., & Hanel, R. (2017). Genomic differentiation and demographic histories of Atlantic and Indo-Pacific yellowfin tuna (Thunnus albacares) populations. Genome Biology and Evolution, 9(4), 1084–1098. https://dx.doi.org/10.1093/gbe/evx067 Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics, 30(15), 2114–2120. Campana, M. G., Parker, L. D., Hawkins, M. T., Young, H. S., Helgen, K. M., Gunther, M. S. et al. (2016). Genome sequence, population history, and pelage genetics of the endangered African wild dog (Lycaon pictus). BMC Genomics, 17(1), 1–10. https://dx.doi.org/10.1186/s12864-016-3368-9 Cane, M. A. & Molnar, P. (2001). Closing of the indonesian seaway as a precursor to east african aridification around 3–4 million years ago. *Nature*, 411(6834), 157–162.

348 Chan, I. K. K., Chan, D. K. O., Lee, S. C., & Tsukamoto, K. (1997). Genetic variability of the 349 Japanese eel Anguilla japonica (Temminck & Schlegel) related to latitude. Ecology of 350 Freshwater Fish, 6(1), 292 45–49. https://dx.doi.org/10.1111/j.1600-0633.1997.tb00141.x 351 Chang, Y.-L., Miyazawa, Y., & Béguer-Pon, M. (2016). Simulating the oceanic migration of silver Japanese eels. PLoS ONE, 11(3), e0150187. 352 353 https://dx.doi.org/10.1371/journal.pone.0150187 354 Clark, P. U., Dyke, A. S., Shakun, J. D., Carlson, A. E., Clark, J., Wohlfarth, B. et al. (2009). 355 The last glacial maximum. science, 325(5941), 710–714 356 Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A. et al. (2011). 357 The variant call format and VCFtools. *Bioinformatics*, 27(15), 2156–2158. 358 https://dx.doi.org/10.1093/bioinformatics/btr330 359 Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O. et al. (2021). 360 Twelve years of samtools and beftools. *Gigascience*, 10(2), giab008. 361 Dannewitz, J., Maes, G. E., Johansson, L., Wickström, H., Volckaert, F. A., & Järvi, T. 362 (2005). Panmixia in the European eel: a matter of time.... Proceedings of the Royal Society. 363 B, Biological Sciences, 272(1568), 1129–1137. https://dx.doi.org/10.1098/rspb.2005.3064 364 Dong, F., Kuo, H.-C., Chen, G.-L., Wu, F., Shan, P.-F., Wang, J. et al. (2021). Population 365 genomic, climatic and anthropogenic evidence suggest the role of human forces in 366 endangerment of green peafowl (Pavo muticus). Proceedings of the Royal Society. B, 367 Biological Sciences, 288(1948), 20210073. https://dx.doi.org/10.1098/rspb.2021.0073 368 Dussex, N., von Seth, J., Knapp, M., Kardailsky, O., Robertson, B. C., & Dalén, L. (2019). 369 Complete genomes of two extinct New Zealand passerines show responses to climate 370 fluctuations but no evidence for genomic erosion prior to extinction. Biology Letters, 15(9),

20190491. https://dx.doi.org/10. 1098/rsbl.2019.0491

371

374

375

376

379

384

385

386

391

394

395

Fuentes-Pardo, A. P. & Ruzzante, D. E. (2017). Whole-genome sequencing approaches for 373 conservation biology: Advantages, limitations and practical recommendations. *Molecular* Ecology, 26(20), 5369–5406. https://dx.doi.org/10.1111/mec.14264 Gallagher, S. J., Kitamura, A., Iryu, Y., Itaki, T., Koizumi, I., & Hoiles, P. W. (2015). The Pliocene to recent history of the Kuroshio and Tsushima Currents: a multi-proxy approach. 377 Progress in Earth and Planetary Science, 2(1), 1–23. https://dx.doi.org/10.1186/s40645-378 015-0045-6 Gattepaille, L., Günther, T., & Jakobsson, M. (2016). Inferring past effective population size 380 from distributions of coalescent times. Genetics, 204(3), 1191–1206. 381 https://dx.doi.org/10.1534/genetics. 115.185058 382 Gong, X., Davenport, E. R., Wang, D., & Clark, A. G. (2019). Lack of spatial and temporal 383 genetic structure of Japanese eel (Anguilla japonica) populations. Conservation Genetics, 20(3), 467–475. https://dx.doi.org/10.1007/s10592-019-01146-8 Gong, X. L., Ren, S. J., Cui, Z. K., & Yue, L. J. (2014). Genetic evidence for panmixia of Japanese eel (Anguilla japonica) populations in China. Genetics and Molecular Research, 387 13, 768–781. https://dx.doi.org/10.4238/2014.January.31.3 388 Han, Y.-S., Hung, C.-L., Liao, Y.-F., & Tzeng, W.-N. (2010). Population genetic structure of 389 the Japanese eel Anguilla japonica: panmixia at spatial and temporal scales. Marine 390 Ecology Progress Series, 401, 221–232. https://dx.doi.org/10.3354/meps08422 Han, Y.-S., Tzeng, W.-N., Liao, I.-C. et al. (2009). Time series analysis of Taiwanese catch 392 data of Japanese glass eels Anguilla japonica: possible effects of the reproductive cycle 393 and El Niño events. Zoological Studies, 48(5), 632–639 Harris, R. S. (2007). Improved pairwise alignment of genomic DNA. Ph.D. thesis, The Pennsylvania State University

- Hawkins, M. T., Culligan, R. R., Frasier, C. L., Dikow, R. B., Hagenson, R., Lei, R. et al.
- 397 (2018). Genome sequence and population declines in the critically endangered greater
- bamboo lemur (*Prolemur simus*) and implications for conservation. *BMC Genomics*, 19(1),
- 399 1–15. https://dx.doi.org/10. 339 1186/s12864-018-4841-4
- 400 Hu, Y., Thapa, A., Fan, H., Ma, T., Wu, Q., Ma, S. et al. (2020). Genomic evidence for two
- phylogenetic species and long-term population bottlenecks in red pandas. *Science*
- 402 *Advances*, 6(9), eaax5751. https://dx.doi.org/10.1126/sciadv.aax5751
- Humble, E., Dobrynin, P., Senn, H., Chuven, J., Scott, A. F., Mohr, D. W. et al. (2020).
- 404 Chromosomal level genome assembly of the scimitar-horned oryx: Insights into diversity
- and demography of a species extinct in the wild. *Molecular Ecology Resources*, 20(6),
- 406 1668–1681. https://dx.doi.org/10.1111/1755-0998.13181
- Hung, C.-M., Shaner, P.-J. L., Zink, R. M., Liu, W.-C., Chu, T.-C., Huang, W.-S. et al.
- 408 (2014). Drastic population fluctuations explain the rapid extinction of the passenger
- pigeon. Proceedings of the National Academy of Sciences, 111(29), 10636–10641.
- 410 https://dx.doi.org/10.1073/pnas.1401526111
- 411 Igarashi, Y., Zhang, H., Tan, E., Sekino, M., Yoshitake, K., Kinoshita, S. et al. (2018).
- Whole-genome sequencing of 84 Japanese eels reveals evidence against panmixia and
- support for sympatric speciation. *Genes*, 9(10), 474.
- 414 https://dx.doi.org/10.3390/genes9100474
- 415 Ishikawa, S., Aoyama, J., Tsukamoto, K., & Nishida, M. (2001). Population structure of the
- Japanese eel Anguilla japonica as examined by mitochondrial DNA sequencing. Fisheries
- 417 *Science*, 67(2), 246–253. https://dx.doi.org/https://doi.org/10.1046/j.1444-
- 418 2906.2001.00227.x

- 419 Kimura, M. (2000). Paleogeography of the Ryukyu Islands. *Tropics*, 10(1), 5–24.
- 420 https://dx.doi.org/ 10.3759/tropics.10.5
- 421 Kotake, A., Arai, T., Okamura, A., Yamada, Y., Utoh, T., Oka, H. P. et al. (2007). Ecological
- 422 aspects of the Japanese eel, *Anguilla japonica*, collected from coastal areas of Japan.
- 423 Zoological Science, 24(12), 1213–1221. https://dx.doi.org/10.2108/zsj.24.1213
- Kotake, A., Okamura, A., Yamada, Y., Utoh, T., Arai, T., Miller, M. J. et al. (2005). Seasonal
- variation in the migratory history of the Japanese eel Anguilla japonica in Mikawa Bay,
- Japan. *Marine Ecology Progress Series*, 293, 213–221.
- 427 https://dx.doi.org/10.3354/meps293213
- 428 Lande, R. (1993). Risks of population extinction from demographic and environmental
- stochasticity and random catastrophes. *The American Naturalist*, 142(6), 911–927.
- 430 https://dx.doi.org/10.1086/ 285580
- 431 Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-
- 432 MEM. *arXiv* preprint arXiv:1303.3997
- Li, H. & Durbin, R. (2011). Inference of human population history from individual whole-
- genome sequences. *Nature*, 475(7357), 493–496. https://dx.doi.org/10.1038/nature10231
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N. et al. (2009). The
- sequence alignment/map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079.
- https://dx.doi.org/10.1093/bioinformatics/btp352
- 438 Liu, L., Bosse, M., Megens, H.-J., de Visser, M., AM Groenen, M., & Madsen, O. (2021).
- Genetic consequences of long-term small effective population size in the critically
- endangered pygmy hog. *Evolutionary Applications*, 14(3), 710–720.
- 441 https://dx.doi.org/10.1111/eva.13150

- Lord, E., Dussex, N., Kierczak, M., Díez-del Molino, D., Ryder, O. A., Stanton, D. W. et al.
- 443 (2020). Pre-extinction demographic stability and genomic signatures of adaptation in the
- woolly rhinoceros. *Current Biology*, 30(19), 3871–3879.
- 445 https://dx.doi.org/10.1016/j.cub.2020.07.046
- Lu, L., Zhao, J., & Li, C. (2020). High-quality genome assembly and annotation of the big-
- eye mandarin fish (*Siniperca knerii*). G3: Genes, Genomes, Genetics, 10(3), 877–880.
- 448 https://dx.doi.org/10. 1534/g3.119.400930
- Mather, N., Traves, S. M., & Ho, S. Y. (2020). A practical introduction to sequentially
- 450 Markovian coalescent methods for estimating demographic history from genomic data.
- 451 *Ecology and Evolution*, 10(1), 579–589. https://dx.doi.org/10.1002/ece3.5888
- 452 Mays Jr, H. L., Hung, C.-M., Shaner, P.-J., Denvir, J., Justice, M., Yang, S.-F. et al. (2018).
- Genomic analysis of demographic history and ecological niche modeling in the
- endangered Sumatran rhinoceros *Dicerorhinus sumatrensis*. Current Biology, 28(1), 70–
- 455 76. https://dx.doi.org/10.1016/j.cub.2017. 11.021
- 456 Minegishi, Y., Aoyama, J., Yoshizawa, N., & Tsukamoto, K. (2012). Lack of genetic
- heterogeneity in the Japanese eel based on a spatiotemporal sampling. *Coastal Marine*
- 458 *Science*, 35(1), 269–276. https: 392 //dx.doi.org/10.15083/00040662
- Morin, P. A., Archer, F. I., Avila, C. D., Balacco, J. R., Bukhman, Y. V., Chow, W. et al.
- 460 (2021). Reference genome and demographic history of the most endangered marine
- mammal, the vaquita. *Molecular Ecology Resources*, 21(4), 1008–1020.
- 462 https://dx.doi.org/10.1111/1755-0998.13284
- 463 Nadachowska-Brzyska, K., Li, C., Smeds, L., Zhang, G., & Ellegren, H. (2015). Temporal
- dynamics of avian populations during Pleistocene revealed by whole-genome sequences.
- 465 *Current Biology*, 25(10), 1375–1380. https://dx.doi.org/10.1016/j.cub.2015.03.047

- 466 Nakamura, Y., Yasuike, M., Mekuchi, M., Iwasaki, Y., Ojima, N., Fujiwara, A. et al. (2017).
- Rhodopsin gene copies in Japanese eel originated in a teleost-specific genome duplication.
- 468 Zoological Letters, 3(1), 18. https://dx.doi.org/10.1186/s40851-017-0079-2
- Nei, M., Maruyama, T., & Chakraborty, R. (1975). The bottleneck effect and genetic
- variability in populations. *Evolution*, 29, 1–10. https://doi.org/10.2307/2407137
- Nikolic, N., Liu, S., Jacobsen, M. W., Jónsson, B., Bernatchez, L., Gagnaire, P.-A. et al.
- 472 (2020). Speciation history of European (*Anguilla anguilla*) and American eel (*A. rostrata*),
- analysed using genomic data. *Molecular Ecology*, 29(3), 565–577.
- 474 https://dx.doi.org/10.1111/mec.15342
- Pike, C., Kaifu, K., Crook, V., Gollock, M., & Jacoby, D. (2020). Anguilla japonica. The
- 476 IUCN Red List of Threatened Species 2020. https://dx.doi.org/10.2305/IUCN.UK.2020-
- 477 3.RLTS. T166184A176493270.en
- 478 Pujolar, J. M., De Leo, G. A., Ciccotti, E., & Zane, L. (2009). Genetic composition of
- 479 Atlantic and Mediterranean recruits of European eel Anguilla anguilla based on EST-
- linked microsatellite loci. *Journal of Fish Biology*, 74(9), 2034–2046.
- 481 https://dx.doi.org/10.1111/j.1095-8649.2009.02267.x
- 482 R Core Team (2021). R: A Language and Environment for Statistical Computing. R
- 483 Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
- Reed, D. H. & Frankham, R. (2003). Correlation between fitness and genetic diversity.
- 485 *Conservation Biology*, 17(1), 230–237. https://dx.doi.org/10.1046/j.1523-
- 486 1739.2003.01236.x

- 487 Sang, T.-K., Chang, H.-Y., Chen, C.-T., & Hui, C.-F. (1994). Population structure of the
- Japanese eel, *Anguilla japonica*. *Molecular Biology and Evolution*, 11(2), 250–260.
- https://dx.doi.org/10.1093/oxfordjournals.molbev.a040107
- 490 Santini, F., Kong, X., Sorenson, L., Carnevale, G., Mehta, R. S., & Alfaro, M. E. (2013). A
- multi-locus molecular timescale for the origin and diversification of eels (Order:
- 492 Anguilliformes). *Molecular Phylogenetics and Evolution*, 69(3), 884–894.
- 493 https://dx.doi.org/10.1016/j.ympev.2013.06.016
- Schiffels, S. & Durbin, R. (2014). Inferring human population size and separation history
- from multiple genome sequences. *Nature genetics*, 46(8), 919–925
- Sharko, F. S., Boulygina, E. S., Tsygankova, S. V., Slobodova, N. V., Alekseev, D. A.,
- Krasivskaya, A. A. et al. (2021). Steller's sea cow genome suggests this species began
- 498 going extinct before the arrival of Paleolithic humans. *Nature Communications*, 12(1), 1–8.
- 499 https://dx.doi.org/10.1038/s41467-021-22567-5
- 500 Spence, J. P., Steinrücken, M., Terhorst, J., & Song, Y. S. (2018). Inference of population
- history using coalescent HMMs: Review and outlook. Current Opinion in Genetics &
- 502 Development, 53, 70–76. https://dx.doi.org/10.1016/j.gde.2018.07.002
- Tørresen, O. K., Brieuc, M. S., Solbakken, M. H., Sørhus, E., Nederbragt, A. J., Jakobsen, K.
- S. et al. (2018). Genomic architecture of haddock (*Melanogrammus aeglefinus*) shows
- expansions of innate immune genes and short tandem repeats. *BMC Genomics*, 19(1), 240.
- 506 https://dx.doi.org/10.1186/ s12864-018-4616-y
- Tseng, M.-C., Kao, H.-W., Hung, Y.-H., & Lee, T.-L. (2012). A study of genetic variations,
- 508 population size, and population dynamics of the catadromous Japanese eel Anguilla
- japonica (Pisces) in northern Taiwan. *Hydrobiologia*, 683(1), 203–216.
- 510 https://dx.doi.org/10.1007/s10750-011-0958-z

511 Tseng, M.-C., Tzeng, W.-N., & Lee, S.-C. (2003). Historical decline in the Japanese eel 512 Anguilla japonica in northern Taiwan inferred from temporal genetic variations. 513 *Zoological Studies*, 42(4), 556–563 514 Tseng, M.-C., Tzeng, W.-N., & Lee, S.-C. (2006). Population genetic structure of the 515 Japanese eel Anguilla japonica in the northwest Pacific Ocean: evidence of non-panmictic 516 populations. Marine Ecology Progress Series, 308, 221–230. 517 https://dx.doi.org/10.3354/meps308221 518 Tseng, M.-C., Tzeng, W.-N., & Lee, S.-C. (2009). Genetic differentiation of the Japanese eel. 519 In: Eels at the edge: American Fisheries Society Symposium. American Fisheries Society 520 Bethesda, MD, volume 58,pp. 59–69 521 Tsukamoto, K., Aoyama, J., & Miller, M. (2009). Present status of the Japanese eel: 522 resources and recent research. In: Eels at the edge: American Fisheries Society Symposium. 523 American Fisheries Society Bethesda, MD, volume 58, pp. 21–35 524 Ujiié, H. & Ujiié, Y. (1999). Late Quaternary course changes of the Kuroshio Current in the 525 Ryukyu Arc region, northwestern Pacific Ocean. Marine Micropaleontology, 37(1), 23–40. 526 https://dx.doi.org/ 10.1016/S0377-8398(99)00010-9 527 Westbury, M. V., Hartmann, S., Barlow, A., Wiesel, I., Leo, V., Welch, R. et al. (2018). 528 Extended and continuous decline in effective population size results in low genomic 529 diversity in the world's rarest hyena species, the brown hyena. Molecular Biology and 530 Evolution, 35(5), 1225–1237. https://dx.doi.org/10.1093/molbev/msy037 531 Xu, S., Zhao, L., Xiao, S., & Gao, T. (2019). Whole genome resequencing data for three 532 rockfish species of Sebastes. Scientific Data, 6(1), 1–6. https://dx.doi.org/10.1038/s41597-

533

019-0100-z

535

536

537

538

539

540

541

542

543

544

545

546

547

548

549

550

551

Yokouchi, K., Sudo, R., Kaifu, K., Aoyama, J., & Tsukamoto, K. (2009). Biological characteristics of silver-phase Japanese eels, Anguilla japonica, collected from Hamana Lake, Japan. Coastal Marine Science, 33(1), 1–10 Figure Legend Figure 1. Plot of the changes in effective population size N_e over the last 10 My for Japanese eel. Effective population size was estimated from the whole genome re-sequencing data of 11 individuals by using the pairwise and multiple sequentially Markovian coalescent (PSMC and MSMC) methods. (a) Plot presenting the mean values of N_e for all 11 individuals analysed, PSMC = red, MSMC = blue; (b) Plot representing the PSMC results for 10 individuals sequenced in this study (red lines), 1 individual from previously published data (orange line), and the mean (black line); (c) Plot representing the MSMC results for 10 individuals sequenced in this study (blue lines), 1 individual from previously published data (light blue line), and the mean (black line).

