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1 **Models based on best-available information support a low inbreeding load and potential**
2 **for recovery in the vaquita**

3

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28 **Main text**

29 In Robinson et al. (2022), we employed genomic and simulation analyses to
30 demonstrate that the critically endangered vaquita porpoise has a reduced potential for future
31 inbreeding depression (inbreeding load), and is therefore not doomed to extinction by
32 deleterious genetic factors. Garcia-Dorado and Hedrick (2022) (hereafter, GD&H) critique our
33 analysis for not sufficiently demonstrating that the vaquita has a low inbreeding load and a
34 good chance of recovery in the absence of continued incidental mortality in fishing gillnets.

35 Our conclusion that the vaquita likely has a very low inbreeding load is supported, first
36 and foremost, by the finding that there are very few deleterious mutations that can contribute
37 to inbreeding depression segregating in the vaquita due to its small historical population size.
38 There is widespread agreement that inbreeding depression is overwhelmingly due to the
39 exposure of recessive deleterious mutations that are concealed as heterozygotes (Charlesworth
40 and Willis 2009; Hedrick and Garcia-Dorado 2016). In the relative absence of such mutations,
41 there is simply very little fuel for inbreeding depression. For example, our genomic analysis
42 shows that vaquitas have ~17 heterozygous loss of function (LOF) mutations per individual,
43 whereas the blue whale genome has ~248 of such mutations. Loss of function mutations can, in
44 many cases, have severe effects on fitness because they disrupt the function of protein-coding
45 genes (though see MacArthur and Tyler-Smith 2010). Under the assumption that such LOF
46 mutations are (partially) recessive, a low count of heterozygous LOF mutations as observed in
47 the vaquita implies a low inbreeding load, whereas a high count as observed in the blue whale
48 implies a high inbreeding load.

49 GD&H are correct that we do not precisely know the selection (s) or dominance (h)
50 coefficients for putatively deleterious mutations, such that our identification of few segregating
51 deleterious mutations in vaquitas offers only qualitative insight into inbreeding load. This
52 limitation motivated our complementary simulation analysis, where we employ a distribution of
53 s for new mutations that was inferred from our genomic dataset. Although we agree that our
54 model, like any model, makes assumptions that should be critically evaluated, our model is
55 informed by the best-available information on selection and demographic parameters in the

56 vaquita. Below, we review some of the key components of the vaquita and our model that
57 inform our conclusions.

58

59 **What should be considered a “small” historical population size?**

60 GD&H assert that inbreeding load in the vaquita cannot be low since the species has a
61 large historical population size. Although the historical population size of vaquita may seem
62 large relative to the very small recent effective population sizes (N_e) that are observed in
63 endangered species, the historical size of the vaquita population is not large when considering
64 the broader context of long-term N_e in mammals or other taxa. Long term N_e in mammals
65 (defined here as $N_e = \pi / (4\mu)$ where π is heterozygosity and μ is the mutation rate) is typically on
66 the order of tens to hundreds of thousands, and rarely below 5000. For example, using
67 published estimates of genome-wide heterozygosity in 42 species of mammals, we recently
68 estimated a median long-term N_e of 21,875 (Kyriazis et al. 2022). Based on our estimate of the
69 mutation rate in vaquitas of $\mu = 5.8e-9$ and estimate of $\pi = 9.04e-5$ (Robinson et al. 2022),
70 vaquitas are estimated to have a long-term N_e of 3,896, the second lowest of the 42 species in
71 this dataset (Kyriazis et al. 2022). This finding is also supported by fitting more complex non-
72 equilibrium demographic models to genomic datasets for the vaquita, which similarly estimate
73 $N_e < 5,000$ going back tens or hundreds of thousands of years (Morin et al. 2021; Robinson et al.
74 2022). The species with the smallest long-term N_e in this dataset, the Channel Island fox, has
75 been previously shown to exhibit no phenotypic signs of inbreeding depression despite having
76 experienced severe recent bottlenecks (Robinson et al. 2018). By contrast, North American gray
77 wolves have a large long-term N_e of $\sim 92,000$ (assuming $\mu = 4.5e-9$ (Koch et al. 2019) and $\pi =$
78 $1.65e-3$ (Robinson et al. 2019)), which may help explain numerous observed instances of severe
79 inbreeding depression in the species (Fredrickson et al. 2007; Raikkonen et al. 2009; Robinson
80 et al. 2019). Thus, although the historical vaquita population size may seem large relative the
81 current size of the population, it is still vastly smaller than the population sizes observed in
82 most other species of mammals.

83 Although these long-term effective population sizes may not be representative of the
84 current size of many threatened or endangered populations, they are essential for modelling

85 the inbreeding load in a species. This is because long-term demographic processes have a major
86 impact on patterns of segregating (recessive) deleterious variation, the key determinant of the
87 inbreeding load. Though recent declines can influence patterns of segregating variation and
88 inbreeding load, these effects often take tens or hundreds of generations to manifest. For
89 example, as we show in our analysis, the recent and dramatic decline in the vaquita over the
90 past ~35 years or ~3 generations does not appear to have impacted genetic diversity or
91 inbreeding load in the species (Robinson et al. 2022). This is likely the case for many other large
92 mammals that have experienced declines over the past century, given the long generation
93 times typical of these species. Thus, low inbreeding loads in species with long generation times
94 are likely a product of small long-term historical population sizes, rather than recent human-
95 mediated declines. However, the extent to which this is true for a given species will depend on
96 the duration and severity of decline, something that can readily be assessed using simulations.

97

98 **What should be considered a “typical” inbreeding load?**

99 A useful approach for determining whether computational models of inbreeding
100 depression are reasonable is to compare the predicted inbreeding load (B) from such models to
101 those that have been estimated from natural populations (note that we report values in terms
102 of the diploid inbreeding load $[2B]$ in Robinson et al. (2022) but report the haploid inbreeding
103 load $[B]$ here to be consistent with GD&H). GD&H cite an average estimate from O’Grady et al.
104 (2006) of $B = 6$, derived from an analysis of 16 existing inbreeding load estimates, as being a
105 typical inbreeding load for wild populations. Based on this result, they then claim that our
106 model-based prediction of $B = 0.48$ for the vaquita is unreasonably low. However, it has
107 previously been shown that the inbreeding load estimate from O’Grady et al. (2006) is
108 unreliable and upwardly biased, in part due to methodological issues associated with
109 using generalized linear models with a logit link to estimate the inbreeding load (Nietlisbach et
110 al. 2019). Specifically, Nietlisbach et al. (2019) found that the three highest inbreeding load
111 values reported by O’Grady et al. (2006) are overestimates due to biased statistical models or
112 issues with the original datasets (note that many of these same concerns apply to estimates
113 reported in Hedrick and Garcia-Dorado (2016)). Moreover, the average estimate from O’Grady

114 et al. (2006) is also likely to be upwardly biased due to their approach of summing together
115 inbreeding load estimates from different life stages, an approach that ignores widespread
116 pleiotropy for mutations underlying fitness (Pickrell et al. 2016; Boyle et al. 2017). This
117 approach can contribute to upward bias by potentially double or triple counting the effects of
118 recessive deleterious mutations that contribute to inbreeding depression at different life stages
119 (see Kyriazis et al. (2022) for further discussion).

120 Based on 22 estimates that are deemed to be reliable and unbiased, Nietlisbach et al.
121 (2019) instead report a median inbreeding load for survival to sexual maturity in vertebrates of
122 $B = 2.25$. Although our predicted inbreeding load of $B = 0.48$ is somewhat lower than this
123 median, this is expected given the small historical population size and low levels of segregating
124 (recessive) deleterious variation in the vaquita. Importantly, we note that this estimate of $B =$
125 2.25 is based on only 13 species, nearly all of which are birds, and should therefore be
126 interpreted cautiously. Thus, obtaining additional high-quality estimates of the inbreeding load
127 from wild populations represents a key area of future research that will enable better
128 assessment of simulation models (see Kyriazis et al. 2022, for further discussion). Nevertheless,
129 based on available evidence, our model predicts an inbreeding load that is consistent with
130 reliable estimates from natural populations.

131

132 **How should we estimate selection and mutation parameters?**

133 GD&H claim that our selection and dominance parameters are incorrect because they
134 do not agree with estimates of selection and dominance parameters derived from experiments
135 in *Drosophila* (e.g., Simmons and Crow 1977; Pérez-Pereira et al. 2021, 2022). However, such
136 experimental estimates are well known to be biased towards strongly deleterious variation, as
137 mutations with more mild effects cannot be observed in an experimental setting (Davies et al.
138 1999; Eyre-Walker and Keightley 2007). These experimental approaches are also limited due to
139 issues of identifiability for selection and mutation parameters (Lynch et al. 1999; Eyre-Walker
140 and Keightley 2007; Halligan and Keightley 2009). In other words, the distributions of fitness in
141 the experimental populations can be explained by a high mutation rate and a low strength of
142 selection (s) or vice versa, making interpretation of these experimental results challenging.

143 Finally, experimental approaches are also only possible for laboratory organisms such as
144 *Drosophila* or yeast, thus their relevance for understanding deleterious mutation parameters in
145 natural populations of mammals such as the vaquita is unclear.

146 These limitations have motivated the increasing use of sequence-based estimates of the
147 distribution of s , which leverage genetic variation datasets to estimate selection parameters
148 while controlling for demography (Eyre-Walker and Keightley 2007). Such approaches are
149 widely employed in population genetics (Eyre-Walker et al. 2006; Boyko et al. 2008; Ma et al.
150 2013; Chen et al. 2017; Huber et al. 2017, 2018; Kim et al. 2017; Tataru et al. 2017; Huang et al.
151 2021) and give a much more complete picture of the full spectrum of deleterious mutations,
152 from strongly to mildly deleterious. Nevertheless, these approaches do have limitations in that
153 they are not well suited for estimating the proportion of lethal mutations (Wade et al. 2022)
154 and often assume additivity during inference (though see Huber et al. (2018)). Addressing these
155 limitations is an area of ongoing research.

156 Given these limitations of sequence-based approaches, we explored a variety of
157 dominance models in our analysis, as well as models with an additional proportion of lethal
158 mutations (see Fig. S21 and Table S6 in Robinson et al. 2022). We also explored results when
159 assuming a selection and dominance model proposed by Kardos et al. (2021) that is similar to
160 that of Pérez-Pereira et al. (2022) (see Kyriazis et al. 2022, for a detailed comparison of these
161 models). In all cases, we found that recovery was still the likely outcome when assuming a 90%
162 reduction in bycatch mortality rates (Robinson et al. 2022). In fact, we observed much lower
163 predicted extinction rates under the model proposed by Kardos et al. (2021), perhaps due to
164 more efficient purging in models with a high fraction of lethal mutations (Robinson et al. 2022).
165 In sum, models with a higher lethal mutation rate, like those favored by GD&H, also support our
166 main conclusion that recovery is possible.

167 Finally, we note we did not consider the impact of non-coding deleterious mutations, as
168 these mutations are generally inferred to be weakly deleterious (s on the order of $1e-3$;
169 (Torgerson et al. 2009; Murphy et al. 2021; Dukler et al. 2022)) and therefore not highly
170 relevant to modelling inbreeding depression. Indeed, the analysis of Pérez-Pereira et al. (2021)
171 supports this assumption by suggesting that such deleterious mutations, though relevant over

172 evolutionary timescales, are not particularly relevant in a conservation context. Future work
173 should aim to refine estimates of the strength of selection against non-coding deleterious
174 mutations and explore their potential impact on genetic load and extinction risk.

175

176 **How can we validate simulation models?**

177 Although the above verbal arguments serve as justification for the simulation analysis
178 we present in Robinson et al. (2022), several approaches can be employed to more
179 quantitatively validate and compare selection and dominance models informed by
180 experimental versus sequence-based studies. These include comparing proposed models in
181 terms of (1) how well they agree with patterns of genetic variation in sequencing datasets and
182 (2) how well they agree with reliable empirical estimates of the inbreeding load.

183 In Kyriazis et al. (2022), we undertook this task using humans as a focal species. Humans
184 are useful for this exercise because there are extensive genetic variation datasets (Auton *et al.*,
185 2015), published demographic models (e.g., Gutenkunst et al. 2009; Gravel et al. 2011; Li and
186 Durbin 2011; Tennessen et al. 2012), estimates of mutation rates and coding sequence length
187 (Keightley 2012), estimates of the distribution of s (Eyre-Walker et al. 2006; Boyko et al. 2008;
188 Kim et al. 2017), and estimates of the inbreeding load (Bittles and Neel 1994) and number of
189 segregating recessive lethals per individual (Gao et al. 2015). Moreover, humans are much
190 more closely related to the vaquita than *Drosophila* and have a long-term N_e that is typical for
191 mammals ($N_e \sim 17,000$). In Kyriazis et al. (2022), we leveraged these existing estimates of
192 human demography, mutation rates, and coding sequence length to compare proposed
193 selection and dominance models from Pérez-Pereira et al. (2022) and Kardos et al. (2021) to a
194 model we previously presented in Kyriazis et al. (2021), which is similar to that of Robinson et
195 al. (2022). In Kyriazis et al. (2022), we also propose a new model that better incorporates the
196 impacts of recessive lethal mutations (Wade et al. 2022).

197 When comparing these various selection and dominance models, we found that
198 sequence-based models, such as the model used in Robinson et al. (2022), make predictions
199 that are consistent with empirical estimates of the inbreeding load in humans, whereas models
200 based on experimental approaches do not (Kyriazis et al. 2022). For example, our model

201 proposed in Kyriazis et al. (2022) predicts an inbreeding load of $B = 3.2$ and ~ 0.9 recessive lethal
202 mutations per diploid. These predictions are compatible with existing evidence in humans (note
203 that Bittles and Neel (1994) estimate $B = 0.7$ for humans, though this is likely to be an
204 underestimate as it is based only on juvenile mortality). By contrast, the model proposed by
205 Pérez-Pereira et al. (2022) predicts an inbreeding load of $B = 14$, vastly exceeding available
206 estimates in humans. The Pérez-Pereira et al. (2022) model also predicts ~ 12 recessive lethal
207 mutations per individual, whereas available estimates are on the order of 0.6 mutations per
208 diploid (Gao et al. 2015).

209 Comparing predicted patterns of genetic variation from these models to those observed
210 in humans also provides support for sequence-based models. Specifically, the Pérez-Pereira et
211 al. (2022) model predicts a large overabundance of rare mutations, with 72.8% of
212 nonsynonymous mutations predicted to be singletons (variants with frequency $1/2n$ in a
213 sample). However, only 56.8% of such mutations are observed to be singletons in the European
214 sample from the 1000 Genomes dataset (Auton et al. 2015; Kyriazis et al. 2022). This large
215 excess of rare mutations predicted by Pérez-Pereira et al. (2022) is a consequence of the
216 extreme strength of negative selection in the model, which results in deleterious mutations
217 being kept at low frequency. Importantly, the model we propose in Kyriazis et al. (2022)
218 predicts 57.3% of mutations to be singletons, in good agreement with observed patterns of
219 genetic variation in humans (Auton et al. 2015).

220 Overall, these findings are consistent with previous findings that selection parameters
221 derived from experimental studies in *Drosophila* and other taxa are biased towards strongly
222 deleterious mutations (Davies et al. 1999; Eyre-Walker and Keightley 2007; Kyriazis et al. 2022).
223 Thus, this analysis helps validate the use of sequence-based estimates of selection parameters,
224 such as those employed in Robinson et al. (2022), given that they are consistent both with
225 patterns of genetic variation and empirical estimates of the inbreeding load.

226

227 **Other concerns**

228 GD&H critique our simulations for ignoring stochastic environmental and demographic
229 factors and not modelling a loss of adaptive potential in the vaquita. However, our analysis

230 does incorporate demographic stochasticity, as we model survival and reproduction
231 probabilistically. Although we do not model environmental stochasticity or loss of adaptive
232 potential, the threat that these factors pose to the vaquita, if any, is entirely unknown.
233 Moreover, we emphasize that the aim of our analysis was to test the assumption that the
234 vaquita is doomed to extinction by inbreeding depression. We do not interpret our model
235 projections beyond demonstrating the qualitative result that inbreeding depression alone does
236 not impede recovery in the species, as we agree with the general view that population viability
237 models should be interpreted cautiously in terms of their ability to accurately predict future
238 population sizes (Beissinger and Westphal 1998). Though incorporating factors such as
239 environmental stochasticity may influence our model predictions, they would not change our
240 central conclusion that recovery remains possible.

241

242 **Conclusions**

243 In conclusion, we agree with GD&H that predictive models should be critically
244 evaluated. Indeed, the critical evaluation of proposed mutation and selection models that we
245 present in Kyriazis et al. (2022) serves as justification for the type of sequence-based models we
246 employed in Robinson et al. (2022). Moreover, this analysis demonstrates that models based on
247 experimental results, such as those proposed by Pérez-Pereira et al. (2022) and Kardos et al.
248 (2021), are biased towards strongly deleterious variation and are not consistent with patterns
249 of genetic variation or empirical estimates of the inbreeding load in humans. Nevertheless, our
250 analysis in Robinson et al. (2022) found that recovery was still the likely outcome when
251 assuming models with a much higher fraction of strongly deleterious variation. However, we
252 emphasize that all predictive models should be interpreted cautiously, given that there is often
253 a fair amount of uncertainty in parameter estimates that can be challenging to validate with
254 orthogonal sources of information (Beissinger and Westphal 1998). For instance, having field-
255 based estimates of the inbreeding load for the vaquita would represent a valuable source of
256 additional information for our study, however, such data do not currently exist, and may never
257 exist given the perilous circumstances of the species. Future work should aim to validate the
258 sorts of models we employ in Robinson et al. (2022) for wild species where field-based

259 estimates of the inbreeding load exist. Such work could greatly strengthen conclusions drawn
260 from such predictive models.

261 All modelling considerations aside, our conclusion that recovery is possible is also
262 supported by all field surveys since late 1990s, including those in 2019 and 2021, where active
263 healthy vaquitas with calves have been sighted (Rojas-Bracho et al. 2022). Despite an almost
264 certain increase in gillnetting within the small range where vaquitas remain, vaquitas continue
265 to survive at higher numbers than expected (Rojas-Bracho et al. 2022). The possibility of
266 recovery is also supported by numerous examples of recovery for species that have dwindled
267 below 20 individuals (Wiedenfeld et al. 2021), many of which were once thought to be doomed
268 to extinction. Although inbreeding depression may in many contexts contribute to population
269 decline, the naïve assumption that it will inevitably doom small populations is a dangerous view
270 that is harmful to species conservation.

271

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280

281 **Author contributions**

282 C.C.K. wrote the manuscript with input from all coauthors.

283

284 **Conflict of interest**

285 The authors declare no conflict.

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