1   **Costly sons do not lead to adaptive sex ratio adjustment in pilot whales,**

2   ***Globicephala melas***

3

4   **ABSTRACT**

5   Maternal investment in reproduction and parental care is an important determinant of both

6   offspring and maternal fitness. However, optimal investment strategies may differ depending on

7   offspring sex, potentially resulting in a sex-biased distribution of maternal resources or adaptive

8   variation in offspring sex-ratio. We used morphometric and genetic data collected from over 3400

9   long-finned pilot whales *Globicephala melas* in 40 pods to investigate whether females experience

10 differential costs of producing sons and daughters and whether they differentially invest in male and

11 female offspring. We found that male calves grow faster than female calves during the first five

12 years of life, suggesting that sons may require greater investment from lactating mothers. This

13 appears to result in mothers experiencing a higher cost to future reproductive opportunities when

14 producing male offspring as the presence of dependent sons (but not daughters) reduced the

15 probability that a female would be pregnant. Despite these costs, we found no evidence that

16 mothers adaptively adjust their investment in sons and daughters according to their body condition

17 or their social and physical environment. These results suggest that mothers may be constrained

18 from biasing investment in the sexes, or that additional benefits may be masking such costs.

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20 **KEY WORDS:** cetacean, *Globicephala melas*, group size, maternal investment, reproductive costs,

21 reproductive success, sex-ratio

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INTRODUCTION

Maternal investment includes the resources, energy and time used for reproduction. While investment in offspring contributes to maternal fitness through increasing offspring survival, investing highly can impose costs on maternal health, survival, and longevity (Lester, Shuter & Abrams, 2004). Consequently, females are likely to be under selection to adjust their investment in offspring adaptively according to the resources available to them (Clutton-Brock, Albon & Guinness, 1981).

In cases where the reproductive value of male and female offspring is influenced differently by variation in maternal investment, mothers may be selected to bias resource allocation towards offspring of one sex (Charnov, 1982). In polygynous species, females are usually a limiting resource over which males should compete for access, with males in good condition out-competing smaller, poorer quality males for access to reproductive females. Such species often show sexual dimorphism, with males growing faster or for longer than females, and hence attaining a larger adult size (Clutton-Brock, 2007). Accordingly, male offspring may require greater energetic investment from their mothers during gestation and/or lactation. Such differences have been shown in many studies, for example, males often weigh more at birth or receive more milk (Duncan, Harvey & Wells, 1984; Ono & Boness, 1996), or richer milk (Hinde, 2009; Robert & Braun, 2012).

In polygynous species, differential maternal investment often results in differential fitness costs to the mother. For example, red deer hinds *Cervus elaphus* and bighorn ewes *Ovis canadensis* that give birth to a son are less likely to reproduce successfully in the following breeding season compared to those that give birth to a daughter (Bérubé, Festa-Bianchet & Jorgenson, 1996; Clutton-

2

48   Brock, et al., 1981) and female African elephants *Loxodonta africana* have greater inter-birth

49   intervals after weaning sons (Lee & Moss, 1986). Similarly, female bank voles *Myodes glareolus*

50   raising experimentally manipulated male-biased litters had higher energy requirements and

51   produced smaller female offspring in their subsequent litters (Rutkowska, Koskela, Mappes & J.R.,

52   2011). However, some studies have failed to find such effects. For example, studies of two highly

53   polygynous and sexually dimorphic pinnipeds, the Antarctic fur seal *Arctocephalus gazelle* (Lunn &

54   Arnould, 1997) and the northern elephant seal *Mirounga angustirostris* (Kretzmann, Costa & Le

55   Boeuf, 1993) fail to find sex-differences in various measures of maternal investment such as suckling

56   rate, pup growth rate or size at weaning. Instead, male seals may invest more in growth post-

57   weaning in order to attain a larger adult size (Lunn & Arnould, 1997).

58

59   In addition to adjusting the quality of offspring produced, mothers can also adjust

60   investment in the sexes by producing unequal numbers of male and female offspring. Trivers and

61   Willard (1973) proposed that, in species where maternal condition affects the fitness of male and

62   female offspring differently, mothers in good condition should bias their offspring sex ratio in favour

63   of the sex for which condition has the strongest impact on fitness. In polygynous species, mothers in

64   good condition are expected to invest in sons while mothers in poor condition should invest in

65   daughters, for which condition has less impact on fecundity. This model has been applied to a

66   variety of mammalian species including ungulates (Sheldon & West, 2004), marsupials (Robert &

67   Schwanz, 2011) and primates (Brown, 2001). In many cases, observations met the predictions,

68   though studies of other species and even of the same species but at different population densities

69   (Kruuk et al., 1999) have failed to show a response (MacLeod & Clutton-Brock, 2013). This apparent

70   variability has led some to question whether there is a common pattern, with suggestions of

71   publication bias and the possibility that additional factors could affect the relationship between

72   maternal condition and sex ratio (Brown & Silk, 2002; Sheldon & West, 2004).

73

74   In social species, sex-differences in dispersal and cooperation may also impact on the costs

75 and benefits of investing in male and female offspring (Emlen, Emlen & Levin, 1986; Hamilton, 1967).

76 For example, sex-differences in helping behaviour often occur in species that show cooperative care

77 where mothers with few helpers may benefit from overproducing the more helpful sex (Emlen, et al.,

78 1986; West & Sheldon, 2002). However, the benefits of producing philopatric helpers may be

79 counteracted to some extent by local competition for food or mates, leading to the prediction that

80 mothers should invest more in the dispersing (less helpful) sex when in poor condition, or when

81 breeding on poor quality territories (Wild & West, 2007). Here, the benefits of producing extra

82 helpers may be outweighed by the costs of producing more competitors, leading to a density-

83 dependent trade-off between costs and benefits of investment in a particular sex (Emlen, et al.,

84 1986). For example, Seychelles warblers show extreme adaptive modification of offspring sex ratio

85 according to an interaction between resource availability and local competition on the natal territory,

86 overproducing female helpers when on territories of sufficient quality to support additional group-

87 members (Komdeur, Daan, Tinbergen & Mateman, 1997). As a consequence, the potential effects of

88 sex-differences in cooperation and competition should be taken into account when investigating

89 maternal investment in the sexes.

90

91   Cetaceans offer an interesting system in which to study maternal investment. The generally

92 larger, non-social baleen whales show reversed sexual dimorphism with females often attaining

93 larger sizes than males (Clapham, 1996). In contrast, many Odontocetes (toothed whales, including

94 dolphins) are highly social, living in groups of up to several hundred individuals or even more

95 (Connor, Mann, Tyack & Whitehead, 1998). Among Odontocetes, males are often larger than

96 females and social interactions may be complicated and enduring. Thus, killer whales *Orcinus orca*

97 live in small matrifocal groups known as pods, with sons staying with their mothers for their entire

98   lives but mating with females in different pods (Foster et al., 2012). Similar systems appear to

99   operate in closely related pilot whales, though with much larger pod sizes. Social ties appear to be

100 unusually strong, with extended maternal dependence and evidence of post-weaning suckling (Amos,

101 Schlötterer & Tautz, 1993).

102

103   Here, we explore factors influencing maternal investment and sex ratio biases in the long-

104 finned pilot whale, *Globicephala melas*. The long-finned pilot whale is a medium-sized whale that

105 lives in groups of between 10 and 1000 animals (Ottensmeyer & Whitehead, 2003). Like its relative,

106 the killer whale (Parsons, Balcomb, Ford & Durban, 2009; Yurk, Barrett-Lennard, Ford & Matkin,

107 2002), groups appear to be extremely long-lived, with individuals of both sexes apparently remaining

108 in their natal pods all their lives (Amos, Barrett & Dover, 1991; Amos, et al., 1993). Male pod

109 members can generally be excluded as fathers of offspring in their own pod using genetic methods,

110 hence it has been hypothesised that mating is likely to occur between different pods when they

111 meet, or during male prospecting trips (Amos, et al., 1991). Female pilot whales may live as long as

112 60 years, perhaps a third again as long as males, who live to a maximum age of around 45 years

113 (Bloch et al., 1993a). Sexual size dimorphism has been observed in the long-finned pilot whale, with

114 adult males reaching 625cm in length, and adult females reaching 512cm (Bloch, Lockyer &

115 Zachariassen, 1993b). Pods generally have a female-biased sex-ratio (Bloch, et al., 1993a), but it is

116 currently unclear as to the extent to which this is due to sex-differences in survival, dispersal or sex-

117 ratio at birth.

118

119   Here we test the prediction that juvenile male pilot whales will grow faster than female

120 offspring during the period of maternal care, and hence will be likely to require greater investment

121 from lactating mothers. We then investigate whether this in turn leads to mothers experiencing a

5

122 higher cost to future reproductive opportunities of producing male offspring. Finally, we test

123 whether mothers adaptively adjust their investment in sons and daughters according to their body

124 condition and their social and physical environment.

125

126 **METHODS**

127

128 *Sample collection*

129   Data were obtained from a pilot whale drive fishery in the Faroe Islands (Zachariassen, 1993),

130 where until recently pilot whales constituted a vital part of the local diet. Between 1986 and 1989,

131 data were collected from 3470 animals from 40 pods as part of a Faroese government funded

132 research project on the biology of the pilot whale (Bloch, et al., 1993a). The sex and total body

133 length of captured whales were recorded, and age was determined by counting the growth layer

134 groups visible in a tooth section (Lockyer, 1993). There is substantial variation in the age at

135 maturation, with some females reaching sexual maturity at 5 or 6 years old (mean 8.4 years (Bloch,

136 et al., 1993b)), while other individuals (particularly males) do not reach maturity until 11-12 years

137 old (Desportes, Saboureau & Lacroix, 1993). For the purposes of this study, whales were split into

138 two age-classes. Individuals were classed as calves if they were 5 years old or younger, as offspring

139 have been observed to suckle until this age (Desportes & Mouritsen, 1988). As females may give

140 birth at age 6, individuals aged 6+ were considered to be adults and were included as potential

141 mothers in maternity analyses. Due to the extreme cohesion of pilot whale pods, it is believed that

142 pods were sampled in their entirety (Amos, et al., 1993) hence the size and sex-ratio of each pod

143 could be calculated. Only individuals classed as adults were included in the calculations of pod size

144 and sex ratio. Reproductive status was allocated to females via a post-mortem, which assessed the

145 presence, length and the morphological sex of any foetus present. Due to time-constraints when

146   sampling a large number of whales, it was not possible to take every measurement for each

147   individual, hence gaps in the data occurred. Analyses always included the maximum number of

148   individuals for which data were available.

149

150   *Calculating age-controlled length*

151   We assume that animals who are in better body condition, here defined as being relatively

152   long for their age, are growing faster through receipt of greater resources. In order to estimate

153   adult body condition and differences in offspring growth rates, we calculated the age-controlled

154   length of individuals for which length and age data (from tooth sections) were available. Age-

155   controlled length was calculated as the residual from a quadratic regression across individuals of

156   length (cm) on age (years). Since males and females are likely to grow at different rates, separate

157   regressions were carried out for each sex. Similarly, to maximise the fit of the regression during

158   early-life growth, additional regressions were carried out on male and female calves under 6 years

159   old.

160

161   *Maternity assignment*

162   A total of 1758 pilot whales, comprising 95% of individuals from 25 pods, had a skin sample

163   taken for genetic analysis. Genotyping was conducted at a panel of nine highly polymorphic

164   microsatellite loci: 199/200, 417/418, 468/469, 409/470, 415/416, and 464/465 (Amos, et al., 1993)

165   and EV37, EV94, EV1 (Valsecchi & Amos, 1996). A subset of pods for which sampling and

166   morphometric data collection was particularly comprehensive (*N*=841 individuals comprising 13

167   complete pods) were genotyped at an additional seven loci: D14 and D22 (Shinohara, Domingo-

168   Roura & Takenaka, 1997); FCB6/17, FCB3 and FCB1 (Buchanan, Friesen, Littlejohn & Clayton, 1996);

169   SW10 (Richard, Whitehead & Wright, 1996) and Gm8 (Fullard et al., 2000). Although this meant that

170   pods differed in the number of microsatellites used in maternity assignments, accounting for this in

171   our models revealed no effect and it should be subsumed in our mixed-effects models by using pod

172   ID as a random factor. Consequently, in the results presented, we did not include this as a separate

173   factor in our models. Genotyping was carried out as described in Fullard et al. (2000).

174   Maternity analysis was conducted using the computer program Cervus, version 3.0 (Marshall,

175   Slate, Kruuk & Pemberton, 1998), which uses a likelihood-based method to assign the most likely

176   parents of an offspring at a specified confidence level. On average, offspring had 25 candidate

177   mothers (range 0 - 67). All females present in the same pod of the offspring were considered to be

178   potential mothers, excluding females that were less than 6 years older than the offspring. A

179   threshold of 6 years was chosen as this is the earliest age at which females have been observed to

180   give birth. For a minority of individuals (170 whales from 20 pods), age had not been calculated from

181   a tooth section due to time constraints when sampling. Here, age was estimated using a quadratic

182   regression of age against length (conducted for each sex separately). Pilot whales grow rapidly for

183   the first few years of life but then growth plateaus, after which point it is not possible to estimate

184   age from length. Females over 400cm in length were therefore considered to be at least 10 years old,

185   and males over 500cm were considered to be at least 15 years of age. For ten individuals, no age or

186   length data were available. In this case, whales were included as potential mothers when they were

187   present in the appropriate pod.

188

189   Cervus determines the confidence of maternity assignments by conducting simulations to

190   evaluate the confidence in assignment of parentage. As well as using observed allele frequencies,

191   the simulation takes account of the number of candidate mothers, the proportion of candidate

192   mothers sampled, completeness of genotyping and estimated frequency of genotyping error. As

193   pilot whale pods are likely to include relatives, simulations to generate the critical delta (difference

194   in log likelihood deemed adequate for a reliable parentage assignment to be made) allowed for 10%

195   of candidate mothers being related to the true mother by 0.25. Although complete pods were

196   sampled, it is likely that some mothers had died or emigrated since the offspring was born. Hence,

197   we included the conservative estimate of 50% mothers having been sampled. Through re-genotyping

198   a subset of samples (N=45) the genotyping error rate was shown to be low, with 0.012 of alleles

199   incorrectly genotyped across loci. Nevertheless, to maximise the accuracy of maternity assignment,

200   we restricted the analysis to include only individuals that had been genotyped at a minimum of 7

201   markers. Maternity analysis yielded 681 maternities assigned at > 90% confidence, including 497

202   assigned at >95% confidence.

203

204   *Statistical analyses*

205   All statistical analyses were performed in the R statistical package 2.11.1 (R Development

206   Core Team, Vienna, Austria). Data included repeat sampling from pods, so generalized linear mixed

207   models (GLMMs) were used to control for pseudoreplication, with the identity of the pod fitted as a

208   random factor. Normally distributed data were analysed using an identity link function, while

209   binomial data were analysed using a logit link function. Full models (including all second-order

210   interactions) were fitted and then simplified by sequential removal of nonsignificant terms ($P > 0.05$),

211   tested using ANOVA. Once the minimal model was achieved, each dropped term was retested by

212   adding it to the final model. Four GLMMs exploring maternal investment and sex ratio were fitted.

213

214   **Model 1**: *Do sons require greater maternal investment than daughters?*

215   To investigate whether male and female offspring are likely to require differing levels of maternal

216   investment, we explored sex differences in early life growth. Calf length was fitted as the response

217   term in a GLMM with normal error structure, with calf sex fitted as an explanatory term, along with

218   calf age, pod size, pod sex ratio and season of capture. Calf early life growth is not linear, with faster

219   growth in the youngest animals. To account for this, calf age squared was included in the model.

220   Data were available from 740 calves under 6 years old in 36 pods.

221

222   **Model 2**: *Do mothers suffer a greater fitness cost of producing sons than daughters?*

223   To explore the potential fitness costs of raising male versus female offspring, we investigated

224   whether the presence of dependent sons and daughters influenced current reproductive status.

225   Female reproductive state (1=pregnant, 0=not pregnant) was fitted as the response variable in a

226   binomial GLMM. The number of dependent sons and number of dependent daughters (under 6

227   years old) were fitted as potential explanatory factors, along with female age, length, pod size, pod

228   sex ratio and season of capture. Data were available for 559 adult females from 25 pods.

229

230   **Model 3.** *Do mothers adaptively adjust the sex of their offspring?*

231   To investigate whether females adaptively adjust the sex of their offspring prepartum, a binomial

232   GLMM was fitted with foetus sex (1=male, 0=female) as the response variable. Factors that might

233   influence the fitness benefits of producing male or female offspring (pod size, pod sex ratio, season

234   of capture, female age and age-controlled length) were included as potential explanatory factors.

235   Data were available for 200 pregnant females with foetuses of known sex, in 35 pods.

236

237   **Model 4.** *Do mothers adaptively adjust investment in sons and daughters?*

238   To investigate whether females adaptively adjust investment in sons versus daughters, dependent

239   on maternal condition, we fitted a GLMM with normal error structure. The age and sex-controlled

240   length of offspring (a measure of relative offspring size) was fitted as the response variable, and

241   offspring sex, mother's age, age-controlled length, season of capture, pod size and pod sex ratio

242   were fitted as potential explanatory factors. Data were available for 160 calves in 25 pods. Sixteen

243   mothers had two assigned calves aged under the age of 6. In this case, one of the two calves was

244   randomly excluded from analysis in order to avoid pseudoreplication.

245

246   **RESULTS**

247   Pods on average contained 19.1 adult males (± 2.37 SEM), 34.1 adult females (± 3.86 SEM)

248   and 24.6 calves (± 2.87 SEM). The mean sex ratio of calves (0.48 ± 0.02 SEM) was not significantly

249   different from 0.5 (Binomial test: X^2_1=2.09, N = 860, P=0.15). However, the adult sex-ratio was

250   female biased (34% male ± 2 SEM, Binomial test: X^2_1=237.42, N = 1798, P<0.001), possibly due to

251   higher rates of dispersal or mortality among males.

252

253   Male calves were found to be larger at birth and to grow faster than female calves (Model 1;

254   Table 1, Figure 1), suggesting that male offspring require greater maternal investment than do

255   females. Calf growth rate was significantly affected by season (Model 1; Table 1), probably due to

256   variation in food availability and birth rate (Martin & Rothery, 1993). Group size and age also

257   interacted to determine the size of calves, with calves having marginally reduced growth rates in

258   large pods (Model 1; Table 1). Adult females were less likely to be pregnant as the number of

259   dependent sons (under 6 years old) genetically assigned to the female increased (Model 2; Table 2,

260   Figure 2). However, the number of genetically assigned dependent daughters had no significant

261   effect on the likelihood that a female was pregnant. This suggests a greater fitness cost of producing

262   sons in comparison to daughters.

263

264   Despite the likely fitness cost of producing sons, there was no evidence that mothers

265   manipulated the sex ratio of their offspring in relation to their condition (fitted either as mother's

266   age or length controlled for age) or environmental conditions (pod size or sex ratio) (Model 3; Table

267   3). Similarly, there was no evidence that mothers invested differentially in sons and daughters in

268   accordance with mother's age, length controlled for age, pod size or sex ratio (Model 4; Table 4).

269   However, there was evidence that mothers invest in offspring of both sexes according to the

270   mother's age and group size. Young mothers appear to produce small calves, regardless of the size

271   of their pod, whilst older mothers produced relatively large calves in smaller pods but small calves in

272   larger pods (Model 4; Table 4, Figure 3).

273

274   **DISCUSSION**

275   We investigated factors influencing maternal investment in long-finned pilot whales,

276   *Globicephala melas*. We found that male offspring grow faster than female offspring during the first

277   five years of life, implying that sons require greater investment from lactating mothers. This in turn

278   leads to mothers experiencing a higher cost to future reproductive opportunities when producing

279   male offspring as mothers with dependent sons were less likely to be pregnant then mothers with

280   dependent daughters. However, despite these costs, we found no evidence that mothers adaptively

281   adjust their investment in sons and daughters according to their body condition or their social and

282   physical environment.

283

284   Differential growth rates between juvenile male and female pilot whales could arise due

285   to differences in the quantity and/or quality of milk they require (Duncan, et al., 1984; Hinde, 2009;

286   Ono & Boness, 1996; Robert & Braun, 2012). Differences in lactation are possible in pilot whales as

287   weaning appears to begin at a marginally earlier age for daughters (Desportes & Mouritsen, 1988),

288   but differences in suckling rate and milk nutritional value may also occur. Differences in lactation

289   have been found in other cetaceans, for example lactose has been found in the stomachs of sperm

290 whales up to 13 years of age in males and 7.5 years in females (Best, Canham & Macleod, 1984),

291 suggesting that males suckle for longer than females. Maternal care may be particularly important to

292 male success, even in later life. For example, for killer whales over the age of 30, the death of a

293 mother increases mortality risk 13.9-fold in sons and 5.4-fold in daughters in the year after their

294 mother's death (Foster, et al., 2012).

295

296   In pilot and killer whales as well as other species where both sexes remain in the natal group,

297 mothers may be selected to increase the survival of sons because males mate outside of the group,

298 while a daughter's offspring are raised within the group and hence increase local competition for

299 resources (Johnstone & Cant, 2010). This potential need for differential resourcing of sons and

300 daughters may also be a driving force behind the evolution of extended post-reproductive lifespan in

301 cetaceans (Foster, et al., 2012; Johnstone & Cant, 2010). While menopause occurs in short-finned

302 pilot whales *Globicephala macrorhynchus* and killer whales, an extensive period of post-reproductive

303 lifespan has not been shown in long-finned pilot whales, where only 5% of females have a post-

304 reproductive lifespan, compared to 25% in killer whales and short-finned pilot whales (Cohen, 2004).

305 While older female long-finned pilot whales can become pregnant, very few actually do conceive

306 (only 2 of 41 females aged over 40 were carrying foetuses) and many older females lactate for

307 extended periods (Martin & Rothery, 1993). Thus, extended maternal care could still be important

308 in the long-finned pilot whale, even if mothers do not undergo a menopause.

309

310   The level of sexual size dimorphism found in the pilot whale suggests that male size may be

311 important in determining mating success. Such a relationship is likely to occur in a polygynous

312 mating system, where males compete for access to receptive females (Clutton-Brock, 2007).

313 Previous studies suggest that matings occur between pods rather than within pods, with several

males from one pod mating with females from a different pod during inter-pod encounters (Amos,

et al., 1993), hence males could be in competition for mating opportunities with males from their

own pod. An alternative, though entirely speculative, possibility might be that resident males

attempt to guard their female relatives from the attentions of lower quality suitors. However,

relatively little is known about the pilot whale mating system and more research is required to

reveal patterns of mating within and between groups.

In the current study, we found a reduced probability of female long-finned pilot whales

being pregnant as the number of dependent sons they have increases. This suggests that the

presence of sons increases inter-birth interval. Similar relationships have been found in other

mammalian species such as red deer (Clutton-Brock, et al., 1981) where hinds are less likely to give

birth in the year following the birth of a son. However, it is also possible that additional costs of

offspring production occur, for example sons could reduce mother's survival or weight or the

survival of future offspring (Bérubé, et al., 1996). Unfortunately, it was not possible to test these

possibilities in our study due to the lack of long-term data (samples were taken from drive fisheries).

Despite finding that males are likely to be more costly to rear than females, we found no

evidence that mothers preferentially produce sons or daughters depending on factors such as body

condition or age. There are several possible explanations for this lack of evidence for adaptive sex

ratio manipulation. First, there could be physiological constraints on controlling the sex of offspring

(Uller et al., 2007; West & Sheldon, 2002; Williams, 1979), with female pilot whales simply unable to

'choose' one sex over the other. Secondly, our rather simple analysis may provide a distorted

picture of the overall costs and benefits of producing sons versus daughters (Griffin, Sheldon & West,

2005). For example, if early male growth rate is a poor predictor of adult reproductive success, poor

338   body condition would no longer be a reason to preferentially produce daughters. The lack of any

339   evidence of sex-allocation biases by mothers could also be due to hidden costs of producing females.

340   While the dispersal patterns of pilot whales are not fully understood and individuals of both sexes

341   can remain philopatric, the bias in the adult sex-ratio of pilot whale pods suggests that males may be

342   more likely to disperse or die than females. Consequently, local resource competition within pods

343   may mean that mothers in poor condition suffer a long-term fitness cost of producing philopatric

344   daughters, explaining why we found no effect of mother's body condition on sex-allocation. Local

345   resource competition has been shown to impact on sex allocation in other species, for example in

346   the bobuck *Trichosurus cunninghami* and Seychelles warbler *Acrocephalus sechellensis* (Komdeur, et

347   al., 1997), mothers produce more dispersing males in poorer quality habitats (Banks, Knight, Dubach

348   & Lindenmayer, 2008). However, habitat quality is difficult to assess for pilot whale pods which do

349   not appear to be territorial and instead follow their cephalopod food sources (Desportes, et al.,

350   1993).

351

352   Alternatively, it is possible that sex ratio biases do occur but the constraints of the current

353   study mean that no pattern is detected. For example, maternal dominance or body condition pre-

354   conception (which could not be assessed post-mortem) could be important in determining maternal

355   investment in the sexes. In a meta-analysis of sex-ratio effects in ungulates, Sheldon & West (2004)

356   found that physiological measures were poor predictors of sex-ratio biases, especially if data were

357   collected post-conception. Instead, maternal dominance was a much more successful predictor of

358   offspring sex-ratio (Sheldon & West, 2004). Unfortunately, as the current study relied on data

359   collected post-mortem, we were not able to determine maternal dominance or body condition pre-

360   conception. Future studies would benefit from behavioural data collected pre-conception.

361

362   In group-living species, or species where cooperative care occurs, social factors may

363   influence the relative costs and benefits of producing male and female offspring. Despite this, we

364   found no influence of pod size or sex-ratio on sex-biased investment. The lack of a relationship in

365   pilot whales could be because there are no differences in the net benefits of the presence of male

366   and female group members, for example there could be no calf care provided by non-parents.

367   Furthermore, as pod-composition was measured post-conception, changes to the size and sex-ratio

368   of pods post-conception or post-partum may have masked any effect.

369

370   While the size of the pod had no impact on offspring sex-ratio, pod size did influence calf growth;

371   calves were larger in small pods, especially when their mother was older. The impact of pod size on

372   growth could be due to increased competition for food resources in larger groups. The impact of

373   female age on calf growth could be caused by either life history trade-offs or energetic constraints

374   on investment. Previous studies on the long-finned pilot whale have shown that, while older

375   mothers produce fewer offspring than younger mothers, they invest more in lactation. Thus, older

376   mothers have been shown to lactate for approximately three times longer than younger mothers

377   (9.1 years for females aged 40+ compared to 3.05 years in females aged 21-39 (Martin & Rothery,

378   1993)), hence the offspring of older mothers may receive more milk and grow faster as a result.

379   Older females may invest more in lactating for existing calves due to reproductive senescence, or

380   alternatively, older females may adaptively invest in lactation rather than pregnancy, possibly due to

381   a lower probability of surviving for long enough to gestate and wean further offspring.

382

383   **CONCLUSION**

384   Female long-finned pilot whales appear to invest more resources into male offspring than

385   female offspring, as evidenced by faster growth of sons in early life than daughters. Furthermore,

16

386   since a greater number of dependent sons (but not daughters) resulted in a lower probability of the

387   mother being pregnant with further offspring, caring for male offspring imposes a direct fitness cost

388   to an adult female. It is possible that these two findings are linked such that the demonstrated

389   fitness cost is a consequence of greater investment of time and/or resources in sons, but regardless

390   of the mechanism our data suggest that sons are costly to produce.

391   Despite this cost to having sons compared to daughters, we found no evidence for adaptive

392   sex ratio adjustment by mothers. Neither the body condition of the mother nor the biotic or abiotic

393   environment predicted offspring sex. We propose that either long-finned pilot whales are unable to

394   adjust offspring sex ratios due to physiological constraints, or that this study was unable to capture

395   all relevant factors. For instance, males may provide important benefits or females may have costs

396   that were not detected here. Nevertheless, we have presented evidence that sons are relatively

397   costly to mothers, but that this does not influence sex allocation in this species.

398

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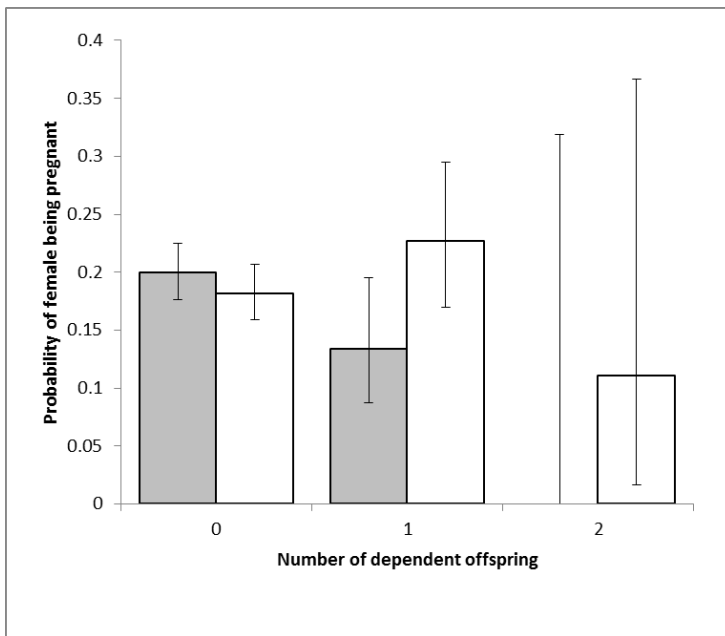524   UK: International Whaling Commission.

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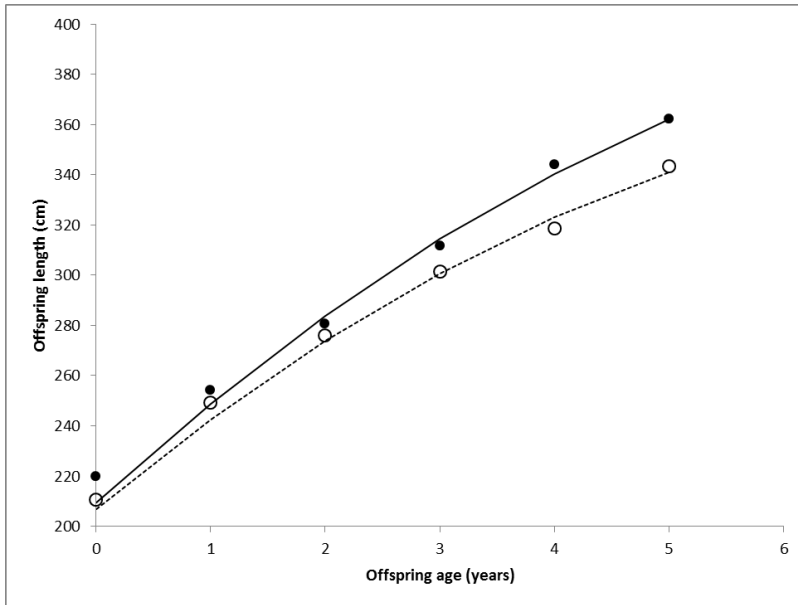
Figure 1. Differences in growth for male and female calves from Model 1, controlling for other factors in the model. Solid line and points represent males and the dotted line and circles represent females.
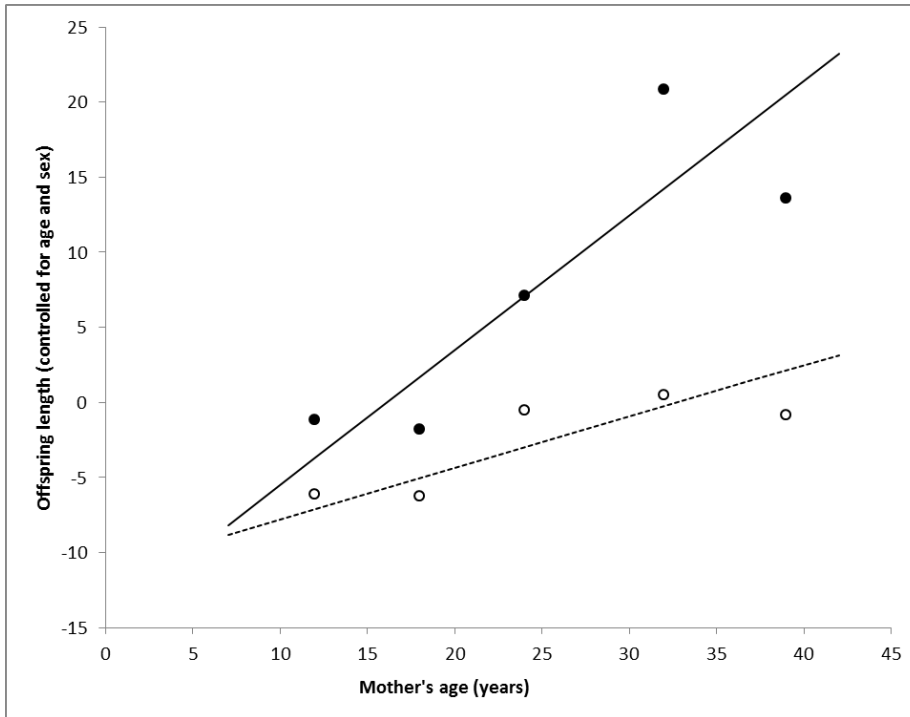
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Figure 2. The effect of dependent sons and daughters on the probability of females being pregnant. Raw data with 80% binomial confidence limits are shown. Shaded bars represent male offspring and unshaded bars represent female offspring.

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538   Figure 3. The impact of the mother's age and pod size on the relative length of calves (controlled for

539   age and sex). Regressions predicted from Model 4, with predicted means (points) controlling for all

540   other factors in the model are shown. Solid lines and points represent large pods (73 individuals) and

541   dotted line and circles represent small pods (20 individuals).

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543

544   Table 1. Summary of Model 1,

Model 1. Do sons require greater maternal investment than daughters?

Response variable: Calf length

Sample size: 740 offspring in 36 pods

Explanatory terms	X^2	Df	P
Season	10.24	3	0.017
Calf age * Calf sex	14.76	1	0.00012
Pod size * Calf Age^2	5.35	1	0.021
Pod sex ratio	0.46	1	0.50
Minimal Model	**Effect Size**		**SE**
Constant	212.49		5.42
Calf age	37.65		3.76
Calf age^2	-1.29		0.69
Calf sex (female)	-2.68		0.057
Pod size	-0.60		0.057
Season (2nd Quarter)	-4.35		3.59
(3rd Quarter)	2.47		3.06
(4th Quarter)	-6.54		3.03
Calf age * calf sex (female)	-3.68		0.95
Pod size * calf age	0.079		0.044
Pod size * calf age^2	-0.019		0.0081

545   Model 1 investigates whether sons require greater maternal investment than daughters. The table

546   shows Chi-squared (X^2), degrees of freedom (df) and P values associated with each term tested,

547   followed by the effect sizes and standard errors (SE) of terms included in the minimal model. Effect

548   sizes are on an identity link scale. All second order interactions between explanatory variables were

549   tested and are included in the table where interactions were significant. Where interaction terms

550   were significant, *P* values for interaction terms, rather than main effects, are presented. Note: where

551   categories are included as explanatory terms in the minimal model (sex and season), effect sizes for

552   the reference category (male and 1st quarter) are always zero.

553

554

555

556   Table 2. Summary of Model 2.

Model 2. Do mothers suffer a greater cost of producing sons than daughters?

Response variable: Female reproductive state (1=pregnant, 0 = not pregnant)

Sample size: 559 females in 25 pods

Explanatory terms	X^2	Df	*P*
Number of dependent sons	6.40	1	0.011
Number of dependent daughters	0.022	1	0.88
Female's age	0.059	1	0.81
Female's length (AC)	2.38	1	0.12
Pod size	0.077	1	0.78
Pod sex-ratio	3.36	1	0.067
Season	5.28	3	0.15
Minimal Model	**Effect Size**		**SE**
Constant	-1.53		0.20
Dependent sons	-0.81		0.35

557   Model 2 investigates whether mothers suffer a greater cost of producing sons than daughters. The

558   table shows Chi-squared (X^2), degrees of freedom (df) and *P* values associated with each term

559   tested, followed by the effect sizes and standard errors (SE) of terms included in the minimal model.

560   Effect sizes are on a logit scale. All second order interactions between explanatory variables were

561   tested but none were significant. AC = age-controlled.

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564

565 Table 3. Summary of Model 3.

Model 3. Do mothers adaptively adjust the sex of their offspring?

Response variable: foetus sex (male = 1, female = 0)

Sample size: 200 pregnant females in 33 pods

Explanatory terms	X^2	df	P
Pod sex-ratio	0.98	1	0.32
Pod size	0.23	1	0.63
Mother's age	0.89	1	0.34
Mother's length (AC)	1.87	1	0.17
Season	5.22	3	0.15
Minimal Model	**Effect Size**		**SE**
Constant	-0.30		0.14

566 Model 3 investigates whether mothers adaptively adjust the sex of their offspring. The table shows

567 Chi-squared (X^2), degrees of freedom (df) and P values associated with each term tested, followed by

568 the effect sizes and standard errors (SE) of terms included in the minimal model. Effect sizes are on a

569 logit scale. All second order interactions between explanatory variables were tested but none were

570 significant. AC = age-controlled.

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574   Table 4. Summary of Model 4.

Model 4. Do mothers adaptively adjust investment in male and female offspring?

Response variable: Age and sex-controlled calf length

Samples size: 160 offspring in 25 pods

Explanatory terms	X^2	df	P
Offspring sex	0.048	1	0.83
Pod sex-ratio	0.16	1	0.69
Season	3.89	3	0.27
Mother's length (AC)	1.88	1	0.17
Mother's age * Pod size	4.074	1	0.044
Minimal Model	**Effect Size**		**SE**
Constant	-15.67		9.81
Mother's age	1.11		0.41
Pod size	0.061		0.14
Mother's age * Pod size	-0.010		0.0051

575   Model 4 investigates whether mothers adaptively adjust investment in male and female offspring.

576   The table shows Chi-squared (X^2), degrees of freedom (df) and *P* values associated with each term

577   tested, followed by the effect sizes and standard errors (SE) of terms included in the minimal model.

578   Effect sizes are on an identity link scale. All second order interactions between explanatory variables

579   were tested and are included in the table where interactions were significant. Where interaction

580   terms were significant, *P* values for interaction terms, rather than main effects, are presented. AC =

581   age-controlled.