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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.

19

20 **KEY WORDS:** cetacean, *Globicephala melas*, group size, maternal investment, reproductive costs,
21 reproductive success, sex-ratio

22

23

24 **INTRODUCTION**

25

26 Maternal investment includes the resources, energy and time used for reproduction. While
27 investment in offspring contributes to maternal fitness through increasing offspring survival,
28 investing highly can impose costs on maternal health, survival, and longevity (Lester, Shuter &
29 Abrams, 2004). Consequently, females are likely to be under selection to adjust their investment in
30 offspring adaptively according to the resources available to them (Clutton-Brock, Albon & Guinness,
31 1981).

32

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

43

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

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

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 than 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

314 males from one pod mating with females from a different pod during inter-pod encounters (Amos,
315 et al., 1993), hence males could be in competition for mating opportunities with males from their
316 own pod. An alternative, though entirely speculative, possibility might be that resident males
317 attempt to guard their female relatives from the attentions of lower quality suitors. However,
318 relatively little is known about the pilot whale mating system and more research is required to
319 reveal patterns of mating within and between groups.

320

321 In the current study, we found a reduced probability of female long-finned pilot whales
322 being pregnant as the number of dependent sons they have increases. This suggests that the
323 presence of sons increases inter-birth interval. Similar relationships have been found in other
324 mammalian species such as red deer (Clutton-Brock, et al., 1981) where hinds are less likely to give
325 birth in the year following the birth of a son. However, it is also possible that additional costs of
326 offspring production occur, for example sons could reduce mother's survival or weight or the
327 survival of future offspring (Bérubé, et al., 1996). Unfortunately, it was not possible to test these
328 possibilities in our study due to the lack of long-term data (samples were taken from drive fisheries).

329

330 Despite finding that males are likely to be more costly to rear than females, we found no
331 evidence that mothers preferentially produce sons or daughters depending on factors such as body
332 condition or age. There are several possible explanations for this lack of evidence for adaptive sex
333 ratio manipulation. First, there could be physiological constraints on controlling the sex of offspring
334 (Uller et al., 2007; West & Sheldon, 2002; Williams, 1979), with female pilot whales simply unable to
335 'choose' one sex over the other. Secondly, our rather simple analysis may provide a distorted
336 picture of the overall costs and benefits of producing sons versus daughters (Griffin, Sheldon & West,
337 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,

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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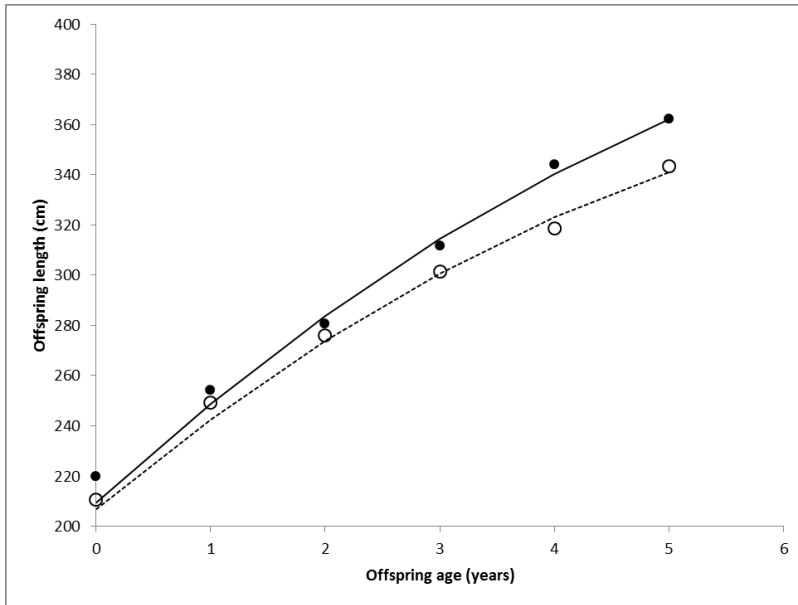
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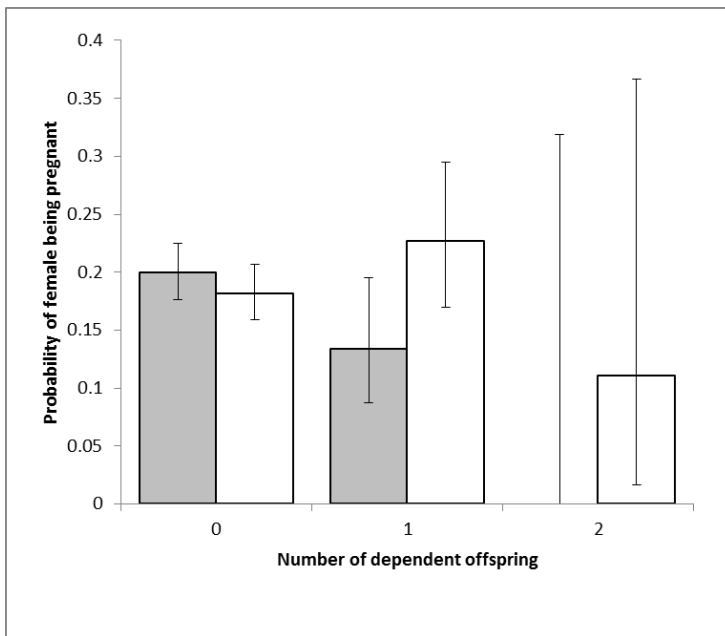
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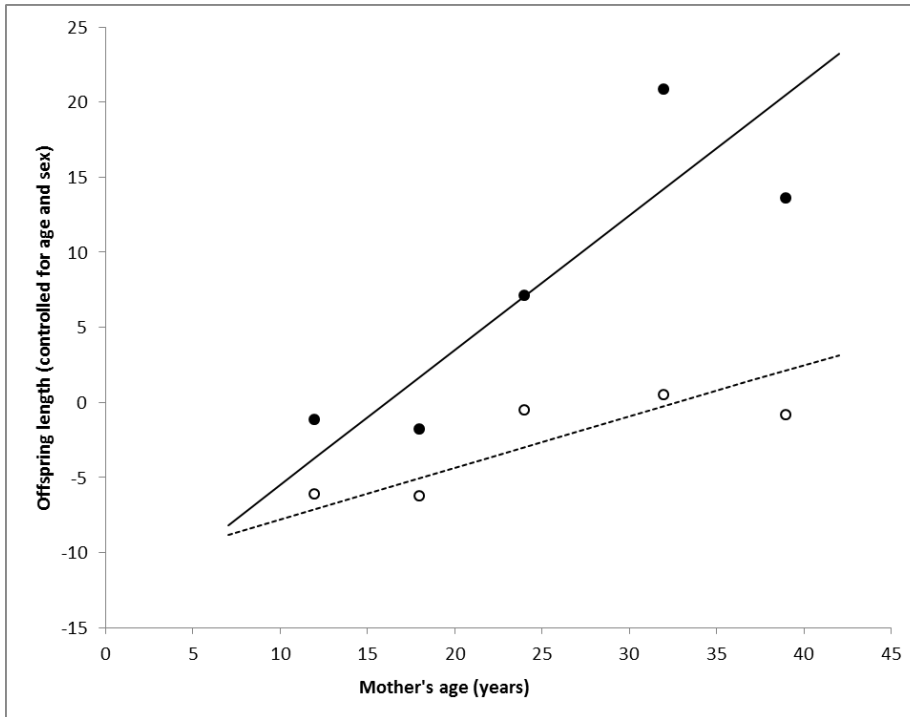
529 Figure 1. Differences in growth for male and female calves from Model 1, controlling for other
 530 factors in the model. Solid line and points represent males and the dotted line and circles represent
 531 females.

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533

534 Figure 2. The effect of dependent sons and daughters on the probability of females being pregnant.
 535 Raw data with 80% binomial confidence limits are shown. Shaded bars represent male offspring and
 536 unshaded bars represent female offspring.



537

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).

542

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	χ^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 (2 nd Quarter)	-4.35		3.59
(3 rd Quarter)	2.47		3.06
(4 th 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 (χ^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

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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	X²	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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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	χ^2	df	<i>P</i>
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 (χ^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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573

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	X²	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.