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

ABSTRACT

Maternal investment in reproduction and parental care is an important determinant of both offspring and maternal fitness. However, optimal investment strategies may differ depending on offspring sex, potentially resulting in a sex-biased distribution of maternal resources or adaptive variation in offspring sex-ratio. We used morphometric and genetic data collected from over 3400 long-finned pilot whales *Globicephala melas* in 40 pods to investigate whether females experience differential costs of producing sons and daughters and whether they differentially invest in male and female offspring. We found that male calves grow faster than female calves during the first five years of life, suggesting that sons may require greater investment from lactating mothers. This appears to result in mothers experiencing a higher cost to future reproductive opportunities when producing male offspring as the presence of dependent sons (but not daughters) reduced the probability that a female would be pregnant. Despite these costs, we found no evidence that mothers adaptively adjust their investment in sons and daughters according to their body condition or their social and physical environment. These results suggest that mothers may be constrained from biasing investment in the sexes, or that additional benefits may be masking such costs.

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

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-

Brock, et al., 1981) and female African elephants *Loxodonta africana* have greater inter-birth intervals after weaning sons (Lee & Moss, 1986). Similarly, female bank voles *Myodes glareolus* raising experimentally manipulated male-biased litters had higher energy requirements and produced smaller female offspring in their subsequent litters (Rutkowska, Koskela, Mappes & J.R., 2011). However, some studies have failed to find such effects. For example, studies of two highly polygynous and sexually dimorphic pinnipeds, the Antarctic fur seal *Arctocephalus gazelle* (Lunn & Arnould, 1997) and the northern elephant seal *Mirounga angustirostris* (Kretzmann, Costa & Le Boeuf, 1993) fail to find sex-differences in various measures of maternal investment such as suckling rate, pup growth rate or size at weaning. Instead, male seals may invest more in growth post-weaning in order to attain a larger adult size (Lunn & Arnould, 1997).

In addition to adjusting the quality of offspring produced, mothers can also adjust investment in the sexes by producing unequal numbers of male and female offspring. Trivers and Willard (1973) proposed that, in species where maternal condition affects the fitness of male and female offspring differently, mothers in good condition should bias their offspring sex ratio in favour of the sex for which condition has the strongest impact on fitness. In polygynous species, mothers in good condition are expected to invest in sons while mothers in poor condition should invest in daughters, for which condition has less impact on fecundity. This model has been applied to a variety of mammalian species including ungulates (Sheldon & West, 2004), marsupials (Robert & Schwanz, 2011) and primates (Brown, 2001). In many cases, observations met the predictions, though studies of other species and even of the same species but at different population densities (Kruuk et al., 1999) have failed to show a response (MacLeod & Clutton-Brock, 2013). This apparent variability has led some to question whether there is a common pattern, with suggestions of publication bias and the possibility that additional factors could affect the relationship between maternal condition and sex ratio (Brown & Silk, 2002; Sheldon & West, 2004).

In social species, sex-differences in dispersal and cooperation may also impact on the costs and benefits of investing in male and female offspring (Emlen, Emlen & Levin, 1986; Hamilton, 1967). For example, sex-differences in helping behaviour often occur in species that show cooperative care where mothers with few helpers may benefit from overproducing the more helpful sex (Emlen, et al., 1986; West & Sheldon, 2002). However, the benefits of producing philopatric helpers may be counteracted to some extent by local competition for food or mates, leading to the prediction that mothers should invest more in the dispersing (less helpful) sex when in poor condition, or when breeding on poor quality territories (Wild & West, 2007). Here, the benefits of producing extra helpers may be outweighed by the costs of producing more competitors, leading to a density-dependent trade-off between costs and benefits of investment in a particular sex (Emlen, et al., 1986). For example, Seychelles warblers show extreme adaptive modification of offspring sex ratio according to an interaction between resource availability and local competition on the natal territory, overproducing female helpers when on territories of sufficient quality to support additional group-members (Komdeur, Daan, Tinbergen & Mateman, 1997). As a consequence, the potential effects of sex-differences in cooperation and competition should be taken into account when investigating maternal investment in the sexes.

Cetaceans offer an interesting system in which to study maternal investment. The generally larger, non-social baleen whales show reversed sexual dimorphism with females often attaining larger sizes than males (Clapham, 1996). In contrast, many Odontocetes (toothed whales, including dolphins) are highly social, living in groups of up to several hundred individuals or even more (Connor, Mann, Tyack & Whitehead, 1998). Among Odontocetes, males are often larger than females and social interactions may be complicated and enduring. Thus, killer whales *Orcinus orca* live in small matrifocal groups known as pods, with sons staying with their mothers for their entire lives but mating with females in different pods (Foster et al., 2012). Similar systems appear to operate in closely related pilot whales, though with much larger pod sizes. Social ties appear to be unusually strong, with extended maternal dependence and evidence of post-weaning suckling (Amos, Schlötterer & Tautz, 1993).

Here, we explore factors influencing maternal investment and sex ratio biases in the long-finned pilot whale, *Globicephala melas*. The long-finned pilot whale is a medium-sized whale that lives in groups of between 10 and 1000 animals (Ottensmeyer & Whitehead, 2003). Like its relative, the killer whale (Parsons, Balcomb, Ford & Durban, 2009; Yurk, Barrett-Lennard, Ford & Matkin, 2002), groups appear to be extremely long-lived, with individuals of both sexes apparently remaining in their natal pods all their lives (Amos, Barrett & Dover, 1991; Amos, et al., 1993). Male pod members can generally be excluded as fathers of offspring in their own pod using genetic methods, hence it has been hypothesised that mating is likely to occur between different pods when they meet, or during male prospecting trips (Amos, et al., 1991). Female pilot whales may live as long as 60 years, perhaps a third again as long as males, who live to a maximum age of around 45 years (Bloch et al., 1993a). Sexual size dimorphism has been observed in the long-finned pilot whale, with adult males reaching 625cm in length, and adult females reaching 512cm (Bloch, Lockyer & Zachariassen, 1993b). Pods generally have a female-biased sex-ratio (Bloch, et al., 1993a), but it is currently unclear as to the extent to which this is due to sex-differences in survival, dispersal or sex-ratio at birth.

Here we test the prediction that juvenile male pilot whales will grow faster than female offspring during the period of maternal care, and hence will be likely to require greater investment from lactating mothers. We then investigate whether this in turn leads to mothers experiencing a higher cost to future reproductive opportunities of producing male offspring. Finally, we test whether mothers adaptively adjust their investment in sons and daughters according to their body condition and their social and physical environment.

METHODS

Sample collection

Data were obtained from a pilot whale drive fishery in the Faroe Islands (Zachariassen, 1993), where until recently pilot whales constituted a vital part of the local diet. Between 1986 and 1989, data were collected from 3470 animals from 40 pods as part of a Faroese government funded research project on the biology of the pilot whale (Bloch, et al., 1993a). The sex and total body length of captured whales were recorded, and age was determined by counting the growth layer groups visible in a tooth section (Lockyer, 1993). There is substantial variation in the age at maturation, with some females reaching sexual maturity at 5 or 6 years old (mean 8.4 years (Bloch, et al., 1993b)), while other individuals (particularly males) do not reach maturity until 11-12 years old (Desportes, Saboureau & Lacroix, 1993). For the purposes of this study, whales were split into two age-classes. Individuals were classed as calves if they were 5 years old or younger, as offspring have been observed to suckle until this age (Desportes & Mouritsen, 1988). As females may give birth at age 6, individuals aged 6+ were considered to be adults and were included as potential mothers in maternity analyses. Due to the extreme cohesion of pilot whale pods, it is believed that pods were sampled in their entirety (Amos, et al., 1993) hence the size and sex-ratio of each pod could be calculated. Only individuals classed as adults were included in the calculations of pod size and sex ratio. Reproductive status was allocated to females via a post-mortem, which assessed the presence, length and the morphological sex of any foetus present. Due to time-constraints when sampling a large number of whales, it was not possible to take every measurement for each individual, hence gaps in the data occurred. Analyses always included the maximum number of individuals for which data were available.

Calculating age-controlled length

We assume that animals who are in better body condition, here defined as being relatively long for their age, are growing faster through receipt of greater resources. In order to estimate adult body condition and differences in offspring growth rates, we calculated the age-controlled length of individuals for which length and age data (from tooth sections) were available. Age-controlled length was calculated as the residual from a quadratic regression across individuals of length (cm) on age (years). Since males and females are likely to grow at different rates, separate regressions were carried out for each sex. Similarly, to maximise the fit of the regression during early-life growth, additional regressions were carried out on male and female calves under 6 years old.

Maternity assignment

A total of 1758 pilot whales, comprising 95% of individuals from 25 pods, had a skin sample taken for genetic analysis. Genotyping was conducted at a panel of nine highly polymorphic microsatellite loci: 199/200, 417/418, 468/469, 409/470, 415/416, and 464/465 (Amos, et al., 1993) and EV37, EV94, EV1 (Valsecchi & Amos, 1996). A subset of pods for which sampling and morphometric data collection was particularly comprehensive (*N*=841 individuals comprising 13 complete pods) were genotyped at an additional seven loci: D14 and D22 (Shinohara, Domingo-Roura & Takenaka, 1997); FCB6/17, FCB3 and FCB1 (Buchanan, Friesen, Littlejohn & Clayton, 1996); SW10 (Richard, Whitehead & Wright, 1996) and Gm8 (Fullard et al., 2000). Although this meant that pods differed in the number of microsatellites used in maternity assignments, accounting for this in our models revealed no effect and it should be subsumed in our mixed-effects models by using pod ID as a random factor. Consequently, in the results presented, we did not include this as a separate factor in our models. Genotyping was carried out as described in Fullard et al. (2000).

Maternity analysis was conducted using the computer program Cervus, version 3.0 (Marshall, Slate, Kruuk & Pemberton, 1998), which uses a likelihood-based method to assign the most likely parents of an offspring at a specified confidence level. On average, offspring had 25 candidate mothers (range 0 - 67). All females present in the same pod of the offspring were considered to be potential mothers, excluding females that were less than 6 years older than the offspring. A threshold of 6 years was chosen as this is the earliest age at which females have been observed to give birth. For a minority of individuals (170 whales from 20 pods), age had not been calculated from a tooth section due to time constraints when sampling. Here, age was estimated using a quadratic regression of age against length (conducted for each sex separately). Pilot whales grow rapidly for the first few years of life but then growth plateaus, after which point it is not possible to estimate age from length. Females over 400cm in length were therefore considered to be at least 10 years old, and males over 500cm were considered to be at least 15 years of age. For ten individuals, no age or length data were available. In this case, whales were included as potential mothers when they were present in the appropriate pod.

Cervus determines the confidence of maternity assignments by conducting simulations to evaluate the confidence in assignment of parentage. As well as using observed allele frequencies, the simulation takes account of the number of candidate mothers, the proportion of candidate mothers sampled, completeness of genotyping and estimated frequency of genotyping error. As pilot whale pods are likely to include relatives, simulations to generate the critical delta (difference in log likelihood deemed adequate for a reliable parentage assignment to be made) allowed for 10%

of candidate mothers being related to the true mother by 0.25. Although complete pods were sampled, it is likely that some mothers had died or emigrated since the offspring was born. Hence, we included the conservative estimate of 50% mothers having been sampled. Through re-genotyping a subset of samples (*N*=45) the genotyping error rate was shown to be low, with 0.012 of alleles incorrectly genotyped across loci. Nevertheless, to maximise the accuracy of maternity assignment, we restricted the analysis to include only individuals that had been genotyped at a minimum of 7 markers. Maternity analysis yielded 681 maternities assigned at > 90% confidence, including 497 assigned at >95% confidence.

Statistical analyses

All statistical analyses were performed in the R statistical package 2.11.1 (R Development Core Team, Vienna, Austria). Data included repeat sampling from pods, so generalized linear mixed models (GLMMs) were used to control for pseudoreplication, with the identity of the pod fitted as a random factor. Normally distributed data were analysed using an identity link function, while binomial data were analysed using a logit link function. Full models (including all second-order interactions) were fitted and then simplified by sequential removal of nonsignificant terms ($P > 0.05$), tested using ANOVA. Once the minimal model was achieved, each dropped term was retested by adding it to the final model. Four GLMMs exploring maternal investment and sex ratio were fitted.

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

To investigate whether male and female offspring are likely to require differing levels of maternal investment, we explored sex differences in early life growth. Calf length was fitted as the response term in a GLMM with normal error structure, with calf sex fitted as an explanatory term, along with calf age, pod size, pod sex ratio and season of capture. Calf early life growth is not linear, with faster growth in the youngest animals. To account for this, calf age squared was included in the model. Data were available from 740 calves under 6 years old in 36 pods.

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

To explore the potential fitness costs of raising male versus female offspring, we investigated whether the presence of dependent sons and daughters influenced current reproductive status. Female reproductive state (1=pregnant, 0=not pregnant) was fitted as the response variable in a binomial GLMM. The number of dependent sons and number of dependent daughters (under 6 years old) were fitted as potential explanatory factors, along with female age, length, pod size, pod sex ratio and season of capture. Data were available for 559 adult females from 25 pods.

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

To investigate whether females adaptively adjust the sex of their offspring prepartum, a binomial GLMM was fitted with foetus sex (1=male, 0=female) as the response variable. Factors that might influence the fitness benefits of producing male or female offspring (pod size, pod sex ratio, season of capture, female age and age-controlled length) were included as potential explanatory factors. Data were available for 200 pregnant females with foetuses of known sex, in 35 pods.

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

To investigate whether females adaptively adjust investment in sons versus daughters, dependent on maternal condition, we fitted a GLMM with normal error structure. The age and sex-controlled length of offspring (a measure of relative offspring size) was fitted as the response variable, and offspring sex, mother's age, age-controlled length, season of capture, pod size and pod sex ratio were fitted as potential explanatory factors. Data were available for 160 calves in 25 pods. Sixteen mothers had two assigned calves aged under the age of 6. In this case, one of the two calves was randomly excluded from analysis in order to avoid pseudoreplication.

RESULTS

Pods on average contained 19.1 adult males (± 2.37 SEM), 34.1 adult females (± 3.86 SEM) and 24.6 calves (± 2.87 SEM). The mean sex ratio of calves (0.48 ± 0.02 SEM) was not significantly different from 0.5 (Binomial test: X^2_1=2.09, $N = 860$, P=0.15). However, the adult sex-ratio was female biased (34% male ± 2 SEM, Binomial test: X^2_1=237.42, $N = 1798$, P<0.001), possibly due to higher rates of dispersal or mortality among males.

Male calves were found to be larger at birth and to grow faster than female calves (Model 1; Table 1, Figure 1), suggesting that male offspring require greater maternal investment than do females. Calf growth rate was significantly affected by season (Model 1; Table 1), probably due to variation in food availability and birth rate (Martin & Rothery, 1993). Group size and age also interacted to determine the size of calves, with calves having marginally reduced growth rates in large pods (Model 1; Table 1). Adult females were less likely to be pregnant as the number of dependent sons (under 6 years old) genetically assigned to the female increased (Model 2; Table 2, Figure 2). However, the number of genetically assigned dependent daughters had no significant effect on the likelihood that a female was pregnant. This suggests a greater fitness cost of producing sons in comparison to daughters.

Despite the likely fitness cost of producing sons, there was no evidence that mothers manipulated the sex ratio of their offspring in relation to their condition (fitted either as mother's age or length controlled for age) or environmental conditions (pod size or sex ratio) (Model 3; Table 3). Similarly, there was no evidence that mothers invested differentially in sons and daughters in accordance with mother's age, length controlled for age, pod size or sex ratio (Model 4; Table 4). However, there was evidence that mothers invest in offspring of both sexes according to the mother's age and group size. Young mothers appear to produce small calves, regardless of the size of their pod, whilst older mothers produced relatively large calves in smaller pods but small calves in larger pods (Model 4; Table 4, Figure 3).

DISCUSSION

We investigated factors influencing maternal investment in long-finned pilot whales, *Globicephala melas*. We found that male offspring grow faster than female offspring during the first five years of life, implying that sons require greater investment from lactating mothers. This in turn leads to mothers experiencing a higher cost to future reproductive opportunities when producing male offspring as mothers with dependent sons were less likely to be pregnant then mothers with dependent daughters. However, despite these costs, we found no evidence that mothers adaptively adjust their investment in sons and daughters according to their body condition or their social and physical environment.

Differential growth rates between juvenile male and female pilot whales could arise due to differences in the quantity and/or quality of milk they require (Duncan, et al., 1984; Hinde, 2009; Ono & Boness, 1996; Robert & Braun, 2012). Differences in lactation are possible in pilot whales as weaning appears to begin at a marginally earlier age for daughters (Desportes & Mouritsen, 1988), but differences in suckling rate and milk nutritional value may also occur. Differences in lactation have been found in other cetaceans, for example lactose has been found in the stomachs of sperm whales up to 13 years of age in males and 7.5 years in females (Best, Canham & Macleod, 1984), suggesting that males suckle for longer than females. Maternal care may be particularly important to male success, even in later life. For example, for killer whales over the age of 30, the death of a mother increases mortality risk 13.9-fold in sons and 5.4-fold in daughters in the year after their mother's death (Foster, et al., 2012).

In pilot and killer whales as well as other species where both sexes remain in the natal group, mothers may be selected to increase the survival of sons because males mate outside of the group, while a daughter's offspring are raised within the group and hence increase local competition for resources (Johnstone & Cant, 2010). This potential need for differential resourcing of sons and daughters may also be a driving force behind the evolution of extended post-reproductive lifespan in cetaceans (Foster, et al., 2012; Johnstone & Cant, 2010). While menopause occurs in short-finned pilot whales *Globicephala macrorhynchus* and killer whales, an extensive period of post-reproductive lifespan has not been shown in long-finned pilot whales, where only 5% of females have a post-reproductive lifespan, compared to 25% in killer whales and short-finned pilot whales (Cohen, 2004). While older female long-finned pilot whales can become pregnant, very few actually do conceive (only 2 of 41 females aged over 40 were carrying foetuses) and many older females lactate for extended periods (Martin & Rothery, 1993). Thus, extended maternal care could still be important in the long-finned pilot whale, even if mothers do not undergo a menopause.

The level of sexual size dimorphism found in the pilot whale suggests that male size may be important in determining mating success. Such a relationship is likely to occur in a polygynous mating system, where males compete for access to receptive females (Clutton-Brock, 2007). 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 body condition would no longer be a reason to preferentially produce daughters. The lack of any evidence of sex-allocation biases by mothers could also be due to hidden costs of producing females. While the dispersal patterns of pilot whales are not fully understood and individuals of both sexes can remain philopatric, the bias in the adult sex-ratio of pilot whale pods suggests that males may be more likely to disperse or die than females. Consequently, local resource competition within pods may mean that mothers in poor condition suffer a long-term fitness cost of producing philopatric daughters, explaining why we found no effect of mother's body condition on sex-allocation. Local resource competition has been shown to impact on sex allocation in other species, for example in the bobuck *Trichosurus cunninghami* and Seychelles warbler *Acrocephalus sechellensis* (Komdeur, et al., 1997), mothers produce more dispersing males in poorer quality habitats (Banks, Knight, Dubach & Lindenmayer, 2008). However, habitat quality is difficult to assess for pilot whale pods which do not appear to be territorial and instead follow their cephalopod food sources (Desportes, et al., 1993).

Alternatively, it is possible that sex ratio biases do occur but the constraints of the current study mean that no pattern is detected. For example, maternal dominance or body condition pre-conception (which could not be assessed post-mortem) could be important in determining maternal investment in the sexes. In a meta-analysis of sex-ratio effects in ungulates, Sheldon & West (2004) found that physiological measures were poor predictors of sex-ratio biases, especially if data were collected post-conception. Instead, maternal dominance was a much more successful predictor of offspring sex-ratio (Sheldon & West, 2004). Unfortunately, as the current study relied on data collected post-mortem, we were not able to determine maternal dominance or body condition pre-conception. Future studies would benefit from behavioural data collected pre-conception.

In group-living species, or species where cooperative care occurs, social factors may influence the relative costs and benefits of producing male and female offspring. Despite this, we found no influence of pod size or sex-ratio on sex-biased investment. The lack of a relationship in pilot whales could be because there are no differences in the net benefits of the presence of male and female group members, for example there could be no calf care provided by non-parents. Furthermore, as pod-composition was measured post-conception, changes to the size and sex-ratio of pods post-conception or post-partum may have masked any effect.

While the size of the pod had no impact on offspring sex-ratio, pod size did influence calf growth; calves were larger in small pods, especially when their mother was older. The impact of pod size on growth could be due to increased competition for food resources in larger groups. The impact of female age on calf growth could be caused by either life history trade-offs or energetic constraints on investment. Previous studies on the long-finned pilot whale have shown that, while older mothers produce fewer offspring than younger mothers, they invest more in lactation. Thus, older mothers have been shown to lactate for approximately three times longer than younger mothers (9.1 years for females aged 40+ compared to 3.05 years in females aged 21-39 (Martin & Rothery, 1993)), hence the offspring of older mothers may receive more milk and grow faster as a result. Older females may invest more in lactating for existing calves due to reproductive senescence, or alternatively, older females may adaptively invest in lactation rather than pregnancy, possibly due to a lower probability of surviving for long enough to gestate and wean further offspring.

CONCLUSION

Female long-finned pilot whales appear to invest more resources into male offspring than female offspring, as evidenced by faster growth of sons in early life than daughters. Furthermore, since a greater number of dependent sons (but not daughters) resulted in a lower probability of the mother being pregnant with further offspring, caring for male offspring imposes a direct fitness cost to an adult female. It is possible that these two findings are linked such that the demonstrated fitness cost is a consequence of greater investment of time and/or resources in sons, but regardless of the mechanism our data suggest that sons are costly to produce.

Despite this cost to having sons compared to daughters, we found no evidence for adaptive sex ratio adjustment by mothers. Neither the body condition of the mother nor the biotic or abiotic environment predicted offspring sex. We propose that either long-finned pilot whales are unable to adjust offspring sex ratios due to physiological constraints, or that this study was unable to capture all relevant factors. For instance, males may provide important benefits or females may have costs that were not detected here. Nevertheless, we have presented evidence that sons are relatively costly to mothers, but that this does not influence sex allocation in this species.

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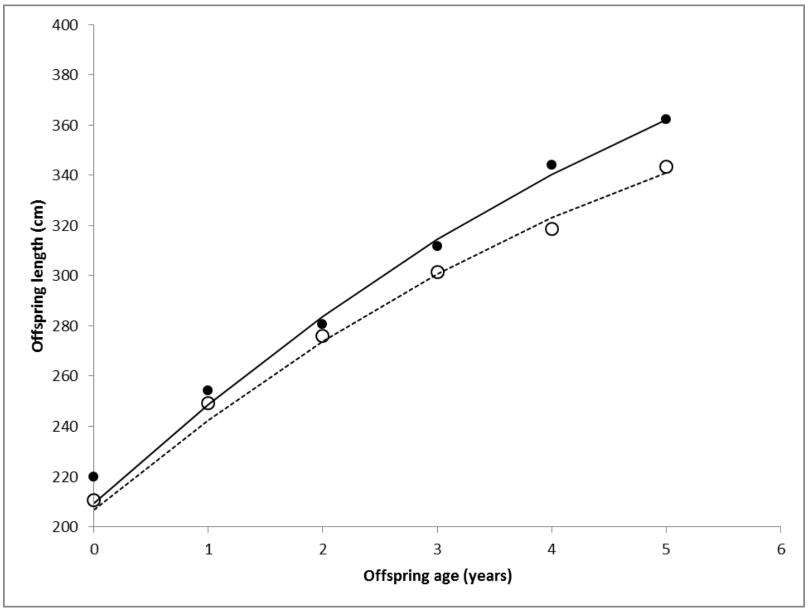

Figure 1. Differences in growth for male and female calves from Model 1, controlling for other factors in the model. Solid line and points represent males and the dotted line and circles represent females.

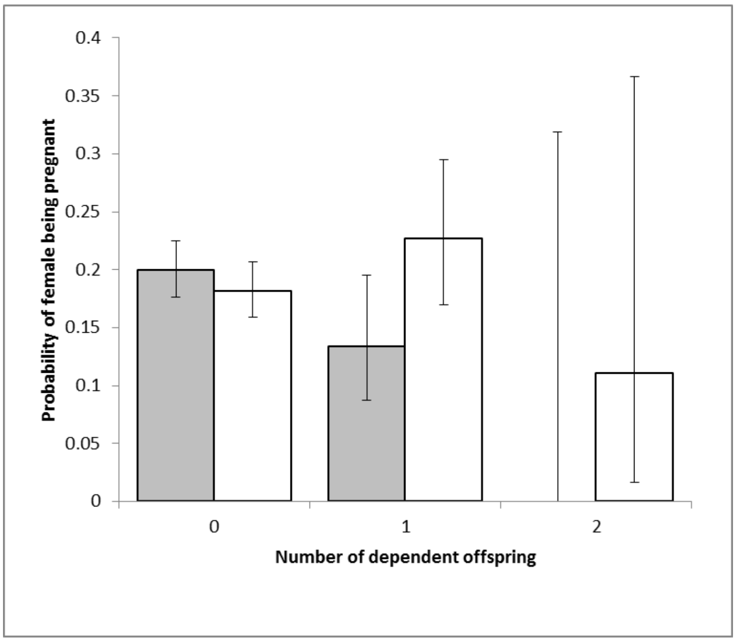

Figure 2. The effect of dependent sons and daughters on the probability of females being pregnant. Raw data with 80% binomial confidence limits are shown. Shaded bars represent male offspring and unshaded bars represent female offspring.

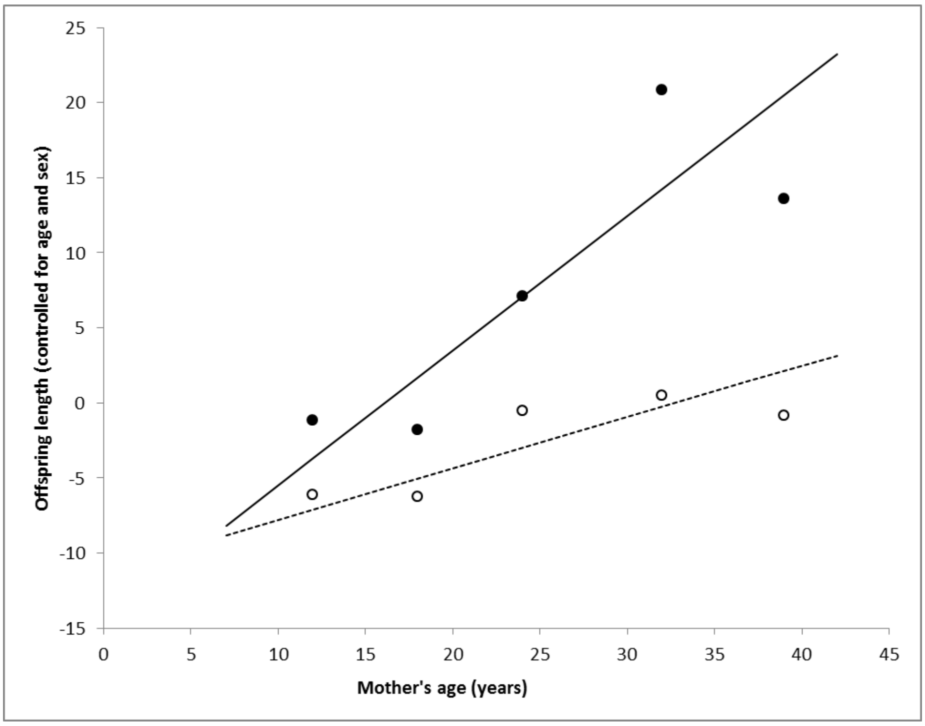

Figure 3. The impact of the mother's age and pod size on the relative length of calves (controlled for age and sex). Regressions predicted from Model 4, with predicted means (points) controlling for all other factors in the model are shown. Solid lines and points represent large pods (73 individuals) and dotted line and circles represent small pods (20 individuals).

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

Model 1 investigates whether sons require greater maternal investment than daughters. The table shows Chi-squared (X^2), degrees of freedom (df) and *P* values associated with each term tested, followed by the effect sizes and standard errors (SE) of terms included in the minimal model. Effect sizes are on an identity link scale. All second order interactions between explanatory variables were tested and are included in the table where interactions were significant. Where interaction terms were significant, *P* values for interaction terms, rather than main effects, are presented. Note: where categories are included as explanatory terms in the minimal model (sex and season), effect sizes for the reference category (male and 1st quarter) are always zero.

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

Model 2 investigates whether mothers suffer a greater cost of producing sons than daughters. The table shows Chi-squared (X^2), degrees of freedom (df) and *P* values associated with each term tested, followed by the effect sizes and standard errors (SE) of terms included in the minimal model. Effect sizes are on a logit scale. All second order interactions between explanatory variables were tested but none were significant. AC = age-controlled.

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

Model 3 investigates whether mothers adaptively adjust the sex of their offspring. The table shows Chi-squared (X^2), degrees of freedom (df) and *P* values associated with each term tested, followed by the effect sizes and standard errors (SE) of terms included in the minimal model. Effect sizes are on a logit scale. All second order interactions between explanatory variables were tested but none were significant. AC = age-controlled.

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

Model 4 investigates whether mothers adaptively adjust investment in male and female offspring. The table shows Chi-squared (X^2), degrees of freedom (df) and *P* values associated with each term tested, followed by the effect sizes and standard errors (SE) of terms included in the minimal model. Effect sizes are on an identity link scale. All second order interactions between explanatory variables were tested and are included in the table where interactions were significant. Where interaction terms were significant, *P* values for interaction terms, rather than main effects, are presented. AC = age-controlled.