High oxytocin infants gain more mass with no additional maternal energetic costs in wild grey seals (*Halichoerus grypus*)

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Abstract

Maximising infant survival requires secure attachments and appropriate behaviours between parents and offspring. Oxytocin is vital for parent-offspring bonding and behaviour. It also modulates energetic balance and neural pathways regulating feeding. However, to date the connections between these two areas of the hormone's functionality are poorly defined. We demonstrate that grey seal (*Halichoerus grypus*) mothers with high oxytocin levels produce pups with high oxytocin levels throughout lactation, and show for the first time a link between endogenous infant oxytocin levels and rates of mass gain prior to weaning. High oxytocin infants gained mass at a greater rate without additional energetic cost to their mothers. Increased mass gain in infants was not due to increased nursing, and there was no link between maternal mass loss rates and plasma oxytocin concentrations. Increased mass gain rates within high oxytocin infants may be due to changes in individual behaviour and energy expenditure or oxytocin impacting on tissue formation. Infancy is a crucial time for growth and development, and our findings connect the oxytocin driven mechanisms for parent-infant bonding with the energetics underlying parental care. Our study demonstrates that oxytocin release may connect optimal parental or social environments with direct physiological advantages for individual development.

Keywords

Maternal bonding; infant bonding; infant development; positive feedback loop; mass gain; parental investment

1. Introduction

Parental attachment and care giving behaviours are of fundamental importance to reproductive success in many species. Throughout the mammalian clade, maternal bonding and nurturing

behaviours are of particular importance, and infant survival is frequently solely dependent on how mothers interact with their offspring. Mothers cannot succeed in raising offspring without some degree of co-ordination between parties to accomplish the common goal of infant survival to independence (Fleming et al., 1999). Cognitive and physiological systems that promote behavioural synchrony across parent-infant dyads play a vital role in this co-ordination. However, any mechanism that enables parent-infant interactions must function despite changing infant cognitive abilities as they develop across the period they are dependent on their parent(s) (Rice and Barone, 2000). Therefore, in infants, physiological systems mediating behavioural expression may be key to keeping dependent offspring with their parents and ensuring infants act appropriately towards them and other conspecifics.

The neuropeptide hormone oxytocin (OT) is vital for both social and parental bonding, plays a key role in the initiation of maternal behaviour and in some species mediates the continuance of good quality infant care throughout the dependent period (Gimpl and Fahrenholz, 2001; Ross and Young, 2009; Rilling and Young, 2014). At birth, a mother's OT release initiates bonding with her infant and maternal care (Gimpl and Fahrenholz, 2001; Ross and Young, 2009). It has been theorised that OT then acts in a positive feedback loop within mother-infant pairs to develop secure attachment between the two and to mediate maternal behaviour directed towards the infant (Rilling and Young, 2014; Nagasawa et al., 2012). A mother's OT feedback loop is initiated via filial infant stimuli causing additional OT release in the mother after birth (Strathearn et al., 2009). This OT expression has been shown to trigger care giving behaviours towards human infants while activating dopamine 'reward' systems a mother's brain (Strathearn et al., 2009), and in humans there is high co-expression between OT and dopaminergic receptor genes to facilitate this (Quintana et al., 2019). Then, by performing care giving behaviours towards her infant, a mother is more likely to be exposed to additional infant stimuli that causes even more OT release

in the mother, perpetuating the 'loop' and generating elevated OT concentrations within securely attached mothers (Rilling and Young, 2014). This positive feedback loop is also theorised to exist in the infant, with good quality maternal care causing infant attachment to the mother and OT release due to parental stimuli (Kojima et al., 2012), generating high OT concentrations in the infant. Therefore, if double positive OT feedback loops exist in mother-infant pairs, with one loop in each individual, high OT mothers should also have high OT infants (Rilling and Young, 2014). Experiments using non-filial socially bonded individuals show that positive OT feedback loops exist across individuals in social contexts (Nagasawa et al., 2015). However, there is no evidence to date that such loops exist within mother-infant pairs, due to a lack of data on infant OT responses alongside their mother's OT concentrations.

While the effects of changing OT concentrations within mothers is well studied (Gimpl and Fahrenholz, 2001), impacts on infants, or the physiology of peripheral tissues, remain poorly understood. There is evidence from laboratory manipulation studies that OT influences the development of a variety of peripheral tissues (Uvnäs-Moberg et al. 1998; Elabd et al., 2014; Colaianni et al., 2015; Rault et al., 2015) and exposure to OT during infancy can have long term impacts on weight gain (Uvnäs-Moberg et al. 1998), as this time period is crucial for body growth and formation (Metcalfe and Monaghan, 2001). In humans (*Homo sapiens*) problems with infant nutrition and development are estimated to cause 45% of deaths in children under five years old globally, with suboptimal breastfeeding, growth stunting and wasting critically affecting child development and survival in the first 1000 days of life (Black et al. 2013). Current interventions to overcome infant 'failure to thrive' in humans, such as complimentary feeding, only show modest success in tackling these problems (Dewey and Adu-Afarwuah, 2008) and understanding physiological mechanisms driving an infant's ability to gain weight and mature is therefore of great importance. If the mass changes induced via OT manipulations in laboratory settings can be

detected in natural systems, then elevation of infant OT through successful bonding and interacting with maternal figures would be a fundamental driver of an infant's ability to thrive and reach independence.

Grey seals (*Halichoerus grypus*) are colonially breeding marine mammals, with females that produce one pup per year. The pups are nursed on high fat milk while mothers fast before weaning abruptly approximately 18 days post-partum (Pomeroy et al., 1999). They present an excellent model system to study maternal behaviour and physiology as blood samples can be collected from both adults and infants, mothers are solely responsible for raising pups to independence, are individually identifiable and the entire dependent period can be observed in a relatively short time period for a large mammal. Additionally, of the few OT systems studied in animal species in the wild, to date the most is known about grey seals (Robinson et al., 2014; 2015; 2017). In this study mother-pup pairs were monitored to assess whether mothers with high OT concentrations produced pups with high OT concentrations, and whether the variation in OT concentrations within mothers and pups were correlated to patterns of mass change across the dependent period.

2. Materials and Methods

2.1 Study sites and animals

Field work was conducted on the island of North Rona (NR), Scotland (59◦06'N, 05◦50'W) and the Isle of May (IoM), Scotland (56◦11'N, 02◦33'W), both grey seal breeding colonies with long term research projects. Data and samples were collected from both colonies during the winter breeding season in 2010 and 2011. Across the two study years, plasma samples were collected from 66 mothers and their pups (36 from NR, 30 from the IoM). 20 mothers occurred in both

119 study years (11 from NR, 9 from the IoM). Mothers were identified by unique markings (natural
120 pelage patterns, or applied tags or brands (Smout et al., 2011)). Sampling was restricted to
121 mothers first seen either pre-partum or with newborn pups. We attempted to capture mother-pup
122 pairs twice during the lactation period to obtain plasma samples at 1–7 days after the pup's birth
123 ('early lactation') then 9–15 days after the first sampling event ('late lactation') (Robinson et al.,
124 2015a). We also attempted to re-capture as many pups post-weaning as possible during the
125 natural 1-4 week post-weaning fast in this species (Reilly 1991), and sampled 43 weaned study
126 pups (15 from NR, 28 from the IoM).

127

128 *2.2 Mass Measurements, Plasma and Milk Sampling and Analysis*

129 Grey seal mothers with pups were approached, captured, weighed and sampled as previously
130 described (Pomeroy et al., 1999; Robinson et al., 2015a). The use of chemical immobilization
131 ameliorates physiological stress responses to capture and handling in phocid seals (Harcourt et al.,
132 2010), and prior validation studies have shown that in grey seals, there was no change in plasma
133 OT with handling time (Robinson et al., 2014; 2015b) and no difference in extracted plasma OT
134 levels across chemically immobilized or physically restrained seals (Robinson et al. 2014).
135 Plasma samples were collected by venipucture, transported to a field laboratory and stored frozen
136 at -20ºC as described in Robinson et al. (2014; 2015). Our capture protocol meant that there was
137 always a 10-minute wait for mothers to become immobilised before a plasma sample could be
138 collected. This wait would eliminate any plasma OT peaks triggered by pre-capture nursing as OT
139 has a short half-life in plasma (Robinson et al., 2014). It is typically only possible to obtain milk
140 samples from seal mothers after an intravenous OT injection, however this could have
141 confounded endogenous OT concentrations in the milk collected. Using plastic 20ml syringes
142 adapted for drawing milk, two milk samples were successfully collected from grey seal mothers
143 without the use of exogenous OT. The analysis protocol for milk samples supplied with the OT

144 ELISA (see above) was followed with two alterations, detailed in the supplementary materials
145 (Appendix A. Methods), to prevent the high fat content of the milk (60%, (Iverson et al., 1993))
146 interfering with the assay.
147
148 Plasma was analysed for OT in duplicate using an ELISA (produced by Assay Designs Inc. at the
149 time of this analysis, ELISA kit is currently produced by Enzo Life Sciences but uses a different
150 antibody) with each sample undergoing solid-phase extraction prior to analysis following
151 methodology previously validated for detecting phocid plasma OT (Robinson et al., 2014). Plates
152 were read using a BioTek ELx800 reader. The standard curve and assay results for all plates were
153 fitted using the calibFit package (Haaland et al., 2011) in R version 2.15.0 (R Development Core
154 Team, 2012). Recovery rates for the extraction and ELISA procedure were 107.2% (n=10), inter-
155 assay coefficient of variance (COV) over the 14 plates used in this study was 16.1% and intra-
156 assay COV for this assay was 3.5%.
157
158 *2.3 Statistical Analysis*
159 All analyses were performed using the statistical package R 3.4.1 (R Development Core Team,
160 2012).
161
162 Plasma concentrations for mothers and their pups in early and late lactation were compared using
163 a one-way ANOVA. The data were analysed after a natural log transformation as the original data
164 were not normally distributed (Shapiro Wilk test, $p<0.001$). Basal plasma OT concentrations were
165 also calculated for the 43 post-weaning pups that we were able to locate on the colony. The OT
166 concentrations from these individuals during early lactation (with mother), late lactation (with
167 mother) and post-weaning (without mother) were compared using a one-way ANOVA. The data

were analysed after a natural log transformation as the original data were not normally distributed (Shapiro Wilk test, $p<0.001$).

GAMMs (Wood, 2006) were used to analyse variables affecting the OT concentration detected in dependent pups and for exploring the relationships between variables affecting mass gain in pups and mass loss in mothers. Details of model construction, selection process and the final model coding are given in the supplementary materials (Appendix A. Methods), For the GAMMs investigating pup mass gain and mother mass loss, rates of mass change were calculated in kg/day for all mother-pup pairs which had mass measurements and were sampled for plasma OT detection in both early and late lactation (n = 58 mother-pup pairs). Larger grey seal mothers lose mass at a faster rate than smaller mothers (Iverson et al., 1993); therefore, the rate of mass loss (kg/day) for all mothers was transformed by dividing mass loss rates by the mother's mass at first capture, during early lactation. This gave individual mass specific rates of mass loss for all mothers for use in subsequent analysis. In pups, plasma OT concentrations detected in early and late lactation were significantly positively correlated ($r = 0.54$, $p < 0.001$, 95% CIs [0.32, 0.7], Appendix A. Methods, Figure A.1) and therefore a mean of the two values was used to correlate with mass gain. Mother plasma OT concentrations across the early and late sampling points were not significantly correlated ($r = 0.12$, $p = 0.37$, 95% CIs [-0.14, 0.37], Appendix A. Methods, Figure A.2) and therefore concentrations from early and late lactation were analysed separately with the transformed mass loss rate.

3. Results

3.1 OT concentrations in mothers and pups

Basal plasma OT concentrations in pup plasma were significantly higher than those detected in mothers throughout early and late lactation (Figure 1, ANOVA: $F_{3,232}= 141.4$, $p<0.001$). No

193 significant differences were detected between pups in early and late lactation (mean ±SE: 21.9
194 ±1.5 pg/ml and 19.9 ±1.4 pg/ml respectively, Tukey honest significant difference test, $p = 0.5$) or
195 mothers in early and late lactation (mean ±SE: 8.2 ±0.6 pg/ml and 7.6 ±0.5 pg/ml respectively,
196 Tukey honest significant difference test, $p = 0.7$). Maternal plasma OT concentrations ranged
197 from 3.5 - 25.5 pg/ml in early lactation and 3.5 – 16.9 pg/ml in late lactation. Pup plasma OT
198 concentrations ranged from 11.5 – 48.1 pg/ml in early lactation and 8 – 52.2 pg/ml in late
199 lactation. There was a significant positive relationship between pup plasma OT concentration and
200 that of its mother (Figure 2, GAMM: $R2 = 0.34$, $p=0.02$, Appendix B. Table B.1). Pups from NR
201 also had significantly higher plasma OT concentrations than pups from the IoM (Figure 2,
202 $p<0.001$).

203

204 3.2 *Maternal presence vs. milk OT as drivers of high infant OT*

205 To explore whether maternal presence may be driving elevated OT levels in pups, samples of
206 plasma OT from as many pups as possible were collected after weaning, when mothers were
207 absent during the natural 1-4 week post-wean fast that occurs in this species (Reilly, 1991). Pups
208 that had weaned from their mothers had significantly lower plasma OT concentrations (10.9
209 ±0.9pg/ml) than when they were with their mothers in both early or late lactation (Figure 3,
210 ANOVA: $F2,126= 37.18$, $p<0.001$, Tukey honest significant difference test, $p=0.5$ between early
211 and late pup groups and $p<0.001$ between weaned pups and all non-weaned pup groups).

212

213 To explore whether pups may be ingesting and absorbing OT from their mothers' milk, milk
214 samples were collected from as many grey seal mothers as possible (n=2) to estimate
215 concentrations of OT that pups ingest from milk consumption. The two milk samples collected
216 contained 128.9 and 95.6 pg/ml OT, giving a mean of 112.2 ±16.6 pg/ml (SE) in phocid milk.

217

218 *3.3 OT concentrations, maternal mass loss and pup mass gain rate*

219 Pup mass gain rate was linked to mean pup plasma OT concentrations across the lactation period
220 (GAMM: R2 = 0.38, p = 0.016, Appendix B. Table B.2) with the two being significantly
221 positively correlated (r = 0.35, p = 0.007, 95% CIs [0.1, 0.6], Figure 4). A mother's rate of mass
222 loss was independent of maternal OT concentrations in both early and late lactation (GAMM: R2
223 = 0.31, p = 0.17 and p = 0.11 respectively, Appendix B. Table B.3).

224

225 **4. Discussion**

226 *4.1 High OT mothers produce high OT pups*

227 The results for this study support the existence of positive OT feedback loops within mothers and
228 pups in both of the seal colonies studied. Maternal and pup plasma OT concentrations were
229 significantly higher on average than those detected in non-breeding female grey seals (4.3 ±0.5
230 pg/ml, Robinson et al., 2015a), but there was great variation in individual values, especially
231 within pups. Data on infant plasma OT levels are currently scarce, however, two studies
232 measuring newborn OT plasma levels exist for humans and laboratory mice that mirror the OT
233 patterns reported in this study. Human newborns had elevated plasma OT concentrations
234 compared to adults in a study monitoring them for the first 4 days of life (Leake et al., 1981),
235 while weaned human children have plasma OT concentrations comparable to those in adults
236 (children 6-11 years: 1.2pg/ml (Modahl et al., 1998), adults: <2pg/ml (Szeto et al., 2011).
237 Laboratory mice pups approaching and at the point of weaning also have high plasma OT levels
238 compared to other developmental stages (Higashida et al., 2010). Elevated OT levels are known
239 to trigger proximity seeking behaviours in adult and infant grey seals (Robinson et al., 2015a;
240 2017), If stimuli from the presence of the mother/pup is causing the high OT concentrations

241 recorded across the pair, the mother-infant positive feedback loop system proposed by Rilling and
242 Young (2014) can be constructed with our data from a natural population (Figure 5).

243

244 By documenting infant OT concentrations alongside their mother's levels, we provide the first
245 evidence, to our knowledge, of double OT loops in mother-infant pairs, with one loop in each
246 individual but dependent on each other's presence for their continuation (Figure 5). Such loops
247 would act to keep mothers and offspring together, synchronising them behaviourally and
248 physiologically towards the common goal of infant survival. The structure and function of OT is
249 widely conserved across the mammalian clade (Gimpl and Fahrenholz, 2001; Feldman et al.,
250 2016; Jurek and Neumann 2018). Thus far, grey seals have been shown to posses an OT system
251 that is directly comparable to other domestic or captive animal species and humans, as their basal
252 plasma concentrations, plasma clearance rates and maternal patterns of plasma OT expression
253 match those detected in laboratory model species and humans (Robinson et al., 2014; 2015).
254 Therefore, it is likely that the evidence for positive OT feedback loops across mother-infant pairs
255 from grey seals would be present in other species.

256

257 The relevance of peripheral OT concentrations compared to central OT concentrations, and
258 whether any meaningful correlations exist between the two is still debated (Valstad et al., 2017).
259 However, peripheral and central release of OT due to stimuli from dependent infants has been
260 documented in humans and rodents, including nursing, sounds and sight of the infant and
261 interacting with the infant (Strathearn et al., 2009; Uvnäs-Moberg et al., 1998). Peripheral OT
262 concentrations are also arguably more relevant to measure when investigating links between the
263 hormone's concentrations in relation to mass changes in peripheral tissues, such as adipose
264 deposits or skeletal muscle.

265

266 *4.2 Maternal presence as a driver of high OT in pups*

267 Our study found that pup plasma OT concentrations remain consistently high throughout the
268 dependent period, only decreasing once they weaned and the mother was no longer present. A
269 pup's developmental stage and the fasting state weaned pups enter as soon as the mother leaves
270 could theoretically influence plasma OT levels. However, OT concentrations in individual grey
271 seal pups show no variation across two weeks of fasting (Robinson et al., 2015b) and remain
272 consistent when pups leave the breeding colony and start feeding at approximately one month of
273 age, and throughout their first year of life (8.3 ± 0.6 pg/ml, Robinson et al., 2014). There is also
274 no change in plasma OT levels across the various developmental stages either side of weaning, as
275 levels in newborns are comparable to pups approaching weaning (see results section 3.1), pups
276 that have been fasting for 3 days are comparable to those who have fasted for several weeks
277 (Robinson et al. 2015b) and fasting pups are comparable to all other developmental stages in the
278 first year of life (Robinson et al., 2014). Pup OT decreases significantly and consistently in the
279 first three days of the mother leaving, regardless of the age at time of weaning (Robinson 2014).
280 Pup plasma OT levels are subsequently stable for weeks despite undergoing sustained fasting and
281 substantial developmental changes, and do not change as pups shift from fasting to feeding or
282 undergo all the developmental changes that occur in their first year. It is more likely that some
283 aspect of maternal presence is driving elevated OT in dependent pups, because once the mother
284 leaves and this stimulus is removed, pup OT levels fall.

285

286 Ingestion of OT from breast milk has been proposed as a route of neonatal exposure to this
287 hormone in humans (Uvnäs-Moberg et al., 1998; Carter, 2003) and mice (Higashida et al., 2010).
288 Higashida et al. (2010) attribute ingested milk as the cause of high OT levels in mouse pups due
289 to the sheer quantity of OT present in mouse milk. However, this interpretation must be viewed

with caution, as the OT levels reported from that study indicate that unextracted substrates were used in the analysis which gives high, inaccurate results (Robinson et al., 2014; Leng and Sabatier, 2016). Higashida et al. (2010) also only detect the amount of OT in mouse milk, without putting this value into context with how much mice pups actually drink. Other studies state that physical barriers to absorption and uptake and chemical degradation in the digestive tract make ingested milk an unlikely source of significant amounts of OT in infants (Fiellestad-Paulsen et al., 1995). Even when medical trials have given high buccal doses of OT to humans, their ability to raise plasma OT concentrations is limited (Dawood et al., 1980; Landgraf 1985). When put into context with the OT we detected in seal milk and the volumes of milk a seal pup ingests daily, it is apparent that the OT levels in seal milk are not high enough to impact on plasma concentrations (see Appendix C and Table C.4 for these calculations). The low number of milk samples that were obtained (n=2) is a potential limitation of this study, as additional mothers may yield samples with higher OT concentrations. However, even if the questionably high OT levels detected in laboratory rat milk from Higashida et al. (2010) is used to calculate whether ingested OT could impact on pup plasma levels, these milk concentrations are still far too low to raise infant plasma levels significantly (see Appendix C and Table C.4 for these calculations). It is therefore plausible that aspects of the mother's presence other than her ability to provide milk are driving elevated OT in pups, potentially including the scent, sounds and sight of the mother.

Conspecific stimuli from individuals that are bonded to each other have already been shown to cause elevations in peripheral OT (Strathearn et al., 2009, Nagasawa et al., 2015). Other findings from this study also lend support to the theory that it is a mother's presence driving high pup OT levels. The inter-colony differences in mother-pup OT levels show that NR mother-pup pairs had significantly higher plasma OT than IoM pairs. Mothers on NR spend more time in close proximity to their pups than mothers on IoM (Redman et al., 2002) primarily due to topographical

315 differences at the two colonies affecting access to water (Caudron et al., 2001; Redman et al.,
316 2002). According to the positive loop theory, more time in close proximity equates to greater OT
317 release and concentrations in bonded individuals, and the OT results from the two colonies agree
318 with this (Fig 2).

319

320 An endocrinological system that stimulates synchrony of both physiology and behaviour across
321 individuals has the potential to act on other important bonds outside of maternal ones. There is
322 evidence from social insects that complex social traits evolve from co-opting systems acting on
323 maternal behaviour and physiology (Amdam et al., 2006), and it seems likely this has happened
324 with the positive OT loop mechanism. There is already direct evidence that positive OT loops
325 stimulate pro-social behaviour and elevate OT concentrations across socially bonded, but
326 unrelated pairs even across species boundaries (Nagasawa et al., 2015). Therefore, this unique
327 mechanism could enable the co-ordination of a number of individuals' physiology, across pairs or
328 groups. By aligning group members' motivation to perform specific behaviours, OT may
329 stimulate group synchrony even when faced with individual risks such as serious injury or death
330 (Samuni et al., 2016). The existence of co-operative behaviour has generated much research into
331 theoretical reasons for its development and perpetuation in individuals, populations and species;
332 however, the underlying physiological mechanisms driving such behaviour remain relatively
333 poorly understood (Soares et al., 2010). The OT loop system acting both within individuals and
334 across group or bond members is a promising area for future work, uncovering how individuals
335 can be motivated to act against their own interests in high risk or low reward contexts.

336

337

338

4.3 High OT pups gain mass faster

High OT concentrations were associated with greater pup growth rates without extra energetic cost to their mothers, as no differences in relative maternal mass loss rates were detected. Two results suggest that the difference in mass gain rates between high and low OT pups is not due to variation in how much milk pups ingest. First, behavioural data was collected from the NR mother-pup pairs in this study, and their plasma OT concentrations showed no relationship with variation in nursing bout frequency or duration (Robinson et al., 2015a). Second, if high OT pups were achieving their additional mass gain by ingesting more milk from their mothers, those mothers would show greater mass loss rates per day than low OT mothers, which was not observed. OT is known to modulate feeding in mammalian species (Gaetani et al., 2010; Atasov et al., 2012) and has been shown to reduce food intake in several animal species (reviewed in Olszewski et al., 2010). This may explain why infants with elevated OT concentrations are not motivated to nurse more from their mothers. However, it does not explain how high OT infants are able to gain mass at a higher rate, without ingesting additional milk.

The variation in mass gain across high to low OT pups may be due to behavioural differences impacting individual metabolism and fat accumulation in pups. The elevated OT concentrations in pups are likely indicative of successful mother-pup attachment, and elevated OT would trigger pups to remain close to their mothers (Robinson et al., 2015a; 2017). This may reduce energetic expenditure in pups by preventing excursions away from their mother, which would elevate metabolic rate and initiate conflicts with adjacent seals. It is also possible that by encouraging pups to remain close to their mothers, high OT pups are more sheltered from strong winds (McCafferty et al., 2005), experiencing a microclimate that reduces their thermal output and lowers metabolic overheads. OT manipulations in laboratory rats indicate that the hormone triggers huddling behaviour (Alberts 2007) and modulates the function of brown adipose tissue,

364 directly impacting on thermoregulation in infants (Harshaw et al., 2018). Therefore, rather than
365 actively stimulating mass gain, elevated OT concentrations in pups may reduce activities that
366 divert resources away from growth prior to weaning.

367

368 With the growing body of evidence linking OT to the development of several tissue types, it is
369 also possible that elevated OT in pups stimulates physiological pathways that cause increased
370 mass development. Experiments giving OT to rat pups promoted weight gain in adults via
371 increased deposition of adipose tissue (Uvnäs-Moberg et al., 1998) and when given to young pigs
372 (*Sus scrofa domesticus*), OT reduced mass lost during weaning events (Rault et al., 2015). OT has
373 also been linked to skeletal muscle development in mice (*Mus musculus*) (Elabd et al., 2014) and
374 bone mass accumulation in mice and humans (Colaianni et al., 2015). Physiological pathways for
375 increased OT concentrations influencing mass changes independent of food intake have been
376 proposed (Rault et al., 2015; Colaianni et al., 2015), such as OT causing the stimulation of
377 digestive activity and fat storage by linking increases in plasma cholecystokinin, insulin and
378 adipose tissue in OT treated rats (Uvnäs-Moberg et al., 1998). More research is needed to identify
379 which biological tissues are affected by OT, so that the developmental consequences for exposure
380 to high or low OT levels due to variation in social or parental stimuli can be determined.

381

382 Grey seal mothers fast while nursing their pups and lose up to 40% of their body mass at
383 parturition during this time (Pomeroy et al., 1999), using approximately 80% of their energetic
384 reserves to produce milk and sustain themselves on the colony (Fedak and Anderson, 1982). The
385 ability to wean at as large a mass as possible is the most important factor affecting grey seal pup
386 survival in its first year of life (Hall et al., 2001). That OT facilitates mass gain or slows mass loss
387 in dependent pups with no additional energetic cost to the mother is of great importance in a true
388 capital breeding species which has rapid offspring mass gain and abrupt termination of maternal

389 care. Any physiological factors enabling efficient mass gain in infants will be highly selected for
390 as it would increases the probability of success for a mother within that breeding episode without
391 additional investment costs.

392

393 Steady mass gain postpartum is crucial for successful infant development and survival in all
394 animal species, including humans (Black et al., 2013; Shields et al., 2012). Any factors that
395 increase infant mass gain while minimising the energetic costs to parents is highly advantageous
396 in any species exhibiting parental care. All organisms must give their offspring the best
397 developmental start in life while attempting to balance the negative costs to themselves; any
398 factor reducing the conflict between these two contrasting demands on an organism will impact
399 on their survival, their current and future reproductive success. A link between good maternal
400 care, high OT and increased infant mass gain has been previously proposed in rodents based on
401 manipulating OT levels experimentally (Uvnäs-Moberg et al., 1998). Additionally, a study
402 investigating weight gain and massage therapy in preterm human babies theorised that elevated
403 plasma OT in babies receiving massages indicated a role for the hormone in mediating infant
404 weight gain (Field, 2001). To our knowledge, our study provides the first evidence of an OT-mass
405 gain relationship in wild mother-infant pairs and highlights the importance of understanding the
406 hormone's role in mediating mother-infant bonds, care giving behaviour and physical
407 development in infants.

408

409 **5. Conclusions**

410 Our study provides the first evidence that positive OT loops acting across bonded individuals
411 exist in mother-infant pairs in natural environments, and that they are linked to the promotion of
412 infant development without additional energetic costs to mothers. Including energetic benefits in

413 the proposed loop mechanism highlights how such systems physiologically give selective
414 advantage to securely bonded mother-infant pairs (Figure 5). OT facilitates and regulates parental
415 and social bonds throughout the mammalian clade, with OT-like peptides in bird
416 (Chokchaloemwong et al., 2013) and fish (O'Connell et al., 2012) species fulfilling similar roles
417 in other vertebrate groups. OT loops and the associated fitness benefits linked to them may
418 therefore be a widespread mechanism for connecting optimal parental or social environments
419 with direct physiological advantages for individual development. Understanding the mechanisms
420 by which OT and OT–like peptides affect interactions between the bonded individuals and infant
421 mass gain has wide ranging implications for animal husbandry practises, medical interventions,
422 advice to human parents, societal understanding of how health and relationships are linked and
423 studying the energetic constraints of parental care.

424

425 **Acknowledgements**

426 We would like to thank William Patterson, Hannah Wood, Simon Moss, Matthew Bivins, Paula
427 Redman, Theoni Photopoulou, Johanna Baily and everyone who assisted with the sample
428 collection on North Rona and the Isle of May during the 2010 and 2011 seasons. The help and
429 cooperation of Scottish National Heritage, HM Coastguard and the Northern Lighthouse Board
430 are gratefully acknowledged.

431

432 **Funding**

433 The UK's Natural Environmental Research Council (NERC) funded the long-term program of
434 research on grey seals at North Rona and the Isle of May. PPP and SDT were in receipt of NERC
435 grant NE/G008930/1 and PPP was in receipt of Esmée Fairburn Foundation grant 08-1037 during
436 the work. This paper formed part of KJR's PhD funded by the UK Natural Environment Research

Council (NERC) grant NE/H524930/1 and by SMRU Marine, St Andrews, UK. The funding bodies had no role in the design of the study, the collection of samples, the analysis of samples or data and the interpretation of the data.

Ethical approval

All animal procedures were performed under the UK Home Office project license #60/4009 and conformed to the UK Animals (Scientific Procedures) Act, 1986. All research received prior ethical approval from the University of St Andrews Animal Welfare and Ethics Committee and the School of Biology's Ethics Committee.

Data Availability

The dataset supporting the conclusions of this article is included within the article and its Appendices (Appendix D).

Competing interests

Declarations of interest: none

Authors' contributions

KJR conceived the study; KJR, SDT and PPP collected samples in the field; KJR performed all sample and data analysis; NH provided essential laboratory equipment; KJR wrote the manuscript; all authors critically revised the manuscript and gave final approval of the version to be published.

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Figure Legends

Figure 1. OT concentrations in mothers and pups. Mean basal plasma oxytocin (pg/ml) in grey seal mothers and their pups during early and late lactation with median, upper and lower quartiles, 1.5x interquartile range and outliers shown. Significant differences at the $p<0.001$ level between groups are denoted by asterisks.

Figure 2. Mother - pup plasma oxytocin relationships. Prediction plot showing the GAMM output of the relationship between mother and pup plasma oxytocin concentration (pg/ml) on North Rona (solid line) and the Isle of May (dashed line).

Figure 3. Maternal presence as drivers of high infant OT. Mean basal plasma oxytocin (pg/ml) pups during early lactation, late lactation and post-weaning with median, upper and lower quartiles, 1.5x interquartile range and outliers shown. Significant differences at the $p<0.001$ level between groups are denoted by asterisks.

629 **Figure 4. OT concentrations and pup mass gain rate.** The significant positive relationship
630 between pup plasma oxytocin concentrations (pg/ml) and the mass a pup gains per day while still
631 with its mother (kg/day) with the Pearson's correlation significance value.

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633 **Figure 5. Positive mother – infant OT loops and infant mass gain.** Proposed double positive
634 feedback loop involving oxytocin (OT) release, mother-pup bonding and behaviour and mass
635 changes in grey seals.

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Appendix Files for "High oxytocin infants gain more mass with no additional maternal energetic costs in a natural system" by Kelly J. Robinson, Neil Hazon, Sean D. Twiss and Patrick P. Pomeroy.

Appendix A. Methods

Milk Sample Analysis

The protocol supplied with the oxytocin ELISA was followed for analysing the two milk samples with the following alterations;

1. In addition to the clarification protocol given with the ELISA, milk samples then underwent solid-phase extraction with the same protocol used to extract plasma samples (Robinson et al., 2014).
2. The two milk samples were run on the ELISA plate diluted to 1:2.

Statistical Analysis

All analyses were performed using the statistical package R 3.4.1 (R Development Core Team, 2012).

GAMM for investigating oxytocin concentrations detected in dependent pups

Biologically plausible explanatory variables used in this GAMM (Wood, 2006a) model was plasma oxytocin concentration of the pup's mother, sample timing during the season (early or late lactation), the pup's sex, the colony the pup was born on (NR or IoM) and the year of sampling (2010 or 2011). The model was fitted using the multiple generalized cross validation library mgcv (Wood, 2012). The identities of the mothers were fitted as a random effects smooth (Wood, 2006b) to control for pseudo-replication in the dataset from using some of the same individuals

over the two years of the study and to control for consistent individual differences in behaviour (Twiss et al, 2012; Robinson et al., 2015a). The smoothing parameters were set by maximum likelihood to reduce the risk of over fitting associated with other methods (Wood, 2011). The model was fitted with a Gamma error distribution. Model selection was done by backwards stepwise elimination through examination of R^2 values, AIC values, QQ and residual plots to identify the best model for the data. During the selection process, the 'year of sampling' variable was discarded to improve the model's fit to the data and the 'plasma oxytocin concentration of the mother', 'timing during season' (early/late), 'pup sex' and 'colony' variables were retained.

Final GAMM code for investigating oxytocin concentrations detected in dependent pups;

```
GammOutput1 <- gam(PupOxytocin ~ MotherOxytocin + EarlyLateLactation + PupSex +
                   Colony + s(ID, bs="re"), family=Gamma(link="log"), method="ML",
                   data=GreySealOxytocinData)
```

GAMMs for investigating mass gain in pups and mass loss in mothers

Biologically plausible explanatory variables used in these GAMM models (Wood, 2006a) were plasma oxytocin concentration of the pup or mother (mean of early/late concentrations in pups for pup model, early and late concentrations separately for mother model) and the pup's sex. The models were fitted using the multiple generalized cross validation library mgcv (Wood, 2012). The identities of the mothers were fitted as a random effects smooth (Wood, 2006b) for the same reasons given above. The colony the mother-pup pair belonged to (NR or IoM) was also fitted as a random effect smooth based on the results of the first GAMM model described above. The smoothing parameters were set by maximum likelihood to reduce the risk of over fitting associated with other methods (Wood, 2011). Models were fitted with a Gaussian error distribution. Model selection was performed by backwards stepwise elimination through examination of R2 values, AIC values, QQ and residual plots to identify the best model for the

data. When selecting variables for the model of pup mass gain rate, removing the 'pup sex' variable improved the model's fit to the data. During the selection process for the models of maternal mass loss, the 'pup sex' variable was removed to improve the model's fit to the data.

Final GAMM code for investigating mass gain in pups;

```
GammOutput2 <- gam(PupMassGain ~ PupOxytocinMean + s(ID, bs="re")+s(Colony, bs="re"),
                   method="ML", data=GreySealOxytocinMassData)
```

Final GAMM code for investigating mass loss in mothers;

```
GammOutput3 <- gam(MotherMassLossTransformed ~ MotherOxytocinEarlyLactation +
                   MotherOxytocinLateLactation + s(ID, bs="re") + s(colony, bs="re"),
                   method="ML", data= GreySealOxytocinMassData)
```

Figure A.1

Pup oxytocin concentrations in early and late lactation (pg/ml).

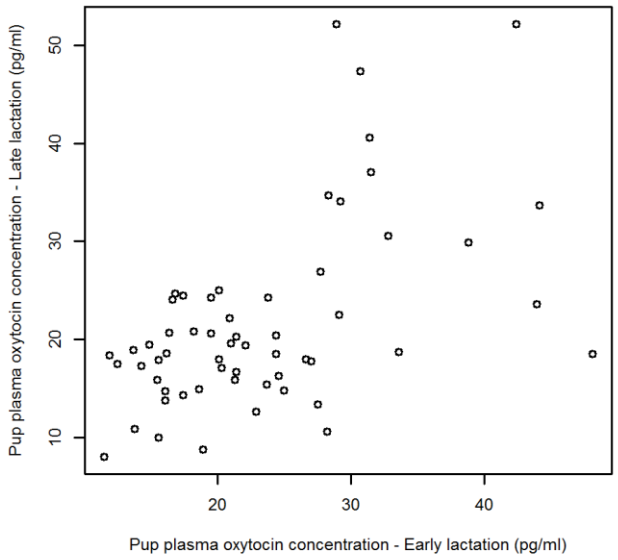
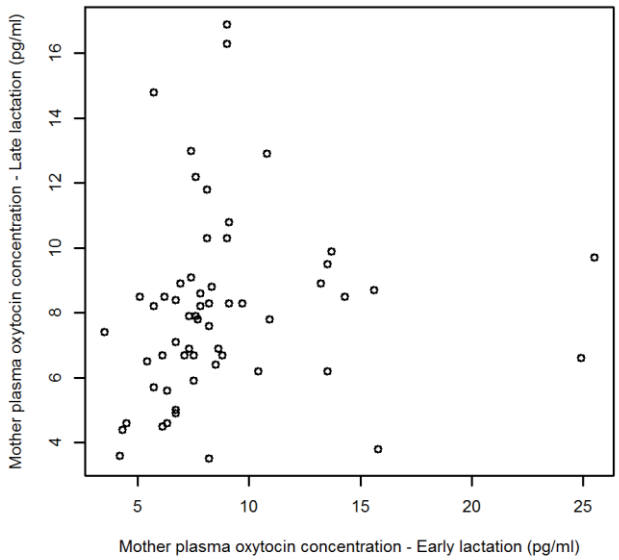


Figure A.2

Mother oxytocin concentrations in early and late lactation (pg/ml).



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Appendix B. Tables

Table B.1

GAMM output for variables affecting pup oxytocin concentrations in plasma, their estimates, standard errors and p values.

Dependent variable	Explanatory variable	Estimate	Standard Error	P value
Pup oxytocin concentration (pg/ml)	Maternal plasma oxytocin concentration (pg/ml)	0.019	0.0083	**0.02**
	Sample timing during the season (early/late)	-0.052	0.053	0.33
	Pup sex (male/female)	0.077	0.065	0.24
	Colony (North Rona/Isle of May)	0.34	0.071	**<0.001**
	Smooth term for mother's identity	Na	Na	**0.02**

Table B.2

GAMM output for variables affecting pup mass gain rate, their estimates, standard errors and p values.

Dependent variable	Explanatory variable	Estimate	Standard Error	P value
Rate of mass gain in pups (kg/day)	Mean pup oxytocin concentration (pg/ml)	0.02	0.007	**0.016**
	Smooth term for colony (North Rona/Isle of May)	Na	Na	0.07
	Smooth term for mother's identity	Na	Na	0.06

Table B.3

GAMM output for variables affecting mother mass gain rate, their estimates, standard errors and p values.

Dependent variable	Explanatory variable	Estimate	Standard Error	P value
Rate of mass loss in mothers (kg/day) transformed by maternal size close to parturition	Maternal oxytocin concentration during early lactation (pg/ml)	0.00017	0.00012	0.17
	Maternal oxytocin concentration during late lactation (pg/ml)	0.00030	0.00018	0.11
	Smooth term for colony (North Rona/Isle of May)	Na	Na	0.25
	Smooth term for mother's identity	Na	Na	0.07

Appendix C. Buccal OT doses, peripheral OT concentrations and seal milk ingestion

When put into context with the volumes of milk a seal pup ingests daily, it is apparent that the OT levels in seal milk are not high enough to impact on plasma concentrations. The mean volume of milk a grey seal pup ingests is 3030ml/day (Iverson et al., 1993). Using the mean OT concentration in grey seal milk detected in this study (112.2pg/ml), a grey seal pup ingests approximately 339966pg of oxytocin per day, or 0.48% of the lowest buccal dose that has been shown to have no effect on plasma OT levels (Table C.4). Furthermore, in seals this intake is split into approximately five suckling bouts in a 24-hour period (Iverson et al., 1993). Therefore, on average pups only consume 67993.2pg of OT per suckling bout, or 0.01% of the buccal dose which had no demonstrable effect on plasma OT levels (Table C.4). Pups would have to drink far greater quantities of milk than they actually consume within a two-hour period to approach the doses proven to significantly raise plasma OT concentrations. As this study had only two milk samples to calculate ingested OT from, the high milk OT values from mice reported in Higashida et al. (2010) can also be used to demonstrate that their levels would still not be high enough to impact pup plasma levels. Mouse milk from Higashida et al. (2010) contained approximately 1,200pg/ml OT, which would mean if a seal pup had a mother producing comparable levels of OT in her milk, the pup would ingest 3,636,000pg of OT per day, or 5% (1% if splitting the ingestion over five suckling bouts per day) of the lowest buccal dose that has been shown to have no effect on plasma OT levels (Table C.4). Therefore, it is unlikely that ingested milk is the source of the high plasma OT concentrations found in pups consistently throughout early and late lactation. Other aspects of the mother's presence, potentially including scent, sounds and sight of the mother, are more credible stimuli for release of the hormone within the pup.

Table C.4 Experimentally tested buccal doses of oxytocin for adult humans and their success rates.

Buccal dose given (units as stated in source)	Frequency administered	Total dose given in picograms	Successful?	Reference
70μg	Once	70,000,000pg	No	Landgarf, 1985
200 IU	Every 20 minutes for 2 hours	2,400,000,000pg	No	Dawood et al., 1980
400 IU	Every 20 minutes for 2 hours	4,800,000,000pg	Yes (majority elevated to 24 - 50pg/ml)	Dawood et al., 1980

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Appendix D. Original data. For 'Pup post-wean OT (pg/ml) column, codes for individuals not sampled are as follows: NA: not applicable, SCO: single capture only, NRC: not re-captured

Mother ID	Pup OT (pg/ml)	Mother OT (pg/ml)	Colony	Year	Early or late lactation	Pup sex	Pup mass gain rate (kg/day)	Transformed mother mass loss (mass specific rate, kg/day)	Pup post-wean OT (pg/ml)
R	15.1	9.5	NR	2010	E	M	SCO	SCO	NRC
S	27.7	7.4	NR	2010	E	M	2.18	0.021538462	6.7
S	26.9	9.1	NR	2010	L	M	NA	NA	NA
T	11.9	6.7	NR	2010	E	F	2.254545455	0.0120012	NRC
T	18.4	8.4	NR	2010	L	F	NA	NA	NRC
U	16.4	8.3	NR	2010	E	F	2.795833333	0.022610405	NRC
U	20.7	8.8	NR	2010	L	F	NA	NA	NRC
V	33.6	7.5	NR	2010	E	M	2.177777778	0.017364248	NRC
V	18.7	6.7	NR	2010	L	M	NA	NA	NRC
W	21	15.8	NR	2010	E	F	1.84	0.026219512	NRC
W	19.6	3.8	NR	2010	L	F	NA	NA	NRC
A	28.2	25.5	NR	2011	E	M	2.89	0.025224327	8.2
A	10.6	9.7	NR	2011	L	M	NA	NA	NA
H	21.4	24.9	NR	2011	E	M	2.3	0.022289258	12.6
H	20.3	6.6	NR	2011	L	M	NA	NA	NA
J	30.7	7.6	NR	2011	E	F	2.6	0.026589595	10.8
J	47.4	12.2	NR	2011	L	F	NA	NA	NA
X	31.5	8.2	NR	2011	E	F	2.255	0.022246456	NRC
X	37.1	8.3	NR	2011	L	F	NA	NA	NRC
Y	36.2	4.3	NR	2011	E	M	SCO	SCO	NRC
L	28.3	6.9	NR	2011	E	F	2.354545455	0.024467649	15.1
L	34.7	8.9	NR	2011	L	F	NA	NA	NA
M	29.2	13.2	NR	2011	E	F	1.7	0.018356589	14.1
M	34.1	8.9	NR	2011	L	F	NA	NA	NA
N	43.9	15.6	NR	2011	E	M	2.325	0.022449336	NRC
N	23.6	8.7	NR	2011	L	M	NA	NA	NRC
P	44.1	13.7	NR	2011	E	M	2.36	0.02452381	20.5
P	33.7	9.9	NR	2011	L	M	NA	NA	NA
O	22.1	3.3	NR	2011	E	F	SCO	SCO	NRC
U	31.4	7.4	NR	2011	E	M	2.745454545	0.01338091	NRC
U	40.6	13	NR	2011	L	M	NA	NA	NRC

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Mother ID	Pup OT (pg/ml)	Mother OT (pg/ml)	Colony	Year	Early or late lactation	Pup sex	Pup mass gain rate (kg/day)	Transformed mother mass loss (mass specific rate, kg/day)	Pup post-wean OT (pg/ml)
W	15.6	6.3	NR	2011	E	M	1.73	0.026209677	13.9
W	17.9	5.6	NR	2011	L	M	NA	NA	NA
T	27.5	9.7	NR	2011	E	F	2.3	0.02213762	NRC
T	13.4	8.3	NR	2011	L	F	NA	NA	NRC
Z	22.1	9	IOM	2010	E	M	2.066666667	0.021982414	38.9
Z	19.4	10.3	IOM	2010	L	M	NA	NA	NA
AA	23.8	5.7	IOM	2010	E	F	2.222222222	0.021668472	9.6
AA	24.3	5.7	IOM	2010	L	F	NA	NA	NA
BB	13.7	8.1	IOM	2010	E	M	1.866666667	0.020150419	25.2
BB	18.9	10.3	IOM	2010	L	M	NA	NA	NA
CC	14.9	6.7	IOM	2010	E	M	2.25	0.020120898	11.4
CC	19.5	4.9	IOM	2010	L	M	NA	NA	NA
DD	16.6	13.5	IOM	2010	E	F	1.671428571	0.020493912	NRC
DD	24.1	6.2	IOM	2010	L	F	NA	NA	NRC
EE	27	7.7	IOM	2010	E	M	2.314285714	0.022569164	24.5
EE	17.8	7.8	IOM	2010	L	M	NA	NA	NA
FF	26.6	13.5	IOM	2010	E	M	1.616666667	0.023644388	11.7
FF	18	9.5	IOM	2010	L	M	NA	NA	NA
GG	28.9	10.4	IOM	2010	E	M	1.872727273	0.025245782	12.9
GG	52.2	6.2	IOM	2010	L	M	NA	NA	NA
HH	24.6	8.2	IOM	2010	E	M	1.969230769	0.02572482	18.3
HH	16.3	7.6	IOM	2010	L	M	NA	NA	NA
II	21.3	6.1	IOM	2010	E	F	2.125	0.023020258	14.3
II	15.9	6.7	IOM	2010	L	F	NA	NA	NA
JJ	18.2	9.1	IOM	2010	E	F	2.111111111	0.027543789	13
JJ	20.8	10.8	IOM	2010	L	F	NA	NA	NA
KK	24.4	8.8	IOM	2010	E	M	2.4	0.029495472	13.5
KK	18.5	6.7	IOM	2010	L	M	NA	NA	NA
LL	18.9	7.3	IOM	2010	E	F	2.181818182	0.027156041	20.9
LL	8.8	7.9	IOM	2010	L	F	NA	NA	NA
MM	12.5	5.7	IOM	2010	E	F	1.66	0.017397078	11

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Mother ID	Pup OT (pg/ml)	Mother OT (pg/ml)	Colony	Year	Early or late lactation	Pup sex	Pup mass gain rate (kg/day)	Transformed mother mass loss (mass specific rate, kg/day)	Pup post-wean OT (pg/ml)
MM	17.5	8.2	IOM	2010	L	F	NA	NA	NA
NN	13.8	9.1	IOM	2010	E	M	0.8	0.016148207	NRC
NN	10.9	8.3	IOM	2010	L	M	NA	NA	NRC
EE	23.7	7.8	IOM	2011	E	F	1.842857143	0.025065354	10.9
EE	15.4	8.2	IOM	2011	L	F	NA	NA	NA
OO	15.6	3.5	IOM	2011	E	M	1.1	0.016336634	10.4
OO	10	7.4	IOM	2011	L	M	NA	NA	NA
FF	18.6	6.2	IOM	2011	E	M	1.533333333	0.022793054	10.6
FF	14.9	8.5	IOM	2011	L	M	NA	NA	NA
II	15.5	5.4	IOM	2011	E	F	2.14	0.024508671	11.5
II	15.9	6.5	IOM	2011	L	F	NA	NA	NA
CC	11.5	4.3	IOM	2011	E	F	1.86	0.020927602	NRC
CC	8	4.4	IOM	2011	L	F	NA	NA	NRC
HH	25	6.7	IOM	2011	E	M	1.166666667	0.020134228	7.4
HH	14.8	5	IOM	2011	L	M	NA	NA	NA
PP	21.4	9	IOM	2011	E	F	1.371428571	0.025718962	10.1
PP	16.7	16.9	IOM	2011	L	F	NA	NA	NA
KK	20.3	8.6	IOM	2011	E	M	1.742857143	0.028613507	12.4
KK	17.1	6.9	IOM	2011	L	M	NA	NA	NA
QQ	17.4	7.6	IOM	2011	E	M	1.371428571	0.022911051	7.7
QQ	14.3	7.9	IOM	2011	L	M	NA	NA	NA
JJ	20.1	6.7	IOM	2011	E	F	2.2	0.025756953	6.9
JJ	18	7.1	IOM	2011	L	F	NA	NA	NA
RR	16.2	8.1	IOM	2011	E	M	2.228571429	0.030819434	7.2
RR	18.6	11.8	IOM	2011	L	M	NA	NA	NA
GG	22.9	7.1	IOM	2011	E	F	1.276923077	0.017932987	14
GG	12.6	6.7	IOM	2011	L	F	NA	NA	NA
SS	20.9	5.1	IOM	2011	E	F	1.553846154	0.021249469	7.8
SS	22.2	8.5	IOM	2011	L	F	NA	NA	NA
TT	16.1	5.7	IOM	2011	E	F	1.836363636	0.026677353	4.9
TT	13.8	14.8	IOM	2011	L	F	NA	NA	NA
LL	16.1	10.8	IOM	2011	E	F	1.709090909	0.021577644	8.8

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