Social olfaction in marine mammals: wild female Australian sea lions can identify their pup's scent

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Historically, anatomical evidence has suggested that marine mammals are anosmic or at best microsmatic, i.e. absent or reduced olfactory capabilities. However, these neuroanatomical considerations may not be appropriate predictors for the use of olfaction in social interactions. Observations suggest that pinnipeds may use olfaction in mother–pup interactions, accepting or rejecting pups after naso-nasal contact. Such maternal–offspring recognition is a favourable area for investigating the involvement of odours in social recognition and selectivity, as females are evolutionarily constrained to direct resources to filial young. However, there is no experimental, morphological or chemical evidence to date for the use of olfaction in social contexts and for individual odour recognition abilities in pinnipeds. Here, we report unequivocal evidence that Australian sea lion (*Neophoca cinerea*) females can differentiate between the odour of their own pup and that of another, in the absence of any other distinguishing cues. This study demonstrates individual olfactory recognition in a free-ranging wild mammal and is clear evidence of the social function of olfaction in a marine mammal.

Keywords: olfaction; individual recognition; mother–offspring; communication; *Neophoca cinerea*; marine mammal

1. INTRODUCTION

Based on neuroanatomical and histological evidence, marine mammals have historically been categorized as anosmic or at best microsmatic, that is having no or reduced brain structures devoted to the processing of olfactory information [1–4]. Due to their permanent adaption to an aquatic lifestyle the olfactory nerve is absent in odontocetes and greatly reduced in mysticetes [2], while manatees retain a rudimentary olfactory system [5]. Similarly, early studies concluded that owing to their adaptation to an amphibious lifestyle pinnipeds had lost some olfactory capabilities,

but less than cetaceans [2,5]. However, neuroanatomical criteria may not be fully predictive of the actual use of olfaction in social interactions. Maternal–offspring recognition is a favourable area for investigating the involvement of odours in social recognition and selectivity, as females are evolutionarily constrained to direct resources to filial young [6]. Anecdotal evidence suggests that in pinnipeds, as in many mammals, mothers accept or reject pups after naso-nasal investigations [2,7]. However, there is no experimental, morphological or chemical evidence so far for the use of olfaction in social contexts, and for individual odour recognition abilities in pinnipeds [2,5,7].

Recent studies in captivity have shown that South African fur seals can differentiate artificial odours [8,9], and the existence of a high sensitivity to dimethyl sulphide in harbour seals [10]. Furthermore, genetic evidence suggests that unlike other marine mammals, Steller sea lions, which maintain a terrestrial component to their life history, have a comparable proportion of functional olfactory receptor genes to terrestrial mammals [11]. While these studies show a genetic and perceptual basis for refined olfactory capacities there is as yet neither experimental evidence of their actual use, nor of any natural function of olfaction in pinnipeds.

In Australian sea lions, mothers and pups are frequently separated throughout nursing and are thus expected to have evolved an efficient individual recognition system. Their breeding cycle lasts approximately 17–18 months [12] and mothers alternate 1–2 days in the colony nursing the pup with 1–2 days at sea foraging [13]. As in other otariids, females have long been suspected of using a combination of vocal and olfactory cues to identify their pup when returning to the colony [14]. Australian sea lion mother and pup vocalizations are individual-specific [15] and used to facilitate mother–pup recognition [16,17], but like other pinnipeds these competencies function in a multimodal context.

We tested the ability of wild female Australian sea lions to differentiate between the scent of their own offspring and a non-filial young, using a choice test between two model pups impregnated with scent collected from the pups' heads. If females can discriminate between the scents, and scent provides functional cues, we expect to see different responses to the scent of the females' own offspring than to the non-filial pup.

2. MATERIAL AND METHODS

This experiment was conducted on wild Australian sea lions living on Olive Island, South Australia (32°43' S, 133°58' E). Experiments were carried out during February 2009. Pups of approximately two months of age with black pelage were used. Twelve females who were observed to be exclusively nursing a pup of the targeted age were indiscriminately selected from those present in the colony. Pups used for the non-filial pup scent cues were matched in age.

Four models, each 65 cm long, approximately the size, shape and colour of a 2-month old pup were used. The ventral side of the model was plain black cotton, while the dorsal side consisted of black polyester faux fur. Models were stuffed with polyester filling to simulate the shape of a pup. The swabs used to collect the scent samples were made using two pieces of black cotton flannelette stitched to form a pocket shaped to fit over the head of the model. These swabs were washed three times in water, without detergent, at over 60°C. Once dried each swab was sealed in an airtight polyethylene bag. To avoid contamination, swabs were only handled with vinyl gloves. Each swab was used once only.

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Non-filial pup scent samples were collected immediately prior to an experiment, and filial pup samples were collected when the pup was removed from the subject female, both within 10 min of presentation to the female. The pup was restrained by an experimenter while another collected the scent samples. Wearing gloves, the experimenter rubbed the swab over the head of the pup collecting saliva, nasal mucous, lachrymal fluid and general skin/fur secretions (see the electronic supplementary material, video S1). Both surfaces of the swab were rubbed over all locations of the pup's head to ensure an even spread of the potential scent sources across the entire swab. The swab was placed over the head of a model with a polyethylene bag between the swab and the model to ensure no scent was transferred onto the model. The two models used in each experiment were selected from the four available.

The target female was separated from her pup, whereupon she would begin searching for it, and the pup removed to a safe distance (10–20 m). After scent collection, the pup was held in a canvas bag, out of sight of the mother, in line with her and the models. The pup and the bag were held on the ground, approximately 10 m from the models, and as far as possible, out of the wind. The two models were placed on the ground 2–4 m in front of the female. Models were placed approximately 50 cm apart to allow the female to sniff both from the same position but requiring unequivocal orientation towards each model. The side to which either model was placed was randomized in each trial. The pup was silenced by holding its muzzle when the female was at the models, but induced to call by uncovering its head, to draw the female to the model. We counted the number and duration of sniffs the female directed to each model during 90 s after her first sniff. Approximately 10–15 min elapsed between the capture of the pup and its release. All mothers and pups were successfully reunited following the experiment. The number and duration of sniffs by each female directed to the models were scored from video by an observer blind to the olfactory identity of the models, and compared with the observations of another researcher recorded *in situ* with 93.6 per cent concordance.

3. RESULTS

Twelve females were tested, however two of them failed to approach within 5 m of the models and were excluded from the analysis. Females showed no preference for the model they first approached (chi-squared test of first model approached: own pup six, non-filial four, $\chi^2_{1,n=10} = 0.40$, $p = 0.53$) showing that there was no discrimination between models prior to sniffing them. The duration of individual sniffs did not vary between model types (paired *t*-test of mean duration of sniffs: own pup (mean $\pm$ s.d.) 0.48 ± 0.11 s, range 0.3–0.64, non-filial pup (mean $\pm$ s.d.) 0.52 ± 0.11 s, range 0.3–0.6; $t_5 = -0.593$, $p = 0.579$). However, females sniffed the model with their own pup's scent significantly more times than the model with the non-filial pup's scent (paired *t*-test of number of sniffs: own pup (mean $\pm$ s.d.) 4.6 ± 3.06 , range 1–10, non-filial pup (mean $\pm$ s.d.) 1.3 ± 1.57 , range 0–5; $t_9 = 4.337$, $p = 0.002$; see the electronic supplementary material, video S2), demonstrating a clear ability to discriminate their own pup's scent from another's. Nine out of the 10 females investigated the scent of their own pup more frequently, the other investigated both equally. Critically, no female investigated the model of the non-filial pup a greater number of times. In addition, two females picked up and drew the model impregnated with their own pup's scent closer to them, and one female aggressively rejected the non-filial pup model by biting, shaking and throwing it. Both these behaviours are commonly seen when female otariids assess pups while searching for their offspring [14,18].

4. DISCUSSION

This experiment demonstrates the use of olfactory cues in individual recognition for a marine mammal species. Australian sea lion females can, and critically do, differentiate the scent of their own pup from that of a non-filial pup.

The results of this study support the suggestion that pinnipeds may use multimodal signals during individual recognition tasks and in particular, mother–offspring reunions. In all species observed, mothers and pups use vocal signals to attract attention, locate and recognize one another during reunions [7]. While these signals can be efficient at long ranges, e.g. individuals are often observed calling from the water when approaching the colony, it is not uncommon for a number of individuals, particularly pups, to respond. The use of olfactory cues as a close range recognition mechanism allows mothers to further confirm their pup's identity before accepting it to suckle. Such double-checking of identity helps to ensure that mothers limit misdirected parental care.

In contrast to recent olfactory studies in pinnipeds [8–11] which showed the presence but not a natural function of olfaction in pinnipeds, the present study shows that wild Australian sea lions use their olfactory abilities in a functional manner, by discrimination between the scents of their own offspring and a non-filial pup. Critically, in contrast to previous studies [8–10], this study involved untrained, wild individuals. Thom and Hurst [19] point out that there are limitations to studies involving training regimes. Such studies are highly removed from a natural context, and only test if an odour, or difference in odours, is detectable, not its relevance to the animal. In addition, with sufficient training, mammals can detect odour differences to which they are not normally sensitive [19]. By investigating wild, untrained individuals we avoided these limitations. Therefore, this study not only shows olfactory abilities, but also demonstrates that these olfactory abilities are used in the social context of mother–offspring recognition and reunions, a situation that has long been hypothesized but until now never experimentally examined. The use of multiple sensory modalities (i.e. acoustic and olfactory) may provide signal redundancy in individual recognition and may allow Australian sea lion mothers to minimize ambiguity as to the individual identity of their young. Further histological and chemical investigations are needed to determine the chemo-emissive structures and odorants involved in individual identity signalling and recognition process.

This research was approved by the Department for Environment and Heritage, South Australia (Scientific permit E24934) and their Wildlife Ethics Committee, Approval 61/2005. All experimental procedures followed the Australian code of practice for the care and use of animals for scientific purposes.

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- 1 Harrison, R. L. & Kooyman, G. L. 1968 General physiology of pinnipedian. In *The behaviour and physiology of pinnipeds* (eds R. J. Harrison, R. C. Hubbard, R. S. Peterson, C. E. Rice & R. J. Schusterman), pp. 212–296. New York, NY: Appleton-Century-Crofts.
- 2 Lowell, W. R. 1980 Flanigan, marine mammal chemoreception. *Mamm. Rev.* **10**, 53. (doi:10.1111/j.1365-2907.1980.tb00233.x)
- 3 Scheffer, V. B. 1958 *Seals, sea lions and walruses: a review of the pinnipeds*. Stanford, CA: Stanford University Press.
- 4 Wartok, D. & Ketten, D. R. 1999 Marine mammal sensory systems. In *Biology of marine mammals* (eds J. E. Reynolds III & S. A. Rommell), pp. 117–175. Washington, DC: Smithsonian Institution Press.
- 5 Mackay-Sim, A. M., Duvall, D. & Graves, B. M. 1985 The West Indian manatee (*Trichechus manatus*) lacks a vomeronasal organ. *Brain Behav. Evol.* **27**, 186. (doi:10.1159/000118729)
- 6 Wells, R. S., Boness, D. J. & Rathburn, G. B. 1999 Behavior. In *Biology of marine mammals* (eds J. E. Reynolds III & S. A. Rommell), pp. 324–422. Washington, DC: Smithsonian Institution Press.
- 7 Insley, S. J., Phillips, A. V. & Charrier, I. 2003 A review of social recognition in pinnipeds. *Aquat. Mamm.* **29**, 181. (doi:10.1578/016754203101024149)
- 8 Laska, M., Svelander, M. & Amundin, M. 2008 Successful acquisition of an olfactory discrimination paradigm by South African fur seals, *Arctocephalus pusillus*. *Physiol. Behav.* **93**, 1033. (doi:10.1016/j.physbeh.2008.01.019)
- 9 Laska, M., Lord, E., Selin, S. & Amundin, M. 2010 Olfactory discrimination of aliphatic odorants in South African fur seals (*Arctocephalus pusillus*). *J. Comp. Psychol.* **124**, 187. (doi:10.1037/a0018189)
- 10 Kowalewsky, S., Dambach, M., Mauck, B. & Dehnhardt, G. 2006 High olfactory sensitivity for dimethyl sulphide in harbour seals. *Biol. Lett.* **2**, 106–109. (doi:10.1098/rsbl.2005.0380)
- 11 Kishida, T., Kubota, S., Shirayama, Y. & Fukami, H. 2007 The olfactory receptor gene repertoires in secondary-adapted marine vertebrates: evidence for reduction of the functional proportions in cetaceans. *Biol. Lett.* **3**, 428. (doi:10.1098/rsbl.2007.0191)
- 12 Higgins, L. V. 1993 The nonannual, nonseasonal breeding cycle of the Australian sea lion, *Neophoca cinerea*. *J. Mamm.* **74**, 270. (doi:10.2307/1382381)
- 13 Higgins, L. V. & Gass, L. 1993 Birth to weaning: parturition, duration of lactation, and attendance cycles of the Australian sea lions (*Neophoca cinerea*). *Can. J. Zool.* **71**, 2047. (doi:10.1139/z93-290)
- 14 Marlow, B. J. 1975 The comparative behaviour of the Australasian sea lions *Neophoca cinerea* and *Phocarctos hookeri* (Pinnipedia: Otariidae). *Mammalia* **39**, 159. (doi:10.1515/mamm.1975.39.2.159)
- 15 Charrier, I. & Harcourt, R. G. 2006 Individual vocal identity in mother and pup Australian sea lions (*Neophoca cinerea*). *J. Mamm.* **87**, 929. (doi:10.1644/05-MAMM-A-344R3.1)
- 16 Charrier, I., Pitcher, B. J. & Harcourt, R. G. 2009 Vocal recognition of mothers by Australian sea lion pups: individual signature and environmental constraints. *Anim. Behav.* **78**, 1127. (doi:10.1016/j.anbehav.2009.07.032)
- 17 Pitcher, B. J., Ahonen, H., Harcourt, R. G. & Charrier, I. 2009 Delayed onset of vocal recognition in Australian sea lion pups (*Neophoca cinerea*). *Naturwissenschaften* **96**, 901. (doi:10.1007/s00114-009-0546-5)
- 18 Harcourt, R. 1992 Maternal aggression in the South-American fur-seal in Peru. *Can. J. Zool.-Revue Canadienne De Zoologie* **70**, 320. (doi:10.1139/z92-048)
- 19 Thom, M. D. & Hurst, J. L. 2004 Individual recognition by scent. *Ann. Zool. Fenn.* **41**, 765.