Final Thesis

Are Cape fur seals (*Arctocephalus pusillus*) able to detect fish rich in oil by the use of olfaction?

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Contents

1. Abstract

Aquatic mammals are traditionally believed to have a poorly developed sense of smell. Behavioral observations, however, suggest that pinnipeds such as fur seals may use olfaction both for foraging and food selection as well as for social communication.

It was therefore the aim of this study to develop a behavioral method to test olfactory capabilities in Cape fur seals *Arctocephalus pusillus*. This method was then used to test if the seals are able to discriminate between odors and, in particular, between the odors of fish that differed in their content of oil.

Using a food-rewarded operant conditioning paradigm the seals succeeded in discriminating between fish- and non-fish odors and between the odors of fish with salmon oil vs. fish without salmon oil. Furthermore, the animals mastered positive and negative transfer tasks and demonstrated long-term (two weeks) memory for the reward value of odors.

These results suggest that Cape fur seals can use olfaction to detect fish rich in oil.

Key words: Olfaction, discrimination, fish odors, non-fish odors, Cape fur seals

2. Introduction

In general, little attention has been drawn to olfaction in marine mammals and the few studies that have been performed so far yielded contradictory results regarding the significance of the sense of smell in this group of animals (Kowalewsky et al. 2006). While other senses such as hearing, vision and somatosensation have been more carefully studied (Renouf 1991) there is an inconsistent picture of the olfactory capacity of pinnipeds (Kowalewsky et al. 2006). Some authors stated that seals have a poor sense of smell (King 1964) whereas other authors reported the nasal olfactory epithelium of fur seals to be of the typical mammalian structure (Kowalewsky et al. 2006).

Early anatomical descriptions of peripheral chemosensory and/or central structures in the brain of pinnipeds suggest that they are basically similar to those of terrestrial carnivores but that they have somewhat reduced olfactory areas (Hoelzel 2002). The olfactory areas are thought to be more reduced in phocids than in otariids (Kowalewsky et al. 2006).

All species of pinnipeds that have been studied so far possess a vomeronasal organ (Hoelzel 2002).

Olfaction is likely to play a substantial role in the behavior of pinnipeds while the animals are on land. It has been proposed as an important social indicator and as a means of mother-pup recognition in several otariid and phocid species (Renouf 1991). Anecdotal reports claim that they can detect a human hundreds of feet away with their sense of smell when on land (Riedman 1990) which suggests that olfaction may play a role in predator detection (Brown 1985).

Mother and pups are often separated in species that breed in colonies. Therefore they must be able to discriminate one another from conspecific seals to reunite and ensure correct placement of maternal effort and reduce unattended amount of time of the pup. South American fur seals *Arctocephalus australis* were found to use vocal cues for recognition while visual cues play a minor role. However, it appears that the mothers distinguish their pup by olfactory cues as well because before apparent acceptance of the pup naso-nasal contact occurs (Phillips 2003). This type of recognition has also been shown in species such as Antarctic fur seals *Arctocephalus gazella* (Dobson & Jouventin 2003) and harp seals *Phoca groenlandica* (Kovacs 1995).

Scent communication is used by most mammals and it can serve a number of functions, for example that of marking territories (Ryg et al. 1992). During the rut in ringed seals, *Phoca hispida*, the males have an enlargement and heightened activity in their sebaceous and apocrine glands in the facial regions producing a strong odor (Hardy et al. 1991). The secretion contains organosulphur and nitrogen-containing compounds. These secretions could possibly be used as a marking of the males´ territories, most likely around the breathing hole and in their lairs. The secretion contains polyunsaturated fatty acids and a type of methyl ester that possibly can prevent it from being diluted in the water and thereby work as scent mark at the breathing hole (Ryg et al. 1992). Male fur seals secrete a scent that may attract females during rutting season and also work as a mark for their territories (Hamilton 1956). A musky odor observed in seals is thought to originate in the glands opening into each hair canal. At moulting and breeding seasons there is increased activity in those glands which suggests a sexual scent gland function (King 1964).

However, the role that olfaction plays in recognition and other aspects of communication in pinnipeds is essentially untested (Insley et al. 2003). During the breeding season in New Zealand fur seals, *Arctocephalus forsteri*, the males attempt to sniff frequently at the perineal and facial regions of females. The females can use open-mouthed threats and jabs to resist the approach from an investigating male. To avoid this the males regularly sniff where the female has her place of rest when she has left it. These olfactory investigations have been suggested to be the only means for the males to assess the female's reproductive state (Miller 1974).

In a study performed by Ross (1972) on nuzzling behavior, that is naso-nasal contact, in captive fur seals this behavior occurred between the dominant individual and the other animals when there was agitation in the enclosure. Nuzzling also occurred after separation of male and females when the male was introduced to the females again. The nuzzling behavior changed prior to parturition and copulation which may be related to changes in the animal's hormonal state. Nuzzling also occurred between male and female when the female was pregnant and also three to four days after the birth (Ross 1972).

For many pinnipeds food resources change throughout the year (Riedman 1990). Food resources are patchily distributed at open sea and seals can spend several days at sea on foraging trips. Olfaction has been suggested to play an important role in pinniped orientation (Kowalewsky et al. 2006). Areas of high marine productivity have been described as foraging grounds for harbour seals because their prey is likely to be found there (Thompson & Miller 1990). Elevated atmospheric concentration of dimethyl sulphide (DMS) is a reliable indicator for such productive zones (Bürgmeister et al. 1990). It reflects the pattern of primary production because plankton and fish occurs there (Sims & Quayle 1998).
A recent study (Kowalewsky et al. 2006) showed that pinnipeds (in this case *Phoca vitulina*) have an extraordinarily high olfactory sensitivity for a substance (DMS) potentially relevant to their sensory ecology.

The olfactory sensitivity to DMS is well tuned to the concentrations found in their marine habitat and this can help them to locate or identify foraging grounds. This suggests that olfaction may play a significant and until now underestimated role in pinnipeds (Kowalewsky et al. 2006).

The present study is about Cape fur seals (*Arctocephalus pusillus*). These Otariids are opportunistic feeders eating fish, cephalopods, and crustaceans. 24 species of fish have been found in their stomachs (Harrison et al. 1968). Some feed alone while others cooperate in small groups to locate and exploit schools of fish and if the school is large they herd them. They have learned to steal from commercial fishing nets at sea (Riedman 1990). Grey seals *Halichoerus grypus* have often been observed to appear downwind the oil slick resulting from another seal surfacing and consuming a captured salmon (Arne Fjälling, pers.comm.)[1].

[1] pers.comm., Arne Fjälling Institute of Coastal Research /The National Board of Fishery, Sweden

By the west coast of Sweden fishermen have large problems with harbour seals (*Phoca vitulina*) stealing from their eel hoop nets (Königson et al. 2000). In a study by Königson et al. (2000) they found that harbour seals prefer eels over cod and herring that also get caught in the net while in another study they preferred cod, herring and flatfish while rejecting eel (Lunneryd 2001). It has been suggested that individual foraging specialization could be the answer to the problem of stolen eels (Lunneryd 2001). The eel is richer in energy than the other fish species caught in the net and in the eel hoop nets they are easier for the seals to catch (Königson et al. 2000).

It has been shown that different species of fish produce different strong aromas, for example mackerel has a strong total aroma while blackback flounder has a weak one (Prell & Sawyer 1988, Morita et al. 2003). This raises the possibility that seals may use olfactory cues to select preferred species of fish.

2.1 Aim of the project

The aim of this study is to develop a method to test olfactory capabilities in Cape fur seals. With this method I will also test if the seals are able to discriminate between odors and in particular between the odors of fish that differ in their content of oil and if they have a long-term odor memory.
Hypotheses to be tested:
Hypothesis 1: Cape fur seals are able to discriminate between different odors.
Hypothesis 2: Cape fur seals are able to discriminate between odors of fish with salmon oil and fish without salmon oil.
Hypothesis 3: Cape fur seals have a long-term memory for odors.

3. Material and methods

3.1 Animals and management

The study was conducted at Kolmården Wild Animal Park, Sweden. Seven Cape fur seals, *Arctocephalus pusillu*s, were used. Two were males and five were females. The two males were 4 years old while the females were 13 and 14 years old.

The seals were kept in an outdoor aquarium (Bråddjupet) with large underwater windows for public display.

The Cape fur seals were fed twice every day and during feeding they were kept in a house in individual cages.

3.2 Experimental set-up

In this study the Cape fur seals had to choose between two different odors. The odors were presented to the seals in two containers which they had to investigate by sniffing to find the rewarded odor. The variable measured was the percentage of correct choices that the seals made per session.

For the presentation of odor stimuli I used containers (Rubbermaid Cooling bag, Clas Ohlson) with a bottom width of 17 cm and length 32 cm, top width 23 cm and length 37 cm (Figure 1). They had a height of 34 cm and the total volume was 20 liter. The external layer was removed to prevent that most of the scent would spread between the two layers and not reach the animals' nose. Holes with 3 mm in diameter placed in intervals of even distance forming a circle with ~ 7.5 cm were drilled 6.5 cm up in the middle of the containers in an exact pattern on each container.

Battery powered ventilators (Clas Ohlson) in the size 60x60x25 mm were placed in the lid of each container providing an ingoing airflow of 0.58 m^3 min^{-1}.

Figure 1: The picture on the left shows the experimental setup from the trainer´s view with two containers while the right picture shows the experimental setup from the seal´s view with the two odorports.

The containers were screened off from the seals behind a plastic board with an area of 100x50 cm. In the plastic board odor ports with a diameter of 7.5 cm were made with a space between them of 42.5 cm and 46.5 cm above the floor. To prevent the olfactory cues to mix, a wooden board with the width of 16.3 cm was placed in the middle of the plastic board.

White plastic boxes (14.5 x 19 x 9.5 cm) were placed in the containers to prevent the odor stimuli from being visible to the seals.

The containers and boxes were cleaned after every test session with hot water.

A yellow plastic target was used to guide the animals to the two odor ports in the beginning of the training. The target was attached to an L-shaped metal bar allowing the trainer to operate it from behind the containers.

A mirror was placed up on the cage above the animals with the purpose to enable the trainers to observe the seals during the sessions.

3.3 Odor stimuli

Essential oils of clove *Syzygium aromaticum*, myrtle *Myrtus communis,* and black pepper *Piper nigrum* were used as odor stimuli. 2-3 drops of essential oil (Aroma Creativ) were placed in a petri dish with water almost covering its bottom. Four capelins (with 12.7 % musclefat), one herring (17.7 %) and one mackerel (9.5 %), either with or without ~ 5 ml salmon oil (Salmopet, Pure Norwegian salmon oil), were together also used as an odor stimulus. The same species of fish, in similar size, were in the box without salmon oil. Two squids, Argentine shortfin squid *Ommastrephes argentinus*, were used in the final test as odor stimuli.

3.4 Learning procedure

The training and experiments started in the beginning of November and continued until the beginning of February. The training and study were conducted twice per day with approximately 20 trials per session.

All seven animals were used in the initial part of the training and testing but in the end only the results from four of them were included in the analysis (because the others did not cooperate reliably).

The seals have been trained before, and know some basic husbandry behaviors, such as stationing where the trainers want them to and they are also able to execute some behaviors used during the public display when the zoo is open. They have not gotten any training similar to what was needed in the present study.

The containers were presented daily to the seals about one month prior to the start of the actual training. Then they were introduced to the plastic board that was positioned in front of the containers. Before the actual training started the reaction of the seals to the ventilators was also noted to see if they would respond to them in any negative way, which they did not.

3.5 Initial training

Training proceeded according to the following steps:
Step 1: In this step the seals were rewarded with fish through the two odor ports training them to approach the two odor ports.
Step 2: In this step the seals had to station against the trainer´s hand by each odor port.
Step 3: In this step the seals had to learn to open their nostrils when stationing by the odor port. To this purpose the trainer rubbed their nose for a short period of time.
Step 4: The hand was here replaced with a small target against which they had to station their nose and approach with open nostrils.
Step 5: A container with fish and salmon oil was presented for them in this step with the ventilator on. The container was presented at both odor ports. The seals were led with the target to the container where they had to station and approach with open nostrils. When the training proceeded the demands on the seals increased. They had to station in the correct odor port for longer periods of time, for more than one second. The nose also had to be better centered in the odor port. They were not allowed to stay for a long period of time in the incorrect odor port, more than 1 second.
Step 6: A second container was introduced without the ventilator on. This container was empty (no odor) and was presented simultaneously with the first container which held an odor. Also in this step the animals were led to the correct container which was the container with fish and salmon oil. The side where the correct container was placed was changed pseudorandomly.
Step 7: The two containers were presented (Figure 2) in the same way as in step six but here the animals had to make their own choice. Fish and salmon oil with ventilator on in one container and one empty container with the ventilator off was presented for the seals.

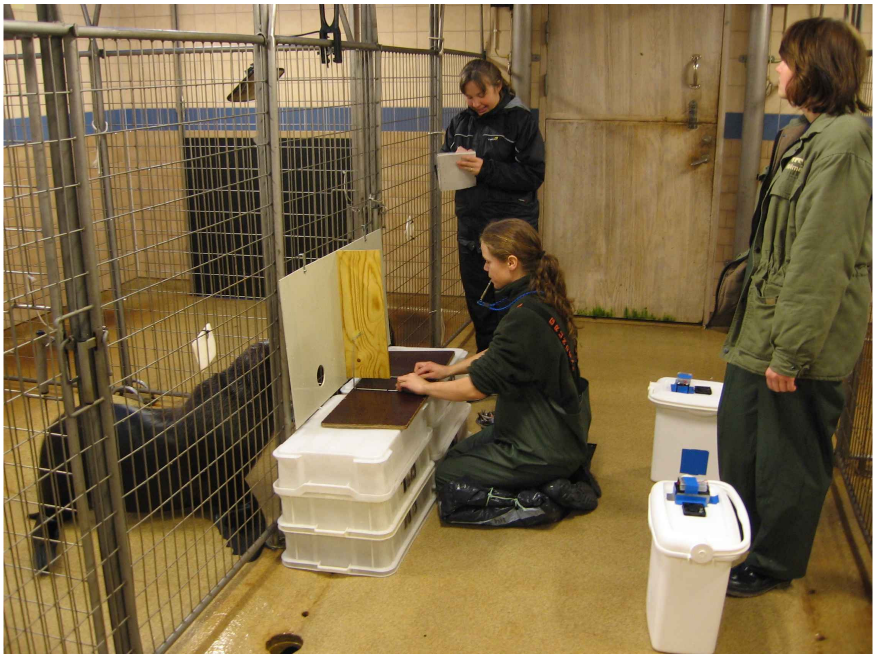


Figure 2: The complete experimental setup from the side before the start of a session. The two containers with the ventilator and the battery on the lid and the mirror above the cage.

The seals indicated their choice for one of the two options by placing their nose in one of the odor ports (Figure 3). There were some exceptions where they showed an obvious choice but the nose was not perfectly placed in the odor port. For example, one of the males made his choice by poking twice at the correct container with his nose not perfectly centered in

Figure 3: One seal is making a choice by placing its nose in one of the odor ports.

the odor port. When the animals made a correct choice they were rewarded with fish through the correct odor port but when they made an incorrect choice the containers were quickly removed and no reward was provided.

3.6 Experimental program

The study was divided into seven experiments with different stimulus combinations (see table 1) and in these experiments the ventilator was on in both containers.

Table 1: The critical experiments included the following stimulus combinations.

Rewarded stimuli (S+)		Unrewarded stimuli (S-)
1: Fish + salmon oil	vs.	Empty container
2: Fish + salmon oil	vs.	Clove (essential oil)
3: Fish + salmon oil	vs.	Black pepper (essential oil)
4: Fish + salmon oil	vs.	Myrtle (essential oil)
5: Fish + salmon oil	vs.	Fish without salmon oil
6: Squid	vs.	Fish without salmon oil
7: Empty container	vs.	Empty container

Experiment 1 was performed to demonstrate that seals have the capability to perceive the odor and to respond correctly.
Experiment 2 was performed to demonstrate that seals can discriminate between fish and non-fish odor.
Experiment 3 was performed to demonstrate that seals can make a negative transfer. A negative transfer means that the positive stimulus is kept constant wheras the negative stimulus is exchanged for another one.
Experiment 4 was performed to demonstrate that seals can make another negative transfer.
Experiment 5 was performed to demonstrate that seals can discriminate between two fish odors that differ by the presence or absence of a fish oil.
Experiment 6 was performed to demonstrate that seals can make a positive transfer. A positive transfer means that the negative stimulus is kept constant wheras the positive stimulus is exchanged for another one.

Experiment 7 was performed as a control to detect if the seals use non-olfactory cues.

The criterion for an animal to be regarded as successfully discriminating between two odors was set at 75 % correct decisions in two consecutive sessions.

During training and the actual test the seals were not able to see the trainer behind the plastic board and thereby any given cues from the trainer to the animal revealing the correct choice were excluded. A study showed that Cape fur seals are able to use gazing (head direction) and pointing to select an appropriate target object (Scheumann & Call 2004). I observed their behavior and noted their choice beside the setup but the seals did not seem to glance at me and thus were not likely to receive any cues.

During most of the sessions only me and the two trainers were present but during some sessions only one trainer was present and then I had to move the containers behind the trainer and at the same time note the seals´ choice. During some sessions other people were also present but that did not seem to bother the animals in their performance.

Because of shifting need for food and other unexpected events there was a different number of trials during some sessions.

Interference from other animals and level of noise did not seem to disturb the performance of the performing animal which was focused on the given task.

To exclude different effects of the ventilators I changed them during one session with two animals.

Two longer breaks of 7 and 14 days between sessions allowed me to assess if the animals remembered the reward value of the odors tested.

3.7 Statistics

For each individual Cape fur seal, the percentage of correct choices from each session was calculated.

Significance levels were determined by calculating binomial z-scores corrected for continuity (Siegel & Castellan 1988) from the number of correct and false responses for each individual and stimulus. The alpha level was set at 0.05. This corresponds to 15 out of 20 decisions correct.

4. Results

4.1 Experiment 1: Fish + salmon oil(S+) vs. empty container(S-)

In this experiment only one reached the criterion but none of the other three animals reached the criterion within 8-10 sessions. Although they were given help by the trainers (they were allowed to change from incorrect odor port to correct) their performance was inconsistent.

One animal (Jocke) reached the criterion in the first two sessions but that was with alote of help. Two animals (Flisa, and Tinny) performed over 75 % in one session (with help) while one animal (Villma) never reached this percentage of correct choices (Figure 4a,b,c,d).

In this experiment only one animal (Tinny) showed some kind of searching behavior while the other three animals (Flisa, Jocke and Villma) did not show any kind of searching behavior.

4.2 Experiment 2: Fish + salmon oil (S+) vs. Clove (S-)

All four animals succeeded in discriminating between a fish odor and a non-fish odor.

Flisa reached the criterion after six sessions (Figure 4a). Jocke reached the criterion after two sessions (Figure 4b). Tinny reached the criterion after twelve sessions (Figure 4c). Villma reached the criterion after five sessions (Figure 4d), (Binomial test, $p < 0.05$).

In this experiment there was a general improvement in performance with all animals but to different degrees. Two animals (Flisa and Tinny) were not constant in their searching behavior while one animal (Villma) in this experiment began with searching behavior in some sessions. One animal´s (Jocke´s) performance improved immediately when the non-fish odor was introduced.

4.3 Experiment 3 (negative transfer): Fish + salmon oil (S+) vs. black pepper (S-)

All four animals succeeded in this first negative transfer task.

Flisa reached the criterion after four sessions in this experiment (Figure 4a). Jocke only needed two sessions to reach the criterion (Figure 4b). Tinny reached it after three sessions (Figure 4c) while Villma reached it after five sessions (Figure 4d), (Binomial test, $p < 0.05$).

The experiment led to an improvement in the searching behavior in three of the animals (Flisa, Tinny and Villma) while one animal (Jocke) continued with his searching behavior in the same way as in the previous experiment.

4.4 Experiment 4 (negative transfer): Fish + salmon oil (S+) vs. myrtle (S-)

All animals that participated in this experiment succeeded with the second negative transfer.

Flisa reached the criterion after two sessions (Figure 4a). Jocke did not participate in this experiment. Tinny reached the criterion after two sessions (Figure 4c) while Villma reached the criterion after two sessions (Figure 4d), (Binomial test, $p< 0.05$).

All three animals that participated in this experiment were searching well.

4.5 Experiment 5: Fish + salmon oil (S+) vs. fish without salmon oil (S-)

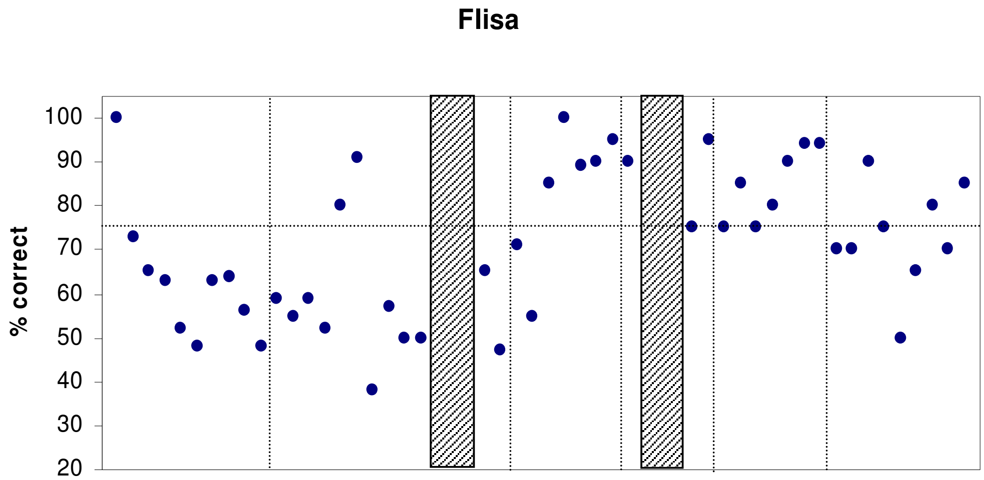
All four animals succeeded in discriminating between two fish odors that differed by the presence or absence of a fish oil in this experiment.

In this experiment Flisa reached the criterion after two sessions (Figure 4a). Jocke reached the criterion after two sessions (Figure 4b), Tinny reached it after two sessions (Figure 4c) and Villma after four sessions (Figure 4d), (Binomial test, $p< 0.05$).

In the first two sessions with Flisa the fish was in the container from the previous experiment. Her results were generally better after the change to the new container. Flisa was also searching well in this experiment except in session three where she got help and she was choosing randomly. Jocke had the fish in the container from the previous experiment for seven sessions. Jocke did not score below 80 % correct in any of his sessions. His results became more constant between the different sessions after the change to the new container. With the contaminated container he had results spread between 86 % and 100 % while in the new container the results were between 95 % and 100 %.

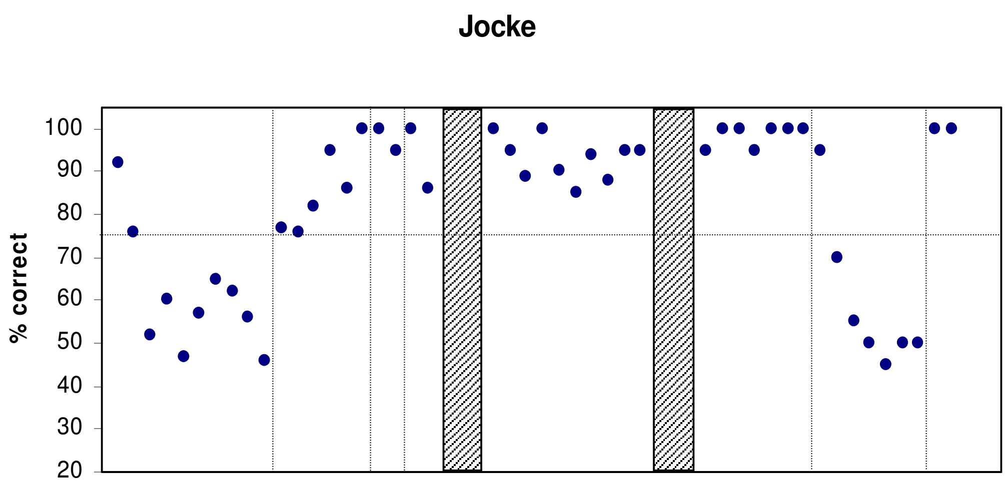
Tinny had the contaminated container for four sessions. Her results improved after the change. Tinny had below 80 % correct choices in the first two sessions of the experiment but then her searching behavior improved.

a)



Experiment: 1 2 B1 2 3 4 B2 4 5 6

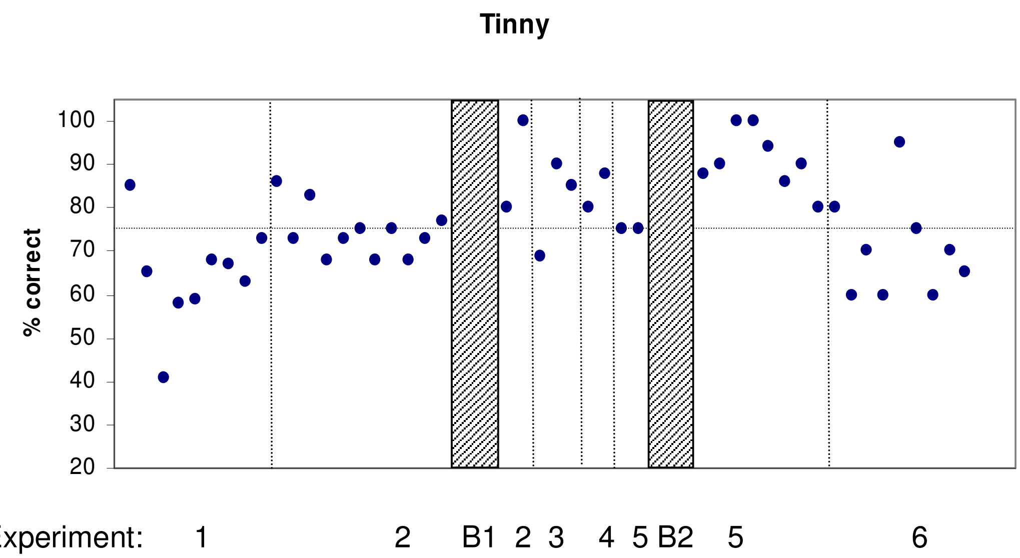
b)



Experiment: 1 2 3 5 B1 5 B2 5 6 5

Figure 4 a+b: Percentage of correct choices for each session with Flisa (a) and Jocke (b).The numbers 1-6 indicate the different experiments and the vertical lines show where they started and ended. B1 and B2 indicate the two breaks during training. The horizontal line shows the criterion for an animal to be regarded as successfully discriminating between two odors. It had to be reached in two consecutive sessions.

c)



d)

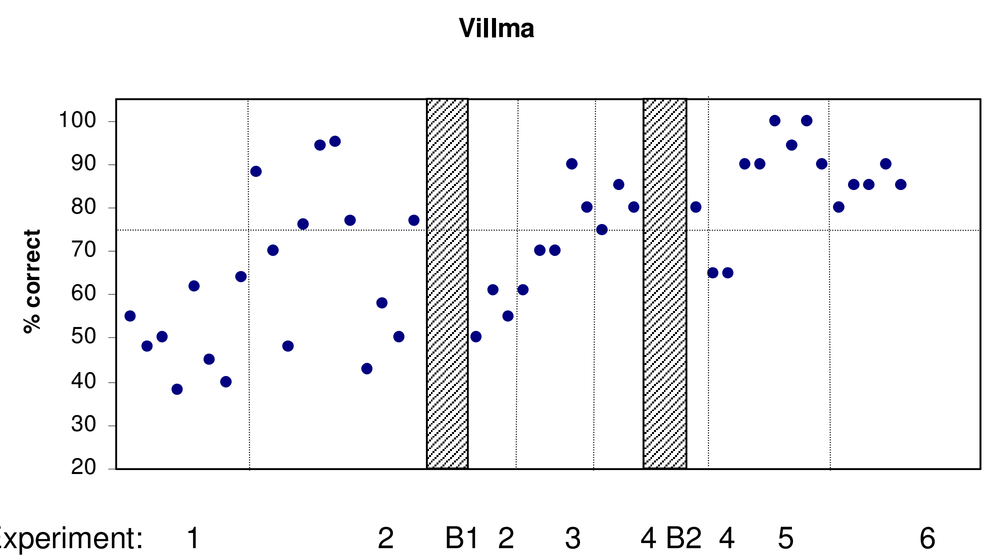


Figure 4 c + d: Percentage of correct choices for each session with Tinny (c) and Villma (d). The numbers 1-6 indicate the different experiments and the vertical lines show where they started and ended. B1 and B2 indicate the two breaks during training. The horizontal line shows the criterion for an animal to be regarded as successfully discriminating between two odors. It had to be reached in two consecutive sessions.

Villma also had the contaminated container for four sessions and her results also improved after the change. Villma´s searching behavior was not constant in the first two sessions but after that her results were at 80 % or above in every session.

4.6 Experiment 6 (positive transfer): Squid (S+) vs. fish without salmon oil (S-)

Three animals (Villma, Tinny and Flisa) reached the criterion in this experiment and completed the positive transfer while one animal (Jocke) did not reach the criterion.

Flisa reached the criterion after four sessions (Figure 4a). Jocke had 95 % correct in his first session but after that his results were decreasing for almost every session (Figure 4b). Tinny reached the criterion after five sessions (Figure 4c) while Villma reached the criterion after two sessions (Figure 4d), (Binomial test, $p < 0.05$).

Two animals (Flisa and Tinny) were not performing a constant searching behavior in this experiment. Jocke was searching but then he mostly chose the incorrect odor port. During the two last sessions of the project Jocke was allowed to return to experiment 5 to build up his motivation again. He immediatly performed at 100 % correct in two consecutive sessions (Figure 4b).

4.7 Experiment 7 (test control): Empty container vs. empty container

The control tests were interspersed between the other trials in experiment five in two sessions.

Two animals (Jocke and Tinny) were searching fast and then chose one odor port while the other two (Flisa and Villma) were searching without making a choice.

Flisa was moving back and forth between the two odor ports. In the first two trials she chose one odor port while in the other trials she was searching without making a choice.

Jocke was searching very fast and then chose one odor port and it was evenly divided on both sides.

Tinny chose one odor port every trial but she showed a searching behavior in the last trials of the two control sessions.

Villma chose one odor port (right) the first trial but the other trials she was moving back and forth without making a choice for an odor port.

4.8 Memory test

After two breaks of 14 and seven days between sessions (indicated as B1 and B2 in figure 4 a-d) testing was resumed, allowing me to assess if the animals remembered the reward value of the odors tested.

Before the break of 14 days (B1) Flisa performed at 50 % correct and after the break she performed at 65 %. Before the break of seven days (B2) Flisa performed at 90 % and after she performed at 75 % (Figure 4a).

Before the 14 days break (B1) Jocke performed at 86 % and after he performed at 100 %. Before the seven days break (B2) he performed at 95 % and after he also performed at 95 % (Figure 4b).

Before the 14 days break (B1) Tinny performed at 77 % and after the break she perforemd at 80 %. Before the seven days break (B2) she performed at 75 % and after at 88 % (Figure 4c).

Before the first break (B1)Villma performed at 77 % and after she performed at 50 %. Before the second break (B2) she performed at 80 % and after she performed at 80 % (Figure 4d).

The animals were generally performing at the same level as before the breaks or in some cases even better after the break. This suggests that they are able to memorize the reward values of odors for at lest two weeks.

4.9 Control test: Change of ventilators

When I changed the ventilators between the two containers there was no visible change in the performance of the animals. Jocke had 95 % correct during that session and he had 95 % before and after that session. Villma had 90 % correct during the session and she had 100 % before and 80 % after.

None of the animals showed any sign of being affected in its performance by changing the ventilators.

5. Discussion

The results of the present study demonstrate that Cape fur seals are capable of acquiring a food-rewarded olfactory discrimination paradigm. The animals succeeded in discriminating between fish- and non-fish odors and between the odors of fish with salmon oil versus fish without salmon oil. Furthermore, the Cape fur seals mastered positive and negative transfer tasks and showed long-term memory for the reward value of odors.

Prior to discussing the results of the present study, it seems appropriate to discuss the advantages and disadvantages of the method used and to compare it to other methods used for studies of olfactory discrimination abilities.

5.1 Discussion of the method

There are five different groups of paradigm that are commonly used for testing olfactory capabilities in animals (Doty 1975).

The approach paradigm is the most commonly used paradigm. Here, two or more odorants are placed at different locations within a test chamber. The dependent measure is the duration and/or frequency of investigation of the odorants. It is easy to set up and it can be performed within the subject´s homecage, in the field or in a laboratory. Other advantages are that various odorants can be tested and training is not necessary because of the subject´s exploratory or foraging behavior. The disadvantage can be that the odors can diffuse and mix together, the animals can smell an odor without approaching it and there can be a position preference. The biggest disadvantage is that the animals may not show a preference and in such a case no conclusion is possible.

The forced approach-avoidance paradigm measures both the attractive and aversive aspects of an odorant. Here the subject moves away from an aversive odorant and approaches the attractive odorant. The advantage with this paradigm is that both aversive and attractive qualities of an odorant can be determined. The disadvantage is that only one odorant can be tested at a time and, to decrease the error variance, a large number of subjects must be used. The preference resulting from this paradigm is different from the preference in paradigms where two odorants have been compared. In this paradigm too, as in the approach paradigm, the biggest disadvantage is that the animals may not show a preference.

In the odor trail paradigm the animals have to follow scent trails laid by the experimenter. This paradigm is most appropriate in ethological studies of homo- and hetero-specific social and sexual attraction. The disadvantage with this paradigm is that only social odors and, in some cases, food odors can be tested and only certain species can be tested.

The sniff-rate analysis paradigm measures the number, duration, and intensity of sniffs in an odor-controlled environment. This paradigm usually requires a considerable amount of electric equipment to record and analyze the sniffing patterns. A disadvantage with this paradigm is that it can be unclear what the increases and decreases in sniffing rate signify.

The operant conditioning paradigm generates learning in which an event as the delivery of food is made contingent when a response occurs. The operant is a behavior that occurs due to intrinsic causes. That the operant is controlled by a specific stimulus is a result of the conditioning that produces the learning (Bolhuis & Giraldeau 2005). A great advantage of the paradigm is that the dependent variable is objective and measurable. By using the operant behavior as a dependent variable the paradigm allows the reinforcing properties of various odorants to be determined. Another advantage with this paradigm is that various parameters of the odorant presentation can be independently manipulated with minimal difficulties. Bypassing of the recording system is not possible as in the approach paradigm because here the subject must perform a discrete behavior to smell an odorant and the animal´s aversion to an odorant by requiring it to turn off the odorant can be determined. A possible disadvantage with this paradigm is that there can be inadequate cleaning of equipment involved with the odorants. Furthermore, the method requires training to teach the animals to perform an operant behavior.

In this study I used the operant conditioning paradigm. The advantage with this paradigm is that it is easy to test with a simple design and that the sessions were performed in the animals´ homecages eliminating the problems of testing them in a new environment. In this paradigm there is no reliance on spontaneuos preferences and motivation has little impact on the animal´s performance. The discrete behavior of the seals (that they poke with their nose) was the dependent variable.

There were no complex mechanical and electrical components in the experimental set-up except the ventilator and the battery that would have been easy to replace if needed. Training was neccessary to teach the seals to place their nose correctly in the odor port and position preference occurred during some sessions in the beginning of the training but not in the later sessions. The apparatus that was used was not difficult to clean because there were no small parts. With the containers and the wooden board I tried to prevent the two stimuli from diffusing together. The method used in this project gave successful results and it may be suitable to use in future olfactory studies.

5.2 Discussion of the results

All four Cape fur seals were capable of acquiring a food-rewarded olfactory discrimination paradigm. This kind of method has not been used in an olfactory study in pinnipeds so far. A similar two-choice discrimination method has been used successfully in a study of the visual capability in Cape fur seals *Arctocephalus pusillus* and South American fur seals *Arctocephalus australis* by Busch and Dücker (1987). The animals had to discriminate between a black circle of decreasing size and a white plate. They also had to discriminate between horizontal and vertical stripes that were varying in width. With this the authors determined the seal´s visual angle (α) (Busch & Dücker 1987).

The successful discrimination between fish with salmon oil and non-fish odors suggests that it may be important for seals to be able to discriminate between fish odors from non-fish odors in their natural behavior. Seals should be able to detect and discriminate fish odors from other odors in order to successfully forage and select potential food.

All four animals succeeded to discriminate fish with salmon oil from non-fish odors and fish without salmon oil. For the seals to be able to discriminate one odor from another they must be able to detect the odor. The results of this study show that Cape fur seals are able to detect fish with salmon oil and to discriminate it from fish without salmon oil and also from the non-fish odors. This suggests that they may be able to detect torned fish in, for example, fishing gear.

All four animals completed the negative transfers. This shows that the seals are able to learn the reward value of a new non-rewarded odor that is presented to them while the rewarded odor stays the same. It also supports the notion that the seals are actually able to detect the odor of fish with salmon oil and discriminate it from other odors. The negative transfers also helped in the learning procedure by increasing the animal´s searching behavior for the correct odorant.

The memory test also gave successful results in this study. The ability to remember how to get the benefits and the rewards that food brings can be important and it may be one answer to why it is thought to be specialists that steal from fishing gear (Lunneryd 2001) because they have learned a behavior and remember it. Odor memories are said to be longlived and it has been demonstrated in various species, for example the squirrel monkey *Saimiri sciureus*, that they possess a robust memory for odors (Laska et al. 1996).

Even though only three out of four animals (Villma, Tinny and Flisa) succeeded with the positive transfer I consider it as a successful result. The fact that the male did not complete the positive transfer could be due to the fact that he is the only one that does not eat squid and maybe he could have been successful if he had been allowed to perform more sessions.

Behavioral relevance is suggested to be an important determinant of a species´ olfactory sensitivity to an odor stimulus (Laska et al. 2005). Pinnipeds have somewhat reduced olfactory areas (Hoelzel 2002) but that does not necessarily mean that their olfactory sensitivity to odors is low. In pigtail macaques *Macaca nemestrina* the sense of smell is believed to have only little, if any, behavioral relevance. Pigtail macaques, as pinnipeds, have reduced olfactory bulbs and in comparison with other primates a lower number of functional genes coding for olfactory receptors. Despite this, pigtail macaques have a well-developed olfactory sensitivity for the odorants of monomolecular odorants of aliphatic aldehydes present in a large variety of fruits that are included in their diet and are thought to have behavioral relevance for them (Laska et al. 2003). Harbour seals *Phoca vitulina* also have an extraordinarily high olfactory sensitivity to an odorant with behavioral relevance, dimethylsulfide (DMS) (Kowalewsky et al. 2006) even though they, as all pinnipeds, have reduced olfactory areas. Procellariform seabirds live a similar life to that of many pinnipeds with patchily distributed food resources which they can search for over hundreds and even thousands of kilometers. Field studies have been performed on these seabirds and their olfactory sensitivity for DMS-scented vegetable oil slicks. These slicks were paired with plain vegetable oil slicks. The DMS slicks attracted in some species of procellariforms twice as many visits. They also had cod-liver-scented slicks which attracted the seabirds in a similar pattern as the slicks with DMS (Nevitt 2000). This may suggest that procellariforms have a high olfactory sensitivity to DMS and cod-liver scents, two odorants with behavioral relevance for them. That the seals in this study could detect the salmon oil suggests that pinnipeds, even though they have reduced olfactory areas, have the ability to perceive salmon oil.

In a field study with baited buoys performed by Beszczýnska (2005) on Baltic grey seals *Halichoerus grypus* the results indicated that the seals´ olfaction is of minor importance in their foraging behavior. Nevertheless grey seals *Halichoerus grypus* have often been observed to appear downwind the oil slick resulting from another seal surfacing and consuming a captured salmon (Arne Fjälling, pers.comm.)[2] and now the results from this study raises the possibility that seals can use olfaction in their foraging behavior, for example to locate fishing gear and/or discriminate between different species of fish.

Based on my results the next step should be to investigate the seals´ capacity to discriminate between the odors of different species of fish.

[2] pers.comm., Arne Fjälling Institute of Coastal Research /The National Board of Fishery, Sweden

There should also be a more thorough investigation measuring the seals´ longterm memory with longer breaks than in this study.

The main conclusion from this study is that Cape fur seals are able discriminate between odors. They are also able to discriminate between fish with salmon oil and fish without salmon oil and thereby they are able to detect salmon oil. This suggests that the olfactory capacity of seals is higher than previously believed and well-tuned to an odor (salmon oil) of behavioral relevance. Another conclusion is that seals can be trained to discriminate between odors.

These results also suggest that seals may have a long-term odor memory for at least 14 days, if longer is yet to be determined.

The method used gave successful results and may be useful in future studies on olfactory performance in seals.

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