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### Olfactory experiments on Cory's shearwater (*Calonectris diomedea*): The effect of intranasal zinc sulphate treatment on short-range homing behaviour

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# Olfactory experiments on Cory's shearwater (*Calonectris diomedea*): The effect of intranasal zinc sulphate treatment on short-range homing behaviour

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## ABSTRACT

Experiments were carried out with anosmic Cory's shearwaters to verify whether these birds, which are strictly nocturnal in their activity on land, use olfactory cues to localize individual nest burrows. Both the experimental birds, subjected to intranasal injection of zinc sulphate solution, which produces a reversible impairment of the olfactory mucosae, and unmanipulated controls were captured at their breeding colony on the island of Linosa (Pelagian Islands, Italy). Releases were carried out on the coast nearly 2 km away from the breeding colony. Recoveries at the nests showed that experimentals achieved significantly poorer homing success than controls. The view that the birds were disturbed in a non-specific way by experimental manipulation is weakened by the fact that injection of the same zinc sulphate solution into the coanae, a method only marginally involving the olfactory receptors, does not influence homing behaviour. Our results indicate that in Cory's shearwaters the search for individual burrows is based on, or at least complemented by, olfactory cues.

**KEY WORDS:** *Calonectris diomedea* - Cory's shearwater - Homing - Olfaction.

## ACKNOWLEDGEMENTS

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## INTRODUCTION

Procellariiforms include marine long-range fliers whose ability to locate their nest site at night has been investigated by several authors. Only a relatively small number of species has been investigated, however, and in most cases without conclusive findings. Among the possible cues leading to the exact burrow location, visual information seems to be used by a number of shearwater species [*Puffinus assimilis*, *P. carneipes*, *P. tenuirostris* (Warham, 1955, 1958, 1960), *P. puffinus* (James, 1986)], though it cannot be excluded that vision might be supplemented by other kinds of cues. An involvement of both olfactory and visual cues, for example, can be hypothesized from the results of tests carried out by Shallenberger (1975) on wedge-tailed shearwaters (*P. pacificus*). On the other hand, olfactory information is found to play a fundamental role in the system guiding Leach's storm petrels (*Oceanodroma leucorhoa*) to individual nest burrows (see Bang & Wenzel, 1985, for references).

Wink and his co-workers (1980) supposed that echolocation assists Cory's shearwaters (*Calonectris diomedea*) in locating their nest site; this hypothesis, however, has not yet been tested by experimental analysis. To contribute to the knowledge of the sensory information which assists this bird species in localizing the nest site, we have carried out a series of experiments to verify the possible role of olfactory cues.

## MATERIALS AND METHODS

### *The birds*

Cory's shearwaters used in our experiments were captured on the islet of Linosa (Pelagian Isles: 35°52'N, 12°52'E, 5.4 km<sup>2</sup>), where a breeding colony of 10 000 or more pairs was estimated by Massa & Lo Valvo (1986). Our manipulations of the birds did not affect their breeding success given that our tests were carried out with shearwaters which were not incubating a true egg but a chalk one. It must be explained here that local people, following an illegal tradition, collect eggs from shearwaters' nests. This regrettable practice does not prevent the birds from remaining faithful to the empty nest for a period of 15 to 30 days, as we observed several times. Moreover, we strengthened their nest fidelity by providing them with chalk-eggs, whose size and shape were similar to the natural ones. The birds were captured early in the morning by pulling them out of their nest burrows and placing them in individual cloth bags.

### *Bird treatments*

Treatments were carried out on the morning of capture. The birds were banded for individual identification with rings supplied by the Istituto Nazionale di Biologia della Selvaggina, and checked for bill measurements and body weight. The experimentals, chosen randomly, were then treated with a solution of zinc sulphate heptahydrate (2%). In 1989, 2.5 ml of solution were injected into each coana, using a short curved needle and keeping the bird's head turned upside down to allow the solution to flow out from the nostrils. This method, successfully used with homing pigeons (Benvenuti *et al.*, 1992), proved to be totally ineffective in depriving shearwaters of olfactory perception. This has not simply been inferred from subsequent behavioural results, but has been verified by injecting a

coloured solution and inspecting the etmoidal region by surgical operation. It was thus observed that the coloured pathway of the solution had by-passed the nasal conchae and had not involved the olfactory mucosae or, at best, had achieved only marginal contact.

In the tests carried out the following year, the zinc sulphate solution was injected into each nostril by using a slightly curved 45-mm-long needle, which, according to our previous inspection in two operated shearwaters, allows the solution to flow out from the coanae after coming into contact with the olfactory membranes. Soon after treatment the experimental birds, as well as the unmanipulated controls, were placed in individual wooden containers until they were released (a detailed description of the containers is reported in *Massa et al.*, 1991).

The experimental treatment may have had an irritating effect, as testified by the lachrymation in some of the birds, a factor which has never been observed in homing pigeons. This lachrymation, however, could have been caused not by the zinc sulphate, but by the mechanical disturbance related to the insertion of the needle into the nostrils. In any case, this disturbance did not prevent the birds from exhibiting, at release, normal flight behaviour indistinguishable from that of unmanipulated controls.

#### Bird releases

The wooden containers with the control and experimental birds were placed on top of a car and transported to the release site. Releases were carried out in the late afternoon, a few hours after treatment, at a site on the sea coast at an approximate distance of 2 km from the breeding area. In 1989 releases were carried out on the 3rd of June. In 1990 the experiments were carried out on two days (21st and 23rd June); 4 out of 14 shearwaters released on the latter date had already been used as controls in the previous release. They were used again after they had been subdivided randomly into 2 controls and 2 experimentals. The birds were released singly, alternating the two treatments (controls and experimentals), and were followed with 10 × 40 binoculars until they vanished from sight; vanishing time and vanishing bearings were recorded using a stop-watch and compass.

Homing success was ascertained by recoveries of the released birds in their nest burrows, which had been accurately mapped. In 1989, nests were visited only once on the morning following release; a high percentage both of controls and experimentals was, in fact, recovered (experimentals exhibited even better homing success!), rendering any further collection of data superfluous. Conversely, inventories were recorded for several days in 1990, in which a difference in homing success between controls and experimentals was observed (the frequency of our visits to the colony is indicated in Fig. 2).

#### Statistical methods

Differences in the homing performance between control and experimental birds were tested using the  $\chi^2$  or Fisher exact probability test (depending on the sample size), and the Mann-Whitney U test (Siegel, 1956).

## RESULTS

At release all the birds flew off toward the open sea and vanished in a few minutes in directions at right angles to the coastline. The homing data are summarized in Figure 1. In 1989 (1st series of releases), inventories of homed birds carried out on the following morning revealed that 8 controls (57.1%) out of 14, and 9 experimentals (64.3%) out of 14, had homed during the night (shearwaters approach the breeding colony during the night, provided that the moon is not visible). This difference

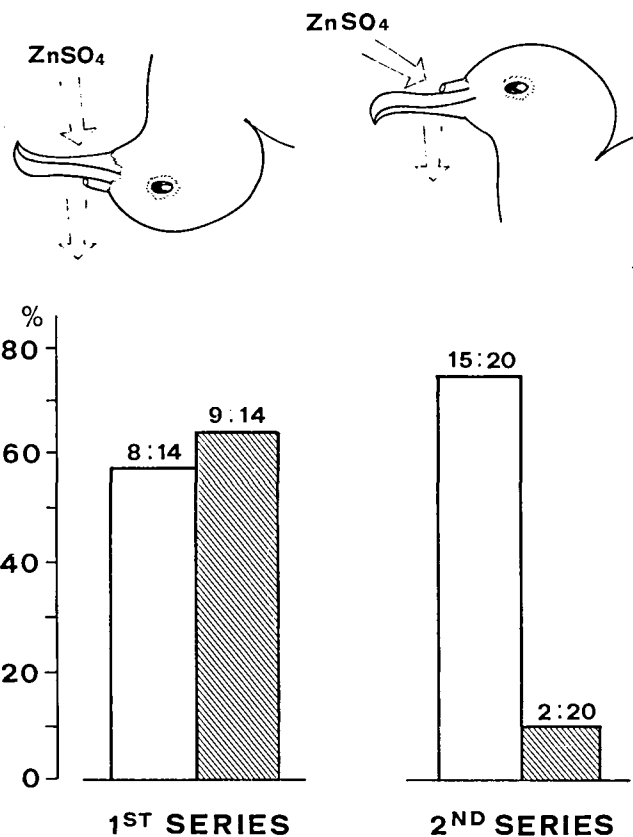


Fig. 1 - The length of the columns is proportional to the percentage of birds which homed during the first night after release. Open and shaded columns refer to control and experimental birds, respectively. The numbers above the columns indicate the actual number of birds that homed, followed by the total number of birds released. The pairs of columns showing the results for 1989 (1st series of release experiments) and 1990 (2nd series of release experiments) are supplemented by illustrations displaying the treatments to which experimentals were subjected [injection of zinc sulphate solution into the coanae, flowing out from the nostrils (1989), or the reverse (1990)].

between the treatments is not significant (Fisher test:  $P > 0.10$ ), besides being contrary to our expectation based on the idea that homing process may be guided by olfactory information.

In 1990 (2nd series of releases), 20 controls and 20 experimentals (13 and 13 on 21st June, 7 and 7 on 23rd June) were released: 15 controls (75.0%) and 2 experimentals (10.0%) were recovered in their nests on the following morning. This difference between the treatments is significant ( $\chi^2$ ,  $P < 0.001$ ). Conversely, no significant difference was revealed between the homing success of control birds released in 1989 and those released in 1990, nor between experimentals tested in 1989 and controls tested in 1990 (Fisher test,  $P > 0.10$  in both comparisons), whereas 1990 experimentals exhibited significantly poorer homing success than 1989 ones ( $P < 0.01$ ).

In 1990, only one control and three experimentals were lost: many birds, in fact, were subsequently recovered in their nests which were monitored for

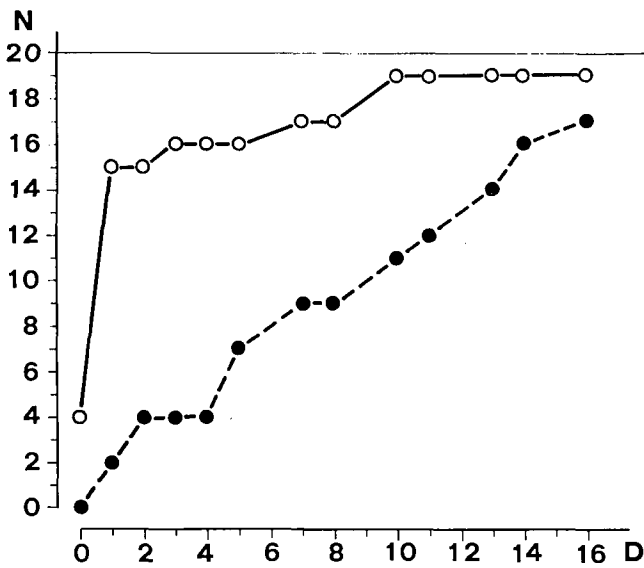


Fig. 2 - Homing performance data: the height of each dot on the abscissa axis is proportional to the number ( $N$ ) of controls (open dots) and experimentals (solid dots) that had homed at the corresponding number of days ( $D$ ) elapsing between release and recovery in the nest burrows.

several days after release. Figure 2, which reports the results of inventories carried out in the experimental period, shows that controls had shorter homing times than experimentals (as measured by the number of days elapsing between release and recovery at nests). According to the Mann-Whitney U test, this difference is highly significant ( $P < 0.001$ , one-tailed test).

## DISCUSSION

Our study on the short-range homing mechanism of Cory's shearwaters is not based on orientational data, given that these birds tend to fly toward the open sea when they are released on the sea shore, with a small degree of scattering in the vanishing bearings, following a course at right angles to the coastline. Thus, the initial orientation appears to be based on local topography and does not depend on the birds' position with respect to home. In this, our birds exhibited the same behaviour as that reported by Massa *et al.*, (1991) in a study on the long-range homing mechanism of the same species.

On the other hand, homing success data are of crucial importance; the poor homing success of birds treated with zinc sulphate in 1990, compared to the good homing ability of controls, suggests that homing Cory's shearwaters rely on olfactory perception. As it has been reported that zinc sulphate treatment causes a temporary and variable impairment of olfactory perception (see Cancalon, 1982, for references), we suppose that experimental birds got back home as soon as their olfactory perception regained a minimal functional level. This conclusion is not surprising if we consider that these birds

are strictly nocturnal in their activities over land and that they tend to approach the breeding colony during the moonless part of a night; the nests, moreover, are often placed in very deep branching burrows in nearly black volcanic rocks. Under these circumstances, it appears unlikely that visual cues can constitute reliable information leading to the exact nest location. We have often observed that when birds circle close to the ground during the landing phase they may hit an unexpected obstacle (the experimenter's body!).

Our explanation of the results may be challenged on account of the fact that we have no direct evidence that our experimental birds were actually deprived of olfactory perception. Moreover, their poor homing success might have been induced by a non-specific disturbance of the experimental treatment, regardless of the effectiveness of our method in depriving the birds of olfactory perception. These alternative explanations of our results are weakened by the fact that, in the 1st and 2nd series of releases – carried out in 1989 and 1990, respectively – shearwaters were subjected to two similar kinds of manipulations: injection of zinc sulfate into the coanae and into the nostrils. Only in the latter case, however – in which the solution actually contacted the olfactory mucosa – was the homing behaviour of the birds affected.

Another reason in favour of our explanation is based on the results of analogous experiments carried out on related bird species. Grubb's (1974) investigation on Leach's petrel (*Oceanodroma leucorhoa*) has shown that this bird relies on an olfactory guidance system in nest burrow location. In a Y-maze, moreover, captive breeding petrels were attracted by an air current coming from their own nest material, and disregarded the one coming from similar materials. Also in the wedge-tailed shearwater (*Puffinus pacificus*) it has been supposed that olfactory cues supplement visual information in the homing process (Shallenberger, 1975).

These bird species, including Cory's shearwater, belong to a systematic group (order Procellariiformes) which, according to the reports of Bang (1966, 1971), exhibits highly developed olfactory bulbs and mucosae; these may be used to detect the smell of food (references in Jouventin & Robin, 1984) and/or of the breeding site.

From our results it is not clear whether the guidance system of Cory's shearwater to individual nest burrows is entirely dependent on olfactory information or whether the homing process is assisted by complementary factors. We cannot exclude the latter possibility, through the suggestion of Wink and his co-workers (1980), that in this bird species echolocation is used to localize the nest site, has not yet been tested by experimental analysis. This idea, however, conflicts with the fact that landing shearwaters often hit unexpected obstacles.

In recent years, it has been shown that olfaction is a basic sensory input for pigeon navigation. Thus, it is reasonable to set our results alongside the model proposed for the pigeon homing mechanism (see Papi

1989 for references). In our opinion, the olfactory information allowing pigeons to home after displacement over long distances is part of a mechanism which is not comparable with the short-range guidance system of petrels and shearwaters to individual nest burrows. Unlike pigeon navigation, the latter ability is an osmotaxis which requires an apparent direct sensory (olfactory) contact with the goal.

It has been reported that several species of Procellariiformes are able to home after experimental or active displacement over hundreds or thousands of kilometers; the sensory information underlying this ability is still unknown. The results recently presented by Massa *et al.*, (1991) suggest that long-distance homing ability of Cory's shearwater is not based on magnetic information. Given that olfactory deprivation disrupts short-range homing ability in this species, it would seem quite difficult to investigate whether long-distance navigation, too, is based on olfactory cues, unless the experimenter has access to some kind of long-distance tracking device.

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