

Navigation in Procellariiform Seabirds



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Abstract

Much of what is known about avian navigation results from half a century of displacement experiments with the homing pigeon, the main model in avian navigation. Whether the same processes underpinning navigation in the homing pigeon are also integral to navigation in other species remains an open question. In particular, how Procellariiform seabirds, which routinely travel huge distances over open ocean, might navigate is of interest because of the contrast in sensory environment compared to terrestrial species. In this thesis, I have three aims. First, to investigate whether the mechanisms involved in homing pigeon navigation might also guide Procellariiform seabirds over the ocean. Second, to establish whether GPS-tracked, free-ranging Procellariiform seabirds might be able to offer insights into avian navigational mechanisms. Third, to investigate some of the ecological drivers of long-distance movement facilitated by these navigational mechanisms in Procellariiform seabirds. By combining novel experimental and analytical approaches, I attempt to answer some of these questions. I find that many of the mechanisms thought to guide homing pigeons also appear to underpin Procellariiform navigation. In particular, the involvement of information from the time-compensated sun-compass, central to structuring spatial representation in terrestrial species, seems to be very similar to that observed in pigeons. Furthermore, like pigeons, olfaction seems to play a specific role in Procellariiform seabirds' map sense. However, there are differences too. I make the tentative discovery that, perhaps unlike pigeons, shearwaters might be able to derive information exocentrically about their location at sea in the form of a vector of direction and distance from the home colony, implying a geometric cognitive representation of large-scale environmental gradients. Finally, in terms of ecological drivers of long-distance movement, I find that Manx shearwaters' migratory phenology is probably constrained by their environment and not by their condition, in-keeping with the idea that long-distance movement comes at a low cost for Procellariiform seabirds.

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Author contributions

All of the work in this thesis is primarily my own.

13 other people co-author one or more of the data chapters in this thesis. Their contributions are as follows:

Tim Guilford contributed to the conception, fieldwork and analysis of all data chapters.

Sarah Bond and Annette Fayet contributed ideas and feedback to chapters 2 and 6, and participated in fieldwork.

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“In the most remarkable of the prewar tests Lockley took two Manx Shearwaters by aircraft to Venice where he released them. Birds of this subspecies never normally visit the Mediterranean and do not cross dry land in their migrations. Yet upon release, one of the birds turned and headed west in the direction of the Italian Alps instead of flying south toward the sea. It reached its own burrow in Skokholm in 341 hours 10 minutes.

Dr. G. V. T. Matthews of the Department of Zoology, University of Cambridge, has been conducting further homing experiments with Manx Shearwaters. He has had birds released at many points on the British mainland and has enjoyed a startling series of returns. In many cases there was strong evidence of an initial sense of homeward orientation, because the birds started on a direct compass bearing for Skokholm. Attempts at sending birds to America had failed because the long journey by steamer exhausted them.

During a conversation with Mr. Lockley at Skokholm on June 1, I happened to say something concerning my anticipated return by air to Boston on the morning of June 3. Lockley at once realized the opportunity for quick transport and asked me to take two birds to Boston for release. Accordingly, I was given a carton containing two Manx Shearwaters which Dr. Matthews had taken from their burrows and banded. I left Tenby, Pembrokeshire, that evening via sleeper train for London. The birds caused no little wonder and merriment to the people in the adjoining rooms, who could not understand the origin of the crowing and cackling sounds which came from my room in the late evening. The next day the birds remained in the carton, each in its own compartment, and in the evening I enplaned for America with the birds under my seat. Only one survived the journey.

On the morning of June 3, immediately after landing and passing through Customs, I was driven in a TWA truck to the easternmost point of Logan International Airport on Boston harbor. There, with the kind cooperation of a TWA employee, I released Shearwater AX6587 at the edge of the water, 100 yards from the point and on the southern shore of a tiny peninsula. It flew directly over the water to the end of the peninsula and then abruptly turned eastward over the ocean. This was at 8:15a.m., E.D.S.T., June 3, 1952.

On June 20 I was surprised to receive the following cable: "MAZZEO, SYMPHONY HALL, BOSTON, MASS. No. AX6587 back 0130 BST 16th stop--FANTASTIC--MATTHEWS." A letter subsequently received said that Matthews on making his second round of the night of June 15/16, was "completely flabbergasted" to find AX6587 in its own burrow. He continued, "I read the ring several times, and then put the bird back and blocked the entrance. I wanted to make sure it would still be in when I took Peter Conder along to verify my observation. As we had not then had your letter, I was convinced that you must have run into trouble with our customs and released the bird at London. The boat came over that morning with your letter--there was no gainsaying the result then! A pretty touch, the bird beating the mail?"

The bird travelled more than 3200 land miles in 12 days, 12 hours, and 31 minutes, or an average of 250 miles per day."

Rosario Mazzeo, Boston, writing in *The Auk*, 1953.

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Table of Contents

Chapter 1	General Introduction	13
Chapter 2	In situ clock-shift reveals that the sun-compass contributes to orientation in a pelagic seabird	47
Chapter 3	Anosmia affects homing orientation but not foraging in shearwaters	77
Chapter 4	False release site experiments with a wild Procellariiform seabird: a pilot study	115
Chapter 5	An extrapolated map for navigation in an ocean wandering seabird	147
Chapter 6	Carryover effects and movement decisions: a controlled experiment	183
Chapter 7	Future Directions	225

Chapter 1

General Introduction

Introduction	15
General methodologies	25
Chapter summary	32
References	34

Introduction

Navigation is fundamental to most animal movement (Gallistel, 1989) and the advent of controlled, goal-oriented movement marks a critical step in the evolution of mobility in early single-celled life (Bingman et al. 2003). Perhaps with the exception of planktonic animals, control over the direction of movement is critical for animals to exploit environmental resources, find a mate and, for many species, to migrate (Holland 2014). Navigation has been perhaps most succinctly defined as “the ability to organize behaviour adaptively to move from one place to another, appear[ing] early in the evolution of animals and occur[ring] in all mobile species” [AmE] (Wiener et al., 2010). For the purpose of this thesis, navigation is defined very simply as “controlled movement relative to a goal”. This definition presupposes a level of organisation that excludes some directed movement, for example, the diel vertical migration of many marine organisms and random-walk prey searching by predators, but includes plume following by albatrosses (Nevitt, Losekoot, & Weimerskirch, 2008), since the bird’s movement is actively adjusted relative to a target.

Many of the best examples of navigation come from birds, which are arguably nature’s greatest navigators. The vast annual migration of birds between the two hemispheres of earth (Alerstam, 1993), the pinpointing of remote oceanic islands by pelagic Procellariiformes (Bonadonna, Benhamou, and Jouventin 2003) and, on a local scale, the recovery of seeds from a cache by corvids (e.g. Wiltschko and Balda 1989) represent impressive navigational feats in a diverse array of ecological circumstances. Despite almost a century of intensive study, the processes facilitating goal-oriented movement are not fully understood. In particular, how animals are able to orient after displacement to unfamiliar areas without outward journey information or cues emanating directly from the goal, known as true navigation (Phillips et al. 2006), has become an enduring question in animal behaviour. However, control of goal-oriented movement within an animal’s familiar areas is also similarly enduring, and the precise nature of the mechanisms controlling goal-oriented movement within familiar areas are unknown (Guilford and Biro 2014; Armstrong

et al. 2013), though different processes do appear to govern navigation depending on a bird's familiarity with a navigational task (Biro et al. 2007). It may be that navigational mechanisms vary hierarchically depending on scale, varying by species depending on its phylogeny and migration ecology (Bingman and MacDougall-Shackleton 2017), but there is little extant knowledge about the interplay between different 'types' (Griffin, 1952) of navigation.

While this thesis concerns itself with the navigation of birds, the overwhelming majority of mobile organisms will routinely depend on goal-oriented movement to perform ecological tasks, and a wide variety of taxa are known to be capable of true navigation; from bats and eels to lobsters (Holland 2007; Boles and Lohmann 2001; Naisbett-Jones et al. 2017; Pittman et al. 2014). While of course the mechanistic solutions for the task of navigation that have evolved will vary depending on the phylogenetic inertia, ecology and anatomical constraints of any given taxon (Wiener et al., 2010), the conceptual problems that different kinds of goal-orientated movement represent, and that are discussed in this thesis, are each likely to apply to large subsets of, if not all, animals (Guilford and Taylor 2014).

The phenomenon of true navigation is not easily explained by stimulus-response (SR) learning (Jacobs, 2003, 2012), and thus its mechanistic basis has perplexed researchers for many decades. In behavioural research, the study of true navigation has focussed either experimentally on its sensory basis (e.g. Gagliardo 2013; Phillips and Jorge 2014) or conceptually on how learning of environmental cues could facilitate the phenomenon (e.g. Benhamou 2003; Wallraff 2000). Fundamental to much of the current theory for how birds might navigate is the idea of an extrapolated map (Wallraff 2000, 2003, 2015), an inconsistently defined concept but is summed up by – an ability of a bird to learn the spatial operation of cues within a familiar area, and then make use of spatial information in these cues beyond that area. The nature or even existence of such 'maps' remains unknown, and true navigational ability seems to vary depending on species (e.g. Thorup et al. 2007; Kishkinev et al. 2016) and age (Perdeck, 1958, 1967).

A (very) brief history of behavioural experiments on animal navigation

While theories for how the homing pigeon, *Columba livia*, might navigate were postulated in the late 19th Century (e.g. Darwin 1873; Vigui r 1882 in Wiltschko and Wiltschko 2003), the rigorous study of bird navigation began in earnest in the 1950s with Griffin (1952) being the first to recognise navigation from a previously unvisited site as perhaps requiring a distinct mechanism (Holland 2014). Matthews' (1953) releases of Manx shearwaters, *Puffinus puffinus*, from Cambridge University, were among the earliest studies to systematically record the homeward orientation of birds after release from a site unequivocally beyond their familiar area. Around the same time, Perdeck (1958) also showed with ringing recoveries that adult birds could interpret their direction from home following displacement. By displacing ringed juvenile and adult starlings, *Sturnus vulgaris*, at the start of migration, Perdeck (1958) showed that adult birds were able to compensate for displacement, but that juvenile birds maintained their innate migratory orientation. Birds could thus determine the direction to a goal following displacement, but not innately.

The finding that starlings and homing pigeons, released with phase-shifted endogenous clocks (known as clock-shifting) had deflected homeward orientation related to the movement of the sun's azimuth resulted in the discovery of the time-compensated sun-compass (Kramer, 1961; Kramer, Pratt, & St. Paul, 1957; Schmidt-Koenig, 1958, 1960, 1961). Its use is now known to be central to navigation across a broad range of diurnal taxa (Able & Dillon, 1977; Dacke, el Jundi, Smolka, Byrne, & Baird, 2014; Schmidt-Koenig, 1960). Deflected orientation of clock-shifted birds not only demonstrated use of the sun-compass, but also that birds must be using geocentric cues to navigate (but see Guilford and Taylor 2014), and that such cues must not directly emanate from the goal (Wiltschko and Wiltschko 2003). This led to Kramer's map-and-compass hypothesis (Kramer 1953; Kramer et al. 1957) which asserted that navigation from unfamiliar areas by birds was the combination of modularly separate map and compass mechanisms. These

early experiments birthed the conceptual framework for the study of navigation in modern ethology and the map and compass model has received little contestation since.

Releasing pigeons from beyond their familiar area after sensory manipulation (e.g. Keeton, Kreithen, and Hermayer 1977; Benvenuti and Gagliardo 1996; Benvenuti and Wallraff 1985) has become one of two central experimental paradigms in the search for the mechanistic basis of not only birds' compass sense, but their position-finding ability too (the map sense). The second, the Emlen funnel (or "orientation cage", Emlen & Emlen, 1966), used for testing the migratory orientation of songbirds, works similarly since the directional programming of a bird can be measured in an ink-lined (or more recently, thermal paper, Bianco et al., 2016) inverted cone. Confining nocturnally migrating birds in Emlen funnels during periods of night-time migratory restlessness, called *Zugunruhe*, either *in situ* or after displacement showed that a directional preference could be measured (Emlen & Emlen, 1966). Emlen funnel experiments thus permitted the extension of studies of birds' map and compass senses to a wider range of species and allowed manipulation of putative celestial and magnetic cues (Emlen, 1975a), resulting in the discovery of compasses based on both celestial cues (Emlen, 1975b) and the magnetic field of earth (Wiltschko and Wiltschko 1976), the latter of which is probably based on magnetic inclination sensed by a light-dependent radical pair process in the visual system (Wiltschko, Wiltschko, & Ritz, 2011). The paradigm has more recently been used to test map cues by measuring the Emlen funnel response of reed warblers, *Acrocephalus scirpaceus*, inside apparatus that simulated a displacement by recreating the geomagnetic properties of a site 1000 km east of the test site (a 'virtual' magnetic displacement, Chernetsov, Pakhomov, Heyers, & Mouritsen, 2015).

In 1971, Papi and colleagues discovered, remarkably, that pigeons deprived of their olfactory sense by nerve section were unable to home from an unfamiliar site but that birds with intact olfactory nerves returned normally (Papi, Fiore, Fiaschi, & Benvenuti, 1971). The olfactory navigation hypothesis has subsequently become the most compelling explanation for the sensory

basis by which birds determine the direction of displacement to unfamiliar sites without outward journey information, but is still hotly debated (see Wallraff 2015; Phillips and Jorge 2014; Gagliardo 2013). The magnetic field of earth, known to provide compass information for night-time migrating songbirds (Wiltschko and Wiltschko 1972), might also provide positional information but has proved much more difficult to tie down (Komolkin et al. 2017; Wiltschko and Wiltschko 2013) though some virtual magnetic displacements have suggested a role of magnetic map-type information in the migratory orientation of songbirds (Chernetsov et al., 2015). A recent paper by Chernetsov et al. (2017) has provided evidence that the apparent ability of songbirds to correct for virtual magnetic displacement longitudinally might be facilitated by birds attending to systematic east-west changes in magnetic declination (the angle made between true and magnetic north) .

The commonality of the olfactory navigation hypothesis and the magnetic map hypothesis is that both are theorised to provide position information beyond an animals experience by having predictable properties over space that are stable in time allowing an animal to use knowledge of the cues from its familiar area to infer its position after displacement to an unfamiliar site. There are several geomagnetic field elements that vary systematically with location and might be used in hypothesised magnetic map. The most frequently implicated is magnetic intensity, which is putatively sensed by magnetically sensitive cells at the base of the bird's bill and relayed to the brain via the trigeminal ganglion (Wiltschko & Wiltschko, 2013), though declination (the angle between true north determined by the rotation of the stars and magnetic north determined by inclination) and inclination could also putatively provide positional cues (Lohmann, Lohmann, & Putman, 2007). By learning how gradients in the geomagnetic field operate, a bird would be able to extrapolate its position beyond its experience.

While still based on putative environmental gradients, the most frequently discussed version of olfactory navigation hypothesis is thought to work slightly differently (though there are various

interpretations: Able, 2001; Benhamou 2003, see Chapter 5). Wallraff (1990) suggested that ratios of volatile molecules in the atmosphere might vary systematically over large distances and as such, birds might, in a learning phase, attend to the direction of the wind that brings different compositions of odours to the loft and thus infer their direction of displacement by comparing the odour at the release site with those remembered (Gagliardo, 2013).

Few other theories for how animals might gain positional information in a map sense have been hypothesised and those that have, such as large-scale variation in infrasound (Kreithen & Quine, 1979) and spatial variation in the strength of earth's gravitational field (Blaser et al., 2014; Dornfeldt, 1991), have failed to find more than circumstantial support (Hagstrum, 2000; Quine, 1982). Complete navigation based on the timing and movement of celestial cues (the sun-arc hypothesis) was advocated early on by Matthews (1953b, see Chapter 2), but was considered unviable because of the high level of precision that an endogenous clock would need to keep in order to provide useful positional information by comparing the time that the sun reached its zenith with the time remembered at home, and additionally a lack of evidence for animals attending to the height (in addition to the azimuthal position) of celestial objects (Baker, 1978). In the absence of other putative map-type cues, considerable debate has arisen between advocates of the olfactory navigation hypothesis and the magnetic map hypothesis, though some authors have proposed that both might operate facilitating navigation at different scales (Bingman & Cheng, 2005).

The neurological basis for animal navigation

This thesis particularly focuses on behavioural mechanisms underpinning navigation, though animal navigation is undoubtedly a cognitive phenomenon and thus it is important to make reference to its neurological basis. Neurobiological studies, particularly of the brains of mammals, have investigated the cognitive basis for the emergent navigational phenomena that are difficult to explain by primitive stimulus-response (SR) or sensory guidance processes (Best & White,

1991), such as the computation of novel short-cuts (Thinus-Blanc 1987; O'Keefe and Nadel 1978). The conceptualisation (Tolman, 1948) of a cognitive map, a neurological structure with Euclidean properties, provided one mechanism that might facilitate such computations. The discovery that 'place cells' in the hippocampus fired when rats were in different locations in a way that was geometrically isomorphic to an animal's position (O'Keefe and Dostrovsky 1971), led to an enduring cognitive model for how mammals might navigate (O'Keefe and Nadel 1978). The cognitive map has received much criticism, in part due to diverse and inconsistent use of the term (e.g. Gallistel 1989, who interpreted the use of any geometric relationship in the environment as evidence that all animals orient 'in a larger spatial framework' and thus must be in possession of a 'cognitive map' [Bennett, 1996]). Now the term is used primarily to refer to the O'Keefe and Nadel (1978) model, though this has also been met with scepticism. Bennett (1996) argued that novel short-cuts had not, at the time, been demonstrated in any animal satisfactorily since previous experiments could be explained by simpler cognitive processes such as orientation towards tall landmarks associated with targets, recognised by associative generalisation (e.g. in honey bees, *Apis mellifera*, Gould, 1986) or short-cutting by dead reckoning. Subsequent evidence for short-cutting has remained mixed (Grieves & Dudchenko, 2013; Menzel, Geiger, Joerges, Mu, & Ller, 1998), the principle difficulty perhaps being that in fact primitive SR behaviours are difficult to rule out experimentally, and near-ubiquitous in animal behaviour. Nonetheless research of the cognitive map, while not considered by all to have been convincingly linked to behaviour, has become a massive discipline.

Despite the conceptual leap that cognitive map represents for learning a familiar area representation of space, there is no agreed hypothetical framework for navigation that links learning, neuro-anatomy and effecting goal oriented movement from beyond the familiar area of an animal. In O'Keefe and Nadel's (1978) proposed neurological model for mammalian navigation, learning sensory cues associated with place is dependent initially on path integration: place cells receive inputs from an animal's egocentrically derived movement through path

integration (reviewed by McNaughton et al. 2006). This is by reference to allothetic (geometric relationships and/or compasses) cues (i.e. the ‘navigational system’), which allow sensory information from stimuli in the environment to excite only a particular place cell, thus associating an animal’s environmental location in space with its neurological isomorph. Therefore, novel short-cuts can be computed neurologically before being effected, and after the association with environmental stimuli, this can be done without outward journey information. The model, however, does not explain true navigation since there is no path integration stage when an animal is displaced to a novel location. ‘Parallel map theory’ has attempted to bridge this gap by suggesting that the cognitive map might additionally comprise a second laminar of hippocampal cells that might encode (and predict) environmental gradients on a larger, but coarser scale (Jacobs, 2003, 2012) and thus might facilitate extrapolation. That such a mechanism might also apply to birds is supported circumstantially by evidence of homology in hippocampal function of rats and birds (Healy et al., 1996), and the description in 2006 of place-like and grid-like cells in the hippocampi of homing pigeons (Bingman and Sharp 2006). However, hippocampal function seems only to be involved in navigation in pigeons released in their familiar area (Bingman and MacDougall-Shackleton 2017) and thus, especially in birds, relatively little can be concretely inferred about the neurological basis of true navigation.

The anatomical basis of the cognitive map is somewhat peripheral to the subject of this thesis, but the cognitive map of O’Keefe and Nadel (1978) and its extensions (Jacobs, 2003) provides a useful conceptual framework for considering whether or not the examples of goal-oriented movement that we will explore fit within the extant conceptualisations of how spatial information might be learned, encoded and made use of in a bird’s brain. Just as novel short-cuts might provide behavioural evidence of a cognitive map *sensu* O’Keefe and Nadel (1978), detailed manifestations of birds’ ability to home from beyond their familiar area might provide evidence for the cognitive processes underpinning true navigation. For example, understanding whether a bird can resolve where it is in space, or whether true navigation instead results from a bird’s

ability to (more simply) resolve its most likely direction of displacement imply fundamentally different cognitive properties for any mechanism facilitating true navigation. Furthermore, like short-cutting in an animal's familiar area (Bennett, 1996), whether extant research demonstrates adequately that any behaviours in true navigation cannot be explained by SR learning is questioned by some authors (Guilford and Burt 2017). We thus return loosely to the cognitive map in Chapter 5, but key to introducing this thesis is the idea that behaviour can imply cognitive processes and by extension inform the search for neurological apparatus that might underpin it.

Procellariiform seabirds as a model for avian navigation

Procellariiform seabirds are probably the most wide-ranging avian navigators: many are long-distance, trans-equatorial migrants and even whilst breeding, when they are central place foragers, undertake routine foraging trips of many thousands of kilometres, relocating their island-based colonies after many days at sea (Hamer et al., 2001). There has been long-standing interest in the processes underpinning seabird navigation (e.g. Lack and Lockley 1938; Matthews 1953; Massa et al. 1991). The advent of miniature tracking technology has brought about a revolution in our understanding of pelagic seabirds (Guilford et al. 2011). Ecological questions have thus far been the focus of seabird tracking work. For example, we now know that Manx shearwaters from at least five breeding colonies in the British Isles, separated by up to 650 km, routinely make use of the same foraging areas (Dean et al., 2015); that pairs coordinate their chick-provisioning behaviour (Shoji, Aris-Brosou, Fayet, et al., 2015; Tyson et al., 2017); and we can record in precise detail the vast foraging trips that albatrosses make across the Southern Ocean in search of pelagic resources (Weimerskirch et al., 2002).

During the 20th century displacement experiments were carried out with various seabird species; experiments initially investigated homing ability (Lack and Lockley 1938; Matthews 1953), but later began to manipulate putative navigational information by placement of small rare earth magnets on the heads of Cory's shearwaters, *Calonectris borealis*, (Massa et al., 1991). Similar

experiments on wandering albatross, *Diomedea exulans*, and waved albatross, *Phoebastria irrorata*, were repeated soon after GPS tracking became available (Bonadonna et al., 2005; Mouritsen, Huyvaert, Frost, & Anderson, 2003). Procellariiform seabirds were already known to be in possession of enlarged olfactory bulbs (Bang & Cobb, 1968) and their sensitivity to low concentrations of dimethyl sulphide (a volatile chemical associated with regions of ocean with high productivity) led to the suggestion that, as in pigeons, olfaction might provide spatial information sufficient for navigation over open ocean (Nevitt & Bonadonna, 2005). The discovery that albatrosses and other Procellariiformes use olfactory plumes to locate resources at sea suggested that movement could be controlled in response to odour cues (Dell’Ariccia et al., 2014; Nevitt et al., 2008). The role of olfaction in seabird homing following displacement was finally tested in an impressive release experiment with Cory’s shearwaters, showing that olfaction probably also plays a crucial role in large-scale oceanic navigation unrelated to plume following (Gagliardo et al., 2013).

Seabirds might make excellent models for avian navigation. First, seabirds take well to GPS tracking and have high motivation to home following displacement, especially during incubation (Dell’Ariccia & Bonadonna, 2013; Gagliardo et al., 2013; Matthews, 1953a; Pollonara et al., 2015) but also during chick-rearing (Shiomi, Yoda, Katsumata, & Sato, 2012). Unlike pigeons, results cannot be biased by the training schedule or breeding stock – the selection for rather particular aspects of homing in pigeons might play a small role in explaining some of the problems encountered in generalising discoveries even across different research groups working on pigeons (e.g. Wiltschko and Wiltschko 1998). Furthermore, seabirds are arguably better navigators than homing pigeons. The rate of successful homing in pigeons drops off significantly as the displacement distance increases (Wallraff, 1990). On the other hand, Matthews’ (1953) releases of shearwaters show that this might not be so extremely the case with birds that naturally wander many thousands of kilometres.

Perhaps the most important attribute of seabirds for making them targets for the study of avian navigation is the sheer scale that seabirds routinely navigate. Many Procellariiformes are known to engage in routine foraging trips of many thousands of kilometres. Crucial is that over these different scales, different processes might govern goal-oriented movement (Bingman and MacDougall-Shackleton 2017). A shearwater navigating back to its island colony from a foraging trip a thousand kilometres away, thus, might in the same journey traverse two or three different mechanistic scales. Taken together with the predictable advent of visual information as seabirds reach the coast (determined primarily by the spherical geometry of earth and the elevation of coastal topography), there are key, discrete, transition points in seabird navigation that it might be possible to elucidate. By analysing birds' orientation with respect to these, it might be possible to infer much about the cognitive processes underpinning long-distance movement in Procellariiform seabirds. Thus, this thesis aims to build on extant knowledge of seabird navigation by developing experimental protocols and tools for its investigation.

General methodologies

In this thesis I have worked on two species: the Manx shearwater, *Puffinus puffinus* and the Scopoli's shearwater, *Calonectris diomedea*. These two members of the family Procellariidae are typical Procellariiformes: they are long-lived, monogamous, long-distance migrants laying a single egg annually (Brooke, 1990; Hamer et al., 2001), and, during breeding, undergo long foraging trips, sometimes many hundreds of kilometres from the colony (Guilford et al., 2008). Once adult, they tend to be faithful to single nest site, allowing for multi-year tracking studies (Guilford et al., 2009).

Manx shearwater

The Manx shearwaters is a 400-gram Procellariid, which is near-endemic to the British Isles with the majority of the world's population residing on two British colonies alone (Brooke, 1990). Manx shearwaters are plunge divers, normally foraging within the first 10 m (average 7 m) of the

water column, but occasionally diving to depths of up to ~50 m (Shoji et al., 2015) to catch small, pelagic fish and cephalopods. Unlike many other shearwaters, Manx shearwaters do not follow boats and appear not to be attracted to dimethyl sulphide at sea (Dell’Ariccia et al., 2014). They undergo long migrations, travelling south down the east Atlantic before crossing to the west Atlantic in equatorial waters and spending the winter on the Patagonian shelf, off Argentina (Brooke 1990; Guilford et al. 2009). During the breeding season, they are central place foragers; routinely foraging distances in the order of hundreds to thousands of kilometres from the colony in the open sea. The Manx shearwater data used in this thesis were collected from five island colonies widely sampling the distribution of the species. These were Skomer Island (Wales); Rum (Scotland); Copeland (Northern Island); Lundy (England) and Skokholm (Wales).

Scopoli’s shearwater

The Scopoli’s shearwater is a large, 650-gram Procellariid (Navarro & González-Solís, 2007). Recently split from its congener, the Cory’s shearwater, *Calonectris borealis*, it is an endemic breeding species to the Mediterranean Sea with colonies on many of the Mediterranean Islands, the largest of which being located on Zembra Island, Tunisia (Defos du Rau et al., 2012). As members of the genus *Calonectris*, Scopoli’s shearwaters are primarily surface feeders, feeding on small fish and cephalopod molluscs, which are foraged upon both during the day and nocturnally during moonlit nights (Rubolini et al., 2015). Like Manx shearwaters, Scopoli’s shearwaters undergo large trans-equatorial migrations, spending the winter in the south Atlantic, frequently in the Benguela Current off the coast of Namibia and South Africa. However, some populations may be partial migrants and migration is less stereotyped than in Manx shearwaters (Müller et al., 2014). *Calonectris* shearwaters have become the principal model for studies of olfactory navigation in seabirds and have provided some of the first insights that, as in pigeons, olfaction probably plays an important role in homing (Gagliardo et al., 2013; Pollonara et al., 2015).

GPS and geolocator tracking

Over the past 20 years, miniaturisation of Global Positioning Systems technology and batteries has led to a revolution in the way that mobile animals, especially birds, can be studied (Guilford et al. 2011). For the work relating to this thesis, I have used a single model of small commercially available GPS, which is then adapted for use on seabirds (Figure 1.1). Mobile Action I-gotU gt-120 GPS devices with these adaptations have become widely used on seabirds (e.g. Guilford et al., 2008; Shoji et al., 2015). Their small size, low price, and user-friendly computer interface makes them the go-to choice for seabird tracking work. There are, however, a few disadvantages. The first is that the maximum temporal resolution for recording with these devices is 1 second. The second is that data appear to be filtered (location error as deviation from a straight path is much smaller than location error in a stationary device; very slow movement results in aggregated location estimates). The parameters of the filter are not available to the user. With 1-minute resolution (the maximum used in this thesis), the error is much smaller than the distance moved by the bird and thus for measuring orientation of shearwaters flying at 50 kmh⁻¹, this does not present a problem.

The second type of tracking device used in this thesis are geolocators (Figure 1.1), specifically designed for seabird use, made by two companies: Biotrack and Migrate Technology. These are miniature (2-gram) archival loggers that record light intensity and saltwater immersion of the devices allowing a bird's position to be inferred by analysing the times of sunrise and sunset and its behaviour to be inferred from patterns of saltwater immersion (Fayet et al., 2016; Shoji et al., 2015) which might indicate whether a bird is resting on the water, in flight or foraging.

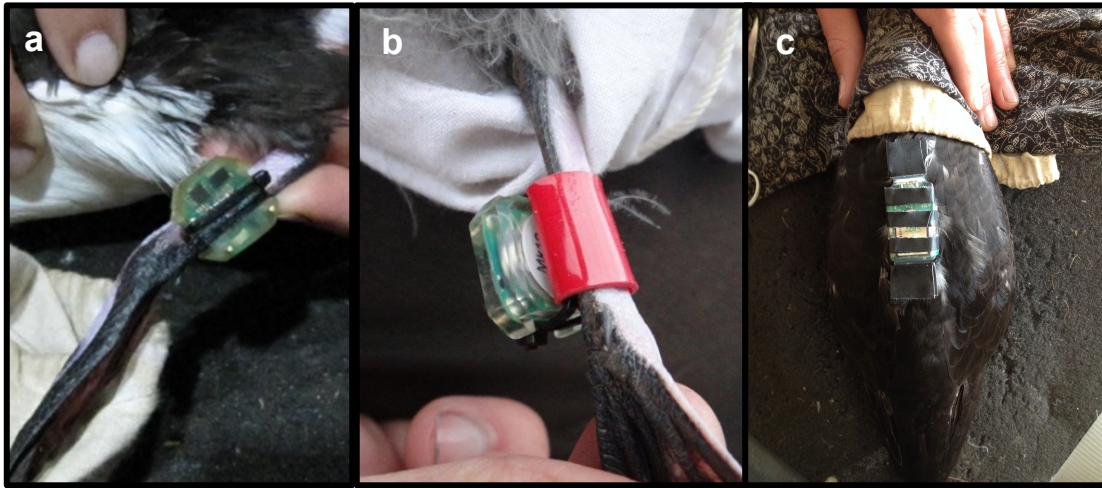


Figure 1.1. a) A Biotrack Mk19 geolocator, similar to those used in this thesis, deployed on the leg of a Manx shearwaters showing the pins which record a voltage and thus detect saltwater immersion. Credit: H. Kirk. b) The attachment to the leg of a geolocator via an elliptical plastic leg ring. Credit: N. McKee. c) Mobile Action I-gotU gt-120 GPS logger, rehoused in heatshrink plastic and deployed on a Manx shearwater using thin strips of Tesa marine tape laid underneath small bunches of contour feathers dorsally on the birds back. Credit: O. Padgett.

Track analysis

GPS data and GLS immersion data contain signals of at-sea behaviour inherent in the time-series datasets that they produce (Freeman et al., 2010). Thus, various analytical techniques have been developed to recognise patterns in these data streams that can help attribute, quantify and map at-sea behaviour. Many of the methods employed to recognise at-sea behaviour assume that a bird operates in a time-series of discrete behavioural states (though some do not e.g. Fauchald and Tveraa 2003), and that each state is characterised by its own probability distribution of speeds and turning angles (emissions), which can be estimated at each time point in GPS data. Several clustering methods have been borrowed from Machine Learning and implemented previously with great success. These can be relatively simple Gaussian mixture models (Freeman et al., 2010), where the most likely mixture of n states is calculated based on the frequency distribution of emissions for the whole dataset and then, treating each datum in the time-series dataset as

independent, calculating its probability of belonging to each of the identified states. More complicated, Bayesian methods such as hidden Markov models (HMMs) have also been used successfully in seabirds (e.g. Boyd et al., 2014; Fayet et al., 2015; Oppel et al., 2015), pigeons (Roberts et al. 2004; Guilford et al. 2004) and bluefin tuna, *Thunnus maccoyii* (Patterson, Basson, Bravington, & Gunn, 2009). HMMs account for the temporal non-independence of behaviour by calculating a probability of belonging to a state, say for a datum at time t that is not only influenced by the emission measured at that datum (speed and turning angle), but by the state assigned for the data at $t - 1$ and $t + 1$. This is encoded by an informative prior (a transition matrix representing the state-switching probabilities), which can be determined either by a supervised approach or by an algorithmic maximum likelihood approach. Validation of these analytical techniques can be achieved by using a second data stream from a subset of birds that have carried time depth recorders in co-deployment (Dean et al., 2012). This way the proportion of dives that fall within periods of time identified in the foraging state can be used to give an indication of the technique's accuracy.

Saltwater immersion data present a more difficult problem, since very low and very high scores are massively over-represented (Dean et al., 2012). At its simplest, the series of saltwater immersion records while a shearwater is resting on the water will be characterised by many consecutive 'wet' detection events, with the reverse true for directed flight. One option is to impose threshold in the number of wet events recorded for each ten-minute interval, which can correspond to resting, foraging and directed flight. Even simple methods such as this have been previously shown to result in behavioural classifications with biological relevance (Shoji et al. 2015). In addition to classifying behaviour from GPS data, HMMs have been used with some success to classify behaviours in saltwater immersion data streams (Dean et al., 2012; Fayet et al., 2016), but such methods are difficult to implement on non-normal data and thus far there has been no comparative analysis that indicates that the success of these methods is greater than less computationally intensive threshold methods. One way of negating these problems is to identify

breakpoints in the cumulative rate of immersion events (or some derived metric) over time (Shoji et al. 2015). At a scale of hours or greater, behaviours tend to resolve linearly in this fashion and so well-trodden line segmentation algorithms or breakpoint analyses (such as piecewise linear regression) can be used to identify contiguous units of behaviour.

For the purpose of the analysis in this thesis, an important analytical technique has been to identify decision points that correspond to when a free-ranging seabird begins homing after a foraging trip (or after a displacement if homing does not commence immediately). One way might be to select sections of GPS track based on its orientation for analysis. However, defining behaviour as homing based on its orientation runs the risk of missing poorly oriented homing behaviour (after experimental manipulation, for example) and would result in short periods of ‘homing behaviour’ during tortuous foraging flight. Techniques that identify breakpoints in behaviour that are not directly related to orientation *per se* have been developed to split animal trajectories into behaviourally consistent segments (Thiebault & Tremblay, 2013) based on small changes in the consistency of speed and turning angle over consecutive GPS fixes. There is much mathematical literature on break point algorithms that goes far beyond the scope of this thesis. However, one algorithm, the Douglas-Peucker algorithm, has been implemented successfully to identify changes in remote behaviour in gannets and is easy to implement (Thiebault & Tremblay, 2013). In this thesis, I modify techniques based on the Douglas-Peucker algorithm to suit the purpose of identifying goal-oriented movement. Where practical, I have then assessed these ‘decision points’ using randomisations to see whether they correspond to changes in behaviour measured by other metrics.

Aims of this thesis

There are three broad aims to this thesis. The first is to explore whether key experiments that have resulted in profound discoveries in pigeon navigation can be successfully replicated on seabirds, and inform about the applicability of those navigational discoveries to birds navigating over an apparently featureless environment. A second aim is to see whether, by identifying oriented flight in free-ranging seabird telemetry, natural, ecologically relevant tracks can be relevant to the study of navigation. A third aim is to apply these quantitative techniques to year-round telemetry data, and in doing so uncover some of the ecological drivers of large-scale movement in seabirds.

The application of decades of pigeon experiments to seabirds has already begun with research into the homing capacity of seabirds that have been denied their sense of smell. However, arguably the most permeating experiment in pigeon navigation has been the clock-shift and so this is where the thesis begins, by attempting what seems, surprisingly, to be the first clock-shift homing experiment of any wild bird (with the exception of an attempted ex-situ clock-shift (in a Cambridge University lab) by Matthews 1955). I then turn to olfactory navigation, attempting to eliminate some of the potential problems with the hypothesis as it applies to seabirds by conducting an experiment on the effects of anosmia in free ranging Scopoli's shearwaters. A second chapter on the olfactory navigation hypothesis reports a pilot experiment recreating the false release site experiment from the literature. In Chapter 5, I then report the analysis of a large, extant GPS dataset of Manx shearwaters in an attempt to tackle some of the cognitive questions in true navigation.

A subsidiary theme of this thesis is in the analysis of seabird tracking data. In particular, identifying break-points in the resulting time-series data that relate to animal decision-making in tracking data might prove a powerful tool for the study of navigation. The ultimate aim in this regard is to decompose free-ranging animal movement into a series of goal-oriented units of behaviour and I hope that this thesis will go some way towards that achieving that, by making use

of break point methods for identifying the homeward leg of foraging trips. In Chapter 6, I have used similar methods to identify key stages in the annual cycle of Manx shearwaters carrying geolocators. While my final chapter focuses on ecological questions, rather than navigation, the linking theme is the analysis and mechanistic control of large-scale movement. Furthermore, I also hope that the analysis in Chapter 6 might highlight the ecological function of the navigational mechanisms that form the basis for this thesis, and exemplify the ubiquity of organised control of animal movement over scales ranging from centimetres to tens of thousands of kilometres.

Chapter summary

This thesis comprises five data chapters originally prepared as manuscripts for publication and adapted for the thesis. Each is a self-contained piece of work, containing its own introduction, materials and methods, results and discussion. Each has been co-authored by collaborators but in each case, the work is primarily my own. Chapter 2 is published in *Current Biology* and Chapter 3 is published in *Scientific Reports*.

Chapter 2 – *In-situ clock-shift reveals that the sun-compass contributes to orientation in a pelagic seabird*

Clock-shift experiments are the acid test of the time-compensated sun-compass, since animals must compensate for the sun's diurnal movement across the sky by reference to an endogenous clock. Clock-shift experiments also have the power to make the cognitive inference as to whether navigation is under the control of a map-and-compass. Here, I develop a methodology for the *in-situ* clock-shift of a wild bird for the first time and show that Manx shearwaters make use of a sun-compass during controlled movement back to the colony after displacement. Furthermore, I show that, as in pigeons, reduced clock-shift effects on orientation can be measured whilst shearwaters are navigating with familiar area information. Using the case of pelagic birds as an example, I develop a new theory (direction-accompanied landmarks) for why sun-compass information is apparently incorporated into birds' familiar area representation.

Chapter 3 – *Anosmia impairs homing but not foraging behaviour in shearwaters*

Induced anosmia from nasal irrigation with zinc sulphate solution has dramatic effects on birds' homing ability, both in the homing pigeon and in pelagic seabirds. However, whether in seabirds this is because of an absence of olfactory information or because of general effects of zinc sulphate remains unknown. In this chapter, I investigate the effect of anosmia on at-sea behaviour and navigation in free-ranging shearwaters. I find that while anosmic birds are able to forage successfully and behave normally at sea, they have impaired homing orientation.

Chapter 4 – *False release site experiments with wild seabirds: a pilot study*

Here I report a pilot experiment, which attempts to repeat the false release site experiment that was originally conducted on pigeons (Benvenuti & Wallraff, 1985) but using Scopoli's shearwaters. The false release site experiment aims to elicit an incorrect directional response from birds after displacement by providing navigationally relevant information from air from a second site located diametrically opposite the release site with respect to the home goal. Here, the experiment was unsuccessful, but nonetheless I found that there is an indication that orientation behaviour is affected by this controlled experimental approach and that such experiments might be worth conducting in the future.

Chapter 5 – *An extrapolated map for navigation in a pelagic seabird*

Because free-ranging animals have access to outward journey information, free-ranging animal tracking data are rarely used to study navigation. However, there is good evidence that even with outward journey information, directed movement is controlled by a map and compass (rather than path integration or beaconing). Here I explore the additional information that can be gained by studying birds that are auto-displaced thousands of kilometres. For the first time, I am able to measure where a bird thinks it is and thus provide novel insights into the cognitive mechanisms that underpin avian navigation.

Chapter 6 – *Carry over effects: a controlled experiment*

The final chapter explores some of the movement decisions of Manx shearwaters from an ecological angle, by examining the effect of a controlled reduction in the cost of chick rearing on subsequent phenology and at-sea behaviour. While not directly relevant to the underlying navigational mechanisms, the chapter examines ecological predictors of the large-scale movement that such processes underpin. The key finding supports previous studies that show that in Manx shearwaters, energetic relief does not result in measurable carry-over effects, and as a consequence we deduce that in this species, the timing of migration is not always under the control of a bird's condition.

Chapter 7 – *Future directions*

Here I summarise the key results of this thesis. I particularly pick out the findings from Chapters 2, 3 and 5 and discuss their implications on our understanding of avian navigation and suggest how these can inform future experiments. I suggest several key experiments that might be informative about the architecture of spatial cognition in seabirds, incorporating some suggestions for the development of analytical techniques.

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Chapter 2

In-situ clock-shift reveals that the sun-compass contributes to orientation in a pelagic seabird

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Abstract	49
Introduction	49
Materials and Methods	51
Results	56
Discussion	61
Acknowledgements	65
References	65
Supplementary Material	69

Abstract

Compass orientation is fundamental to most controlled animal movement, structuring spatial representation and underpinning long-distance navigation. The time-compensated sun-compass is thought to be central to the movement of diurnal animals, but its use by an actively navigating wild bird is yet to be shown. Using the first in-situ clock-shift of a wild bird, we show here that an oceanic navigator, the Manx shearwater, makes use of a time-compensated sun-compass during homeward guidance to the breeding colony after displacement. We applied clock-shift techniques at the nests of incubating birds before releasing them, equipped with GPS loggers, at distant, but likely familiar sites in a controlled experiment. Furthermore, we show that even once shearwaters approach their home colony where they are likely to make use of landmarks to guide them home attention is still paid to the sun-compass, demonstrating that compass control might play an important role in the processes underlying directed movement within the familiar area as well as during true navigation.

Introduction

Compass orientation is central to the control of animal movement from the scale of local food-caching movements around a familiar area in parids (Duff et al., 1998) and corvids (Balda & Wiltschko, 1991; Wiltschko & Balda, 1989) to the first-autumn vector navigation of songbirds embarking on long-distance migration (Holland, 2014; Mouritsen & Mouritsen, 2000; Perdeck, 1958). Compass orientation is at the heart of animal movement across these scales, but precisely how compass information is encoded and incorporated into movement decisions remains unknown. In diurnal organisms, the sun-compass is thought to dominate (Guilford & Taylor, 2014), and has been identified at least under laboratory manipulation in a broad range of taxa from butterflies to birds (Able & Dillon, 1977; Dacke et al., 2014; Guilford & Taylor, 2014; Mouritsen & Frost, 2002). In the study of diurnal birds, where the homing pigeon *Columba livia f. domestica* has been the main model, a time-compensated sun-compass (Schmidt-Koenig, 1961b) is central to the two-step map-and-compass process of navigation from unfamiliar places,

known as “true navigation”, as well as guiding movement via a representation of familiar area landmarks (Armstrong et al., 2013; Biro et al., 2007; Guilford & Biro, 2014; Wallraff et al., 1999). Nevertheless, despite its central position in animal movement, the importance of compass control remains unknown and untested in the movement of wild animals.

Clock-shifting is the acid test of sun-compass use (Guilford & Taylor, 2014). By phase-shifting an animal’s endogenous clock (typically by placing the animal in a light-proof room with exposure to a shifted photoperiod (Giunchi et al., 2003; Munro & Wiltschko, 1993; Schmidt-Koenig, 1960), sun-compass use can be demonstrated when the animal incorrectly consults the sun’s azimuthal position after experimental displacement (Schmidt-Koenig, 1958, 1960, 1961a). Since the sun’s azimuth moves clockwise over the course of a day, under a fast clock-shift, (when a bird’s subjective time is further forward than objective time), the qualitative expectation under sun-compass use is for the bird to show an anticlockwise deflection during oriented movement. For a slow-shifted bird, the reverse is true since the bird’s subjective time lags behind yielding a clockwise deflection. Thus, the predicted deflection in orientation after release from an unfamiliar site for a clock-shift given full sun-compass use is equal to the change in the sun’s azimuth between the objective time and the subjective experimental time (Gagliardo, 2005; Wiltschko et al., 1994).

The navigational challenges which must be overcome by ocean wandering seabirds requires them to be excellent navigators (Bonadonna, Benhamou, & Jouventin, 2003). A key question, therefore, is whether the same mechanisms that underpin terrestrial navigation are also employed for long-distance movements in environments that we assume to have far fewer visual features (Bonadonna et al., 2003). While some studies have investigated whether, as proposed in pigeons, seabirds might make use of olfaction during homing after displacement (Gagliardo et al., 2013; Padgett et al., 2017; Pollonara et al., 2015) no study has tested the compass component of seabird navigation; its mechanistic basis or the extent of its role in underpinning spatial control.

Consequently, how seabirds achieve such remarkable navigation is an enduring question in animal behaviour.

Here we tested for sun-compass orientation in a wild pelagic seabird by clock-shifting Manx shearwaters *Puffinus puffinus* in their natural environment. Manx shearwaters undertake long (one week) incubation stints during which a clock-shift can be applied (Figure 2.1), and incubating birds are highly motivated to home following displacement (Matthews, 1953a). After being clock-shifted while incubating their egg, birds were displaced to distant (but likely familiar) release sites, at sea, 29-48 km from their breeding colony, and their homing movements were tracked with miniature bird-borne GPS loggers. We measured the minute-by-minute deflection of clock-shifted birds' trajectories from the beeline home (instantaneous deflection) as they homed over sea following release in order to test whether the birds' compass orientation responded to manipulation of their endogenous clock. In a second analysis, we evaluated the role that the sun-compass plays as shearwaters get closer to the colony during homing, where many visual landmarks from the adjacent topographic landscape are available.

Materials and methods

Clock-shift Procedure

The study was carried out on Skomer Island, Wales (51°44' N, 5°19'W), probably the largest Manx shearwater breeding colony globally (Perrins et al., 2012), from mid-May to early July, and late May and early June 2015 and early June 2017 respectively. To clock-shift wild birds in situ, we monitored the attendance of incubating pairs of burrow-nesting Manx shearwater at the colony. Like other Procellariiformes, the two members of the pair will alternate incubation stints, which regularly last a week (Brooke, 1990). Birds that switched incubation duty 6 or 7 days before a release date were alternately assigned to a fast clock-shift, slow clock-shift or a control treatment. "Clock-shift mats" which comprised a 35 x 35 cm sheet of 3 mm thick commercial grade rubber with a Rolson 24 LED lamp fitted in its centre were placed over the entrance to each

burrow and held in place using metal pegs (Figure 2.1). Photoperiods were manipulated by turning these lights on and off as in Table 2.1, based on the sunrise and sunset times for the release date. Our “control” (zero shift) clock-shift birds underwent the same procedure but no shift in the photoperiod was imposed. The photoperiod was then maintained with the phase-shifted dawn and dusk for 5-7 days until release, sufficient for the desired clock-shift in homing pigeons (Schmidt-Koenig, 1960).

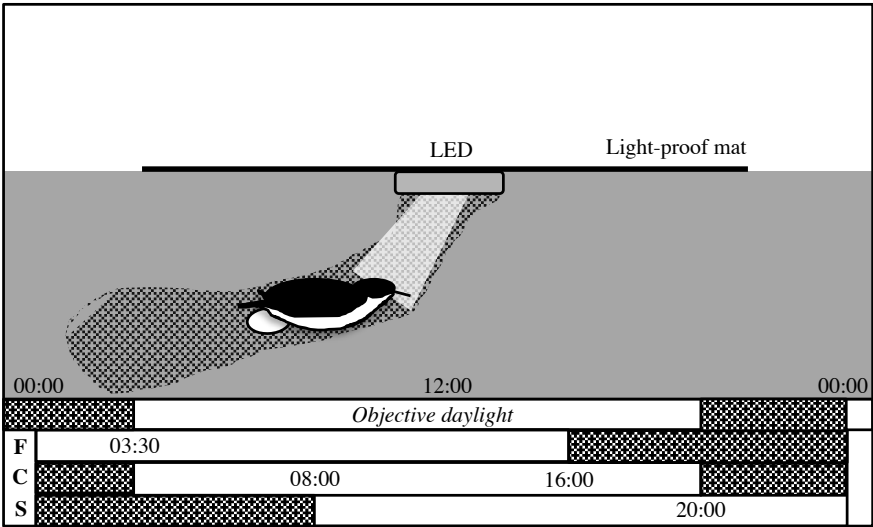


Figure 2.1. The *in-situ* clock-shift setup at the Manx shearwater’s burrow whilst it incubates its egg. Schematic shows the schedule of dark and light photoperiods over the 5-7 days during which the clock-shift was applied. The times shown in the figure are shifted ~30 mins (so that they fit within the arbitrary 24 h period between consecutive midnights). For actual times see text and Table 2.1.

Ethical statement

All work adheres to the ASAB/ABS guidelines for the Use of Animals in Research and was conducted after ethical approval by Natural Resources Wales, Skomer Island Advisory Committee and the University of Oxford’s Local Ethical Review Process. GPS deployments were approved by the British Trust for Ornithology Unconventional Methods Technical Panel (permit C/5311). Similar biologging techniques have been used on this colony since 2007 with no significant effect on reproductive success (Fayet et al., 2015).

Table 2.1. Table of objective and subjective photoperiods to which birds were subject before releases for the control, slow and fast levels of our clock-shift treatment. All times in GMT. (+1) indicates the next day.

Release date	Objective / control		Subjective slow		Subjective fast	
	sunrise	sunset	light on	light off	light on	light off
22nd May 2015	04:18	20:16	08:18	00:16 (+1)	00:18	16:16
11th June 2015	04:01	20:38	08:01	00:38 (+1)	00:01	16:38
9th July 2015	04:12	20:38	08:12	00:38 (+1)	00:12	16:38
29th May 2017	04:09	20:26	08:09	00:26 (+1)	00:09	16:26
4th June 2017	04:04	20:33	08:04	00:33 (+1)	00:04	16:33

Release

61 birds were released over six dates (Table 2.1). Before being transported to the release site birds were fitted with I-gotU GT-120 GPS loggers using strips of TESA 4651 marine tape (Guilford et al., 2008). GPS loggers were programmed to take fixes at 1-minute intervals. Birds were removed from their burrows while both the objective and the subjective time was ‘daytime’ for all subjects. It was ensured that birds did not see the sun’s disc during deployment and transportation. When birds were collected for GPS deployment, their eggs were moved to an incubator in which they were kept at ~37°C until dusk when they were returned to their burrows such that the adults did not arrive at an empty nest in the event that they were not caught upon landing at the colony.

Birds were translocated in individual or partitioned cardboard boxes and taken by boat at a bearing of ~225° southwest as far as possible (sea-state permitting). Releases were 30 – 49 km from the colony. Where possible, birds were released in trios with one fast-shift bird, one slow-shift bird and one control bird (Table S2.1, supplementary material). When released, birds were placed on a wooden board on the side of the boat and allowed to take off into the wind in their own time.

Retrieval

At dusk on the night following release birds were recaptured by hand upon arrival and their GPS devices retrieved before being placed back into their burrows and allowed to resume incubation or to join their partner to complete the incubation changeover if their partner had also returned on the same night.

Track Processing

We identified at-sea behaviour in our homing tracks by using a speed filter empirically derived by fitting a Gaussian mixture model as in other studies (Freeman et al., 2010) (for details, see Figure S2.3, supplementary material). Because our releases necessarily took place earlier in the day than birds would begin homing, we identified the point along the track after release where birds began directed flight towards the colony as the decision to start homing. We used a threshold of 8 minutes continuous flight within a 45° arc of the colony direction. Tracks were cut at the far end either when they reached 8 km from the colony, where much socialising occurs and movement orientation is no longer related to navigation, or at the end of nautical twilight after which time sun-compass use is unlikely (whichever came first). Track-segments with rafting behaviour (i.e. birds sitting on the water) were removed and analysis was therefore conducted on homeward oriented flight sections of track. Instantaneous deflection from the goal was calculated as in several other studies (Armstrong et al., 2013; Filannino et al., 2014) by computing the spherical angle between the current bearing made by the bird and that of the beeline home at each one minute interval along the track (Figure S2.4, supplementary material). We used instantaneous deflection since it is a measure fundamentally associated with compass orientation, unlike other metrics sometimes reported to analyse compass deflections ((virtual) vanishing bearings). A hypothetical bird that consults a deflected compass from a known location will set off at a bearing equal to the homeward direction summed with the induced deflection. If the bird rapidly updates its map, it will readjust its course to its new bearing from home plus its induced deflection. Therefore, a bird with high-resolution map updating would spiral homewards with a rate of change of course related to its map resolution and the imposed compass deflection. In this hypothetical scenario, instantaneous deflection will remain constant, whereas virtual vanishing bearing will decrease as the distance between the release site (where the bearing is measured from) and the bird increases, even for a bird consulting a consistently shifted compass with constant deflection.

Statistics

We used linear mixed models (LMM) to analyse the effect of our clock-shift treatment on the instantaneous deflection of birds as they homed to the colony. Since our data were structured in trios (individual releases) and across five dates that differed in weather, release trio and release date parameters were fitted as nested random (intercept only) effects such that model errors around the effect of treatment were independent. Because our clock-shift treatment was effectively a single treatment with three ordered levels, we fitted an LMM with clock-shift as a continuous predictor (-4, 0 or +4). As a conservative approach (since this tests only for a monotonic response to treatment), and to obtain estimates for each treatment, we also fitted an LMM with treatment level as a factor and conducted post-hoc pairwise tests with p-values adjusted with a Bonferroni correction for the two comparisons (control ~ fast; control ~ slow). For all LMMs, we obtained p-values by comparing a full model including treatment with its corresponding null, nested model with only random effects using a likelihood ratio (LR) test. Instantaneous deflections were highly concentrated and thus their circularity did not cause a violation of the assumptions for LMMs of normal residuals and homogenous variance in error. However, to ensure that circularity did not affect the outcome of our analysis, we performed an intuitive randomisation test that reflected the structure of our data to confirm that the LMM assumptions did not impact our results. To calculate our observed deflections (global response), we first normalised the instantaneous deflection for each release trio as a positive or negative deflection to control, and then normalised the mean of these deflections as a positive or negative deflection for each release date. We next randomised each bird's treatment 100,000 times and recalculated our global response and recorded the probability of the observed deflections or greater by chance, permitted in either direction (two tailed: Slow < Control < Fast; Fast < Control < Slow).

Treatment effect with distance to the colony

To assess whether the deflection induced by our clock-shift treatment changed as shearwaters came closer to the colony, we refitted our LMMs to 2.5 km binned distances from the colony from 8 km to 40 km and > 40 km. This allowed us to obtain an estimate of treatment effect for each bin but taking account of different sample sizes and data structure for different distances (for example, only birds from two releases had homing tracks within the farthest bins). We then used a linear regression to see if the magnitude of the effects varied with distance to the colony.

Analysis of secondary response variables

The effect of treatment on mean homing speed was tested using LMMs fitted with the same random effects as detailed above. A non-parametric Kruskal-Wallis test was employed to test the effect of treatment on the highly non-normal departure times (when birds first undertook a prolonged homing portion of flight).

All LMMs were fitted using the ‘lme4’ package (Bates et al., 2015) in R. Gaussian mixture models were fitted using the ‘Mixtools’ package (Benaglia et al., 2009).

Results

46 of the 61 birds released over five release dates (Table S2.1, supplementary material) returned to the island on the day of the release. The remaining 15 were recovered in subsequent days, two of which had returned to the colony on the day of the release but not been recaptured. Two birds returned after their GPS had fallen off. Analyses were therefore conducted on the flying portions (>2.32 ms⁻¹, Figure S2.3 supplementary material) of 48 birds’ 1-minute resolution GPS tracks (Figure 2.2) of birds under three levels of clock-shift treatment (+ 4 hours (fast shift, n = 20), - 4 hours (slow shift, n = 13) and 0 hours (artificial light, no shift, n = 15) released 29-48 km southwest of their home colony in the Irish sea. Raw deflections for birds are shown in Table S2.2, supplementary material. We used linear mixed models to test for the effect of treatment on instantaneous deflection during homing with random (intercept only) effects fitted to account for

the effect of release trio and release date. Likelihood ratio (LR) tests between the full model and a nested, null case of the model without treatment as a predictor were used to obtain p-values. Treating the clock-shift as a continuous predictor (-4, 0, +4) revealed a highly significant effect of treatment on birds' mean instantaneous deflection to the colony during active homing flight (LR test: $\chi^2 = 21.32$, d.f. = 1, $p < 0.00001$) with an estimated effect of $13.2 \pm 0.62^\circ$ per four hour difference in clock-shift schedule. Modelling instantaneous deflection as a response to treatment as a factor (rather than assuming a linear response of deflection to the direction of shift) yielded similar results ($\chi^2 = 21.40$, d.f. = 2, $p < 0.0001$) with largely symmetrical estimates for the effect of fast and slow treatment relative to controls (fast: $-14.3 \pm 4.8^\circ$; control: $+0.7 \pm 8.0^\circ$; slow: $+12.1 \pm 5.3^\circ$, Figure 2.3). The difference among treatments was upheld at the $\alpha = 0.05$ significance level for models run pairwise for control-fast and control-slow even after p-values were doubled as a Bonferroni correction for the two-way comparison, which conservatively (and in this case incorrectly) assumes that treatment expectations are independent (control – slow: $\chi^2 = 5.19$, d.f. = 1, Bonferroni $p = 0.04$; control-fast: $\chi^2 = 5.82$, d.f. = 1, Bonferroni $p = 0.03$). Release date explained a significant amount of variation in the instantaneous deflection to home (refitted as a fixed predictor and tested with LR test: $\chi^2 = 21.65$, d.f. = 2, $p < 0.0001$). While our concentrated directional data met the assumptions of LMMs, we nonetheless conducted a randomisation to test the probability of observing our result by chance without assuming anything about the residual distribution and autocorrelation in the data. We calculated each treatment's overall deflection by taking the mean difference of each fast and slow bird from the mean control for each date, and then calculating the overall mean. We then randomised the treatment identity of each bird 100,000 times and calculated to resulting overall mean deflections of treatments. We found that fewer than 0.1% of randomisations gave deflections that were equal to or greater than observed in either the F<C<S or the S<C<F directions (two-tailed $p < 0.001$). For visual comparison, tracks transformed such that they have the same start location and rotated such that the home bearing from the start is the same for each are shown in Figure 2.4.

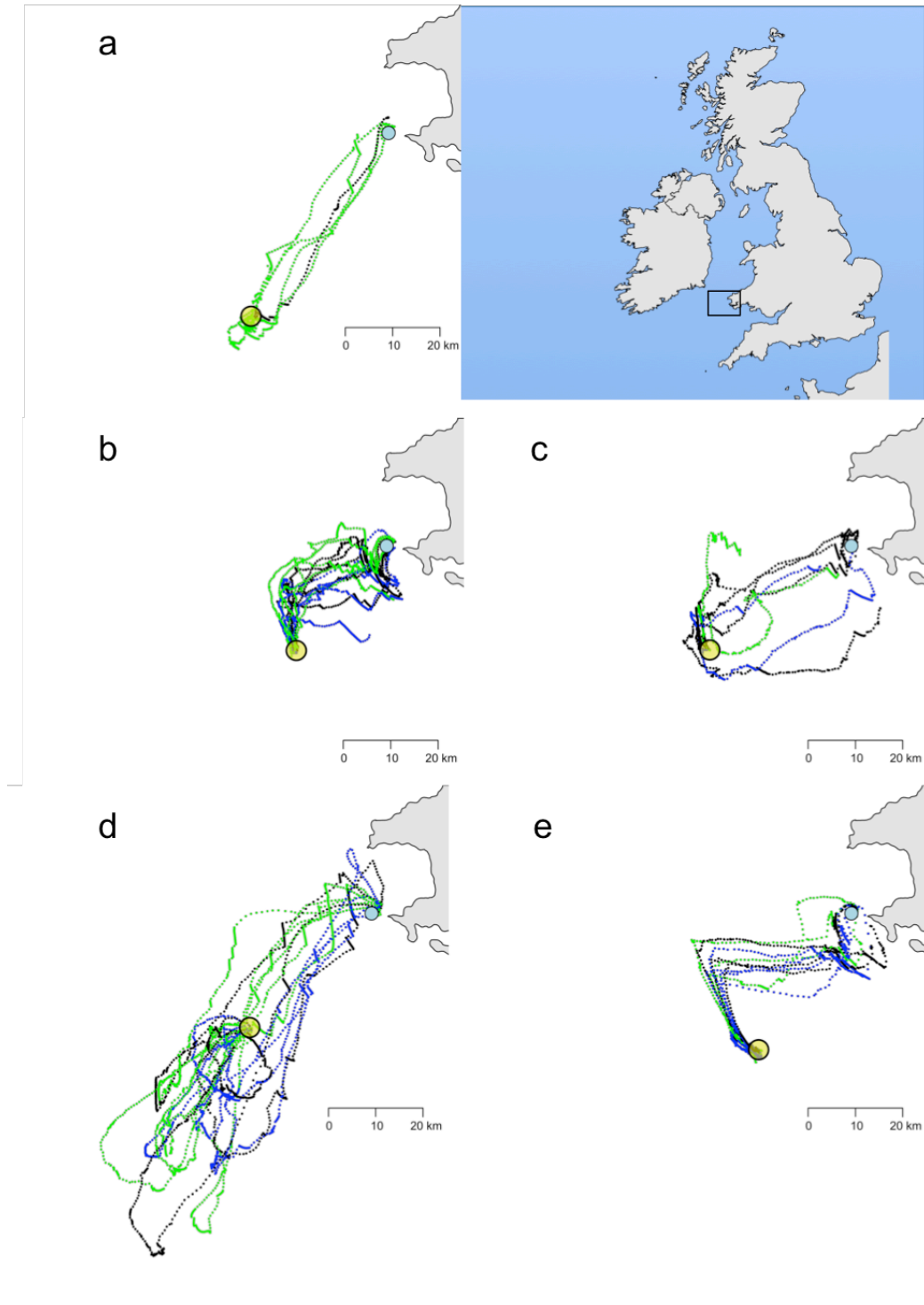


Figure 2.2. Figure shows individual releases in chronological order with control (black), fast (green) and slow (blue). 2015 (a-c) and 2017 (d-e). Release site for each is shown as yellow circle and Skomer Island as a blue circle demonstrating the considerable movement of birds between release and homeward oriented movement. Rafting behaviour can be seen where consecutive 1-minute fixes are close together forming solid lines. Tracks from all releases are shown together in Figure S2.1, supplementary material.

There was no significant difference in mean flight speed (LR test: $\chi^2 = 0.86$, d.f. = 2, $p = 0.65$) or time of departure towards the colony after release (Kruskal-Wallis $X^2=2.79$, d.f. = 2, $p = 0.25$) that might have indicated motivational differences among treatments. There was also no significant change in the magnitude of the modelled deflections for each 2.5 km bin as birds came closer to the colony (slow-control: $F = 0.17$, d.f. = 1,8, $p = 0.69$; fast-control: $F = 0.01$, d.f. = 1,8, $p = 0.93$). Measured deflections are shown as a function of distance to the colony in Figure 2.5.

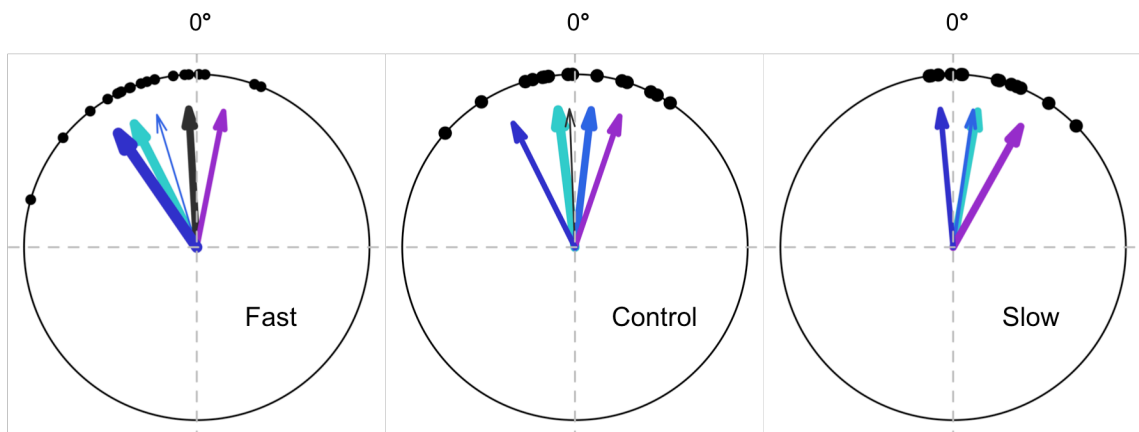


Figure 2.3. Circle plots showing the mean instantaneous deflection of each bird (on the circle perimeter) as it homed to the colony. The colony direction is 0°. Arrows show the second order means for each release date. Arrows representing the same release date are represented in the same colour for comparison. Arrow thickness is the sample size for that release (thickest arrow = 6; thinnest = 1). Measured deflections from the beeline are shown. Modelled deflections are in the main text.

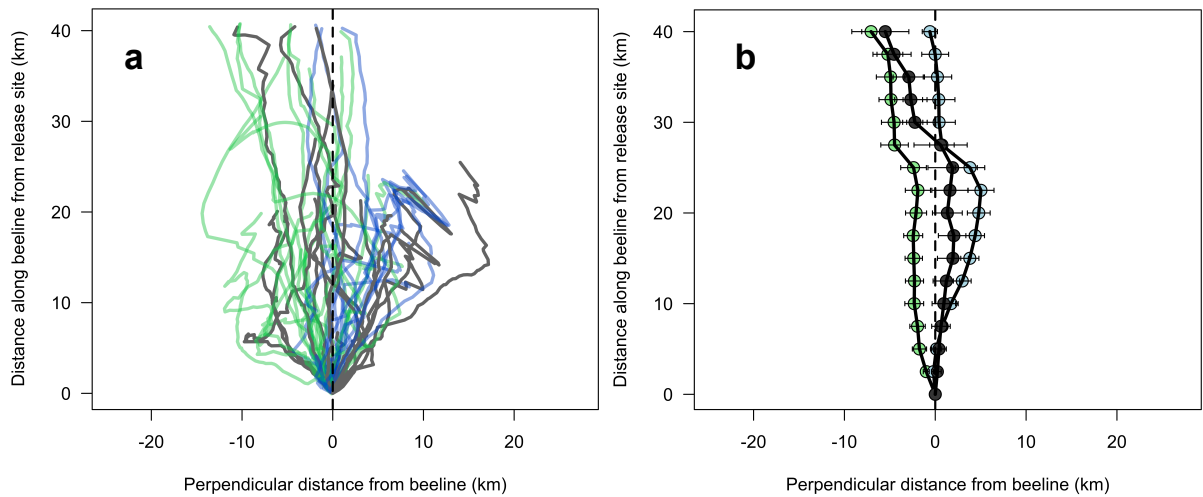


Figure 2.4. Slow (blue), fast (green) and control (dark grey) flight sections of tracks transformed from a North-South map coordinate system such that the start of directed homing flight is at $[0,0]$ and the colony location lies at $[0,n]$, where n differs among tracks depending on the distance to the colony when birds began directed homing flight. Consequently the Great Circle geodesic to the colony is at a bearing of 0° from $[0,0]$ and distances and relative angles along the paths are inherited from the birds' real UTM coordinates by the transformation allowing for a visual comparison of (a) all analysed tracks and (b) their mean perpendicular distance to the beeline for where the path crosses each 2 km interval parallel to the beeline from the original release site. Note that since tracks are normalised from the start of homing behaviour, distances to the colony are not comparable (hence also the differing length of tracks in (a)).

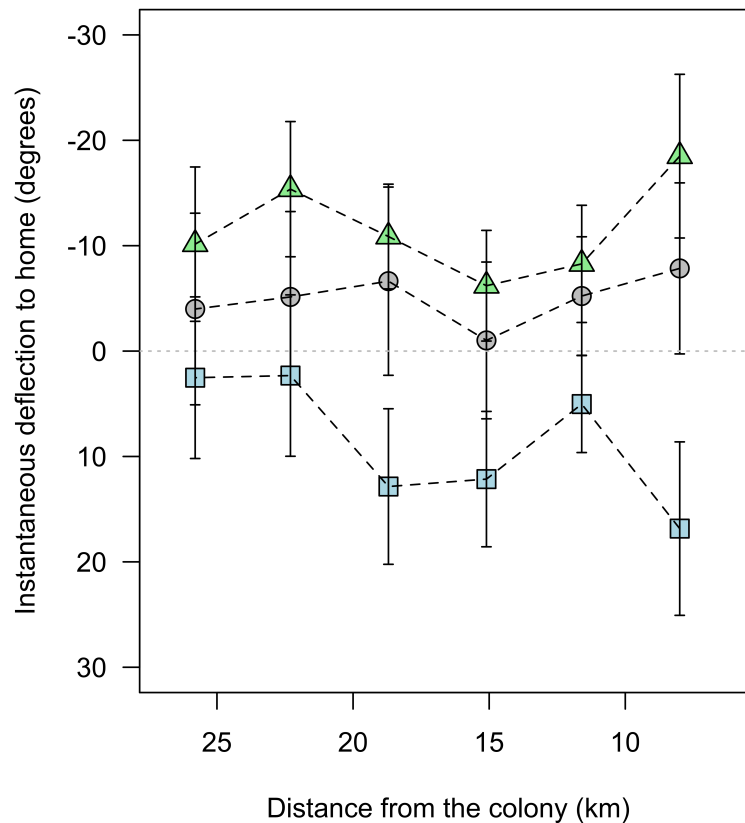


Figure 2.5. Instantaneous deflection as shearwaters got closer to the colony during directed homing flight for control (grey circles), fast (green triangles) and slow-shifted (blue squares) birds. Distance bins in which our full sample of birds undergo active homing flight are shown and for the range of distances where visual information is likely to be available. Means shown are calculated treatment means of each bird's mean deflection for that distance bin. The x and y -axes are reversed (they ascend downward (y) and to the right (x)) such that positive and negative deflections from the beeline are still clockwise and anti-clockwise respectively.

Discussion

The deflections in birds' orientation relative to the goal induced by the clock-shift indicate that Manx shearwaters incorporate guidance from a time-compensated sun-compass into their movement decisions as they home after release from distant but likely familiar areas. Our controlled experimental design, with a clock-shift in both directions, precludes the possibility that

the result obtained here was confounded by some aspect of the clock-shift treatment not related to a manipulation of birds' circadian rhythms. By using a mixed-modelling approach to identify differences among treatments in instantaneous deflection, we were able to disentangle directional bias across release dates from the effect of clock-shift on the compass orientation of birds homing from different starting locations. This revealed that release date strongly influenced the homing orientation of birds (most probably caused by wind direction or the intended rafting destination of birds before making landfall on the colony) but nevertheless that orientation was influenced consistently by the clock-shift treatment even accounting for different starting locations and thus different beeline routes home. We observed no differences in the timing of homing or the speed that birds flew during homeward oriented flight, which suggests that the observed deflections among treatments were not caused by differences in motivation that might have been unwittingly influenced by clock-shift. Navigationally, the observed deflections in this study are consistent only with sun-compass use. If birds were to use the sun's arc as a map (Matthews' Sun-Arc hypothesis (Matthews, 1953b)) instead of a compass, an idea discussed in much detail elsewhere (Baker, 1978), then clock-shifting would result in deflected homing orientations in the opposite direction to that observed in the current study. Under that hypothesis fast-shifted birds would perceive the later-than-expected sunset as displacement to the west (and thus should orient more clockwise in the current study), and show-shifted birds would perceive an earlier sunset as displacement to the east (and thus should orient more counter-clockwise). To our knowledge, these findings therefore represent the first successful attempt to clock-shift an animal in the wild and the first evidence for use of sun-compass orientation in a wild bird during active navigation; an experiment only previously attempted once, unsuccessfully, and without the advantage of GPS telemetry (Matthews, 1953a).

Pure sun-compass guidance is predicted to result in deflected orientation equivalent to the angular displacement of the sun's azimuth between the actual and subjective time of day at release (Figure S2.2, supplementary material) (Gagliardo, 2005; R. Wiltschko et al., 1994). Where pigeons are

released from familiar areas, however, deflection is typically much smaller than this, around half, perhaps indicating compromise with guidance from a magnetic compass or piloting cues from familiar visual landmarks (Wallraff et al., 1999). In birds that are very familiar with their route home (Biro et al., 2007) or are released in sight of their loft (Armstrong et al., 2013) deflections are smaller again, but still present. Thus, whilst the qualitative prediction of opposing deflection under slow and fast shift indicates involvement of the sun-compass in many different situations (in pigeons at least), the way in which it is used varies between different situations and this is reflected in the degree of deflection relative to the expectation for pure sun-compass use. In our experiment, the difference in deflection between fast and slow clock-shift treatments was $\sim 26^\circ$ of an expected $\sim 101^\circ$ under full sun-compass use for the median time of homeward-oriented flight. This represents a substantial but partial effect of clock-shift on the birds' orientation that persisted as birds approached the colony; a journey taking them from areas with no or few landmark cues to 8 km from the colony where much visual information is available from the adjacent topography.

Small deflections under clock-shift are not easy to place into extant theory for bird navigation (Armstrong et al., 2013; Biro et al., 2007; Guilford & Biro, 2014). For example, birds made use of a mosaic map of landmarks with known spatial relationships, sun-compass guidance between these features should be indicated by a full deflection in orientation under clock-shift as measured by instantaneous deflection. Conversely, if birds navigate home by flying towards landmarks associated with getting closer to the colony (known as pilotage (Guilford & Biro, 2014)), or calculate their desired heading by reference to the configuration of landmarks, a clock-shift should not induce a response when landmarks are available. How compass information is relevant to the use of visual information remains unknown. It has recently been suggested (Guilford & Taylor, 2014) that landmarks themselves could be recognised in a time-compensated way since how landmarks appear might vary with the sun's movement over the course of a day. In the same vein, we speculate that for a bird such as a shearwater, being able to see a distant landmark feature with a relatively uniform shape (perhaps a mountain or a small island) might yield only information

about the distance to the object, from its apparent size, and not its precise direction if the object appeared similar from a range of directions. Recognition of the landmark alone would then provide an arc of potential locations at which the bird could be positioned at that given distance. Incorporating the time-compensated angle of the sun relative to the landmark, however, could enable birds to reduce their location uncertainty to a subset of the arc thus enhancing the location information gained from relatively sparse and otherwise uninformative horizon-limited features. This predicts both the persistence of clock-shift deflection and its partiality for shearwaters or pigeons navigating by reference to at least some visual cues.

The partial clock-shift deflections in the current study suggest that the mechanisms guiding seabirds' movements over open-ocean may conform to the same rules that have been suggested to govern navigation in the few terrestrial species tested previously, where the time-compensated sun-compass is central to control of oriented movement. This is perhaps surprising given the different sensory challenge that navigation at sea represents. Our *in-situ* clock-shift of a wild seabird therefore opens up the potential for studying wild birds navigating in scenarios that are more ecologically relevant than those studied previously and thus might in the future yield clues about the unresolved aspects of both oceanic and terrestrial animal navigation.

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We would like to thank the Wildlife Trust of South and West Wales for invaluable support during this project, with thanks especially to Bee Büche and Eddie Stubbings for help on Skomer Island and for thoughtful discussion on bird transportation. We would also like to especially thank Jason Moss and Elisa Miquel Riera for logistical support and for assistance in the field and on boat trips, Sash and Lucy Tusa whose generous donation of their boat and marine expertise made the project possible, and for Dale Sailing for donating boat time and expertise.

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Supplementary material

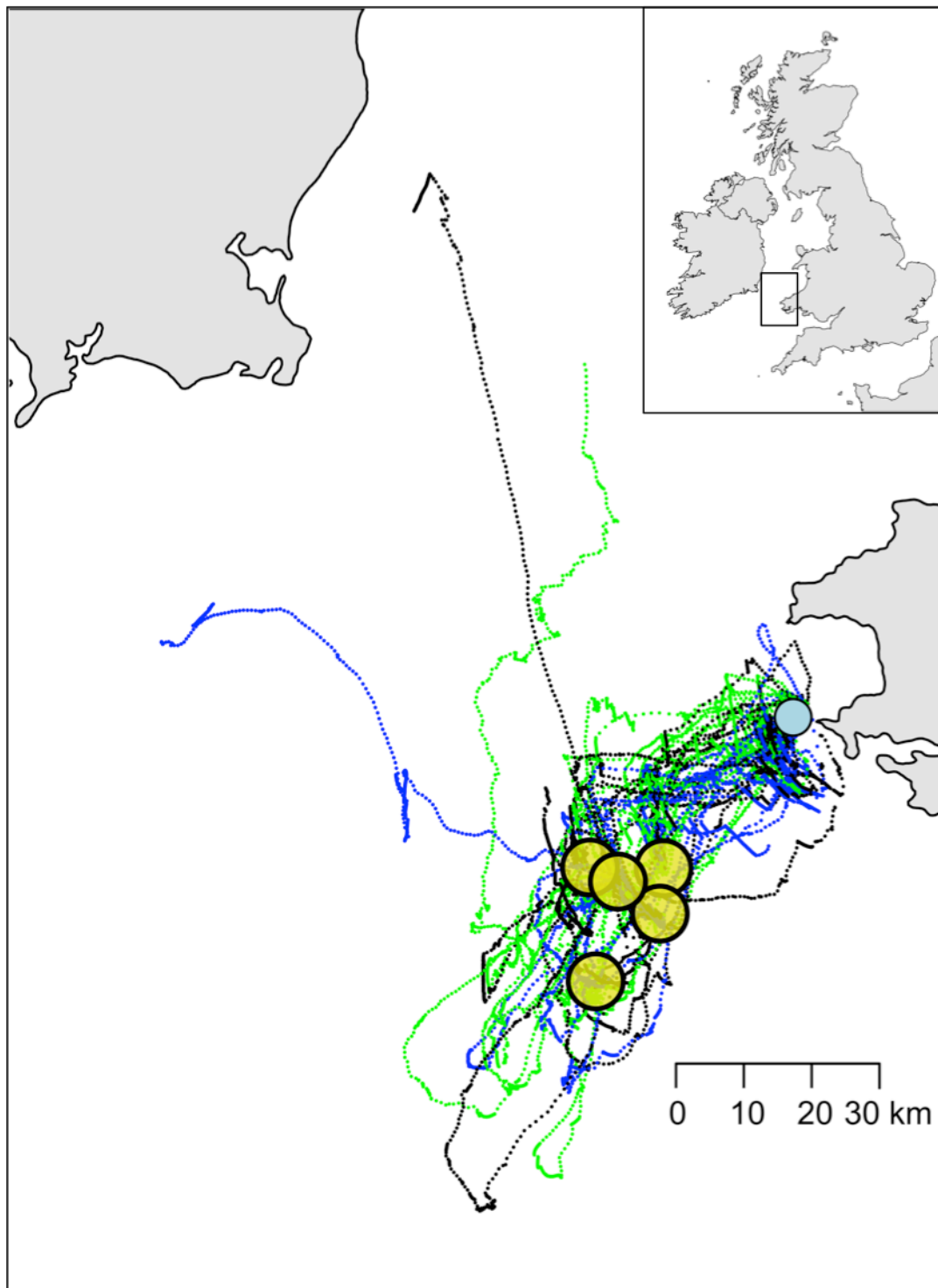


Figure S2.1. The tracks of all displaced birds up to the end of nautical twilight on the day of release. Release sites are shown as yellow circles. Skomer Island is shown as a blue circle. Tracks with no homeward oriented movement on the day of release were excluded for analysis.

Table S2.1. Release information for the five release dates (sample sizes pertain to the tracks analysed).

Release date	Sample sizes			Release coordinates		Release conditions
	n fast	n control	n slow	Latitude	Longitude	
22nd May 2015	4	1	0	51°23'N	5°42'W	Thick fog but sun's disc visible
11th June 2015	5	5	4	51°31'N	5°34'W	Good visibility 1/8 cloud
9th July 2015	1	4	2	51°32'N	5°43'W	Good visibility 4/8 cloud
29th May 2017	6	3	3	51°39'N	5°35'W	Good visibility 7/8 cloud
4th June 2017	3	3	4	51°31'N	5°40'W	Good visibility 1/8 cloud
total	19	16	13			

Table S2.2. Homing information for each bird. Mean deflection for each bird and each treatment for the five releases is shown.

Bird ID	Release Date	Treatment	Mean flight speed (ms ⁻¹)	Start homing	Mean deflection to goal	Mean for release
EK59703	22/05/2015	C	12.5	20:17	-2°	-2°
EK59699	22/05/2015	F	11.4	18:50	1°	
EK59749	22/05/2015	F	11.6	20:40	3°	
EK59833	22/05/2015	F	12.8	20:04	-14°	-3.25°
EK59863	22/05/2015	F	11.4	20:19	-3°	
EB52824	11/06/2015	C	7.1	18:25	26°	
EK59745	11/06/2015	C	7.8	15:43	-33°	
EX93861	11/06/2015	C	7.6	18:23	-1°	-7.2°
EZ01678	11/06/2015	C	6.9	15:43	-11°	
EZ01766	11/06/2015	C	7.5	15:43	-17°	
EK59942	11/06/2015	F	7.5	15:24	-51°	
EZ01651	11/06/2015	F	7.1	16:12	-4°	
FB24686	11/06/2015	F	7.0	15:42	-27°	-26.8°
FB30100	11/06/2015	F	7.7	19:14	-26°	
FB32189	11/06/2015	F	7.1	15:50	-26°	
EK59846	11/06/2015	S	9.6	20:52	3°	
EX93846	11/06/2015	S	6.3	16:08	3°	
EZ01753	11/06/2015	S	6.7	15:43	16°	10.5°
EZ01755	11/06/2015	S	7.4	20:11	20°	
EK59608	09/07/2015	C	11.8	19:10	17°	
EK59806	09/07/2015	C	9.1	18:23	28°	
EZ01643	09/07/2015	C	11.4	17:40	-10°	6.5°
FB32166	09/07/2015	C	11.1	17:26	-9°	
EK59854	09/07/2015	F	9.6	18:42	-17°	-17.0°
EY07276	09/07/2015	S	11.9	20:17	-5°	
EZ01631	09/07/2015	S	10.4	19:45	22°	8.5°
EK59962	29/05/2017	C	11.2	15:07	-15°	
EY07280	29/05/2017	C	9.6	19:20	-49°	-27.0°
EZ72887	29/05/2017	C	13.9	20:23	-17°	
EK59827	29/05/2017	F	12.2	18:23	-31°	
EK59869	29/05/2017	F	12.0	20:18	-19°	
EZ72787	29/05/2017	F	13.4	19:11	-23°	
EZ72873	29/05/2017	F	4.9	16:33	-74°	-34.7°
EZ72875	29/05/2017	F	12.1	19:44	-23°	
EZ72879	29/05/2017	F	8.9	20:07	-38°	
EK59965	29/05/2017	S	11.6	18:30	-7°	
EZ72872	29/05/2017	S	13.3	20:23	-1°	-5.3°
EZ72884	29/05/2017	S	10.7	13:12	-8°	
EK59734	04/06/2017	C	10.3	19:04	7°	
EZ72956	04/06/2017	C	10.4	20:47	16°	18.7°
FB24600	04/06/2017	C	11.6	18:49	33°	
EK59607	04/06/2017	F	11.7	20:13	22°	
EK59747	04/06/2017	F	11.9	20:40	20°	11.3°
EZ72856	04/06/2017	F	13.6	20:09	-8°	
EZ72953	04/06/2017	S	9.9	20:22	15°	
EZ72954	04/06/2017	S	9.7	19:50	23°	
EZ72955	04/06/2017	S	7.7	19:46	45°	29.3°
FB05283	04/06/2017	S	11.6	20:29	34°	

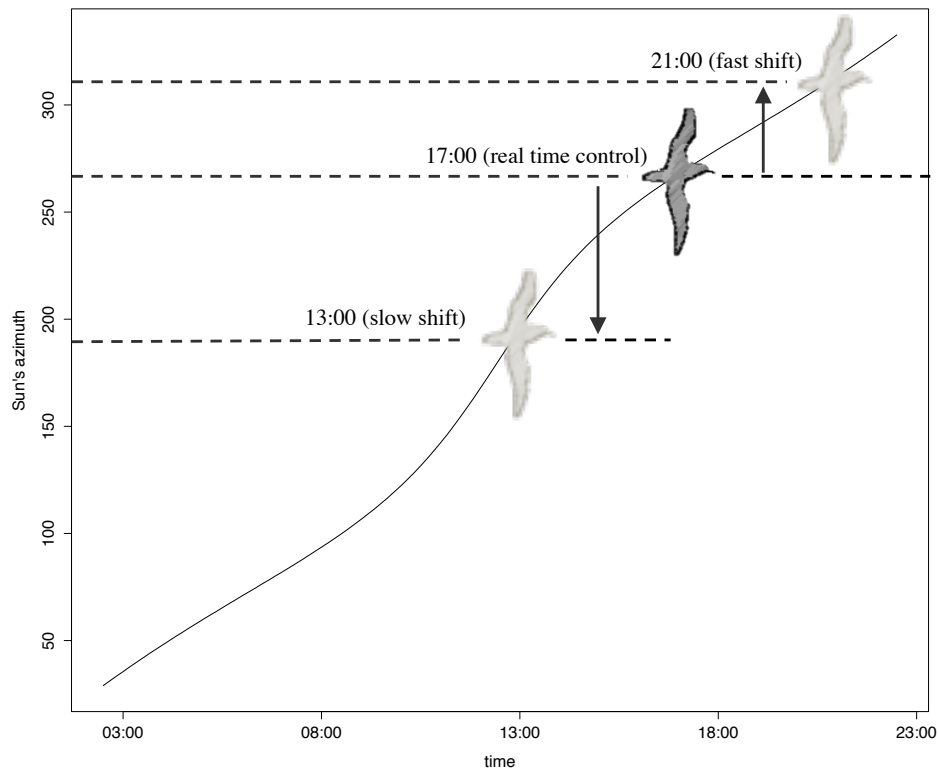


Figure S2.2. Predicted deflections under full sun-compass use are equal to the sun's azimuthal movement between objective (control) and subjective times (fast and slow). The sun's change in azimuth is shown as a line for 09 July 2015. The differences in azimuth among the dashed lines show the magnitude and direction of the expected clock-shift. Throughout the study, we take clockwise to be the positive direction. Azimuth data were downloaded from the NOAA Solar Position Calculator (<https://www.esrl.noaa.gov/gmd/grad/solcalc/azel.html>)

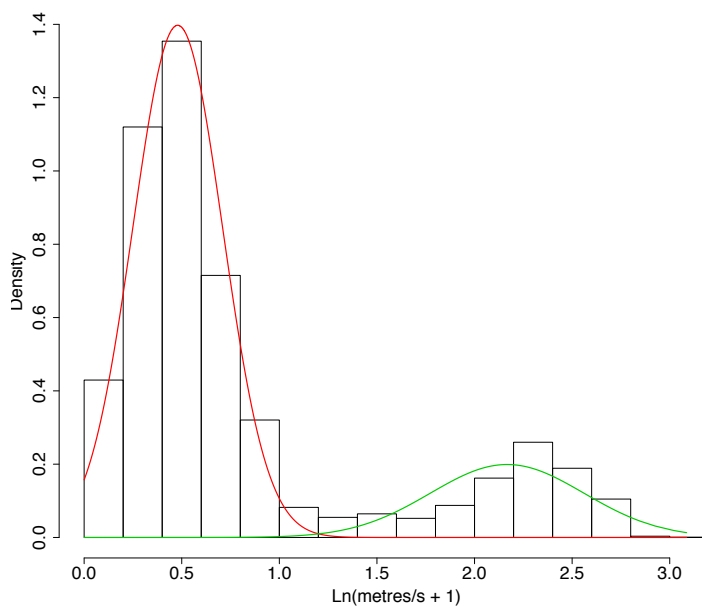


Figure S2.3. Relative probability density of assignment of different log-transformed speeds (no units) to each underlying behaviour. The red line is the probability density of the ‘slow’ behaviour and the green line is the probability density of the ‘fast’ behaviour resulting in the observed emission speed. Tracks were cut from the time of release until the end of nautical twilight on the day of release. We considered homing to start when eight consecutive fixes (one per minute) were high speed and oriented towards Skomer (within 65 degrees). A Gaussian Mixture Model was fitted to log transformed bimodally distributed speed data calculated between consecutive GPS fixes. Data were logged so that a positive probability could not be assigned to a negative speed (Slow peak mean = 0.6597 ms⁻¹ and var = 0.1609. Fast peak mean = 8.3883 ms⁻¹ and var = 9.9180). Fitting was done using Expectation Maximisation in the Mixtools package in R. The resulting cut-off speed was at $\ln(\text{speed} + 1) = 1.2$, hence 2.32 ms⁻¹. Only high-speed fixes were used in analysis because low-speed fixes meant birds were rafting on water and their instantaneous deflection was related to the direction of the water’s current rather than the birds’ orientation decisions.

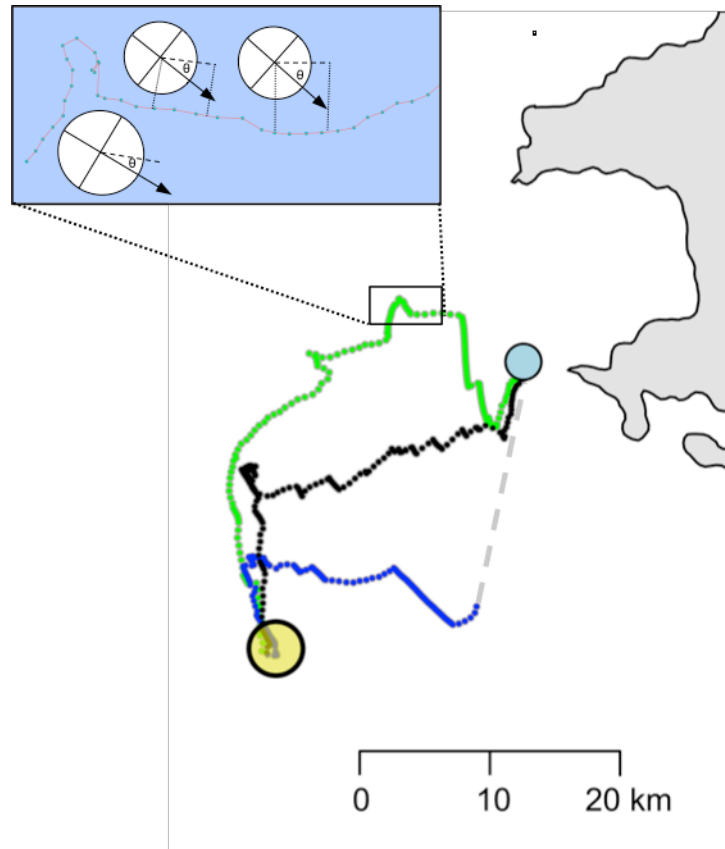


Figure S2.4. Example of 1-minute resolution GPS tracks as birds home from release site 2 (yellow circle). One fast-shifted (green), one slow-shifted (blue) and one control (black) track. Tracks are cut at the end of nautical twilight on the day of release, if they did not arrive within 8 km the colony (blue circle) sooner. To show that the slow-shifted bird reached the colony after dark on the same night, the track has been completed to the colony. Method for calculating instantaneous deflection to the goal (θ) is shown schematically in the magnified example box. Arrows in that box indicates the beeline direction to the colony. The dotted line is the current angular trajectory of the animal's track.

Chapter 3

Anosmia impairs homing orientation but not foraging in shearwaters

Oliver Padget, Gaia Dell’Ariccia, Anna Gagliardo, Jacob Gonzalez-Solis and Tim Guilford

Padget, O., Dell’Ariccia, G., Gagliardo, A., Gonzalez-Solis, J. and Guilford, T. Anosmia impairs homing but not foraging in shearwaters. *Scientific Reports*. **7**. e9668.

Abstract	79
Introduction	79
Materials and Methods	81
Results	87
Discussion	97
Acknowledgements	102
References	102
Supplementary Material	107

Abstract

Shearwaters deprived of their olfactory sense before being displaced to distant sites have impaired homing ability but it is unknown what the role of olfaction is when birds navigate freely without their sense of smell. Furthermore, treatments used to induce anosmia and to disrupt magneto-reception in displacement experiments might influence non-specific factors not directly related to navigation and, as a consequence, the results of displacement experiments can have multiple interpretations. To address this, we GPS-tracked the free-ranging foraging trips of incubating Scopoli's shearwaters within the Mediterranean Sea. As in previous experiments, shearwaters were either made anosmic with 4% zinc sulphate solution, magnetically impaired by attachment of a strong neodymium magnet or were controls. We found that birds from all three treatments embarked on foraging trips, had indistinguishable at-sea schedules of behaviour and returned to the colony having gained mass. However, we found that in the pelagic return stage of their foraging trips, anosmic birds were not oriented towards the colony but that coastal navigation was unaffected. These results support the case for zinc sulphate having a specific effect on the navigational ability of shearwaters and thus the view that seabirds consult an olfactory map to guide them across seascapes.

Introduction

Procellariiform seabirds are some of nature's greatest navigators. The results of two recent displacement experiments have supported the case that, like pigeons over land (Gagliardo, 2013; Wallraff, 2015), Procellariiform seabirds (albatrosses, shearwaters and petrels) might make use of olfactory information to navigate over large distances across seascapes. In the first displacement (Gagliardo et al., 2013), Atlantic Cory's shearwaters, *Calonectris borealis*, breeding in the Azores were released 800 km in the mid Atlantic ocean and either deprived of their olfactory sense by washing of the olfactory mucosa with zinc sulphate or subject to magnetic perturbation by carrying strong neodymium mobile magnets attached to their heads. Whilst control and magnetically manipulated birds homed successfully along straight routes, birds treated with zinc

sulphate wandered in the ocean for thousands of kilometres and were impaired at homing. In a second study (Pollonara et al., 2015), Scopoli's shearwaters, *Calonectris diomedea*, breeding in the Tuscan archipelago were subject to the same standard treatments and displaced in open sea within the Mediterranean Sea's basin. In this case, anosmic shearwaters were able to home, but did so significantly more slowly than controls and magnetically manipulated birds. The authors argue that anosmic birds were able to compensate for a lack of olfactory information by making use of the richer topographic information available in the Mediterranean Sea, an idea which was supported by anosmic birds spending significantly more time within 40 km of the coast during homing.

However, while previous studies have shown that magnetic manipulation has no effect on seabird orientation during free-ranging (Bonadonna et al., 2005; Mouritsen et al., 2003) or homing movement after displacement (Gagliardo et al., 2013; Pollonara et al., 2015) it remains unknown how anosmia might affect birds' orientation during natural, free-ranging movement when given a choice to embark on their own foraging trips or how magnetic or anosmic treatments might affect birds' ability to forage and gain body mass. The reliance of navigation on olfactory cues has never been tested in a natural context and as such the applicability of discoveries from displacement experiments to natural movement is an open question (Thorup & Holland, 2009). A further motivation for examining the effect of navigational treatments (especially anosmia) on free-ranging birds is that disorientation experiments have a limited ability to separate the effect that treatments might have on non-specific factors not directly related to the navigational system that might be the cause of poorer homing performance (Wallraff, 2005). Experiments testing the effect of zinc sulphate treatment on information processing in homing pigeons (Benvenuti & Gagliardo, 1996; Budzynski et al., 1998) have not found evidence of a generalised effect. Similarly in seabirds, Dell'Arciccia and Bonadonna (2013) (Dell'Arciccia & Bonadonna, 2013) showed that anosmic Cory's shearwaters retain motivation to home from short-distance displacements, but return during daylight instead of during the night. This suggests a reliance on

visual cues in the absence of olfactory information and constitutes evidence for impairment in navigational ability without other non-specific responses to zinc sulphate treatment. However, it remains to be elucidated whether there is a more general behavioural effect of the zinc sulphate treatment in free-ranging and foraging pelagic Procellariiform seabirds and over long-distance orientation.

In this study we aimed to address these questions by investigating whether standard navigational treatments used in displacement studies, zinc sulphate induced anosmia (Dell’Ariccia & Bonadonna, 2013; Gagliardo et al., 2013; Holland et al., 2009; Pollonara et al., 2015; Schlund, 1992) and magnetic disruption using strong rare-earth magnets (Massa et al., 1991; Mouritsen et al., 2003; Pollonara et al., 2015), cause disruption of natural foraging trips in a shearwater. Unlike previous studies, treatments were not followed by translocation. Instead, we tracked shearwaters’ free-ranging foraging trips using miniature GPS loggers. We were therefore able to observe whether manipulated birds were motivated to embark on foraging trips, able to forage effectively and could perform normal behavioural tasks. Furthermore, it allowed us to investigate the effect of olfactory deprivation, achieved by the same means as in previous release experiments, on orientation in free-ranging foraging trips for the first time. Our magnetic manipulation was only partially successful because most birds had lost their magnets by the time they returned from foraging, limiting the robustness of inferences about the impact of magnetic disruption on foraging or the role of magnetic cues in orientation.

Materials and methods

Experimental treatments

Scopoli’s shearwaters’ nest attendance was monitored from 25th June 2016 until 21st July 2016 (during the last third of incubation) at the colony of Cala Morell, Menorca, Spain (40°03'19.2" N 3°52'55.6" E) where there is an on-going study of this species, and incubating pairs suitable for manipulation and device deployment were identified. At this stage, new birds were ringed and

morphometric measurements taken. Birds were caught either by hand or with a neck noose and were returned to the entrance to their nest crevice and observed after handling. Birds were assigned to treatments alternately. In our first treatment, shearwaters were made anosmic ($n = 10$) by washing of the olfactory mucosa via the nares (the nostrils on the bill of a bird) with 4 ml of 4% zinc sulphate heptahydrate dissolved in water as in previous studies (Gagliardo et al., 2013; Pollonara et al., 2015). Zinc sulphate treatment acts by causing necrosis of the nerve cells in the olfactory mucosa and birds remain anosmic until the cells of the mucosa have differentiated, matured and connected to the olfactory bulb, a process that takes many weeks in the homing pigeon (Bedini et al., 1976). In our second treatment, birds were disrupted magnetically ($n = 10$) by attachment of a small cylindrical (4 mm x 5 mm) neodymium magnet secured at the end of a 2 cm x 0.4 cm, flattened cylinder of TESA tape (total mass 1 g) and attached to feathers on the top of the head, directly between the eyes. The magnetic end of the TESA cylinder was free to move around its arc to either side of the bird's head. Preliminary observations of the anosmic treatment were made on four birds at their nests to assess whether the zinc sulphate treatment negatively impacted incubation behaviour (three of these later entered the experiment, alternated with controls and magnetically disrupted birds). As in Gagliardo et al. (2013), control birds ($n = 12$) were not sham treated with respect to the zinc sulphate treatment since there is robust evidence that washing the olfactory mucosa with saline solution (Pollonara et al., 2015), physiological solution (Bonadonna & Bretagnolle, 2002; Bonadonna et al., 2001; Dell'Araccia & Bonadonna, 2013), or the non-olfactory nasal mucosa with zinc sulphate (Benvenuti & Ioalè, 1993) does not affect the behaviour of petrels but nonetheless the procedure does inevitably risk some damage to the olfactory mucosa (Benvenuti & Ioalè, 1993) and could produce a partially anosmic control treatment. However, a glass bead was deployed as a sham in place of a magnet on all non-magnetically manipulated birds so that we could compare controls with both other treatment groups and reduce the number of birds used for the experiment.

Before being treated, birds were fitted with Mobile Action I-gotU gt-120 housed in waterproof heatshrink plastic. Devices were attached using TESA tape which was laid underneath small bunches of contour feathers centrally on the back of the birds and wrapped over the device. Housed GPS devices (including TESA) weighed 18 g (3.1% of body mass at the time of deployment, 3.3% including the magnetic and sham treatments) and measured approximately 9 cm x 3 cm x 1.5 cm, which includes tabs of heatshrink housing at each end used for attachment (Guilford et al., 2008). Birds were weighed immediately prior to the treatment and upon retrieval. GPS devices were scheduled to take fixes every 5 minutes. Total handling time was normally less than 15 minutes. All experimental procedures were conducted in accordance with animal welfare regional legislation (BOIB 97 Decret 65/2004) and were approved by the University of Oxford's local ethical review process. Experiments were carried out under licence from the Balearic government (CEP 22/2016).

Identifying the start of homing, at-sea behaviour and foraging success

GPS tracks regularly comprised multiple trips made by the birds between deployment and retrieval. These were split and each trip analysed separately. We employed a multi-step process to identify objectively the homing sections of each trip. Tracks were interpolated by cubic splines such that locations were at exactly 5 minute intervals (Tremblay et al., 2005) before being divided into behaviourally consistent units by implementation of a Douglas-Peucker line segmentation algorithm as in Thiebault and Tremblay (2013) (Thiebault & Tremblay, 2013). By going backward through each trip, we could then identify the first behaviourally consistent segment that resulted in significant homeward movement. We defined the start of that segment as the decision to home. All pre-processing was carried out blind with respect to treatment.

To identify at-sea behaviour, we fitted a Gaussian mixture model to speed and turning angles calculated for the tracks as in Fayet *et al.* (2016) (Fayet et al., 2015). We identified the optimal number of behavioural states by assessing the log-likelihood of 1-10 states before assigning each

GPS location to its most likely state in the best mixture model. Mass gained at sea was determined from the mass change of birds between device deployment and retrieval. Because following deployment birds often continued incubating for several days before departing on a foraging trip, during which time they will have lost mass, we corrected the measured mass change to account for the lower mass of the bird at departure. To do this, we calculated the rate of percentage mass loss for a subset of incubating birds which were weighed more than once during an incubation stint ($n = 8$) and used this to estimate a departure mass for each of our birds, thus giving a more accurate estimate for the mass gained at sea. We report this as corrected mass gained. We then compared the proportion of GPS locations assigned to each behaviour out of the total GPS locations for day-time and night-time sections of track. Night-time was identified as the time between the end of one nautical twilight and the beginning of the next (calculated for the median GPS latitude and longitude on the median date).

Outbound and homing orientation

Outbound orientation was the virtual vanishing bearing (Biro, Freeman, Meade, Roberts, & Guilford, 2007) of each bird's position as it reached 10 km on the outward stage of trips to the Catalanian coast, measured between geographical north, the colony and the bird. Homing orientation was analysed separately for the portion of trips that were either coastal (within 40 km of the Balearic archipelago) or pelagic (beyond 40 km of the Balearic archipelago). 40 km was chosen in line with previously published analyses (Pollonara et al., 2015) as the point at which birds could probably see land. For pelagic homing, inbound orientation was defined as being from the identified start of homing to the point where the bird reached the 40 km threshold. Coastal homing was the remainder of the trips from the moment they passed within 40 km of the Balearic coast until they reached the colony (or the entire trip for birds which did not travel further than 40 km from the coast during the entire trip). For both sections, the bearing with respect to home between consecutive interpolated GPS locations identified as 'flight' was compared among treatments. Track straightness was measured as the path length between the start of homing and

the point where the bird reached 40 km from the Balearic coast divided by the beeline distance (the shortest Great Circle distance between the start and end of the track section).

Statistics

To test for an effect of treatment on outbound orientation, we conducted a circular analysis of variance (Watson-Williams test). To deal with repeated measures caused by multiple trips from each bird, we iteratively sampled one measure from each bird at random and then performed a Watson-Williams test on this subset. This procedure was repeated 5000 times. We present the estimated mean, standard error (s.e.) and estimated p-values of these iterated Watson-Williams tests.

We used linear mixed models (LMMs) to test for an effect of treatment on homing orientation, the relationship between homing departure time and distance to the colony at the start of homing, total distance travelled in each trip, trip duration, the straightness of return tracks and trip repeatability characteristics (see supplementary material). For LMMs, treatment was coded as a three-level factor (anosmic, magnetically manipulated and control). We used binomial generalised linear mixed models (GLMMs) to test for an effect of treatment on the proportion of time spent in each behavioural state, both for daytime and night-time activity (Fayet et al., 2015). Each of the three behavioural states was analysed with a separate GLMM, with the response variable being 0 or 1 for each GPS location with treatment, coded as a three-level factor, as a predictor. LMMs and GLMMs included a random intercept effect to account for repeated measures. This was bird ID for response variables calculated on an entire trip (total trip distance, trip duration, straightness of return track), and was trip ID nested within bird ID for response variables measured at each GPS location (orientation home, behavioural state of each GPS location) to reflect the structure of our data. For the GLMMs testing the effect of treatment on the proportion of fixes in each behavioural state, a second random intercept effect was included to account for date effects such as moon state and weather variation across the tracking period. This

was the Julian date (for models testing day fixes) or the Julian date at the start of the night (for models testing night fixes) when data were recorded.

To obtain p-values from mixed models, we conducted a likelihood ratio (LR) test between each full model (with treatment and random intercepts effects) and a nested, null case of the model (random intercept effects only). For parameter estimates, models were fitted with restricted maximum likelihood but for LR tests they were refitted by maximum likelihood estimation (Zuur, Leno, Walker, Saveliev, & Smith, 2009). Where significant, LMMs were followed by a *post-hoc* Tukey test to evaluate between which levels of treatment significant differences lay with degrees of freedom adjusted by the Satterthwaite method (Satterthwaite, 1946). For LMMs, the assumption of approximately normal residuals was checked by examination of each model's Q-Q plot. GLMMs were checked for over-dispersion by comparing the sum of squared Pearson residuals to the residual degrees of freedom.

A General Linear Model was used to analyse the relationship between mass gained (between device deployment and retrieval) and time spent foraging, which was estimated from the number of GPS fixes identified as foraging (1 GPS location = 5 minutes). To assess whether treatment affected the mass gained per unit time we ran a GLM with treatment (coded as a three-level factor), time spent foraging and the interaction between treatment and time spent foraging as predictors.

All statistics were conducted using R base or the lme4 package (Bates, Maechler, Bolker, & Walker, 2015) in R (ver. 3.2.1). Tukey tests were conducted using the multcomp package and the Watson-Williams test was conducted using the circular package.

Results

Tracking success and impact of tracking on breeding success

Deployments for all three treatments were carried out between the 29th June and 10th July and retrievals were made between the 6th and 20th July. 12 control (C), 10 magnetically manipulated (M) and 9 anosmic (A) birds were tracked resulting in tracks from 10 control, 9 magnetically manipulated and 9 anosmic birds comprising a total of 59 foraging trips (16 control, 23 magnetically manipulated and 20 anosmic; Figure 3.1). A single GPS carried by a magnetically manipulated bird failed to record data for the full duration of the deployment due to its battery discharging. The resulting incomplete trip was not included in the analysis. All birds that were handled, including those made anosmic, resumed incubation immediately following the procedure and continued to incubate normally until embarking on a foraging trip. All birds embarked on foraging trips and all birds except one magnetically treated bird were seen again within the period that the colony was monitored for returns (until 21st July). Anosmic birds were recovered and GPS devices were retrieved within 15 days of treatment and deployment, a duration over which zinc sulphate-treated birds are assumed to have remained entirely anosmic. Therefore, all trips recorded by the GPS were included in our analyses.

There was no difference in the date of deployment (Kruskal-Wallis test: $\chi^2 = 0.35$, $df = 2$, $p = 0.84$), the date of the first outbound trip (Kruskal-Wallis test: $\chi^2 = 2.15$, $df = 2$, $p = 0.34$) or the time spent on the nest between deployment and departure (Kruskal-Wallis test: $\chi^2 = 2.10$, $df = 2$, $p = 0.35$) among treatments. There was also no difference in the number of trips recorded per individual among treatments (Kruskal-Wallis test: $\chi^2 = 2.24$, $df = 2$, $p = 0.33$). Primarily, incubation stint changeovers occurred normally, but birds in all treatments were sometimes recorded leaving the nest 1-3 days before partners returned (1 control, 3 magnetically manipulated and 2 anosmic), a behaviour occasionally observed in crevice-nesting petrels (Boersma & Wheelwright, 1979). Of the head-mounted manipulations, only 4/12 control birds and 3/9 anosmic birds and 4/10 magnetically manipulated birds were carrying their sham- or true magnet

upon retrieval. After conducting exploratory statistics to test for differences between magnetically treated birds that did and did not retain their magnets (Table S3.1, supplementary material), magnetically treated birds were pooled together for all analyses.

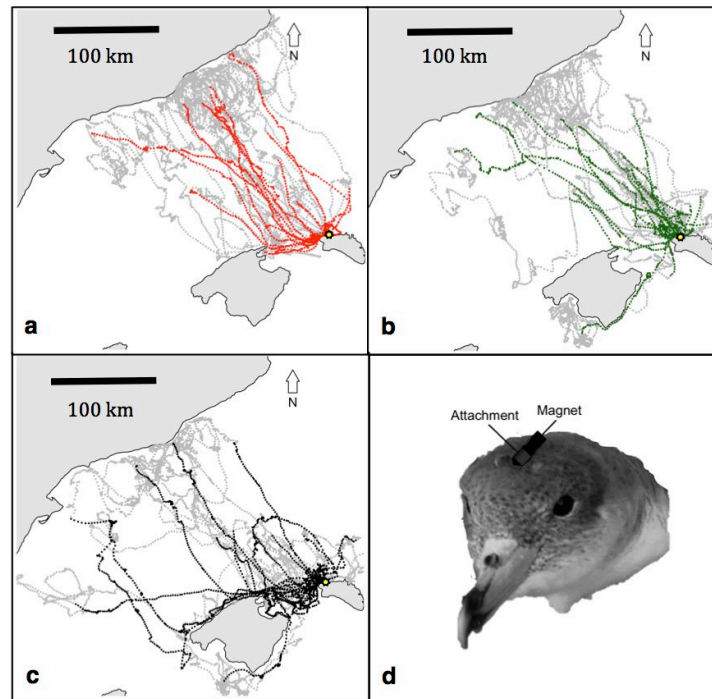


Figure 3.1. GPS tracks (59 trips) of control (a), magnetically manipulated (b) and anosmic (c) birds. The homing portion of the trips are highlighted for control (red), magnetically disrupted (green) and anosmic (black) birds respectively. (d) Schematic showing the TESA magnet attachment to the head of the shearwater consisting of a flattened cylinder of TESA tape attached at the base to the feathers on the top of the head and containing a magnet free to move at the end. Yellow points show the colony position. Maps were generated in R using the “maps” package.

All nests included in the study were checked for breeding status on 24th July, after hatching had begun. Of the 32 nests included in the experiment, 14 failed to hatch their egg (control 4/12; magnetic 6/10; anosmic 4/10), primarily because of egg predation from brown rat, *Rattus*

norvegicus. This did not differ significantly among treatments (Kruskal-Wallis test: $\chi^2 = 2.15$, $df = 2$, $p = 0.34$).

Trip characteristics

The Scopoli's shearwaters in this study made long (3-10 day) foraging trips, typical of incubating *Calonectris* shearwaters (Afán et al., 2014). The trips mostly comprised an outbound and inbound commute, separated by intense foraging in a relatively restricted area where they most likely foraged on small pelagic fish and cephalopods (Neves et al., 2012) caught at or near the surface in plunge dives (Martin, 1986; Ramos et al., 2009). A typical, annotated trip is shown in Figure 3.2. Foraging trips in this study were broadly made to three areas: the Catalanian coast ($n = 20$), coastal destinations within 40 km of the Balearic Islands ($n = 11$) or non-coastal destinations (>40 km offshore) in the Balearic Sea ($n = 27$). Birds which commuted to the Catalanian coast foraged approximately 35 ± 0.2 km (mean $\pm$ s.e.) from the coast between the Ebro Delta ($40^\circ 42' 29.3''$ N $0^\circ 52' 8.0''$ E) in the south and the Muntanyes de Begur National Park in the north (Lat: $41^\circ 58' 46.6''$ N $3^\circ 16' 58.5''$ E), a region known to be a foraging hotspot for Mediterranean Procellariiformes (Cortes, Manuel Arcos, & Gonzalez-Solis, 2017). A single anosmic bird travelled to the coast near Valencia ($40^\circ 4' 1.2''$ N $0^\circ 19' 0.8''$ E). There was no difference in the propensity of the three treatments to undergo trips to the different regions ($\chi^2 = 4.11$, $df = 2$, $p = 0.128$), the duration of foraging trips (LMM estimates $\pm$ s.e.: control: 126 ± 37 h; magnetic: 94 ± 27 h; anosmic: 102 ± 27 h; LR test: $\chi^2 = 0.95$, $df = 2$, $p = 0.62$), or the distance covered during foraging trips (LMM estimates $\pm$ s.e.: anosmic (9 birds, 20 trips) = 867 ± 197 km; control (10 birds, 16 trips) = $1,074 \pm 271$ km; magnetic (9 birds, 23 trips) = 716 ± 267 km; LR test: $\chi^2 = 2.11$, $df = 2$, $p = 0.35$). There was no difference among treatments in the route fidelity between the outbound and inbound sections of birds' trips (Table S3.2, supplementary material) or in individual repeatability characteristics of outbound route and destination for birds that underwent multiple trips during the experimental period (Figure S3.2 and Table S3.3, supplementary material).

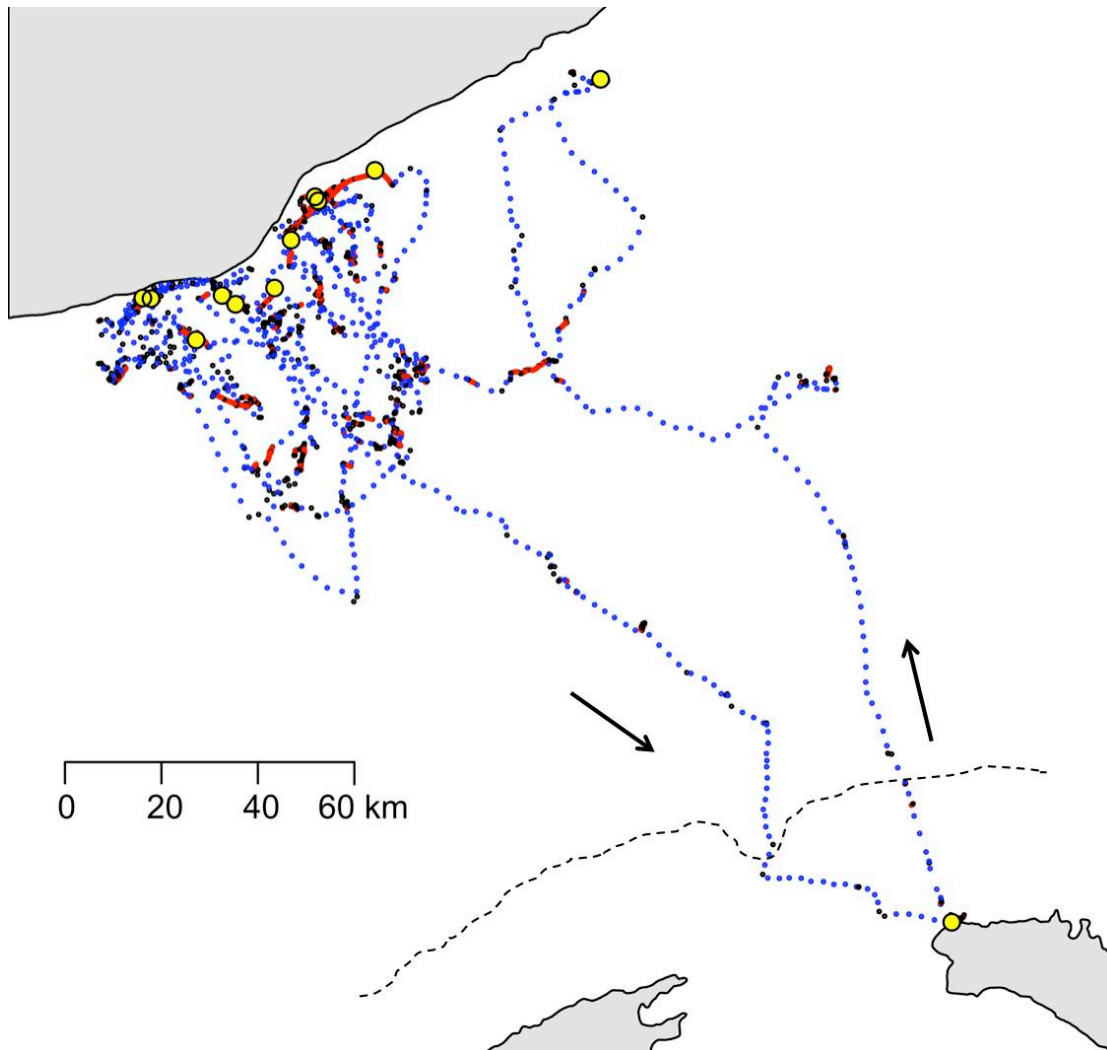


Figure 3.2. A typical foraging trip to the Balearic coast. Behaviours identified by our mixture model are commuting (blue), foraging (black) and resting (red). Yellow dots show where the bird was at midnight on each day of the trip including the night it departed. This magnetically manipulated bird spends 10 days at sea before commuting back to Mallorca. The dotted line shows the 40 km contour from the Balearic Islands. Maps were generated in R using the “maps” package.

At-sea behaviour

Our mixture modelling approach identified three behavioural states (Figure S3.1). Since log-likelihood increases monotonically with the number of states assumed, we chose the number of states indicated by an elbow in the increasing log-likelihood as providing the best explanatory power without over-fitting states to reflect real clustering in the data. We interpreted these three states as resting on the water (mean speed = 0.3 ms⁻¹), directed flight (mean speed = 6.7 ms⁻¹) and foraging (mean speed = 1.8 ms⁻¹). There was no significant difference in the proportion of time spent in any of the identified behavioural states among treatments either for behaviour during the day or during the night (Table 3.1). The diurnal pattern of behaviours was similar across all three treatments (Figure 3.3), with the majority of commuting behaviour occurring around dawn and dusk, and more resting on the water at night.

Table 3.1. The proportion of time spent in each behavioural state for the three treatments during day and night. Proportions shown are the second order means for the treatments $\pm$ s.e., χ^2 and p-values from LR tests are shown.

	Diurnal activity	GLMM		Nocturnal activity	GLMM	
	Mean $\pm$ SE	χ^2	p	Mean $\pm$ SE	χ^2	p
Foraging	Control: 0.37 $\pm$ 0.15	0.26	0.88	Control: 0.27 $\pm$ 0.14	4.30	0.12
	Magnetic: 0.35 $\pm$ 0.16			Magnetic: 0.20 $\pm$ 0.13		
	Anosmic: 0.36 $\pm$ 0.17			Anosmic: 0.22 $\pm$ 0.14		
Resting	Control: 0.31 $\pm$ 0.15	2.89	0.24	Control: 0.60 $\pm$ 0.14	1.00	0.61
	Magnetic: 0.36 $\pm$ 0.16			Magnetic: 0.67 $\pm$ 0.13		
	Anosmic: 0.33 $\pm$ 0.17			Anosmic: 0.66 $\pm$ 0.15		
Commuting	Control: 0.32 $\pm$ 0.15	2.86	0.24	Control: 0.13 $\pm$ 0.10	0.30	0.86
	Magnetic: 0.29 $\pm$ 0.15			Magnetic: 0.13 $\pm$ 0.11		
	Anosmic: 0.31 $\pm$ 0.16			Anosmic: 0.11 $\pm$ 0.11		

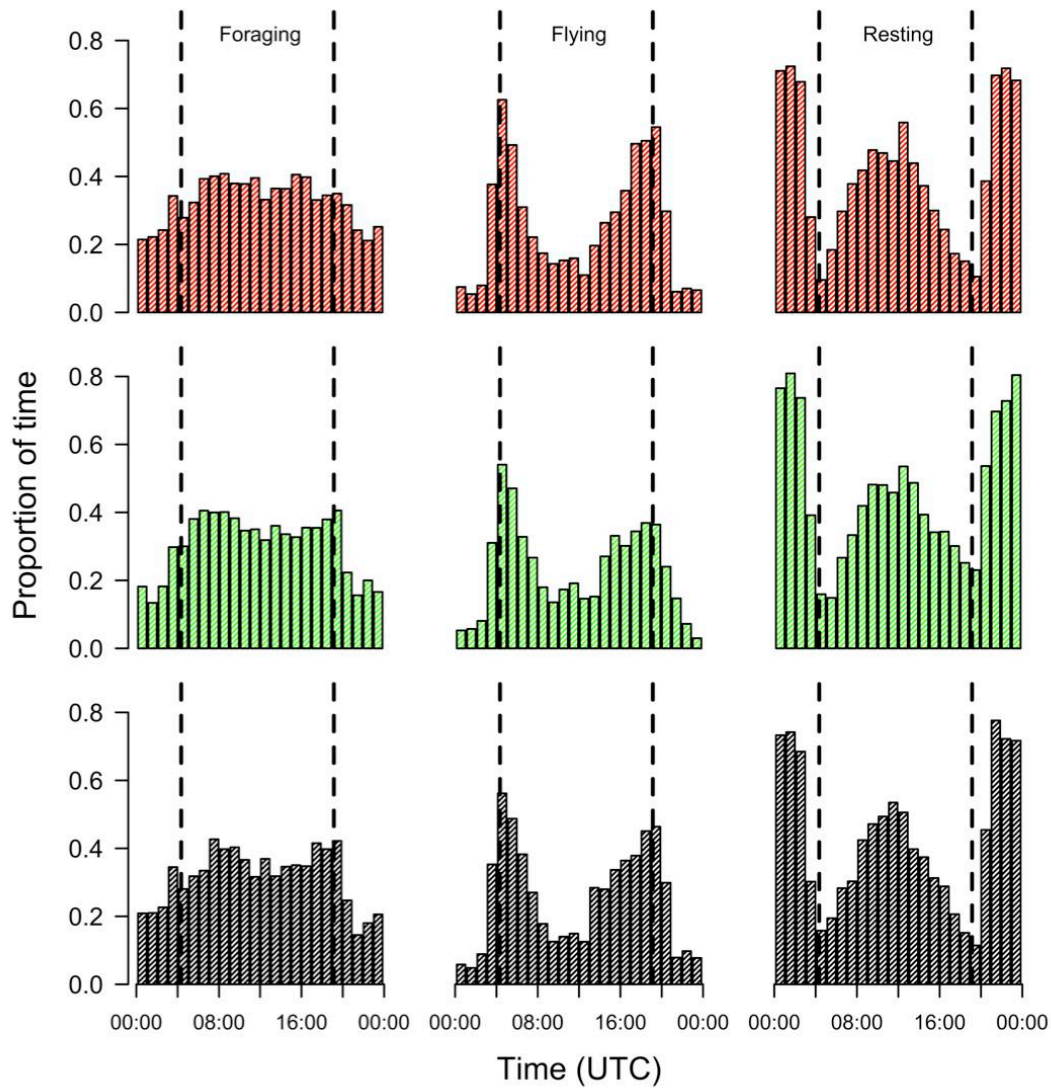


Figure 3.3. Diurnal behaviour patterns for control (red), magnetically manipulated (green) and anosmic (black) birds. Columns, from left to right are foraging, flying and resting on the water. Sunrise and sunset are shown as dotted lines (times shown are in UTC).

Foraging success

Birds from each treatment for which we had pre- and post-deployment mass gained mass on average during the experiment (controls ($n = 9$): 38.7 ± 27.0 g; magnetic ($n = 8$): 70.8 ± 30.1 g; anosmic ($n = 8$): 29.7 ± 13.0 g). There was no significant difference among treatments in the mass gained during the experiment (ANOVA: $F = 0.73$, $df = 2,22$, $p = 0.49$, Figure 3.4a).

Corrected mass gained (taking account of mass lost while incubating before departure) was also not significantly different among treatments (mean $\pm$ s.e.: control (n = 9): 82.0 ± 24.7 g; magnetic (n = 8): 122.9 ± 27.7 g; anosmic (n = 8): 58.6 ± 12.9 g; ANOVA: $F = 1.94$, $df = 2,22$, $p = 0.17$; Figure 3.4a). Corrected mass gained was significantly predicted by the number of GPS locations labelled as being in the foraging state (GLM: $F = 8.57$, $df = 1,23$, $p = 0.01$; Figure 3.4c) but neither treatment nor the interaction between treatment and foraging effort improved the model significantly (treatment: $F = 2.63$, $df = 2$, $p = 0.10$; interaction: $F = 2.78$, $df = 2$, $p = 0.09$) meaning that there was no effect of treatment on the rate of mass gained per unit foraging effort (Figure 3.4b and c).

Outbound orientation

Outbound orientation was not significantly different among treatments (mean directions: control = 316.97° ; magnetic = 321.32° ; anosmic = 285.23° ; 5000 runs of Watson-Williams Test, $p = 0.25 \pm 0.002$, Figure 3.5a). However, anosmic birds were initially more coastal in the outbound sections of their trips, being on average ~ 2 km closer to the coast than magnetically disrupted or control birds as they reached 10 km from the colony (LMM estimates $\pm$ s.e.: control: $9,395 \pm 843$ m; magnetic: $9,637 \pm 761$ m; anosmic: $7,378 \pm 557$ m; LR test: $\chi^2 = 9.73$, $df = 2$, $p = 0.01$; *Post-hoc* Tukey pairwise test: Control-Magnetic: $t = 0.30$, $p = 0.95$; Control-Anosmic: $z = 2.416$, $p = 0.04$; Anosmic-Magnetic: $z = 2.97$, $p = 0.01$; Figure 3.5b).

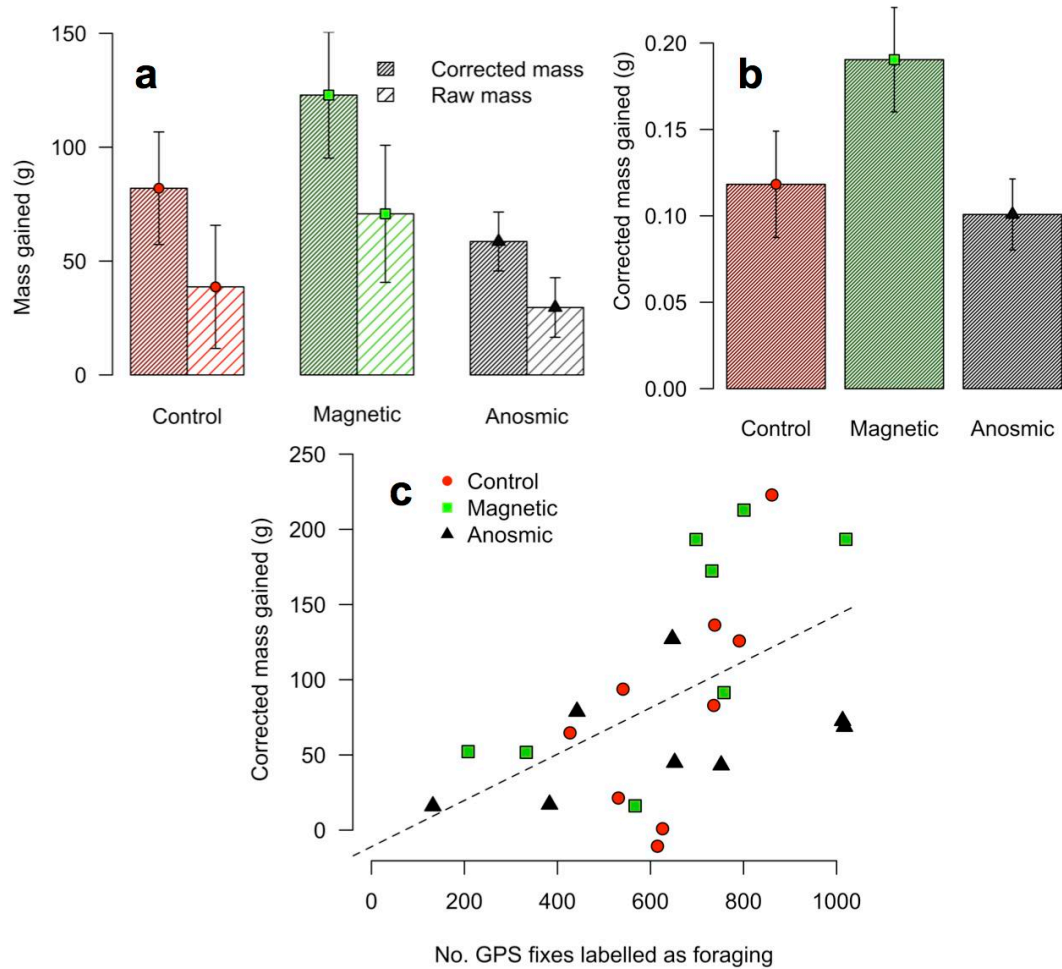


Figure 3.4. (a) Raw mass (mean $\pm$ s.e.) change over the course of the deployment and corrected mass (mean $\pm$ s.e.) taking account mass lost during incubation between weighing and the start of the trip. (b) The corrected mass (mean $\pm$ s.e.) gained per GPS location labelled foraging and (c) the mass gained as related to the amount of time spent foraging (number of GPS locations labelled foraging). For statistics see text.

Timing of homing

The time that birds began homing on the day preceding the night of arrival (Figure 3.5c) was strongly predicted by the distance that they had to travel to reach the colony (LMM LR test: $\chi^2 = 56.40$, $df = 1$, $p < 0.0001$) but neither treatment nor the interaction between treatment and distance significantly improved the model (treatment LR test: $\chi^2 = 1.03$, $df = 2$, $p = 0.60$; interaction LR

test: $\chi^2 = 0.38$, $df = 2$, $p = 0.83$), meaning that the ability to anticipate the distance home did not differ among the three treatments.

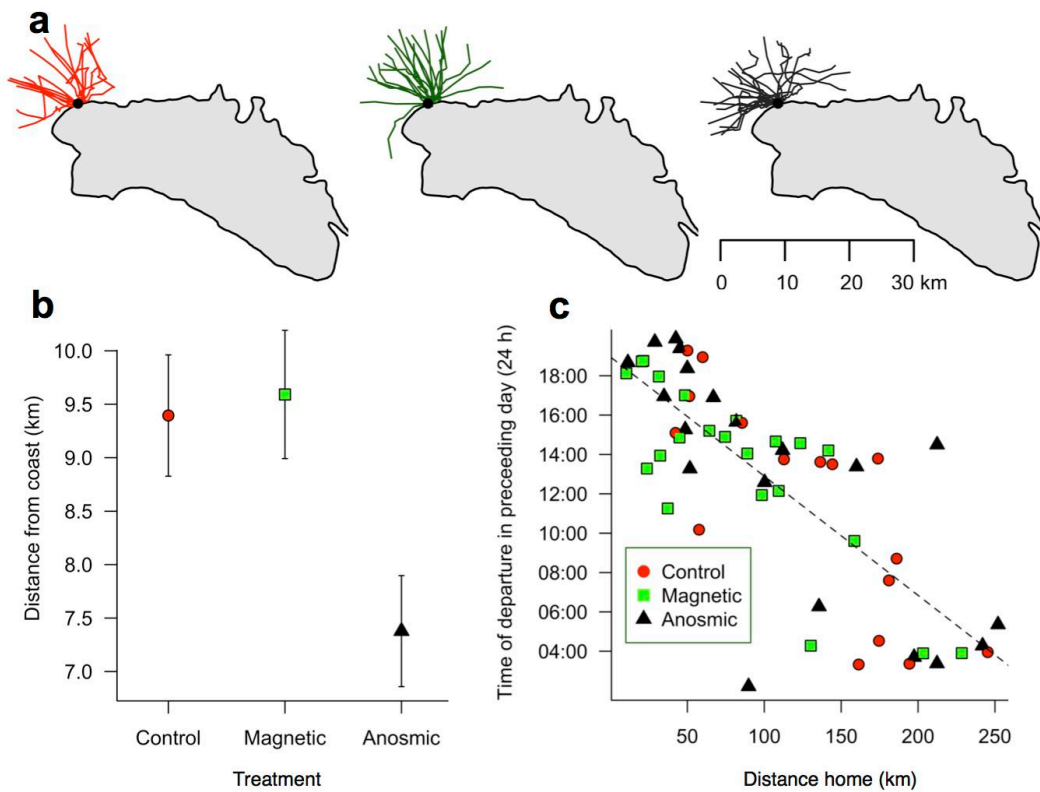


Figure 3.5. (a) Outbound sections of birds' trips up to 10 km from the colony are shown for anosmic (black), magnetic (green) and control (red) treated birds. (b) Distance from Balearic coast as birds reach 10 km from the colony on the outbound sections of trips. (c) Time that birds begin to the home to the colony in the day preceding the night of arrival as a function of distance to the colony at the start of homing. Linear regression is shown (see text for LMM statistics). Maps were generated in R using the “maps” package.

Pelagic orientation home

For the pelagic (>40 km from the Balearic coast) sections of trips identified as homing, orientation relative to the beeline home, measured for each GPS location during homing, differed significantly among treatments (LMM estimates $\pm$ s.e.: control (n = 12) = $4.3 \pm 6.8^\circ$; magnetic (n = 14) = $1.7 \pm 6.7^\circ$; anosmic (n = 12) = $21.9 \pm 5.0^\circ$; LR test $\chi^2 = 10.00$, df = 2, p = 0.0067; Figure 3.6a). A *post-hoc* Tukey test revealed that this difference lay between anosmic and control (t = 2.97, p = 0.040) and anosmic and magnetic (t = 3.02, p = 0.013) but not control and magnetic (t = 0.41, p = 0.912) treatments. For both control and magnetically manipulated birds, the home direction, 0° , lay within the 95% confidence interval for the return orientation whereas for anosmic birds, 0° lay outside of the 95% confidence indicating that during the pelagic homing phase anosmic shearwaters were not oriented toward the colony.

Coastal orientation home

Within 40 km of the Balearic archipelago there was no difference among treatments in homeward orientation (LMM estimates $\pm$ s.e.: control = $8.9 \pm 5.3^\circ$; magnetic = $1.4 \pm 5.0^\circ$; anosmic = $7.0 \pm 3.5^\circ$; LR test: $\chi^2 = 2.23$, df = 2, p = 0.3282; Figure 3.6b). For this coastal phase, the home direction lay within the 95% confidence intervals for all treatments.

Track straightness

There was no significant difference in the straightness of the homing trajectory among treatments during the pelagic phase of homing (control = 0.89 ± 0.04 ; magnetic = 0.93 ± 0.04 ; anosmic = 0.87 ± 0.03 ; LR test: $\chi^2 = 2.22$, df = 2, p = 0.33).

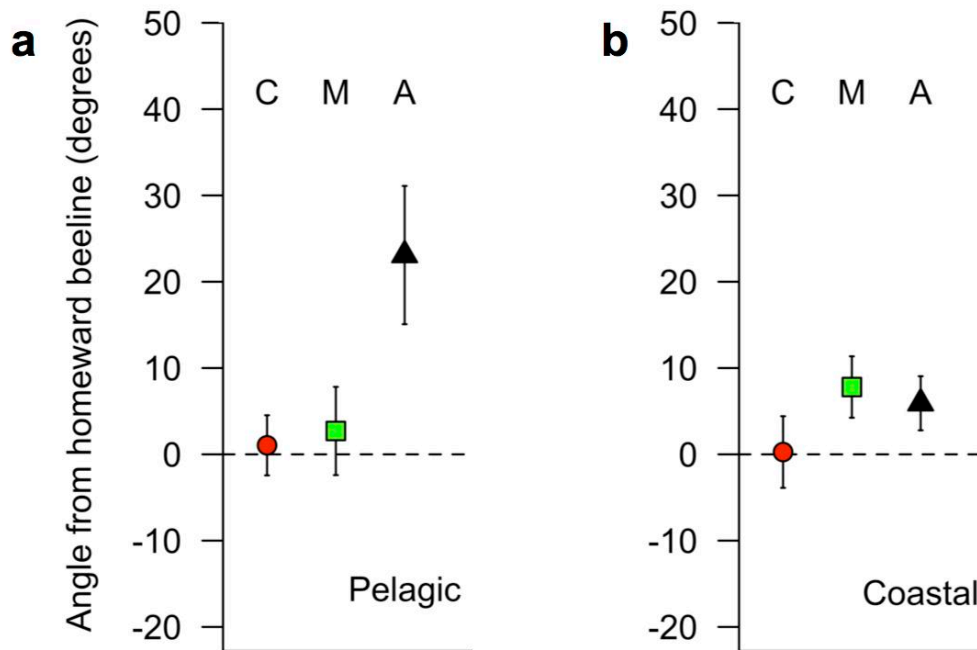


Figure 3.6. Orientation (mean $\pm$ s.e.) during the homing phase (transformed so that homeward direction = 0). Pelagic sections (a) are those from the point of homing until 40 km from the Balearic Archipelago. Coastal (b) is homing within 40km of the Balearic archipelago. For statistics see text.

Discussion

In this study, we investigated for the first time the effect of nasal irrigation with zinc sulphate on the free-ranging foraging trips of a pelagic seabird and the effect of magnetic disruption by attachment of strong rare-earth magnets on birds' ability to forage and gain mass at sea. Both anosmic and magnetically treated shearwaters were able to embark on foraging trips, forage successfully to gain mass, and return to the colony to resume incubation. Given that only four of our magnetically manipulated birds retained their magnets for the entire trip, we cannot draw robust inferences on the effect of the magnetic treatment on the at-sea behaviour of shearwaters in this study. However, it is likely that magnets were retained for the outbound sections of the track which occurred within the first 6 hours of birds leaving the colony and so we confine our inference to effects of the treatment on the outbound sections of birds' trips.

By inferring at-sea behaviour from clustering patterns in speed and tortuosity data, we were able to compare at-sea behaviour among our treatments and show that both anosmic and magnetically manipulated birds were similar to controls in their ability to perform general behavioural tasks. Gaussian mixture models and other similar pattern recognition techniques have previously been employed to identify at-sea behaviour in other shearwater species and their effectiveness at identifying foraging behaviour has previously been validated using concurrently deployed dive-loggers (Dean et al., 2012; Freeman et al., 2010). In the present study, we confirmed our expectation that birds would gain significantly more mass at sea with more time identified as foraging, implying that our mid-speed behaviour was indeed related to foraging behaviour. In this way we were able to measure mass gained per unit of time foraging at sea and compare this among treatments, allowing us to observe that anosmic and magnetically manipulated birds could gain mass at sea and, importantly, that they did not need to spend more time foraging to do so. Whilst magnetically manipulated birds did gain more mass on their foraging trips than control and anosmic birds, this difference was not greater than that expected by chance and, to our knowledge, there are no plausible ways in which magnetic disruption could increase a bird's foraging ability.

The foraging success and similarity of nycthemeral patterns in at-sea behaviour between anosmic birds and the other two treatments suggests that the zinc sulphate treatment did not measurably affect birds' ability to perform normal behavioural tasks. Our choice not to sham-treat control birds with respect to the zinc sulphate treatment does not impact the inferential power of this result, since it is therefore known that control birds did not have reduced foraging ability that could be attributed to the physical procedure of nasal irrigation. The ability of birds treated with zinc sulphate to perform generalised behavioural tasks is an important consideration when interpreting the results of anosmic release experiments (Wiltschko, 1996). The lack of a generalised response to zinc sulphate at sea expands upon the finding that Cory's shearwaters retain motivation for short-distance homing and resume normal incubation after treatment with zinc sulphate (Dell'Arciccia & Bonadonna, 2013) and is consistent with previous experiments in

homing pigeons. When treated with zinc sulphate solution, pigeons retain their ability to perform forced-choice memory tasks (Budzynski et al., 1998), and are able to home when unilaterally (one nare) irrigated with zinc sulphate providing that they are able to detect atmospheric odours through the second nare (S. Benvenuti and Gagliardo 1996). Our finding that anosmic shearwaters are able to time the initiation of their homing trips appropriately according to the distance that they had to travel further suggests that general cognitive processes underpinning navigation remain intact during zinc sulphate treatment. Moreover, that anosmic birds did not differ from controls in their track straightness indicates that anosmic birds' ability to maintain a heading, most probably by reference to a compass (Guilford & Taylor, 2014), also remained intact.

While we did not find any generalised effects of our sensory manipulations, we did find some specific effects of zinc sulphate treatment on pelagic orientation. The homing trajectories of anosmic birds during the pelagic phase of homing differed significantly to control and magnetically treated birds with anosmic birds' orientation not including the homeward direction, 0° , in their 95% confidence interval as they homed to within 40 km from the Balearic coastline. This was a strong effect, present in pairwise *post-hoc* tests with a conservative p-value correction for false positives. The effect of zinc sulphate treatment on orientation was restricted to the homing stage as we found no difference among treatments in the direction of the initial outbound stage of birds' foraging trips. However, anosmic birds were significantly more coastal (7.4 km for anosmic, 9.4 km for control and 9.6 km for magnetically disrupted birds) upon reaching 10 km from the colony as they departed on foraging trips, implying that shearwaters might make use of visual information more in the absence of olfactory cues. Our magnetic treatment had no effect on outbound orientation or distance to the coast relative to controls, during which time we expect that birds were still carrying their magnets; a finding that is consistent with studies reporting no effect of strong rare-earth magnets on free-ranging orientation in albatrosses (Bonadonna et al., 2005; Mouritsen et al., 2003) or homing following displacement in white-chinned petrel or Cory's shearwaters (Benhamou, Bonadonna, & Jouventin, 2003; Gagliardo et al., 2013; Massa et al.,

1991; Pollonara et al., 2015). It is possible that the navigational effect of our anosmic treatment could be caused by the physical discomfort caused by the treatment procedure, since control birds were not nasally irrigated at all. However, the normal behaviour of anosmic birds leading up to the onset of homing extends findings from previous studies which show that there is no impact of nasal irrigation with inert solutions on procellariiform behaviour (Benvenuti & Gagliardo, 1996; Bonadonna & Bretagnolle, 2002; Bonadonna et al., 2001; Dell’Ariccia & Bonadonna, 2013), constituting good evidence that our effect on homing orientation was caused by a lack of olfactory information rather than non-specific effects of the treatment which could include demotivation to home, poor foraging ability or an inability to time daily activities.

The finding that anosmic birds were motivated to embark on normal, successful foraging trips but nonetheless had impaired orientation during directed homing flight supports the view that birds make use of olfactory information during navigation back to their colony after foraging and is consistent with a navigational interpretation of previous displacement experiments in *Calonectris* shearwaters (Gagliardo et al., 2013; Pollonara et al., 2015) and other experiments across a broad range of avian taxa (Gagliardo, 2013; Holland et al., 2009; Wikelski et al., 2015). However, this is the first time that experimental evidence for a role of olfactory information in navigation has been found in a free-ranging bird and thus represents a significant advance in our understanding of pelagic navigation. In this study, we were able to see that olfactory information was not crucial to free-ranging movement within what is likely to be birds’ familiar areas but that an olfactory map is nonetheless consulted for the return leg of these trips even when birds are auto-displaced. Furthermore, that anosmic birds’ orientation recovered as birds came within 40km of the coast and that anosmic birds remain closer to land during outbound sections of foraging trips suggests that in the absence of olfactory information, shearwaters make use of visual topographic landscape features to guide their movement, a finding which is consistent with previous release experiments in which anosmic shearwaters spent significantly more time during homing within 40km of the coast than controls (Pollonara et al., 2015) and the shift from night to day homing

from short-distance displacement in order to find the nest while anosmic (Dell’Ariccia & Bonadonna, 2013). A peculiar result in this study, therefore, is that birds homing from the Catalanian coast were sufficiently well oriented initially to reach the Balearic archipelago but had poorer orientation than controls despite birds regularly embarking on their homeward trips from the Catalanian coast, an area rich with topographic information. This could be indicative of relatively poor spatial resolution in a bird’s visual map further away from the colony, an assertion further supported by the strikingly parallel orientation in five of the six anosmic birds’ tracks whilst homing from the Spanish coast, possibly indicative of birds simply heading away from the coast after foraging. Alternatively, birds might not make use of visual landmarks at all at the beginning of their homeward journey to the colony perhaps resorting to a compass bearing associated with the end of foraging or tied to knowledge of the outbound journey. However, if this were the case, one might expect some within-trip fidelity between the outbound and inbound sections of trips; an expectation not observed in the current study (see supplementary material).

Like other Procellariiformes (Dell’Ariccia et al., 2014; Nevitt, Veit, & Kareiva, 1995), Scopoli’s shearwaters are known to be attracted to dimethyl sulphide at sea and probably locate pelagic resources by olfaction (Dell’Ariccia et al., 2014). Given that anosmic shearwaters in this study were not able to compensate entirely for an absence of olfaction with visual information whilst navigating from the Catalanian coast it is perhaps surprising that they were able to locate food successfully without olfaction. It is possible that shearwaters are able to relocate large-scale foraging areas by making use of learned compass directions or local topographic information when departing from the colony. Once reaching these areas, area-restricted search is probably informed by both olfactory and visual information, such as congregations of other seabirds (Nevitt, 2000), which could allow anosmic birds to locate resources precisely. Over short time periods, this might be sufficient to exploit resources that are moderately ephemeral in distribution and resolve the apparent contradiction. Over longer time-periods than reported in the current study, the ability to locate large-scale resource areas might decline as resource location has to be

found again; a part of the process for which olfaction is likely to be more crucial. Our result is therefore consistent with a complex, multi-sensory search strategy that is likely to begin with a memory-informed outbound departure heading, augmented at different scales by olfaction and vision but the short-term nature of the experiment might preclude a reduction in foraging efficiency of anosmic birds over a longer time scale.

The effect of anosmia on the orientation of shearwaters during homing from distant foraging sites when flying in the absence of local visual cues in this study constitutes further evidence that olfactory information contributes to the map of seabirds navigating over their pelagic environment. These results confirm that in release experiments, differences in anosmic birds' motivation and foraging ability are unlikely to preclude a real navigational effect of being denied olfactory information and so provide a key progression in our understanding of seabird navigation.

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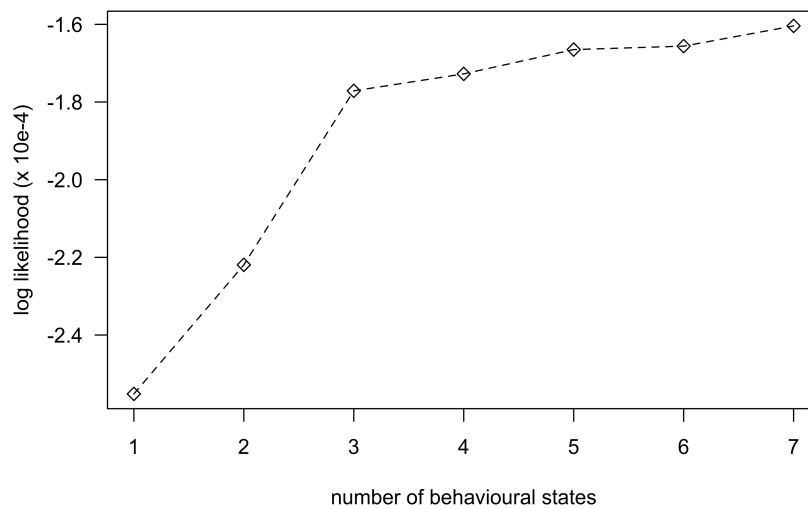
Supplementary Material

Figure S3.1. Log-likelihood of Gaussian mixture models with different numbers of putative states. The elbow indicates that a three state mixture model represents the best compromise between explanatory power and model fit. The states in our chosen mixture model were later identified as foraging, resting and commuting.

Table S3.1. Exploratory statistical analysis between magnetically manipulated birds that retained their magnets (n = 4) until recapture and those that did not (n = 6). The statistical methodology was the same as the main analyses described in the main text.

	Diurnal activity	GLMM		Nocturnal activity	GLMM	
	Mean±SE	X(2)	p	Mean±SE	X(2)	p
Foraging	With magnet: 0.34 ± 0.27 Without magnet: 0.35 ± 0.20	0.30	0.59	With magnet: 0.15 ± 0.21 Without magnet: 0.22 ± 0.17	2.93	0.12
Resting	With magnet: 0.36 ± 0.28 Without magnet: 0.36 ± 0.20	0.02	0.89	With magnet: 0.75 ± 0.21 Without magnet: 0.64 ± 0.17	1.52	0.22
Commuting	With magnet: 0.30 ± 0.26 Without magnet: 0.29 ± 0.19	0.19	0.66	With magnet: 0.10 ± 0.17 Without magnet: 0.15 ± 0.14	0.70	0.40

	Pelagic	GLMM		Coastal	GLMM	
	Mean±SE	X(2)	p	Mean±SE	X(2)	p
Homing orientation	with magnets: -6.07 ± 6.77 without magnets: 5.01 ± 4.00	2.75	0.10	with magnet: -9.74 ± 9.39 without magnetic: 3.63 ± 4.87	2.12	0.15

Entire trip

	Mean±SE	t	p
Mass gained	with magnets: 116.67 ± 42.06 g without magnets: 61.50 ± 36.44	t(5) = -0.97	0.38

Nearest neighbour method

The nearest neighbour distance (NND) gives a metric, in km, which is the average spatial distance between two tracks and therefore relates to the fidelity of two tracks. NND between two tracks, a focal track and a comparison track, is calculated by taking in turn each GPS fix on the focal track and finding the nearest (in space) GPS location on the comparison track. The distance between these two nearest neighbours is measured; giving each GPS fix on the focal track an NND to its nearest neighbour. The mean NND for the focal track is then taken as the nearest neighbour distance for that track from the comparison track. The distance varies slightly depending on which track is the focal and comparison track and so for the two tracks, the process is done reciprocally and averaged.

Fidelity of homing route to outbound route within each trip among treatments

To test whether treatment had an effect on birds' propensity to retrace their outbound route as they returned to the colony, we computed nearest neighbour distances (as above) between the outbound and homing sections of each trip. The homing section was defined as for other analyses by our Douglas-Peucker algorithm. The outbound route began at the colony. However, when choosing a point to define as the end of the outbound section, we wanted to avoid foraging behaviour close to the onset of homing whilst capturing the route that the shearwater had taken leading up to its position at the start of homing. To do this, we chose the first time that the outbound track came within a threshold distance of the position that the bird started homing (shown in Figure S2). For the threshold, we chose 10% of the distance between the colony and the start of homing for each trip since most behaviour outside of this threshold is active flight, rather than foraging or resting. However, since this choice is largely arbitrary, we conducted the analysis at 0% (i.e. all track before the start of homing was compared with all track after the start of homing), 5%, 20% and 50%. We chose to only analyse fixes labelled as in the flying state so that average distances were not heavily weighted by where birds chose to rest. Since the data comprised repeated measures from individual birds, we used linear mixed models to test for an

effect of treatment on the NND (fidelity) between the outbound and inbound sections of track.

The results are shown in Table S3.3.

Table S3.2. Table shows nearest neighbour estimates between outbound and inbound routes for each of the three treatments. The analysis is repeated for different thresholds for determining the end of the outbound section.

Threshold	LMM estimates of inbound-outbound NND	Likelihood ratio test statistics	
		$\chi^2(2)$	p
	Mean $\pm$ s.e.		
0%	Control: 32 ± 9 km	2.91	0.23
	Magnetic: 21 ± 8 km		
	Anosmic: 19 ± 6 km		
5%	Control: 33 ± 9 km	2.71	0.23
	Magnetic: 23 ± 9 km		
	Anosmic: 20 ± 7 km		
10%	Control: 34 ± 10 km	2.59	0.27
	Magnetic: 23 ± 9 km		
	Anosmic: 21 ± 7 km		
20%	Control: 34 ± 10 km	2.24	0.33
	Magnetic: 33 ± 10 km		
	Anosmic: 21 ± 7 km		
50%	Control: 32 ± 10 km	4.42	0.11
	Magnetic: 16 ± 11 km		
	Anosmic: 12 ± 8 km		

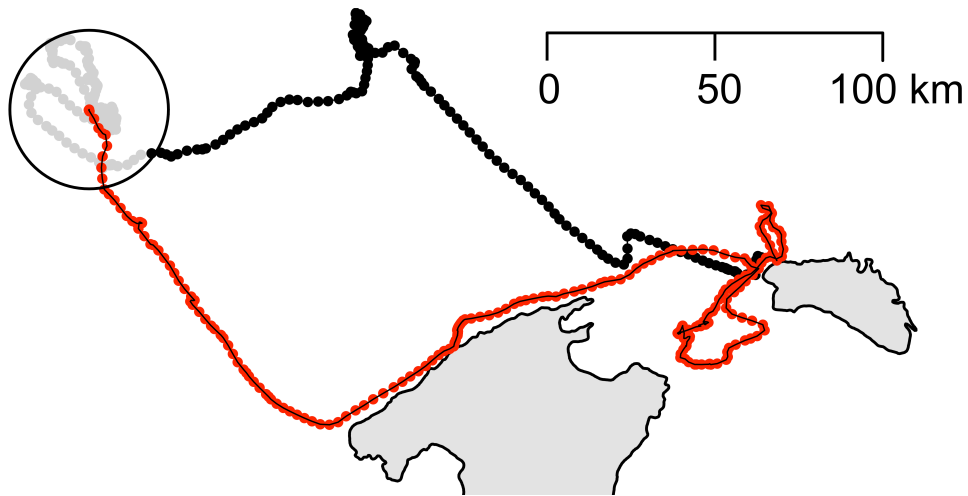


Figure S3.2. An example track is shown with outbound (black) and homing (red) sections of track. The circle represents the 10% distance contour around the start of homing. The end of the outbound section is defined when the bird first comes within the contour. Maps were generated in R using the “maps” package.

Repeat trip fidelity within birds

For individual birds that underwent multiple trips during the experiment, we measured the NND pairwise among each bird's trips. For example, a bird which underwent three trips would have the NND for trip 1 and 2, 2 and 3 and 1 and 3 calculated in both directions (the reciprocal) and averaged to give a measure of that bird's repeat trip fidelity. This was done for the outbound sections of trips, from leaving the colony to the furthest point reached from the colony on that trip since this would capture fidelity in foraging destination. This gave within-bird NND measures that could be compared among treatments. Because only 10 birds underwent multiple trips during the experiment, these are exploratory statistics on a small data set ($n = 13$) but show no evidence of any difference in fidelity across treatments that might have been caused by anosmia.

Table S3.3. Nearest neighbour distances (NND) are shown for repeat tracks from the same birds, and compared among treatments. There was no difference among treatments (GLM: $F = 0.10$, $df = 2,10$, $p = 0.91$)

	Mean $\pm$ s.e.
Control (n=3)	NND: 37.8 ± 11.4 km
Magnetic (n = 6)	NND: 39.6 ± 9.7 km
Anosmic (n = 4)	NND: 35.2 ± 7.5 km

Chapter 4

False release site experiments with a wild *Procellariiform* seabird: a pilot study

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Unpublished manuscript

Abstract	117
Introduction	117
Materials and Methods	122
Results	127
Discussion	132
Acknowledgements	137
References	137
Supplementary Material	143

Abstract

It is well established that anosmic pigeons are unable to home from an unfamiliar release site. One early experiment that showed directly that this is because homing pigeons use information about their direction of displacement from atmospheric odours was the false release site experiment (Benvenuti and Wallraff, 1985). In the false release site experiment, pigeons were allowed to smell air at a ‘false’ release site 25-55 km from the loft, before being transported in charcoal filtered air to a real release site at a similar distance to the loft, located diametrically to the false release site. Pigeons were then made anosmic before release, upon which they flew as though navigating toward the loft from the false release site (thus actually flew in the opposite direction to home). The similar scatter of experimental birds compared to controls, exposed to air for the same schedule at the real release site, suggested that pigeons need only intact air for normal homing orientation after release.

Recent olfactory deprivation experiments have shown that, like pigeons, Procellariiform seabirds might make use of olfactory cues in air for long-distance navigation across the ocean. However, it remains to be tested whether manipulation of olfactory cues can misinform shearwaters about their location after displacement. Here, we attempted to repeat the false release site experiment of Benvenuti and Wallraff (1985) on Scopoli’s shearwaters released at sea equipped with miniature archival GPS loggers to record their orientation. Logistical challenges meant that our experiment was unable to faithfully replicate that of Benvenuti and Wallraff (1985), but nevertheless our results indicate that experimental birds’ orientation might have been affected by the manipulation.

Introduction

The olfactory navigation hypothesis (Papi, Fiore, Fiaschi, & Benvenuti, 1972) is the most compelling explanation for how homing pigeons, *Columba livia*, determine their direction of displacement from unfamiliar sites in the first stage of the two-step map-and-compass process of

true navigation. The hypothesis is that pigeons are able to perform true navigation because in a learning phase, pigeons attend to the odours carried by winds that intersect the loft from different directions. After displacement to an unfamiliar site, they are able to determine their direction of displacement by remembering the wind direction associated with the particular composition of odours at the release site (Gagliardo, 2013). The olfactory navigation hypothesis was first proposed by Papi (Papi et al., 1972) to explain the finding that pigeons with a sectioned olfactory nerve were unable to home from unfamiliar release sites (Papi, Fiore, Fiaschi, & Benvenuti, 1971), though Wallraff (1970) had already proposed that an ‘atmospheric factor’ might be involved in pigeon navigation based on observations that pigeons raised in lofts shielded from the wind, but with a view of the loft locality had poor homing performance (Gagliardo, 2013). In the time shortly after Papi’s discovery, many studies showed that pigeons made anosmic by different procedures were unable to home (reviewed by Wallraff 1983), including by nerve section (Papi et al., 1971; Wallraff, 1980); nare-blocking tubes (Hartwick et al. 1977); local anaesthetic (Wallraff and Foà 1981; Wallraff 1982) and nasal irrigation with zinc sulphate solution (Benvenuti et al. 1992). Wallraff’s (1970) experiments suggested that these findings might be because anosmic pigeons are unable to access olfactory cues in the air, but it was recognised that olfactory deprivation experiments might not rule out a non-specific effect of anosmia on homing. Subsequently, very many studies have provided evidence of a specific role of olfaction in homing pigeon navigation (Gagliardo, 2013). For example, pigeons raised in lofts that automatically detect and reverse the direction of entering wind have reversed vanishing bearings upon release compared to controls raised in identical lofts without wind reversal (Ioale, Papi, Fiaschi, & Baldaccini, 1978). Similarly, pigeons reared in lofts where the wind entering is deflected through 90° have correspondingly shifted orientation after release (Baldaccini, Benvenuti, Fiaschi, & Papi, 1975; Kiepenheuer, 1978). Perhaps more impressively, pigeons exposed to artificial odours associated wind from specific directions could be induced to orient in the reverse direction to home at the release site (Ioale et al., 1990).

In contrast to experiments manipulating the wind direction at the loft during the learning phase, experiments manipulating the olfactory cues available to pigeons at the release site ('olfactory site simulation') have produced mixed results. Attempts to expose pigeons at the release site to air that has been transported from a second site ('air bag experiments') before making them anosmic at release have mostly found that orientation of experimental and control birds differ statistically (Ganzhorn 1990; Ganzhorn and Burkhardt 1991; Kiepenheuer 1985, 1986; Baldaccini et al. 1982), but have often not produced the expected reorientation toward the direction expected from the second site. These experiments are difficult to control (Holland, 2014) and sometimes both experimental and control birds have poorer orientation than nosmic birds, possibly because of changes in the chemical composition of air during transportation or storage (Benvenuti & Wallraff, 1985). Some of these experiments were also confounded (for example, experimental and control birds were released at different sites in Ganzhorn and Burkhardt, 1991) and some involved displacements as little as 7.2 km from the loft, where site familiarity is difficult to rule out.

An experiment that did successfully demonstrate that release site information could misinform pigeons precisely of their location was the false release site experiment of Benvenuti and Wallraff (1985). In an elegant design, three groups of pigeons were transported in cages that were actively ventilated through a charcoal filtered air (the efficacy of this method at removing information relevant to pigeon homing had been shown previously by Wallraff and Foà, 1981). The first (experimental) group to a false release site 25-55 km from the loft, where the charcoal filter was removed and air from the site was drawn through the cages for 3 hours to expose them to site-specific odour information. The second group (control) was transported to a real release site, located at a similar distance to the loft but in the opposite compass direction. Charcoal filters were again removed and air was drawn from the site for during the same 3 hours as the experimental group, after which filtration was resumed. Following exposure to air at the false release site, experimental birds were transported in filtered air to the real release site. A third group of birds

travelled with the experimental birds but the filtration of air was not interrupted. All birds were made anosmic with local anaesthetic (lidocaine) and released. The procedure was reciprocated between the two release sites. The finding that pigeons exposed to environmental air at the false release site (FRS) were oriented in the compass direction of home expected at the FRS provided some of the best evidence that pigeons derive positional information from atmospheric odours specific to a site. These findings, and those of other studies purporting to demonstrate olfactory navigation in pigeons have, however, been strongly disputed (Phillips & Jorge, 2014). The principal objection to the findings from the false release experiment is the olfactory activation hypothesis (Jorge et al., 2009), which suggests that olfactory information primes non-olfactory navigational pathways in the brain. Thus providing false, directional information provides some resolution to this dispute (though see Discussion, this chapter) since the FRS experiment attempts to distil the prediction down not to whether a bird is able to navigate or is motivated to do so, but whether it gleans the correct directional information from air from a particular site.

Given the likely role of olfactory navigation in pigeons, therefore, a key question is whether olfaction also facilitates true navigation in wild birds. The poor homing performance of displaced swifts, *Apus apus*, (Fiaschi et al., 1974) and starlings, *Sturnus vulgaris*, (Wallraff, Kiepenheuer, Neumann, & Streng, 1995) with bilaterally sectioned olfactory nerves suggests that it might. Moreover, a recent displacement experiment showed that anosmic grey cat birds, *Dumatella carolinensis*, displaced 1000 km east during migration were unable to correct their course and instead oriented like naïve juvenile birds, implying that olfaction might also play a role in orientation during large-scale migratory movements (Holland et al., 2009). Similarly, PTT-tracked lesser black-backed gulls, *Larus fuscus*, that had their olfactory nerve sectioned were unable to correct their migratory orientation (Wikelski et al., 2015). In both studies, anosmic, magnetically treated birds showed corrective orientation toward their normal migratory corridor.

The findings of these studies suggest that olfactory navigation might be widespread among birds. However, how far an olfactory map might extend, in what ecological environments it might be useful and how it might be learned in free-ranging birds remains unknown. Two large-scale displacement experiments provide evidence that olfaction might play a role in the orientation of Procellariiform seabirds, even homing over hundreds of kilometres at sea. In the first, Cory's shearwaters, *Calonectris borealis*, either made anosmic, magnetically manipulated by carrying a jostling magnet, were tracked with GPS and PTT devices after displacement in open sea 800 km from the colony in the east Atlantic. While all magnetically manipulated birds and control birds homed successfully, only one anosmic bird returned to the colony. Analysis of its PTT track suggested that it had come within sight of land by chance, and then used topographic features to home. This finding was supported in a second displacement experiment where Scopoli's shearwaters, *Calonectris diomedea*, subject to the same treatments were displaced within the Mediterranean basin. By contrast to the first experiment, 11 out of 14 homed successfully to the colony, but did so slower and by a more coastal route than controls or magnetically manipulated birds.

Thus there is evidence that a wide range of species might make use of olfaction to navigate. However, most evidence from wild birds comes from displacements following olfactory deprivation and it thus remains unknown whether olfactory information can be manipulated in a wild bird to misguide its orientation from a release site. Since manipulating airflow in the home range or at the nest of a wild bird is likely to be impossible, we attempted to see whether site-specific olfactory information provides map-type information in the Scopoli's shearwater by repeating the false release site experiment on GPS tracked Scopoli's shearwaters. In addition to being the first time that such an experiment has been performed on a wild species, this also constitutes the first attempt to conduct a false release site experiment on any species using GPS. Consequently, our inferences about birds' orientation are not confined to the vanishing bearings close to the release site. How orientation changes in the time following release might yield clear

clues about other, non-olfactory factors that might be involved in pelagic navigation in Procellariiform seabirds.

Materials and Methods

Scopoli's shearwaters' were monitored from 25th June 2016 until 21st July 2016, during the last third of incubation, at Cala Morell, Menorca, Spain (40°03'19.2" N 3°52'55.6" E). On 12th July 2016, birds were selected for release if they were on day two or three of their incubation stint such that they had strong motivation to home. Birds were caught either by hand or with a neck noose, and were assigned to treatments alternately as they were caught on the morning of the release. We avoided displacing birds that were on the first day of an incubation stint since Manx shearwaters, *Puffinus puffinus*, tend to exhibit lower homing motivation very early on during incubation (personal observation). Eggs could not be safely removed from the nest during the displacement. While there is a small risk of egg predation during the day, Procellariiform eggs are adapted to withstand periods of chilling (Boersma & Wheelwright, 1979). Thus we considered the risk of egg removal higher.

Immediately after capture, birds were fitted with modified commercially available GPS devices. We used Mobile Action I-gotU gt-120 GPS that with the plastic housing removed. Devices were waterproofed by housing in heat-shrink plastic, and programmed to take fixes at 1-minute intervals, which represented a compromise between resolution and battery longevity. GPS devices were attached dorsally to the birds, directly above their centre of mass using thin strips of TESA marine tape laid underneath small bunches of contour feathers and then wrapped around the device. Total device mass was no more than 18-grams including the tape attachment, which is approximately 2.5-3.5% of the mass of the tracked Scopoli's shearwaters.

Translocation

Birds were housed from capture at the colony in partitioned PVC boxes, lined externally with reflective emergency blanket to prevent over-heating. Boxes were transported by car from Cala Morell to the port of Cala en Bosc in southern Menorca. At the port, air entering the boxes was filtered in fine, activated charcoal, drawn through two cylindrical PVC filters measuring 30 cm x 15 cm tubed in parallel so that each could be removed without interfering with filtration (Figures 4.1 and 4.2). The PVC filters were filled with activated charcoal. Negative pressure to draw air through was provided by a 12-volt car vacuum cleaner attached via a sealed tube to the boxes with an airtight rubber ring at each seal. Boxes were closed and made airtight with 20 cm Parafilm when filtration began. The air inlet during unfiltered aeration was through a PVC tube 2 m above the boat to reduce the potential for pollution with odours from boat fuel.

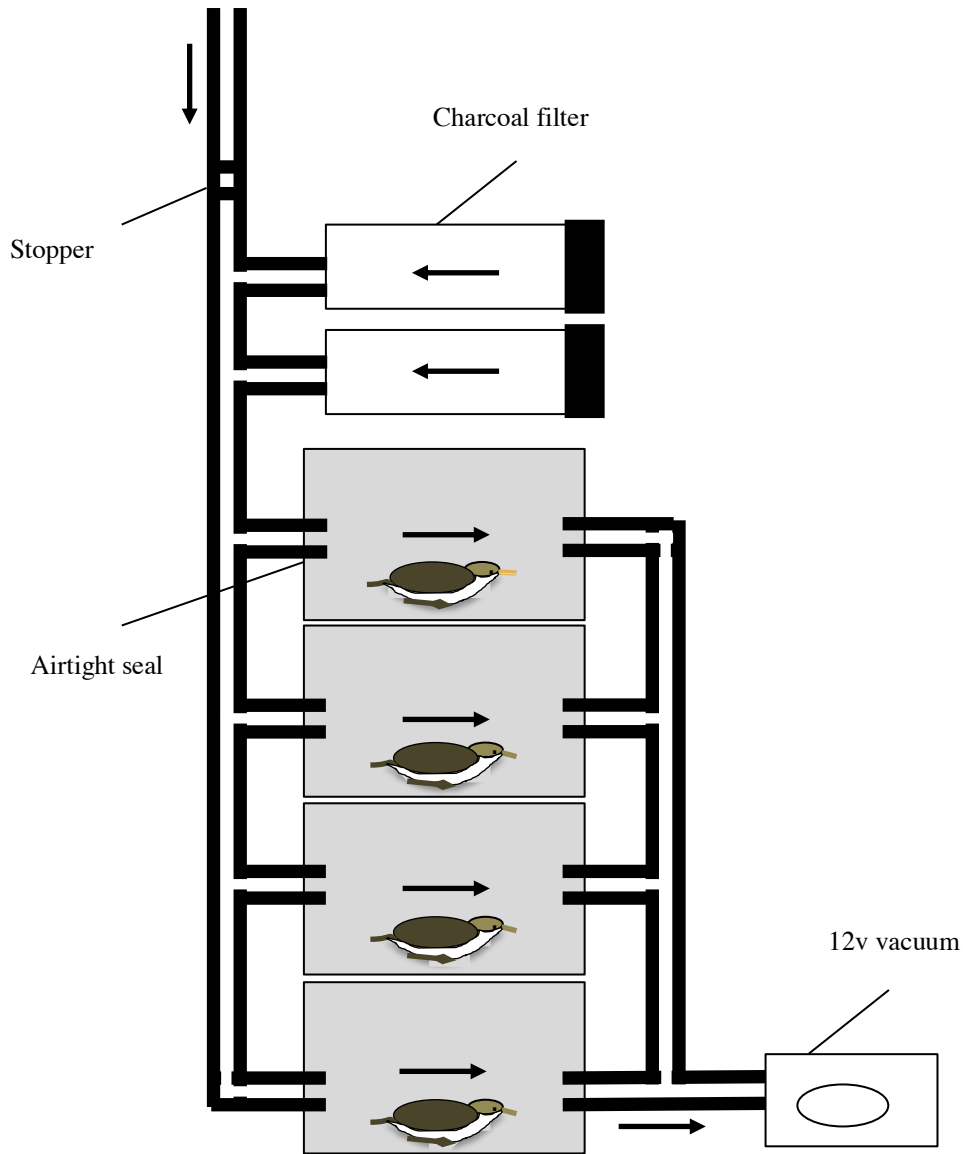


Figure 4.1. The translocation set-up for filtering air during the FRS experiment. The ‘real’ air stopper is shown for the filtered duration of releases, forcing airflow through the charcoal filters above the boxes. The flow of air caused by the vacuum pressure gradient is shown by the arrows.

False release site and release site

The translocation is depicted schematically in Figure 4.3. After being taken by car to Cala en Bosc, birds were taken by boat south to the false release site ($39^{\circ}40'3.2''$ N $3^{\circ}54'33.3''$ E), ~30 km

south of the colony at Cala Morell. Birds were then transported by boat to the real release site ($40^{\circ}19'39.2''$ N $3^{\circ}53'13.0''$ E), ~30 km north of Cala Morell (Figure 4.3).



Figure 4.2. Photograph of the FRS translocation containers, showing the PVC filters and piping to draw flow through the containers. The left-most stack is fitted with the tubing in parallel ready at the negative pressure (vacuum) end. Headtorch for scale.

The control of treatment during the translocation was similar to that by Benvenuti and Wallraff (1985), except for being sequential. The treatment control is outlined schematically in Figure 4.3. For control birds, the birds breathed filtered air throughout the journey. For FRS birds, the birds breathed filtered air throughout the journey, but were allowed 1 hour to breathe unfiltered air drawn through the boxes while the boat was positioned at the FRS. For RRS birds, filtered air was drawn through the boxes until at the real release site, when unfiltered air was drawn through the boxes for 1 hour. FRS and RRS birds were made anosmic immediately after removal from the box by washing of the olfactory mucosa via the nares with 4 ml of 4% zinc sulphate heptahydrate, dissolved in water, as in previous studies (Gagliardo et al., 2013; Pollonara et al., 2015). The zinc sulphate treatment acts by causing necrosis of the nerve cells in the olfactory mucosa. In homing pigeons, recovery of olfactory capacity can take many weeks, since new cells must divide,

differentiate, mature and form a neural connection to the olfactory bulb (Bedini, Fiaschi, & Lanfranchi, 1976). The use of zinc sulphate treatment for testing olfactory navigation has previously been criticised, since the affected area of olfactory mucosa is close (and innervated through), the trigeminal ganglion, one of the putative sites of magnetic reception in birds (Fleissner et al., 2003). Whilst Treiber et al. (2012), showed that putative iron rich structures in the trigeminal ganglion were in fact macrophages and not magnetosensitive nerves, it should be noted that pan-sensory effects of zinc sulphate treatment cannot be ruled out, but seem unlikely given seabirds' normal at-sea behaviour following anosmic treatments (Padget et al., 2017).

Birds were then given 30 minutes to recover from handling before being released individually. All releases were into wind so that birds found it easier to gain loft. Releases were separated by 10 minutes to reduce the chance that birds might form groups. GPS devices were then recovered after recapture at the colony. All experimental procedures were conducted in accordance with regional animal welfare legislation (BOIB 97 Decret 65/2004) and were approved by Oxford University's local ethical review process. Experiments were carried out under licence from the Balearic government (CEP 22/2016). The total time for which birds were incarcerated was less than 13 hours.

Track analysis and statistics

Tracks were analysed from the time of release to midnight on the day of release. Where birds initially rafted, we analysed tracks from the start of directed flight, which was distinguished from resting on the water using a hidden Markov model (HMM). The HMM recognises patterns in underlying speed and turning angle data calculated between consecutive GPS fixes that might relate to different underlying behavioural states and thus can objectively determine whether a bird is most likely to be resting or in flight for each GPS fix (supplementary material and Figures S4.1 and S4.2, supplementary material). Initial orientation was measured as 'virtual' vanishing bearings which were the angle between true north, the point where directed flight began, and a

bird's position as it crossed the arc with a radius of either 1, 5, 10 or 15 km from the position where the bird first underwent directed flight. We performed a Rayleigh test on the initial orientation to see whether shearwaters virtual vanishing bearings differed from random and a Rayleigh test with a specified direction to see whether birds were significantly oriented either toward home or the FRS-predicted directions. We used a Mardia-Watson-Wheeler test and a Wallraff test to examine differences in mean direction and dispersion respectively among our treatments. Secondary response variables that might relate to differences in motivation among treatments (timing of departure, mean flight speed and the proportion of the time spent in active flight) were analysed with an ANOVA or non-parametric Kruskal-Wallis test if the data did not meet the assumption of normally distributed residuals required by an ANOVA. Hidden Markov models were conducted using the “moveHMM” package (Michelot et al. 2017). All other analyses were conducted in the package “circular” (Agostinelli & Lund, 2015) or R base (R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>).

Results

Release success and impact on breeding success

17 Scopoli's shearwaters were released (6 FRS, 6 RRS and 5 control), and homing tracks were obtained from 14 (5 control, 6 false release site and 3 real release site birds, Figure 4.4). 10 birds resumed incubation on the night of release. One bird was seen at the colony on the first night but not subsequently recaptured and another bird was recovered without its device 10 days later. Nesting status was checked on 18th July. By this date, 1/6 FRS birds and 3/6 RRS birds' nests had suffered failure. The remaining control birds were incubating normally. All except one RRS bird travelled to within 15 km of the colony on the day of release. No birds travelled together (Figure S4.4, supplementary material). In our HMM analysis of at-sea behaviour, there was no distinct step in the log-likelihood of HMMs as we added additional states to our two-state model and so two underlying behaviours were best supported as a compromise between accuracy and

complexity (see supplementary material). Our two HMM states corresponded to one high speed with low turning angle and one low speed with very low turning angle. We interpreted these as directed flight and sitting on the water respectively. From this, initial periods of rafting were removed for subsequent analysis. All birds began sustained directed flight within 1 hour of release, except for a single FRS bird that rested on the water for two hours before beginning homing movement.

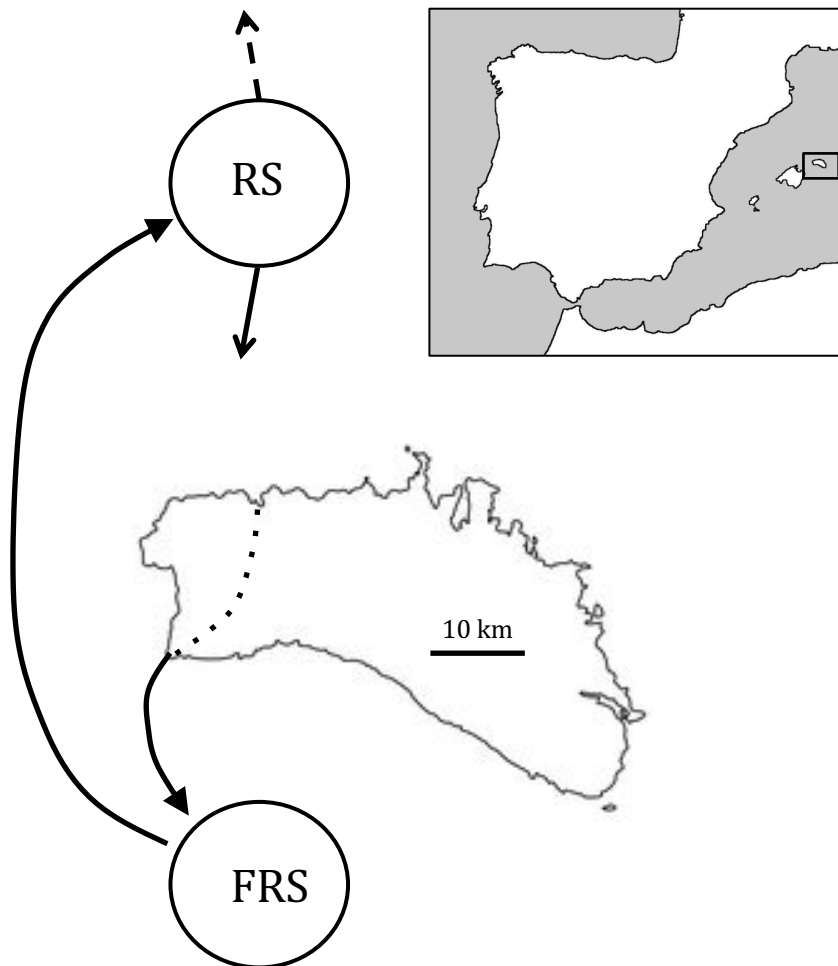


Figure 4.3. Map schematic showing the release site (RS) and false release site (FRS). The route from the colony to the port travelled by car is shown as a dotted line. The route taken by the boat is shown as a solid line. The dotted arrow and dashed arrow from the RS show the predictions for ‘real release site’ birds and ‘false release site’ birds respectively. The FRS and the RRS were 58 km apart.

Initial orientation and scatter

The virtual vanishing bearings in the first 1 km of sustained flight were only significantly different to random orientation for control birds (Rayleigh test: Control: $a = 0.98$, $p = 0.002$; FRS: $a = 0.46$, $p = 0.30$; RRS: $a = 0.44$, $p = 0.60$). Similarly, only controls were oriented towards the colony (Rayleigh's test with specified mean direction: mean: 141° (colony 182°), $a = 0.75$, $p = 0.007$), with the other two treatments being oriented neither towards the colony nor the expected direction for the false release site (FRS birds to false release site; Rayleigh test: $a = 0.05$, $p = 0.44$). There was a significant difference in the mean direction among the three treatments for the first 1 km of directed flight (Mardia-Watson-Wheeler test: $F = 6.33$, d.f. = 2,11, $p = 0.012$). Pairwise tests revealed that this difference lay between control and FRS birds (Mardia-Watson-Wheeler test: $F = 15.95$, d.f. = 1,9, $p = 0.003$), but not between either FRS and RRS (Mardia-Watson-Wheeler test: $F = 2.55$, d.f. = 1,7, $p = 0.15$) or control and RRS (Mardia-Watson-Wheeler test: $F = 0.69$, d.f. = 1,6, $p = 0.43$). This significance of this effect remained for the first 5 km, but was no longer evidence thereafter. The results for the 1, 5, 10 and 15 km virtual vanishing bearings are shown in Table 4.1 and Figure 4.5. Scatter differed significantly among treatments (Wallraff test: Kruskal-Wallis chi-squared = 6.7, df = 2, p-value = 0.03), but the effect was only apparent in the first 5 km of directed flight between controls and false release site birds with FRS birds being significantly more scattered than controls (Wallraff test: Kruskal-Wallis chi-squared = 4.8, df = 1, p-value = 0.03).

Secondary response variables

There was no difference in the time that birds began oriented flight after release (Kruskal-Wallis chi-squared = 0.27, df = 2, p-value = 0.87), the mean speed during directed flight (ANOVA: $F = 0.24$, d.f. = 2,11, $p = 0.79$) or the proportion of time in active flight following release until 20:00 UTC when the first birds arrived at the colony (Kruskal-Wallis chi-squared = 0.15, d.f. = 2, $p = 0.93$).

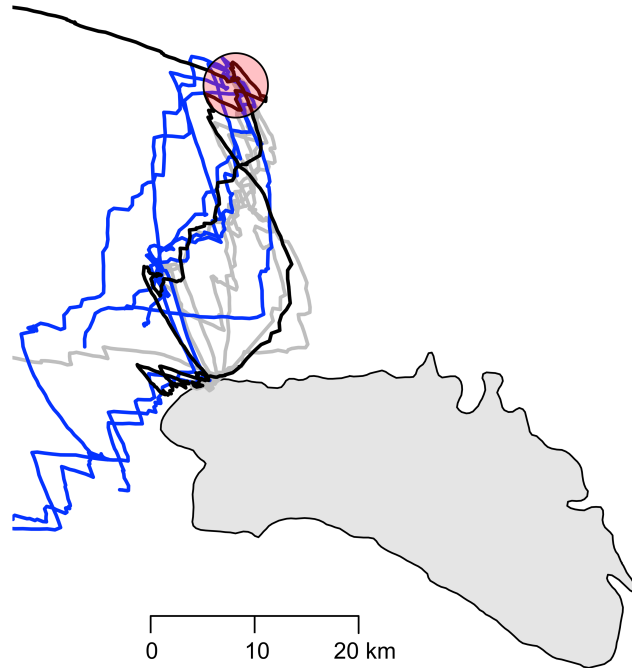


Figure 4.4. Map of release tracks from the time of release until midnight on the day of release. False release site birds (blue), real release site (grey) and control (black) are shown. The release is at the centre of the pink circle. All birds began directed flight within that area.

Table 1. Table shows statistics for the different virtual vanishing bearing radii. Mean is the circular mean bearing of the birds at that distance from the onset of directed flight. P-value is for Rayleigh's test with a specified mean direction (the colony). The homeward direction from the start of directed flight is 182°. Significant p-values (alpha = 0.05) are in bold.

Virtual VB radius	Control			False			Real			Mardia- Watson- Wheeler	
	mean	a	p	mean	a	p	mean	a	p	F	p
1 km	141°	0.98	0.007	273°	0.47	0.51	177°	0.45	0.14	6.63	0.013
5 km	145°	0.99	0.004	225°	0.45	0.48	223°	0.45	0.15	5.8	0.02
10 km	173°	0.99	<0.0001	196°	0.93	<0.0001	212°	0.63	0.1	1.46	0.27
15 km	175°	0.98	<0.0001	197°	0.96	<0.0001	214°	0.64	0.1	1.53	0.26

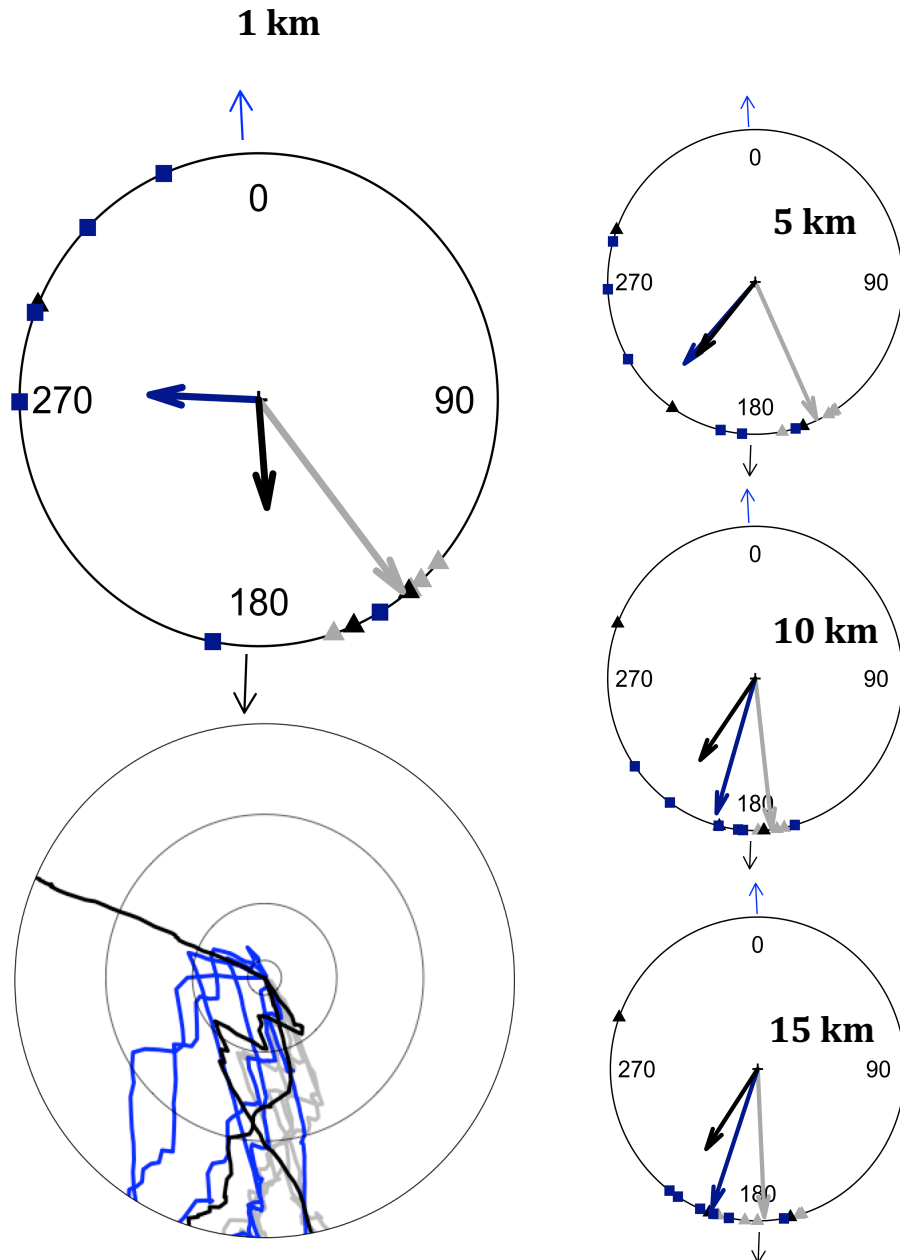


Figure 5.5. Circle plots showing the initial orientation of Scopoli's shearwaters, shown as virtual vanishing bearings with 1, 5, 10 and 15 km radii. For 1 km, control (black triangles), false release site (blue squares) and real release site (grey triangles) birds (circle perimeter) are shown. Vectors show the mean direction of each control group in the same colours. Vector lengths are dispersion (Rayleigh's a). Outer arrows show homeward direction (black) and homeward direction predicted for FRS birds (blue). Tracks adjusted such

that directed flight begins from the same location (0,0) are shown with contours showing the 1, 5, 10 and 15 km virtual vanishing bearing radii.

Discussion

Here, we attempted to perform a false release site experiment on a wild bird for the first time to see whether olfactory information could be manipulated to provide a wild Procellariiform seabird with false information about the direction of displacement. Furthermore, by GPS tracking birds released after being exposed to false release site information we were able to observe the effect of treatment well beyond the initial release. For logistical reasons beyond our control, we were unable to stay for 3 hours at each release site to accurately replicate the experiment of Benvenuti and Wallraff (1985). Furthermore, our sample size was small, we had poor recovery rate of real release site birds and did not manage to travel sufficiently far north to be entirely out of sight of Menorca, where, at 30 km north of Cala Morell, a sliver of land could be seen on the horizon to the south (the topography of Menorca predicts that this should vanish beneath the horizon by 37 km). A further potential issue with our study was that the treatments were not proper cross-controls, since FRS and RRS birds differed not only in the site where they were exposed to natural air, but also in the length of time between exposure to potentially informative cues and release. In this study, the false release site birds had longer than real release site birds between exposure and release and so any effect resulting from this time confound should have been to increase noise rather than produce a false-positive orientation in the expected FRS direction. Thus, despite these inherent problems, we conducted exploratory statistics on the virtual vanishing bearings of our released birds. Anosmic birds mostly homed successfully, and the initial phase notwithstanding oriented homeward similarly to controls. Regardless of the specific manipulation in this experiment, this confirms the expectation (Chapter 3, Pollonara et al. 2015) that after a short displacement, neither olfaction nor outward journey information are crucial to homing.

In addition, however, there was a statistical indication that our treatment had some effect on birds' initial orientation after release over the first 5 km of directed flight, since FRS birds and controls'

orientation differed significantly in the first 5 km of directed flight. Furthermore, visual appraisal of the virtual vanishing bearings suggests that, in this initial stage, four of the six FRS birds were oriented more closely to the expected direction for that treatment under the false release site expectation than the homeward direction, suggesting that the difference in orientation among our treatments here might be because FRS birds incorrectly interpreted their location as being to the south of the colony despite there being a small amount of visual information available. The larger scatter of FRS birds than control birds suggests, however, that their navigational information may have been less reliable than that of control birds. This could be because FRS birds had to remember their location from the false release site (there was a six hour delay between exposure to odours and release), or because FRS birds had incomplete navigational information because they were not exposed to false release site air for sufficiently long.

Our real release site sample was not statistically viable ($n = 3$), and so the mean orientation and dispersion calculated are poor estimates of the population mean and should not be given analytical weight. Since the false release site experiment hinges on the comparison between false and real release site birds, this means that little can be concretely inferred about olfactory navigation from the result of this pilot experiment. However, it is worth considering the orientation of individual RRS birds. The mean orientation of RRS birds was particularly weighted by one bird, which flew northwest and was oriented almost diametrically to the second and third RRS birds. The resulting vector for that treatment was therefore not a good indication of direction for that treatment. The single, poorly oriented RRS bird was the only bird that showed no homeward oriented flight on day of release (all other birds came at least 50% closer to the colony from the release site on the day of release suggesting that at least initially, they were motivated to home) and thus its inclusion in the analysis is conservative. Visual inspection of the three RRS tracks shows that the other two RRS birds were nested within controls and consequently, one tentative explanation is that the RRS birds were well oriented but a single bird did not attempt to home. If this bird is excluded, the two remaining RRS birds seem to have been able to gain sufficient information from the

vacuum drawn air at the real release site to orient properly and had similar initial orientation to controls. If this were to prove robust in future studies, then in Scopoli's shearwaters the process itself of being transported in charcoal filtered air and being made anosmic before release is unlikely to have caused the difference in initial orientation between control and FRS birds and the initial orientation of four of the FRS birds is in striking accordance to our directional expectation.

The difference in orientation between control and FRS birds that we found in our first 1 km virtual vanishing bearing, which was chosen arbitrarily, becomes considerably weaker at 5 km and vanishes at the 10 and 15 km VVB radii as false release site birds become well oriented towards the colony; though qualitatively the orientation of the FRS birds remains poorer than controls throughout the first 15 km of directed flight analysed here. Our analytical choices (primarily which bins to analyse) in this case were decided post hoc and so it is not realistic that we can quantify the probability of observing an effect at one radius and not another by chance with a p-value. Nonetheless, a reasonable biological hypothesis might be that initial orientations of FRS birds were initially oriented based on misinformation from the false release site, roughly as predicted by the olfactory navigation hypothesis. Soon after release, however, birds were able to gather other (perhaps visual) navigational information and re-inform their location, possibly based on a familiar area map of local topography.

The false release site experiment in pigeons was one of the early experiments to show that pigeon orientation can be manipulated by exposure to site-specific olfactory information. There have been successful replications of the result in pigeons (Kiepenheuer 1985; Jorge et al. 2009), though different interpretations exist (Able, 1996; Kiepenhauer, 1986). In a similar experiment, Kiepenheuer (1985) released pigeons that had been driven to a false release site in unfiltered air before being placed in airtight containers and either transported to a second release site in the opposite direction via the loft or transported back to the same release site via the loft. He found

that in general the vanishing bearings of FRS pigeons were statistically in the opposite direction to the loft but argued that a residual northeast tendency across all birds implied that other factors might be involved (Kiepenhauer 1985). Despite a possibility that this can be explained by pigeons from the same loft displaying home-independent orientation after releases (Kiepenhauer et al., 1993; Wallraff 1982), the study is cited as evidence that non-olfactory information is incorporated into homing (Wiltschko, 1996). The apparent discrepancy between the results of airbag experiments and the false release site has also been cited as evidence that the role of olfaction in homing, rather than being the source of map-information, is to prime reception of non-olfactory navigational cues (the olfactory activation hypothesis Jorge et al., 2009; Phillips and Jorge (2014). However, critical experimental evidence for this comes from a single study, in which pigeons primed with odours that provided no navigationally relevant information ('non-sense' odours) restored navigational ability in pigeons that were otherwise transported in filtered air before and subjected to bilateral irrigation with zinc sulphate upon release (Jorge et al., 2009). These results are at odds with the results of the many historic experiments manipulating wind cues at the loft (Gagliardo, 2013), though fuel on-going debate. Gagliardo et al. (2011) found, in a replication of the study of Jorge et al. (2009), using GPS data, that pigeons did in fact require intact environmental air, and that homeward orientation could not be recovered by exposure to non-sense odours drawn through airtight containers housing the pigeons before release. The results supported an earlier finding (Papi 1974) that nonsense odours had a disruptive effect on pigeon homing, but the study has been criticised for providing a high concentration and intermittent schedule (50ml injection every 20 mins) of odours that might have been disruptive for navigation (Phillips & Jorge, 2014), since previous studies have suggested a disruptive effect of odours on homing ability unrelated to navigation (Benvenuti, Fiaschi, & Foà, 1977).

The false release site experiment aims to test whether directional information is gained from air, rather than whether olfactory stimulation is required to facilitate navigation. It thus overcomes some of the objections to conventional displacement experiments raised by proponents of the

olfactory activation hypothesis. Nonetheless, it fails to control completely for the possibility that birds' navigational systems might in fact be primed by odours at the different sites visited, though a wealth of evidence suggests that this is not the case in homing pigeons (Gagliardo, 2013; Gagliardo et al., 2011). On the basis of the results presented here, it is therefore worth considering whether the protocol can be used, in wild Procellariiform seabirds, to investigate whether olfactory information provides similar kinds of positional information as thought to be involved in homing pigeon navigation. The current study highlights that experimental manipulations that have been previously conducted on pigeons might prove informative even in birds that routinely navigate hundreds of kilometres over open water (Bonadonna et al., 2003). In future, a situation could be created experimentally in seabirds where orientation expectations in a FRS experiment differ depending on the structure of the putative olfactory map consulted by birds. For example, the expected orientation of a bird navigating home from the FRS in the current study changes depending on whether we expect olfactory information to be nested in a multi-sensory mosaic map of remembered, patchily distributed odours and landmarks (Papi et al., 1972) or as a gradient map (Wallraff 1980). Under the former hypothesis, we would expect FRS Scopoli's shearwaters to have a greater westwards component in their initial vectors since the most efficient compass direction home would have, in previous experience, involved the avoidance of western Menorca (this issue is discussed in Chapter 5 of this thesis).

The false release site paradigm in Procellariiform seabirds could therefore indicate aspects of how an olfactory map might work that have not been possible in the homing pigeon model. A final positive consideration regarding the use of experimental manipulations of Procellariiform seabirds might be worthwhile is that, in release experiments, homing orientations seem to be better concentrated than in the homing pigeon (in this study the concentration of control birds, measured as dispersion, was 0.98, see also Matthews 1953; Matthews 1955). The scatter inherent even in nosmic homing pigeons can dramatically reduce the statistical power studies. Thus, while the current experiment was not successful at demonstrating that Scopoli's shearwaters make use

of site-specific olfactory cues to orientate, the indication that there was an effect on orientation immediately after release suggests that it might be a worthwhile endeavour in the future, both for extending the discoveries in homing pigeon navigation to ecologically diverse situations in wild birds and for distinguishing between different hypothesised structures that an olfactory map might take.

Acknowledgements

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113–9.

Supplementary Material

Fitting the hidden Markov model

To identify the start of directed flight after release, we split behaviour based on the GPS data into resting on the water and directed flight using a Hidden Markov model (HMMs), similarly to Dean et al. (2012). In this instance, we assumed that some combination of underlying behavioural states give rise to the observed probability distribution of speed and turning angle. Given a number of states, the HMM finds the most likely mixture of distributions to give rise to the overall probability distribution. To decide on the number of states, we ran an HMM with 2-10 states. Given that there is a trade-off between a better model fit with more states (the addition of states always improves model fit) and the explanatory power of each state, we fitted two states based on the fact that no further addition of states drastically improved log-likelihood in the model. For a time-series, a transition probability matrix is calculated and imposed to inform each location's state likelihood thus disambiguating between emissions with similar probability of being attributed to > 1 state. We first interpolated the tracks by cubic splines (Tremblay et al., 2005) to exactly 5 minute intervals (Figure S4.1, supplementary material) and then fitted the HMM to step length and turning angle calculated between consecutive interpolated GPS fixes (Figure S4.2, supplementary material). Step length and turning angle means were initiated using k-means clustering and standard deviations (or concentration parameter for step length) were initially uniform.

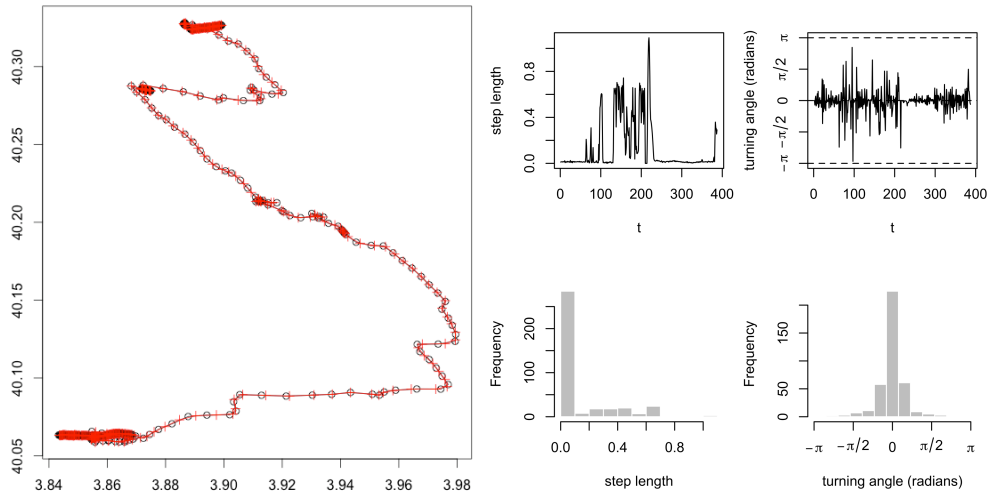


Figure S4.1. Left: Example of interpolation by Cubic Splines (Tremblay et al., 2005). Deviations from one-minute resolution, caused by the time taken for GPS devices to acquire satellites, in the original GPS track (black circles, average interval = 62 seconds) are not tolerated by the HMM processing. Tracks are interpolated to redistributed fixes at exactly 60 seconds (red crosses). Rafting behaviour following release can be seen at the top of the map as a series of GPS fixes close together (low speed, low variation in turning angle). **Right:** Example of step length and turning angles for one of the displaced birds during homing. The HMM assumes that these emissions are given by a sequence of discrete behavioural states, each with a different emission probability distribution. The HMM finds the most likely probability distributions from two states that give rise to these data. These are interpreted as resting and directed flight and are then mapped onto the track with the Viterbi algorithm.

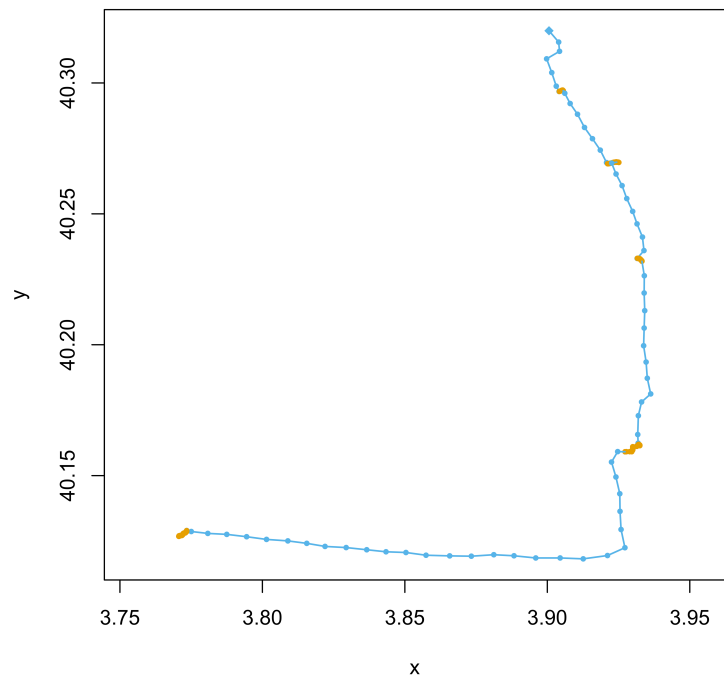


Figure S4.2. Example release track showing periods of direct flight (blue) and rafting on the water (orange).

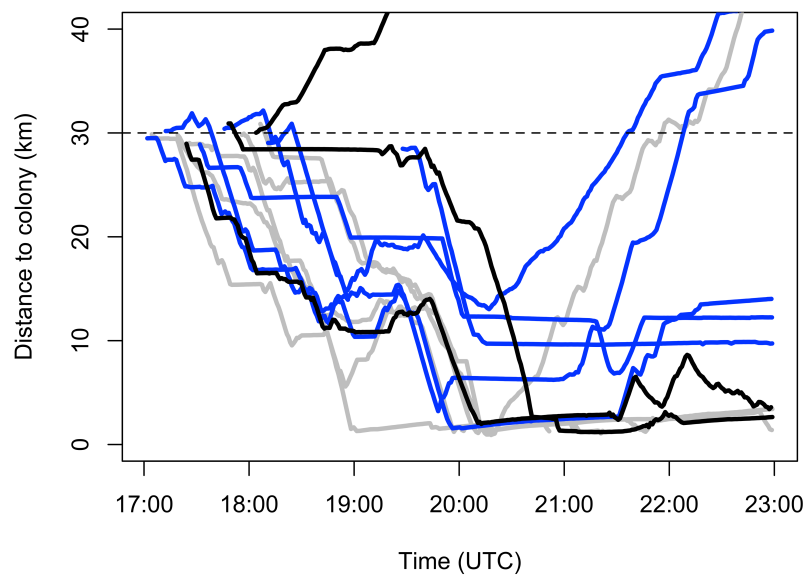


Figure S4.3. Homing schedules of birds following the day of release, with control (grey), FRS (blue) and RRS (black) birds shown. A single RRS bird was the only bird that did not initially move towards the colony. A single FRS bird rafted on the water for two hours before the onset of homing.

Chapter 5

An extrapolated map for navigation in an ocean wandering seabird

Oliver Padget, Geoff Stanley, Jay K. Willis and Tim Guilford

Unpublished manuscript

Abstract149

Introduction149

Materials and Methods152

Results160

Discussion168

Acknowledgements171

References172

Supplementary Material178

Abstract

The ability of animals to home from unfamiliar areas indicates that they may use knowledge of environmental gradients to inform orientation beyond previous experience. However, while much is known about the sensory basis for animal navigation, no study has tested directly the ability of animals to extrapolate environmental cues to infer their position relative to a goal. Here we show that a free-ranging ocean-wandering Procellariiform seabird, the Manx shearwater, *Puffinus puffinus*, extrapolates its position to inform both its direction and distance from home after foraging trips without depending on egocentric information. Because shearwaters do not fly over land, homing that commences beyond landmasses (islands or peninsulas) puts into conflict the route and timing of homing depending on whether they consult a true extrapolated map or a learned familiar location map. We analysed the subset of a large dataset (339 of 732 tracks) of Manx shearwater foraging trips where, at the start of homing, these two mechanisms are put in conflict, and observed that rather than take the shortest route home, shearwaters' initial homing orientations were more often constrained to the Great Circle beeline route implying that they do not anticipate topographic obstacles. Furthermore, shearwaters planned the timing of their journeys home by reference to the beeline distance to the colony at the start of homing. Taken together, this comprises evidence that shearwaters can consult some kind of true extrapolated map, but that they augment this with a visual familiar area map when it is available.

Introduction

Map and compass mechanisms are fundamental to animal movement beyond familiar areas (Kramer et al., 1957; Schmidt-Koenig, 1961; Wallraff, 2003; Wiltschko & Wiltschko, 2003), but how highly mobile animals interpret their location from environmental cues in the map-stage of large-scale movements is not fully understood (Geva-Sagiv et al., 2015; Holland, 2014). While the sensory basis of large-scale navigational maps can be investigated through translocation experiments following sensory deprivation (the displacement paradigm e.g. Papi et al. 1972; Gagliardo et al. 2013), how spatial information inherent in environmental cues is learned and

guides oriented movement remains a more intractable problem and candidate mechanisms are still a theoretic construct (Holland, 2014). Here, we focus on this cognitive architecture rather than the sensory basis of animal navigation.

The observation that many vertebrates (especially birds) and at least one invertebrate are able to orient from previously unvisited sites without cues directly emanating from a goal (Boles & Lohmann, 2001; Matthews, 1953; Phillips et al., 1995; Thorup et al., 2007), known as true navigation (Holland, 2014; Phillips et al., 2006), suggests that birds and other mobile animals might learn how gradients in environmental cues operate in space, allowing them to extrapolate their direction of displacement beyond previous experience (Holland 2014; Wiltschko and Wiltschko 2003; Wallraff 1990; Wallraff 2005). One interpretation of this is a true extrapolated map (Box 5.1), where, after release from an unfamiliar location, an animal could sense the magnitude and direction of cue differences between their location and the target along two or more known cue gradients, providing them the geometric architecture necessary to compute a vector of both direction and distance to the goal at the start of homing (Benhamou, 2003). A unique feature of a true extrapolated map is thus that distance is encoded, a prediction that the popular displacement paradigm cannot resolve since there is no predicted difference in direction amongst the putative cognitive mechanisms.

We used a large multi-colony GPS dataset of free-ranging Manx shearwaters ($n = 732$) undertaking routine foraging trips, sometimes over huge distances of up to several thousand kilometres from the home colony, to investigate whether navigation home from distant at-sea sites is by reference to a true extrapolated map. We assume that shearwaters will, within the constraints of their navigational mechanism, attempt to home after foraging via the shortest route to the colony. Because shearwaters will not naturally fly over land (Brooke, 1990), with the exception of short flights to visit the nest, the shortest route to the colony will not be the beeline if homing begins on the far side of a topographic obstacle (island or peninsula, Figure 5.1). We therefore

identified algorithmically the start of homing behaviour and analysed shearwaters' initial homing orientation when journeys began from such locations ($n = 339$) to see whether shearwaters anticipate obstacles between them and the goal. We then analysed whether shearwaters judge the distance that they must travel at the start of homing journeys by the beeline distance or the shortest over-sea route, since like other shearwaters (Shiomi et al., 2012), Manx shearwaters time their journeys home such that they arrive at the colony shortly before dark (they only attend the colony nocturnally, Brooke 1990). We then investigated the role that visual information might play in orientation and timing of homing by computing the theoretically visible horizon from the start point of each homing track.

Box 5.1: *Putative map structures*

1. **Cue direction** (the 'mosaic map' as proposed by Papi et al., 1972 in the olfactory navigation hypothesis): Distinctive olfactory regions are located around the bird's familiar area. The bird learns the direction of the wind from which particular odour compositions emanate during a learning phase at home. After displacement, the bird reverses the remembered wind direction associated with the composition of odours experienced at the release site and consults a compass to effect homing. There is no encoding of an environmental gradient, and after release to an unfamiliar area, distance cannot be determined.

2. **Cue ratio direction** (Wallraff's 1990 gradient map): An extension of the olfactory navigation hypothesis (Papi et al. 1972) to explain long-distance navigation (Gagliardo, 2013). Two or more odours (or odour information) are distributed systematically along non-parallel axes over large distances (e.g. biogenic odours with latitude). The ratio of the two odours therefor varies predictably with different angles radiating away from the home area. The mechanism from then is as (1). Distance cannot be determined.

3. **True extrapolated map** (Wallraff 1974; Wiltschko and Wiltschko 2003): Two or more olfactory or magnetic gradients are distributed systematically along non-parallel axes. The bird can perceive the sign and magnitude of cue differences between those remembered at the home site and those experienced upon release. Therefore a homeward vector can be computed based on bi-coordinate geometry. The gradient direction of two or more, non-parallel cues must be learned latently by gaining experience of the cues around a familiar area. Distance is encoded.

4. **Generalisation from the best known solution.** The bird learns through exploration, the relative positions of landmarks to home. This could either be learned by rewarded associations of compass cues with learned sensory landmarks or even circumstance (Guilford and Burt de Perera 2017), or by 'cognitive map'-type path integration, associating similarly sensory landmarks with a location that is represented geometrically in the brain (McNaughton et al., 2006). If 'landmark' cues were based on salient sensory stimuli that varied with monotonic or linear predictability over a landscape, animals at an unfamiliar release site might be able to generalise compass directions home from remembered areas with similar stimuli, which by virtue of their spatial distribution, will be closest in Euclidean space to the unfamiliar site (Guilford & Burt de Perera, 2017). Distance cannot be encoded (though distance can be learned within the familiar area).

Materials and methods

Manx shearwater tracking

Manx shearwaters were tracked as part of an on-going study on five islands: Skomer Island (51°44'15.7"N, 5°16'58.8"W) and Skokholm Island (51°41'38.6"N, 5°17'7.5"W), South Irish Sea, Wales; Lighthouse Island (51° 41' 38.5"N, 5°31'32.9"W), Copeland Islands, Northern Ireland; Rum (56°58'59.4"N, 6°17'41.3"W), Hebrides, Scotland; Lundy (51°10'7.6"N, 4°40'7.8" W), Bristol Channel, England. These five islands widely sample the Irish Sea distribution of the species, with Skomer and Rum alone constituting ~50% of the world's population. To track Manx shearwater foraging trips, we used Mobile Action I-gotU gt-120 GPS devices scheduled to take fixes at 5, 10, 15 or 60-minute intervals. Devices were waterproofed in heat-shrink housing before being attached dorsally to the birds using TESA marine tape as in Guilford et al..(2008). Depending on battery configuration, GPS devices weighed 14-19 grams at deployment and comprised no more than 5% of the birds' body mass. Birds were caught by hand from specially adapted study burrows equipped with an inspection hatch, except for on Lundy where they were caught using purse nets. Handling time was normally less than 15 minutes.

Ethical note

The impact of the GPS tagging presented here for the data from 2006-2015 has been previously reported in several studies (Dean et al., 2012, 2015; Fayet et al., 2015; Shoji, Aris-Brosou, Culina, et al., 2015; Shoji et al., 2015) which found no measurable impact of tracking on shearwater productivity compared to a control. All methods were approved by the University of Oxford's local ethical review process (AWERB) and were carried out with under licences from the British Trust for Ornithology (C/6128) in addition to local licences issued by the Northern Ireland Environment Agency and Natural Resources Wales.



Figure 5.1. Schematic map of Britain and Ireland showing Skomer Island (star) and a hypothetical shearwater at the start of homing. The minimum path and beeline route are shown (dotted lines), showing the expected orientation of the shearwater under either path. A shearwater that is consulting a learned map will orient to avoid Ireland, whereas a shearwater extrapolating its position will orient along the beeline until it reaches the Irish coast.

Track processing and identifying the initiation of homing

Tracks were first interpolated to exactly 5 minute intervals using cubic spline interpolation using the “pchip” function in R (Tremblay et al., 2005). We developed an algorithm to objectively and repeatably identify the decision point at which shearwaters start to home after foraging, based on knowledge of a shearwater’s foraging trip and their rate of change of distance from home over time. We first implemented the Douglas-Peucker line simplification algorithm on the each bird’s distance to the colony over time, for the subsection of each trip from the point at which the bird was furthest to the colony, until it came within 20 km of the colony. The Douglas-Peucker algorithm’s sensitivity parameter was incrementally increased from that which gave no

breakpoints until the gradient between last breakpoint and the end of the track was steeper than that of the furthest distance and the end of the track. The breakpoint that gave the steepest remaining section was then identified as the initiation of homing.

Validating the identification of homing initiation

We validated the putative point that homing began by first identifying the at-sea behaviour of the shearwaters and then identifying changes in behaviour that coincide with the identified initiation of homing. We fitted a Hidden Markov Model (HMM) to speed and turning angle (Michelot et al., 2017) to identify clusters in the data which we hypothesise correspond to underlying behavioural states. We calculated the HMM for different numbers of states from 1:10 and then chose the number of states which were the best compromise between model fit and explanatory power by identifying an elbow in the increasing log-likelihood of models (Dean et al., 2012). As in previous studies (Dean et al., 2012; Fayet et al., 2015), three states was the best supported by our data. We interpreted our three states as directed flight, foraging and resting based on their high, mid and low respective speeds. We then tested whether the proportion of directed flight, foraging and resting shifted consistently with our expectations for the start of homing (more flight, less foraging and resting) for a period before after the putative initiation of homing (the first 25% of the homing track and the same time before the start of homing). This was then computed for randomly assigned homing start-points in the final 75% of each track.

Beeline and minimum path distance

The beeline route was defined as the Great Circle distance (Vincenty ellipsoid formula) between each bird's location when it initiated homing and its colony. The minimum path route was the route from the same point that a bird would need to travel to get home flying only over water, navigating around land features (Figure 5.1). This was computed initially by using the 'raster' and 'gdistance' packages in R to find the minimum-cost route over a rasterized map of the northeast Atlantic from the location that homing was initiated to the colony with land set as an

impossibly high cost to traverse. We applied an inbuilt (16-neighbour) correction for Manhattan distance to Euclidean distance (a problem inherent with rasterized distance measurements), but this needed to be refined further and so we then applied an algorithm that iteratively placed Great Circle routes along the resulting path to shorten the path until part of the path crossed land.

Calculating the visible horizon

We used the gridded 30-arc-second GLOBE topographic dataset for the region 49N to 59N and 11W to 1W, encompassing the UK, Ireland, and Brittany (France). Some of the furthest Manx shearwater GPS tracks lay outside of this region, but did not approach within 500 km of land before entering the gridded area. At each grid point we computed a scalar value indicating the amount of visible topographic information a shearwater could theoretically see. This computation comprises two steps: determine what is visible on the horizon, then reduce that to the solid angle of topographic features visible above the horizon for each GPS location.

Let us describe the first step in detail. Suppose we are given an observer location (ϕ_O, λ_O, a) that is the latitude, longitude, and altitude. The arc-distances to the horizon over a perfectly flat sphere of radius R_E is given by

$$S_o = R_E \cos^{-1} \left(\frac{R'_E}{R'_E + a} \right)$$

where $R'_E = (7/6)R_E$ is a first-order approximation for the effective radius used in line of sight calculations owing to refraction in the Earth's atmosphere. Similarly, the arc-distance from a topographic peak of height h to a perfectly flat horizon is given by

$$s_h = R_E \arccos^{-1} \left(\frac{R'_E}{R'_E + h} \right)$$

The peak is above the horizon if the arc-distance from observer to peak is less than $s_o + s_h$. Thus an initial step in the algorithm is to reduce the topographic dataset to just a subset of what could feasibly be seen from the observer's location. The highest peak in the full dataset is selected and S_h computed for that peak. The angle γ subtended on a sphere of radius R_E by this arc-distance S_o

$+ S_i$ was computed. The topographic subset was then given latitude limits of $\phi_O - \gamma$, $\phi_O + \gamma$. A similar procedure sets the longitude limits of the subset but the angle γ' was then computed using a sphere of radius $RE \cos(\max(|\phi_O \pm \gamma|))$, which smallest distance to the Earth's rotation axis within the given latitude limits. This reflects the fact that lines of longitude converge closer to the poles. The topographic subset is now defined. This procedure is repeated iteratively, with the highest peak now chosen to be within the current topographic subset, until a subset is converged upon.

Using this topographic subset, a view of the horizon was now computed, using a modification of Matlab's "viewshed" function. Consider an arbitrary location (ϕ, λ) . The topographic subset was linearly interpolated onto the great-circle C from the observer at (ϕ_O, λ_O) to (ϕ, λ) and beyond to the edge of the topographic subset; this yielded data points (s, h) to 2D Cartesian coordinates (x, z) centred at the observer and oriented with the x-axis pointing from (ϕ_O, λ_O) to (ϕ, λ) , and the z-axis pointing from the Earth's centre through (ϕ_O, λ_O) . The angle α , measured up from the horizon, from the observer to each topographic point is then given by $\alpha = \arctan(z/x)$. The largest such α was then selected, indicating that when looking in this direction, the furthest visible topography (that is not hidden by nearer topography) is at the angle α above the horizon. We do this under the assumption that the contrast between a near and a distant peak is much less than the contrast between a peak and the sky. Note that this choice is irrelevant when the topography is only just visible on the horizon, i.e. there is only one topographic feature above the horizon in that direction. Since it is near the geographic edge of where visual information becomes available to the shearwater that interests us, this choice is therefore of second-order importance. Finally, the nearest topographic data points that went into performing the above linear interpolation are then marked as having had their visibility checked.

This procedure is repeated for each (ϕ, λ) going around the edge of the topographic subset and then performed on any topographic data points that remain to have their visibility checked. The

result is a set of vectors (η, α) where $\eta \in [0, 2\pi]$ is the azimuth—the angle to the line from the observer looking east—and α is the angle above the horizon.

The second step computes the area in steradians of the visible horizon, 360° around. That is, we calculate the area of the polyhedron given by $(\eta, \max(\alpha, 0))$ and the additional points $(0, 0)$ and $(0, 2\pi)$. To assign a horizon value to each of our GPS coordinates, we bilinearly interpolated the resulting grid to each GPS location and extracted its value.

We had to make an assumption about the height that a shearwater would fly. We decided on 3 m since as shearwaters make use of a shear layer above the ocean they are unlikely to routinely fly higher than that. Choosing a much higher value of 20 m has little effect on the overall map of the horizon, since the difference between 20 m and 3 m is small compared to the variation in topography. That is, S_a varies much more than any feasible variation in S_o and visibility is determined by $S_o + S_a$.

Wind data

We used the L3.0 Cross-Calibrated Multi-Platform Ocean Surface Wind Vector V2.0 wind data from NASA (accessed 15-08-2017), giving a wind vector every 6 hours at a grid resolution of 0.25° (Atlas et al., 2011). We bilinearly interpolated wind direction and magnitude in space and then interpolated the resultants in time to give a wind direction and magnitude for the time and position that each of the homing trips in the study began. We computed the components of the wind vector along the headwind-tailwind axis and the crosswind axis relative to the beeline, and the angle of wind incidence on a stationary bird at the location at the start of homing.

Initial orientation

For homing positions that started close to a coast, the coast often constrained the possibility for birds to follow the beeline (i.e. there was only a short distance for which the beeline route was possible) and so we analysed the homing tracks' orientation from the initiation of homing to the

halfway point between that position and the position that the beeline intersected land.

Statistics

Orientation

To analyse whether birds were oriented initially toward the minimum path or the beeline route home, we first normalised the birds' initial homing orientation with respect to our two types of homeward path so that the orientations were positive and negative deflections around 0, first set as the beeline and then as the minimum distance route home. We compared these two distributions of orientations by bootstrapping 95% and 99% confidence intervals of the mean, and then tested whether they were different using a Watson Williams test. We then subtracted each bird's minimum path deflection from its beeline deflection, so that birds closer to the beeline in orientation had negative values and birds closer to the minimum path positive (we call this path closeness). To test if our birds were more frequently oriented closer to the beeline or the minimum path, we recalculated the median path closeness for bootstrapped deflections from beeline and minimum path to give an expected distribution for orientations if they were random with respect to the beeline and minimum path. We repeated this analysis for all tracks where there was a predictive difference between the two routes and then for those tracks where the difference in distance between the two paths was greater than 1% or 5% to observe the effect in the subset with greater predictive differences. Medians were chosen to find the commonest strategy (effectively we were interested in a binary outcome). In addition, some of the birds were poorly oriented with respect to both routes, and using medians prevented the difference between the two paths-types in such cases from influencing the results independently of a bird's orientation behaviour.

Statistics – timing of homing

We used Linear Mixed Models (LMMs) to analyse whether the time that shearwaters began homing was best predicted by the beeline or minimum path distance between the shearwaters location at the initiation of homing and the colony. We first fitted a full model to predict the time

that shearwaters allocated to homing with beeline distance, minimum path distance, island (colony ID), breeding stage (incubation, chick-rearing, failed breeder, or exodus), head-tail wind resultant and topography visible above the horizon as fixed effects and bird ID as a random, intercept only effect. Each parameter was tested for its contribution to the model by a likelihood ratio (LR) test between the full model and the nested case of the model with the parameter dropped. Non-significant parameters were dropped ($\alpha = 0.05$) and the relative contribution of parameters to the final model assessed by evaluating their t statistics and conducting a similar series of LR tests in the final model. Bird ID was retained as a random intercept effect regardless of its contribution to the model to account for repeated measures.

To test what parameters predicted the time that shearwaters allocated to homing (how long they anticipated it would take), we fitted LMMs to the time allocated to homing per distance to the colony, for both the beeline and minimum path distances. If shearwaters were to judge their distance from the colony based on their beeline distance from it, then we would expect that the time allocated per distance of minimum path (i.e. the distance that the shearwater would actually have to fly) would decrease as the discrepancy between the minimum path length and the beeline distance gets larger. We would not expect the same to be true for the time allocated per distance of beeline. To test this, we therefore fitted a similar set of models, with the same starting predictors and used the same model selection process as for timing of homing. Our predictor of interest was therefore the ratio of the beeline path to the minimum path (henceforth *path ratio*, range ~1.0 – 1.35), which was removed to create a nested, null model and a likelihood ratio test performed.

We also used LMMs to test whether shearwaters might path integrate. We tested whether the standard deviation in deflection from the beeline, as a measure of orientation error, was predicted by the time that shearwaters had been out at sea. Colony, breeding stage and bird ID were included as nested random effects. The effect of time at sea was then tested using a LR test between the full model and a model with only random intercepts. This analysis was conducted on shearwaters

where there was no discrepancy between the minimum path and the beeline path, and that initiated homing with no topographic visual information ($n = 102$).

All LMMs were checked for the assumption of approximately normal residuals and homogenous residual variance across the levels of predictors. LMMs were fitted using the “lme4” package in R version 3.2.1.

Results

1535 foraging trips of Manx shearwaters were GPS tracked between 2006 and 2016 on five remote island colonies of the species in the northeast Atlantic. Of these, 732 went out of sight of the home colony and returned before the GPS battery failed (Copeland: 249; Lundy: 29; Rum: 25; Skomer: 411; Skokholm: 18) comprising birds during incubation ($n = 144$), chick-rearing ($n = 533$), pre-laying exodus ($n = 24$), non-breeding immature birds ($n = 30$) and failed breeders ($n = 1$). There were 339 cases where the beeline and minimum path from the bird's position at the start of homing and the colony were in conflict and were thus included in our analysis (Copeland: 101; Lundy: 6; Rum: 22; Skomer: 206; Skokholm: 4). The mean distance of birds at the point identified as the start of homing was 138 km (range: 49 – 765 km). Homing trips are shown in Figure 5.2. Examples of positions identified as the initiation of home are shown in Figure S5.1.

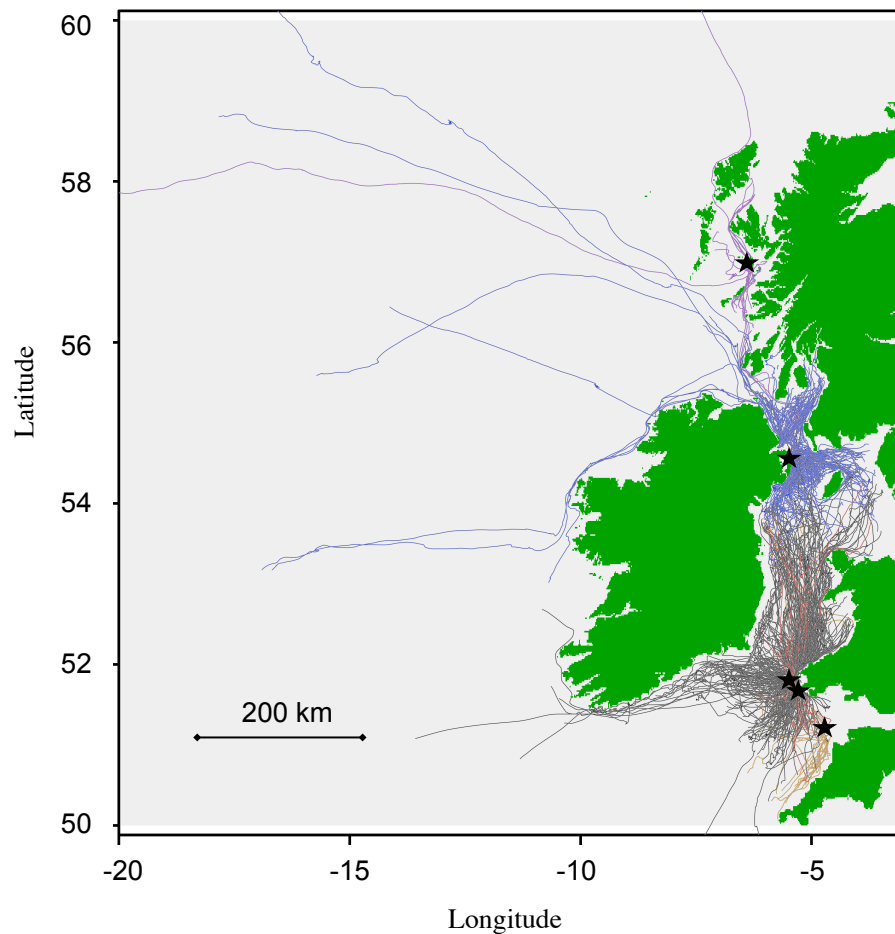


Figure 5.2. Map shows the homing tracks used in this study ($n = 732$) and the location of the five colonies (stars, from north to south: Rum, Copeland, Skomer, Skokholm, Lundy). Here the natural experiment can be seen, as birds begin to home from the west of Ireland following a straight, beeline course.

Validation of the start of homing

We found a distinct change in behaviour between the period before the start of homing and the period after (HMM details are reported in Table S5.2, supplementary material). Birds were significantly more likely to be in active flight (Wilcoxon rank sum: $W = 54421$, $p < 0.0001$) and were significantly less likely to be foraging (Wilcoxon rank sum: $W = 387780$, $p < 0.0001$) or resting (Wilcoxon rank sum: $W = 393640$, $p < 0.0001$) in the period following the onset of homing (Figure S5.2, supplementary material). This was not the case with our randomised homing start points (Figure S5.2, supplementary material) with there being no difference in time spent foraging (Wilcoxon rank sum: $W = 266510$, $p = 0.93$) or commuting (Wilcoxon rank sum: $W = 281360$, p

= 0.08) and a small but significant increase in resting after the randomly placed homing start (Wilcoxon rank sum: $W = 249500$, $p = 0.03$). An example of the computed beelines, minimum paths and visibility of topography above the horizon is shown in Figure 5.3.

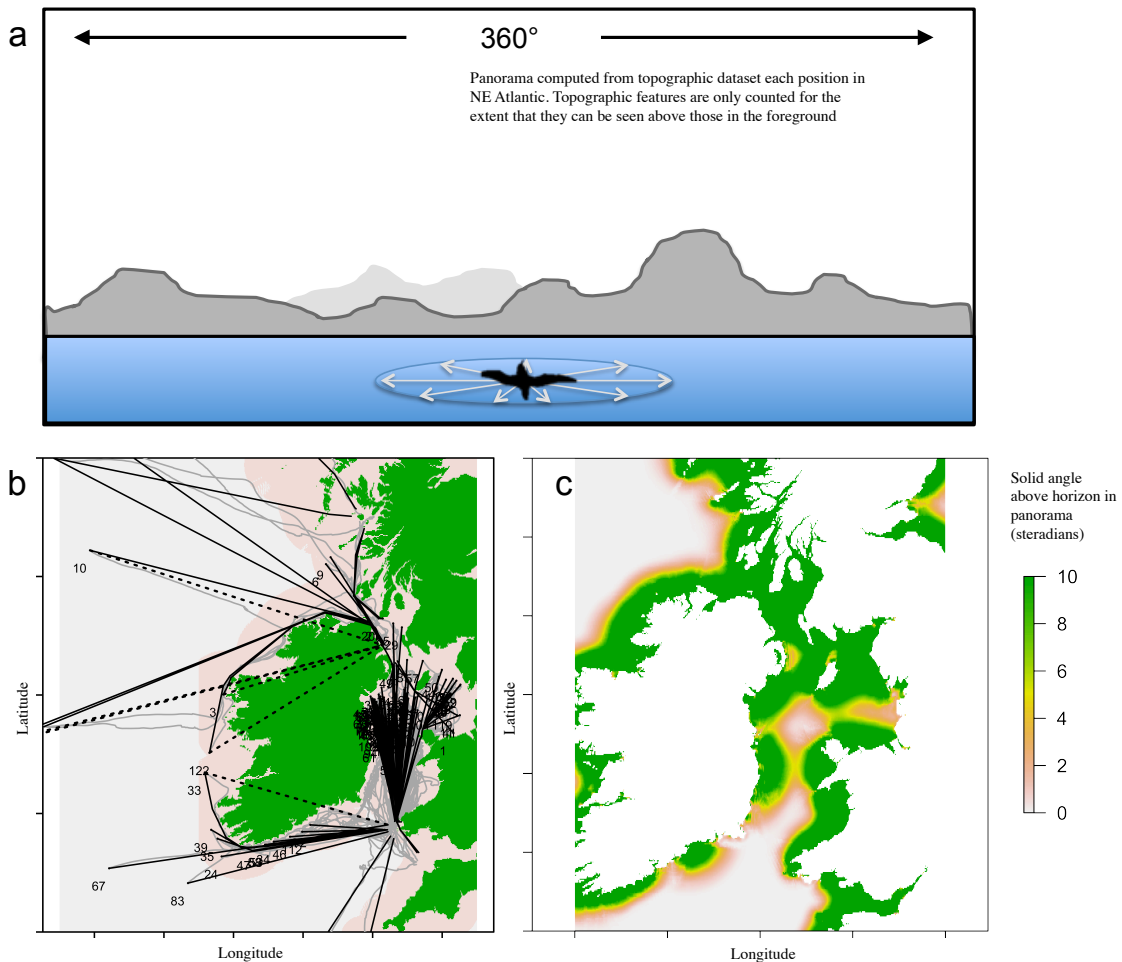


Figure 5.3. a) Birds' eye view of the horizon is shown to visualise the method. Topography above the horizon was computed around the full panorama for each location and reduced down to a scalar value such that locations with different visual information could be compared. b) Examples of the computed minimum paths (solid lines) and beelines (dashed lines) for shearwaters at the start of homing. c) Heat map of the visible horizon for the Irish Sea, measured in steradians (solid angle), showing the considerable amount of variation within the Irish sea in available topographic information.

Orientation along the beeline and minimum path route

For tracks where the beeline and minimum path route differed (Figure 5.3), the mean deflections were closer to the beeline than the minimum path (beeline = -2.57° ; minimum path = -8.16°) and we found a significant difference between the initial orientations with respect to the beeline and the initial orientations with respect to the minimum path ($F_{1,676} = 13.47$, $p < 0.001$, Figure 5.4). We found that 0 lay much closer to the mean deflection from the beeline path than the minimum path, and lay within the 99% confidence intervals of the beeline path only (Table 5.1) though there was an anticlockwise bias in orientation with respect to both routes home. On average, birds were 13.7° deflected from the beeline route home (s.d.).

Our randomisation of minimum path deflections taken from beeline deflections showed that most birds were closer to the beeline in their initial orientation, though not significantly so (10,000 reps, two-tailed $p = 0.18$). However, this became highly significant for those tracks where the beeline and minimum path difference were offset by $>1\%$ (10,000 reps, two-tailed $p = 0.02$, Figure 5.4) or $>5\%$ (10,000 reps, two-tailed $p = 0.003$), where the predictive differences were greater which is consistent with our finding that orientations were significantly closer to the beeline.

Table 5.1. Circular mean and von Mises bootstrapped confidence intervals of deflections from the beeline and from the minimum path.

	Beeline		Minimum path	
	95%	99%	95%	99%
mean	-2.57°		-8.16°	
upper CI	-0.3°	$+0.3^\circ$	-6.3°	-5.7°
lower CI	-4.8°	-5.4°	-10.0°	-10.7°

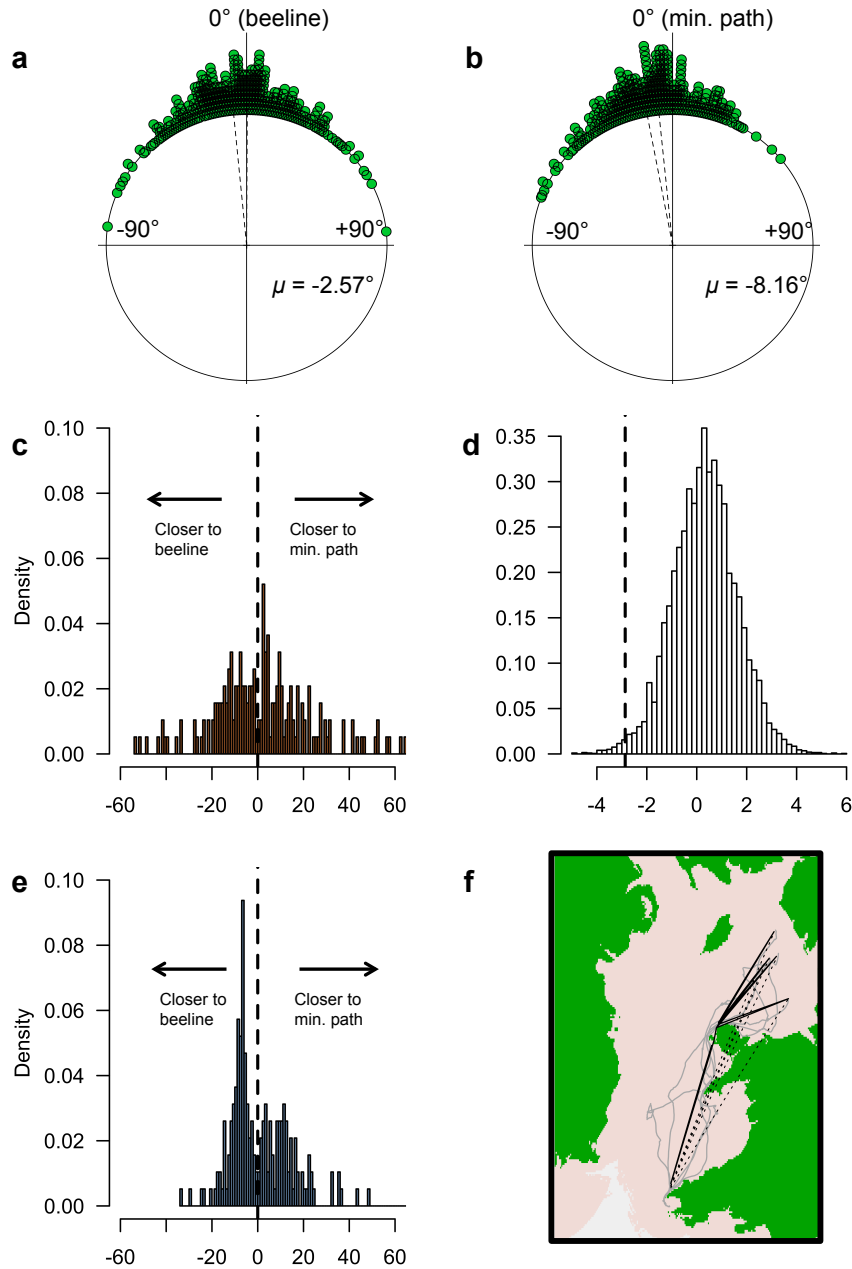


Figure 5.4. Distribution of initial orientations with respect to the beeline route (a) and minimum path route (b) showing 99% confidence intervals of the circular mean. (c) Example distribution of 192 randomly sampled differences between beeline deflections and minimum path deflections for tracks with > 1% predictive difference. (d) The distribution of the 10,000 randomly sampled median differences. The line is the measured median of (b) (two tailed; $p < 0.05$). (e) The distribution of differences between the measured beeline deflection and minimum path deflection in the data ($n = 192$). (f) Map showing example tracks (grey) homing to Skomer Island with minimum path (solid black line) and beeline (dotted black line) drawn for comparison.

The role of visual cues

The visual information measured as the topography that could be seen from a shearwater's starting location above the horizon significantly predicted whether birds were closer to the beeline or the minimum path on their journey home, with birds being closer to the beeline with less visual information, even when controlling for proximity to the coast (LR test: full model: distance to the coast and the amount of visible horizon; nested model: distance to the coast only: $\chi^2 = 16.5$, d.f. = 1, $p < 0.0001$). Proximity to the coast did not predict whether birds were closer to the beeline or the minimum path (LR test: full model: visible horizon only: $\chi^2 = 0.13$, d.f. = 1, $p = 0.72$).

Orientation with respect to the wind

There was no effect of the direction or magnitude of cross wind relative to the beeline on the birds' initial orientation relative to the beeline that birds took (LM: $F_{1,730} = 1.24$, $p = 0.27$).

Timing of homing

All parameters (beeline distance, directed flight before, tailwind, Island and stage) except for the amount of topography that could be seen above the horizon and minimum path distance contributed significantly to our full model (Table 5.2). In the final model, beeline distance was by far the strongest predictor of the timing of homing ($t = 8.41$, $p < 0.0001$, Figure 5.5), with birds allocating 1 min 48 sec minutes per kilometre of beeline to make the journey home. Time spent in directed flight was also a significant predictor ($t = 2.44$, $p = 0.015$), with birds on average allocating an extra 5 min 24 sec to homing for each hour that they had spent in directed flight leading up to the onset of homing, which might have been indicative of their motivation to get home. Wind was also a significant predictor of the timing of homing, with birds beginning homing with a stronger head wind departing for the colony sooner than those with a tailwind ($t = -2.32$, $p = 0.023$), leaving on average 12 min 36 sec later per 1 metre/s increase in headwind.

Table 5.2. Full and final model fitted to the hours allocated to home. χ^2 on two degrees of freedom is shown unless specified for the likelihood ratio test between full and nested null model for each parameter. Retained parameters' p-values are in bold.

Model details					Parameter removal (LR test)	
	Parameter	Estimate	Std. Error	t value	χ^2 (D.o.F)	p-value
Full model	Intercept ^a	7.79	0.94	4.37		
	Beeline	0.09	0.04	2.26	5.00	0.025
	Minimum path	-0.05	0.04	-1.43	1.99	0.159
	Directed flight before	0.10	0.04	2.63	6.66	0.010
	Tail wind ^b	-0.21	0.09	-2.28	4.96	0.026
	Horizon	-0.003	0.01	-0.25	0.10	0.755
	Island - Lundy	-0.38	3.06	-0.15		
	Island - Rum	-6.01	1.77	-3.34		
	Island - Skomer	-4.12	0.92	-4.77	24.90 (4)	< 0.0001
	Island - Skokholm	-7.55	3.70	-2.08		
	Stage - exodus	-0.97	5.11	-0.23		
	Stage - immature	2.70	3.71	-0.89	13.58 (3)	0.004
	Stage - incubation	4.19	1.14	4.18		
	Log Likelihood	-1146.5				
	AIC	2323.0			χ^2 (D.o.F)	p-value
Final model	Intercept ^a	7.58	0.87	8.67		
	Beeline	0.03	0.004	8.44	64.45	< 0.0001
	Directed flight before	0.09	0.04	2.40	5.72	0.017
	Tail wind ^b	-0.21	0.09	-2.30	5.13	0.024
	Island - Lundy	-0.30	3.07	-0.10		
	Island - Rum	-6.20	1.76	-3.52		
	Island - Skomer	-3.98	0.90	-4.41	24.37 (4)	< 0.0001
	Island - Skokholm	-7.55	3.71	-2.04		
	Stage - exodus	-0.51	5.12	-0.10		
	Stage - immature	1.70	3.65	0.47	13.44 (3)	0.004
	Stage - incubation	4.21	1.14	3.69		
	Log Likelihood	-1147.6				
	AIC	2321.2				
	^a Intercept is estimate for Island: Copeland; Stage: chick-rearing					
	^b Tail wind: positive values denote tailwind, negative a headwind					

To analyse which of these effects were incorporated into informing the bird about its anticipated journey time, rather than the time that the bird wanted to arrive back at the colony, we analysed the same predictors with respect to the time allocated per unit distance home, either with respect to the minimum path or with respect to the beeline (full models in Table S5.1, supplementary material). We found that wind was not a predictor of the time allocated to homing per kilometre of minimum path (LR test: $\chi^2 = 2.52$, d.f. = 1, $p = 0.11$, Table S5.1) or beeline (LR test: $\chi^2 = 2.59$, d.f. = 1, $p = 0.12$, Table S5.1), meaning that this influenced the time that they left independently of their distance to travel and so was not incorporated into the decision about travel time. Time spent in flight leading up to the start of homing was incorporated into the decision that the shearwaters made about travel time, but similarly influenced both the time allocated per minimum

path and the time allocated per beeline distance for homing suggesting that this was incorporated as a general adjustment to the birds' schedule but also their perceived distance or perceived time they expected to take to home. As the difference between minimum path distance and beeline increased, shearwaters allocated less time per minimum path for homing but not per distance beeline (Table 5.3, Figure 5.5).

Table 5.3. Final models fitted to the time allocated by shearwaters per km of minimum path and beeline distance. Full model in supplementary material (Table S5.1).

	Model details			Parameter removal (LR test)	
	Parameter	Estimate	Std. Error	t value	X ² (D.o.F) p-value
Time per km of minimum path	Intercept*	0.31	0.07	4.15	
	Path ratio	-0.17	0.07	-2.44	5.85 0.015
	Directed flight before	-0.0005	0.0002	2.34	5.60 0.018
	Island - Lundy	-0.05	0.03	-1.94	
	Island - Rum	-0.05	0.02	-3.02	37.97 (4) < 0.0001
	Island - Skomer	-0.05	0.01	-6.01	
	Island - Skokholm	-0.09	0.03	-2.61	
	Stage - exodus	0.07	0.04	1.68	
	Stage - immature	0.00	0.03	0.13	12.56 (3) 0.005
	Stage - incubation	0.03	0.01	3.26	
	Log Likelihood	-1145.4			
	AIC	2320.8			X ² (D.o.F) p-value
Time per km of beeline	Intercept*	0.25	0.08	3.34	
	Path ratio	-0.12	0.07	-1.62	2.57 0.109
	Directed flight before	-0.0005	0.0002	-2.34	5.62 0.018
	Island - Lundy	-0.05	0.03	-1.93	
	Island - Rum	-0.05	0.02	-3.06	37.75 (4) < 0.0001
	Island - Skomer	-0.05	0.01	-5.98	
	Island - Skokholm	-0.09	0.03	-2.62	
	Stage - exodus	0.07	0.04	1.64	
	Stage - immature	0.00	0.03	0.13	12.16 (3) 0.007
	Stage - incubation	0.03	0.01	3.17	
	Log Likelihood	488.69			
	AIC	-953.39			

* Intercept is estimate for Island: Copeland; Stage: chick-rearing

We found no effect of the time spent out at sea on the standard deviation of the orientation with respect to the beeline for birds initiating homing out of sight of land, and from locations there was no difference between the beeline and minimum path routes home ($n = 102$, $\chi^2 = 2.01$, d.f. = 1, $p = 0.16$).

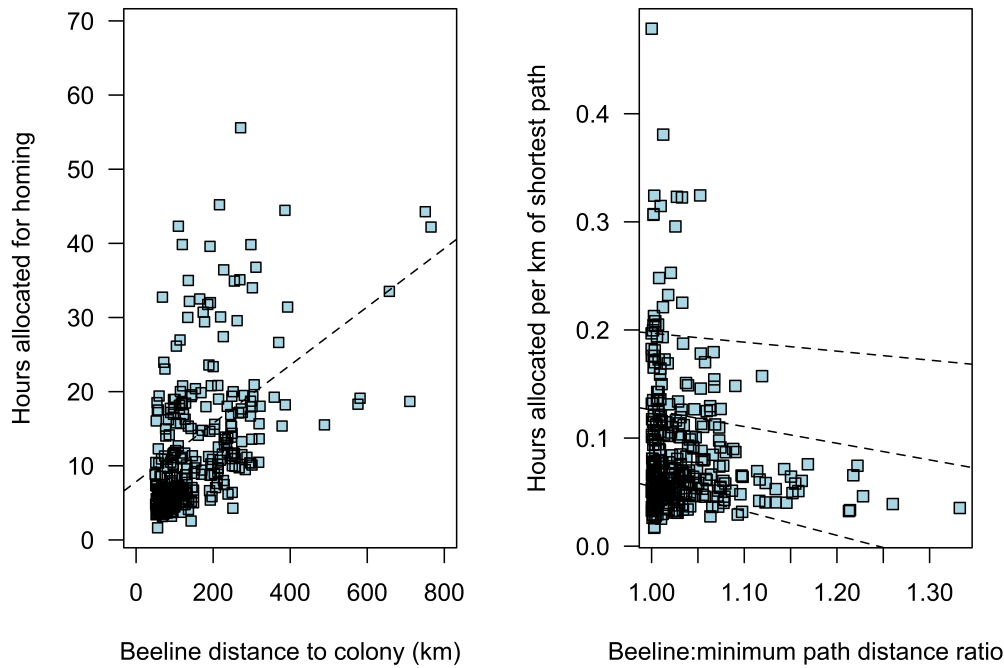


Figure 5.5. Left: The relationship between beeline distance to the colony and the hours before sunset that birds begin homing. The line is the estimated effect of beeline distance from our LMMs (see text). Right: The relationship between the beeline:shortest path distance ratio on the hours allocated per km of the shortest path. The lines show the estimated intercept and the estimated coefficient for path ratio, and the upper and lower 95% CIs for that coefficient. Mixed model statistics shown in text.

Discussion

The orientation and timing of homing of Manx shearwaters in this study suggests that shearwaters must have computed a vector of both direction and distance by which to plan their homing trip, but appear to have done so without reference to a familiar area map and without the use of outward journey information. While a familiar area map might provide information about the distance home and thus allow shearwaters to time their journeys, only an extrapolated map based on a cognitive representation of learned gradients can provide an animal with distance information outside of its familiar area. Whilst arguably an extrapolated map is already among the most favoured theories to explain how birds navigate beyond their familiar area (Benhamou, 2003;

Boström et al., 2012; Chernetsov et al., 2015; Wiltschko & Wiltschko, 2013), we suggest tentatively that this comprises first direct evidence of cue extrapolation in birds or any animal.

There are two potential alternative explanations for the results that we obtained, though we do not think that either has a great deal of support. First, since the shearwaters are wild, and free ranging, we cannot rule out that shearwaters have not visited the locations that they homed from in this study previously, especially since foraging Manx shearwaters probably return to key foraging sites in the Irish Sea (Dean et al., 2015). If orientation and timing of homing were modularly independent, then it might be reasonable to suggest that a shearwater could time its journey home and still orient with respect to the beeline by another form of gradient map (Wallraff, 1980). However, if that were the case, an animal that has learned to time its journey home based on experience of how long it has taken in the past would be expected to judge its distance from the colony by reference to that learned time rather than the time predicted by the beeline distance and thus, it is very likely that the shearwaters in this study are navigating as though from an unfamiliar site.

Second, since the shearwaters in this study had access to outward journey information, it is difficult to know that they did not path integrate. Path-integration could theoretically provide them with an encoded geometric representation of their current location relative to home (Benhamou et al. 2003b). Like an extrapolated map, this would predict that shearwaters would both orient along the beeline home, blind with respect to obstacles, and be able to time their journeys having measured their outward trajectory. While we found no evidence for the key prediction of path-integration, that propagated error would result in poorer homeward orientation with more time at sea, it is worth considering the hypothetical possibility. Animals are known to use idiothetic (vestibular heading recorder) and allothetic (exocentric compass and relational properties of visual cues) modes of path integration (Whishaw & Brooks, 1999). Vestibular path integration accumulates error very rapidly, in the order of metres (Benhamou & Poucet, 1996;

Gallistel, 1989), and so is unlikely to be of use to a shearwater. Allothetic path integration would presumably be frequently broken, since shearwaters will sleep on the water at night and presumably encounter poor weather, during which they are unlikely to be able to accurately interpret their speed. Whether shearwaters could sufficiently judge their movement by optic flow over water remains unknown, but would presumably be necessary (Benhamou et al. 2003b) if path integration were to provide accurate homeward guidance. Finally, Manx shearwaters and other petrels are well known to be capable of homeward oriented movement without any outward journey information and from unfamiliar areas (Benhamou, Bried, et al., 2003; Dall'Antonia et al., 1995; Matthews, 1953) and so it is not unlikely that the same mechanisms are invoked to home from distant sites after foraging trips despite the possibility of the sites having been previously visited.

The finding that shearwaters probably consult a true extrapolated map is relevant to the discussion of the cognitive map, since under the extrapolated map model, birds are able to compute 'novel short-cuts' (McNaughton et al., 2006; O'Keefe & Nadel, 1978). If our finding turns out to be robust, the implication is that at least some birds have a cognitive representation of space to which they have not yet visited, and are able predict attributes of the cues that will occur there (Jacobs, 2012). This is perhaps surprising because of the implied cognitive complexity that it involves compared to other models for avian true navigation. For example, a simpler cognitive process altogether could explain true navigation in the homing pigeon, for which there is a great deal of evidence that true navigation is facilitated by learning the direction of the wind that brings particular odour compositions to the loft (Gagliardo, 2013; Ioale et al., 1978; Wallraff, 1980, 2014) that are later recognised at the release site. This is also likely to be facilitated by large scale gradients (Wallraff, 2000; Wallraff & Andreae, 2000), but the entire set of odour compositions can be learned and thus, while clearly learned latently, can still be explained without cognitive extrapolation.

It is perhaps surprising in itself that shearwaters might perform true navigation by a mechanism that is fundamentally different to that most likely to facilitate true navigation in pigeons, especially considering that in both, navigation is very likely to be based on olfaction (Gagliardo et al., 2013; Padget et al., 2017; Pollonara et al., 2015). One possibility that has already been hypothesised to explain concurrent evidence for both olfactory and magnetic map senses is that different maps operate over different scales (Bingman & MacDougall-Shackleton, 2017). This could also apply within olfactory mechanisms. While the different selection pressures experienced by pigeons and seabirds might have resulted in different map mechanisms, it is equally possible that the taxa do share common navigational processes, but the processes underpinning their respective navigational abilities are being tested at incomparable scales.

There has been recent theoretical work on an extension of the cognitive map model to true navigation (Jacobs, 2003, 2012; Jacobs & Schenk, 2003), which might provide a useful concept for how true extrapolated maps might work neurologically. However, the role of the pigeon hippocampus in homing from unfamiliar areas remains equivocal (Bingman & MacDougall-Shackleton, 2017), casting doubt over the applicability of these models to birds. An interesting line of inquiry in the future therefore might be in the study of bats (Bingman & Cheng, 2005; Holland, 2007; Holland et al., 2006), since they might also be capable of true navigation but have greater homology with the rat, which thus far has been the main model for the neurophysiological basis of navigation. That, in combination with further experiments on free ranging seabirds, might answer the question of how likely an extrapolated map is to really exist, and how widespread it might be.

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Supplementary Material

Table S5.1. Table shows full model estimates and LR test statistics for models predicting the time allocated per kilometre beeline and minimum path distance. The final models are LR tests are reported in the main text. Retained parameters' p-values are in bold ($\alpha = 0.05$).

Model details					Parameter removal (LR test)	
	Parameter	Estimate	Std. Error	t value	χ^2 (D.o.F.)	p-value
Time per km of minimum path	Intercept*	0.29	0.07	3.94		
	Path ratio	-0.16	0.07	-2.19	4.71	0.030
	Directed flight before	-0.001	0.0002	-2.39	5.65	0.017
	Tailwind	-0.001	0.001	-1.60	2.52	0.113
	Visible topography	-0.00004	0.0001	-0.47	0.22	0.642
	Island - Lundy	-0.05	0.03	-1.96		
	Island - Rum	-0.05	0.02	-3.03		
	Island - Skomer	-0.05	0.01	-6.27	39.17 (4)	< 0.0001
	Island - Skokholm	-0.09	0.03	-2.66		
	Stage - exodus	0.06	0.04	1.63		
	Stage - immature	0.001	0.03	0.05	13.42 (3)	0.004
	Stage - incubation	0.03	0.01	3.42		
	Log Likelihood	490.07				
	AIC	-965.56				
					χ^2 (D.o.F.)	p-value
Time per km of beeline	Intercept*	0.24	0.08	3.12		
	Path ratio	-0.10	0.07	-1.37	1.85	0.174
	Directed flight before	-0.0005	0.0002	-2.39	5.68	0.017
	Tailwind	-0.001	0.001	-1.62	2.59	0.108
	Visible topography	-0.00004	0.0001	-0.51	0.26	0.610
	Island - Lundy	-0.05	0.03	-1.96		
	Island - Rum	-0.05	0.02	-3.06		
	Island - Skomer	-0.05	0.01	-6.25	39.05 (4)	< 0.0001
	Island - Skokholm	-0.09	0.03	-2.67		
	Stage - exodus	0.07	0.04	1.59		
	Stage - immature	0.002	0.03	0.05	12.81	0.005
	Stage - incubation	0.03	0.01	3.34		
	Log Likelihood	490.07				
	AIC	-952.13				

* Intercept is estimate for Island: Copeland; Stage: chick-rearing

Table S5.2. Speed and turning-angle parameters for behavioural classifications from our hidden Markov model.

	Step length		Turning angle	
	μ	σ	μ	κ
fly	2.481	1.614	-0.003	20.808
rest	0.165	0.114	-0.0038	98.088
forage	0.455	0.54	-0.0101	1.174

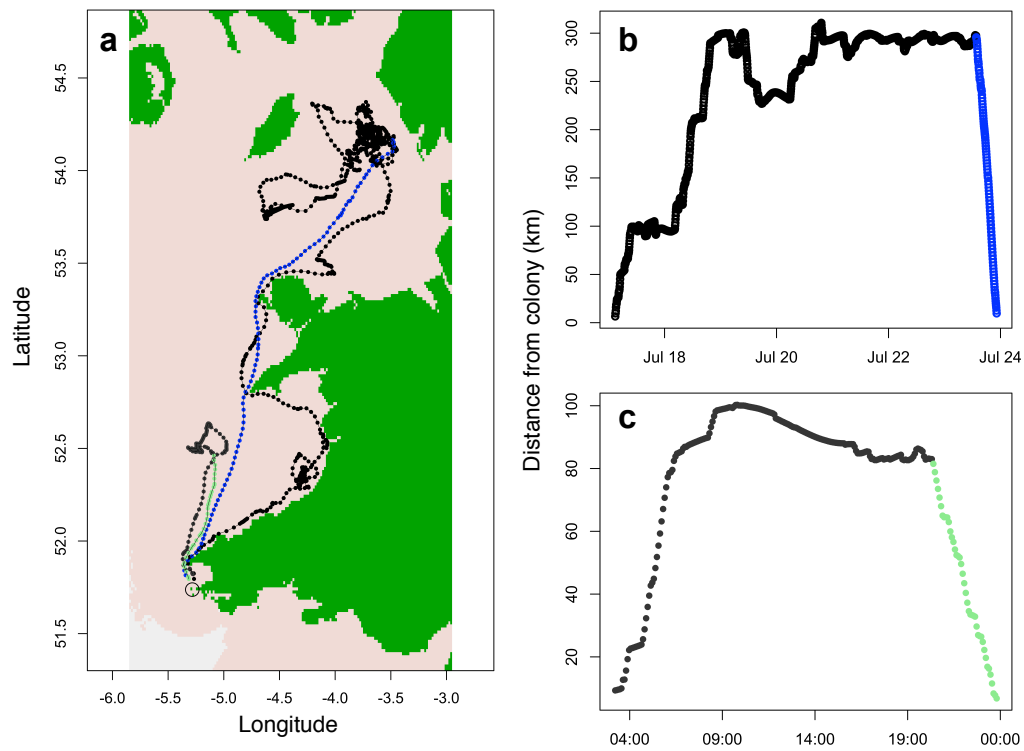


Figure S5.1. (a) Example of a long and short Manx shearwater foraging trip from Skomer Island with homing as blue (long trip) and light green (short trip). (b and c) The same birds' distance from the colony over a long, multi-day trip (b) and short, single-day trip (c), with homing highlighted blue and green respectively.

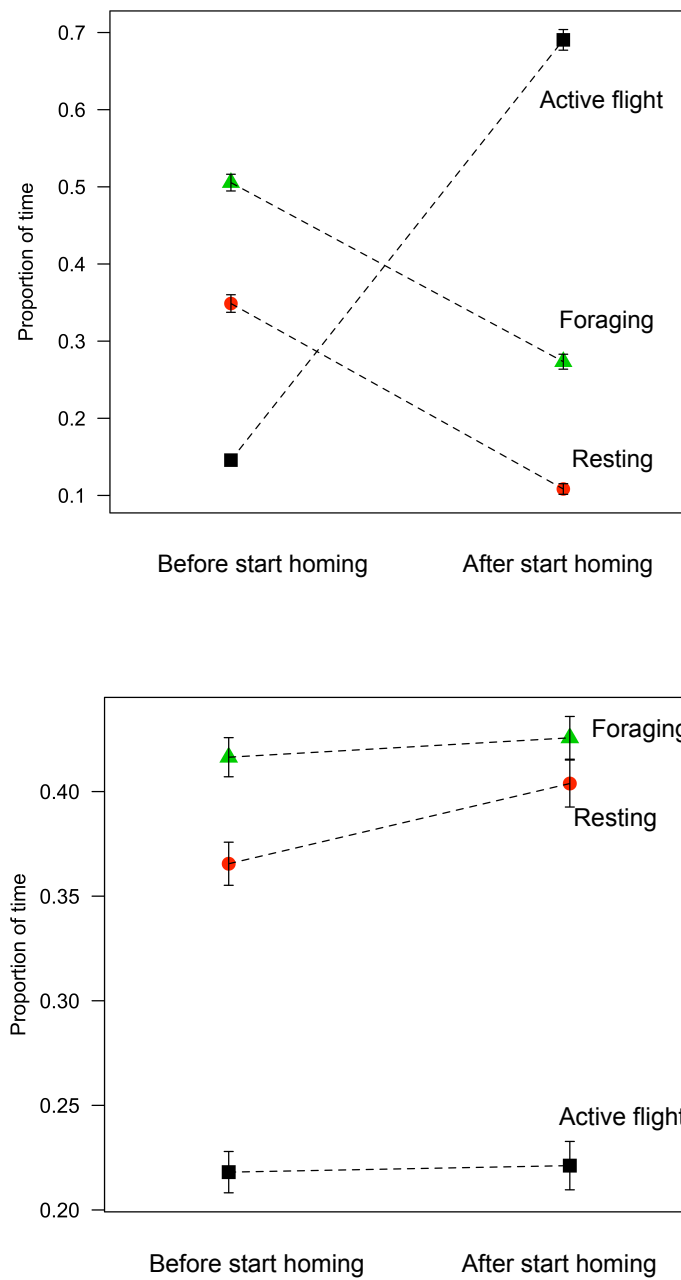


Figure S5.2. The proportion of time spent in active flight, resting or foraging in the first 25% of homing compared to the same time period before the start of homing. Top: for the points identified as homing; Bottom: random points in final 75% of track.

Table S5.3. Transition probability matrix for final hidden Markov model.

		State at time $t + 1$		
		fly	rest	forage
State at time t	fly	0.847	0.002	0.160
	rest	0.003	0.863	0.135
	forage	0.073	0.134	0.792

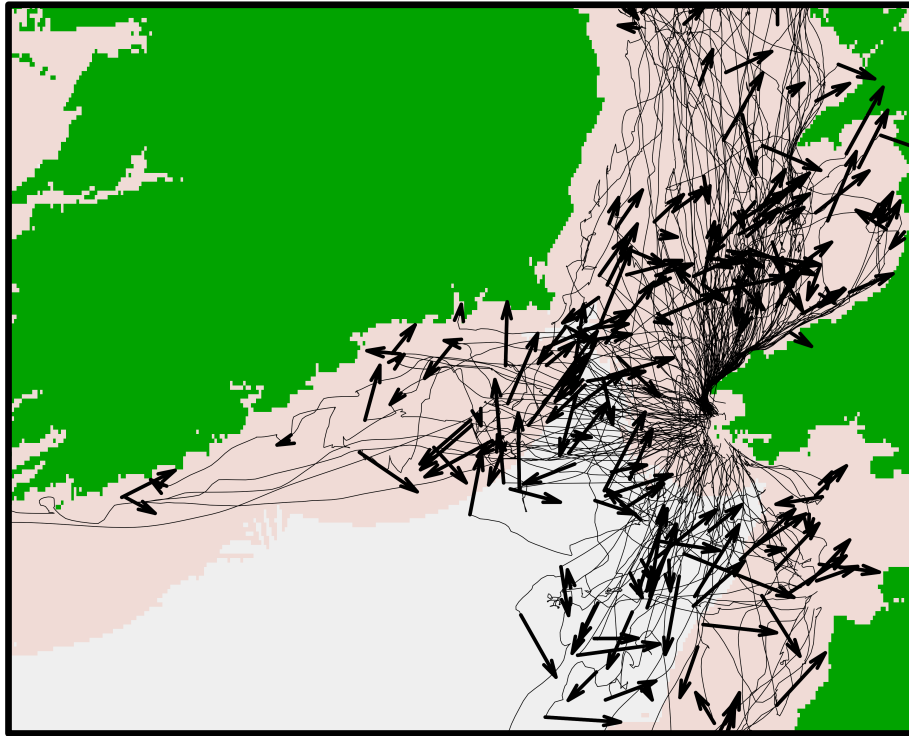


Figure S5.4. An example map of the Celtic Sea with wind vectors superimposed on the start of homing tracks shown in grey. Wind magnitude is only represented relatively as the length of the arrows.

Chapter 6

Carry-over effects in a small, pelagic seabird: a controlled experiment

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Unpublished manuscript

Abstract	185
Introduction	186
Materials and Methods	189
Results	197
Discussion	207
Acknowledgements	213
References	213
Supplementary Material	219

Abstract

Movement and breeding decisions of animals can be influenced by their current internal state and the environmental conditions that they face. In long-lived birds, the internal state might be the result of environmental conditions experienced previously but quantifying such carry-over effects from previous seasons is difficult to do. Here, we aimed to investigate how the energetic demands of chick rearing might influence movement decisions and foraging behaviour in a long-lived Procellariiform seabird by providing a high-energy supplementary diet to Manx shearwater chicks but without shortening the breeding attempt. We controlled for previous breeding success, current breeding phenology and handling during the focal season. We found that during the experiment, the parents of supplemented chicks had normal at-sea behaviour, spending similar proportions of time foraging, resting and flying as controls. Both treatments attended the nest with the same frequency. However, adult shearwaters fed undetectably small quantities of food to chicks that were experimentally fed, and thus conserved an amount of energy roughly equivalent to the energetic content of an average food load each time they attended the nest during the experiment. This energetic relief from chick rearing was not reflected in phenology of the subsequent non-breeding period or breeding success in the following year. However, the adults from experimentally manipulated nests massively increased the proportion of time spent foraging during over winter residency, an unexpected finding in the context of previous studies. We hypothesise that (i) an apparent increase in foraging effort might be caused by changes in foraging behaviour rather than the amount of foraging leading to different rates of detection in saltwater immersion data and (ii) that our lack of carry-over effects, consistent with some other studies, is most likely to be caused by a ceiling effect introduced by other constraints on the phenology of a long distance migrant followed by a strategic withholding of resources for future survival.

Introduction

Carry-over effects (COEs), where environmental conditions in one year affect reproductive performance in subsequent years, are likely to be an underappreciated source of variation in breeding success impacting individual fitness and population dynamics (Harrison, Blount, Inger, Norris, & Bearhop, 2011; Norris, 2005) not accounted for by experience or age (Daunt et al., 2014; Shoji et al., 2015). COEs might also be important for global ecology, since environmental conditions experienced by large populations of migrant animals at wintering or breeding grounds might affect ecosystem dynamics many thousands of kilometres away during subsequent stages of the annual cycle (Catry, Dias, Phillips, & Granadeiro, 2013). COEs are implicitly associated with life-history theory, and frequently discussed in that context (Harrison et al., 2011), since COEs demonstrate the future costs and benefits of current investment decisions, and plasticity in an individual's ability to optimise the life-history trade-off at each breeding event might buffer against the negative impacts of environmental variation (Stearns, 1976, 1992). This could either be flexibly (e.g. in response to parasites; Martin, Weil, & Nelson, 2008) or inflexibly (e.g. in response to expected longevity) across the lifetime of the animal. Life-history trade-offs are thus inextricably linked with the impact and persistence of COEs, and understanding the interplay between an animal's condition and strategic breeding decisions links COEs to life-history theory and might prove important in understanding the evolution of life-history traits (McNamara & Houston, 1996).

COEs are likely to be especially important in influencing life-history strategies in long-lived species (Bize, Roulin, Tella, Bersier, & Richner, 2004; Fayet et al., 2016; McNamara & Houston, 1996). Breeding decisions such as clutch size, investment in egg mass or the decision to attempt breeding at all are, in long lived animals, especially dependent on intrinsic factors (henceforth condition) since, until senescence, future reproduction comprises a considerable proportion of an animal's life-long reproductive output and thus animals should optimise the trade-off between

current and future reproduction by retaining condition after each reproductive episode (Williams, 1966). Furthermore, the condition of long-lived species has greater opportunity to accumulate COEs and as such, the link between previously experienced environmental variation and life-history decisions is likely to influence reproductive performance greatly in any given breeding episode.

There is considerable correlative and experimental evidence that COEs influence reproductive performance in long-lived birds (Inger et al., 2010) as expected by the typical life-history trade-off (Harrison et al., 2011). Swifts (Bize et al., 2004) and seabirds (Catry et al., 2013; Daunt et al., 2014; Fayet et al., 2016; Schultner et al., 2014; Shoji et al., 2015) are especially suited to studies of COEs being at the slow end of the life-history continuum (long-lived, low reproductive output, consequential life-history trade-off) and being strongly seasonal, episodic breeders (Hamer, Schrieber, & Burger, 2001) during which discrete investment and output can be measured. For example, Shoji *et al.* (2015) showed in long-term tracking data of Manx shearwaters, *Puffinus puffinus*, that birds that skip breeding or failed early in their breeding attempt in one year have better reproductive performance in the next than those that have bred successfully, a finding also associated with behavioural changes in the preceding winter. Furthermore, experimental shortening or lengthening of the breeding season of Procellariiform seabirds, either by induced failure (Catry et al., 2013) or cross-fostering of small and large chicks (Fayet et al., 2016), has shown that the effects such as those shown by Shoji *et al.* (2015) are probably mediated by the cost of breeding, since birds with experimentally increased or decreased cost of breeding experienced respective changes in migratory phenology (in some species) and reproductive performance the following breeding season.

Experimental manipulations are crucial for examining COEs, since correlations might easily be confounded by consistent within-individual differences (Catry et al., 2013). However, most

experiments to induce carry over effects in seabirds have thus far been relatively limited in involving only shortening or lengthening the reproductive episode and thus it has been difficult to disentangle effects caused by a bird's condition from those caused by other constraints of breeding such as nest attendance. Lengthening reproductive episodes has been achieved by cross fostering young chicks with older chicks (Fayet et al., 2016), taking advantage of inflexible behavioural mechanisms which result in adults suffering a protracted breeding period to provision the smaller chick until it fledges. Problematically, this potentially maladaptive (Fayet et al., 2016) response to experimentally lengthened breeding episodes might lead to COEs partly unrelated to a birds' condition. For example, the condition of a bird in an experimentally lengthened breeding episode could be, hypothetically, similar to that of a bird with normal breeding phenology but the delay in departure could carry over if time spent in the wintering grounds was constant among individuals. Thus, carry-over effects in phenology and correlated measures of breeding success of subsequent breeding events might not necessarily be related to a bird's state-dependent life-history decisions ('adaptive plasticity' Harrison et al. 2011), but rather a constraint on the species' ability to perform sufficiently well to prepare for subsequent breeding without carrying over an imposed delay in phenology.

Whether life-history trade offs might be actively adjusted in complex responses to a bird's condition is an intriguing question in behavioural ecology (Harrison et al., 2011; Ricklefs & Wikelski, 2002). The experimental demonstrations of COEs are not sufficient, however, to show such adaptive plasticity in response to condition in seabirds. Sabbatical years in Procellariiform seabirds (e.g. Manx shearwaters (Shoji et al., 2015) and wandering albatrosses, *Diomedea exulans* (Weimerskirch & Wilson, 2000)) might be an example of this phenomenon but studies are yet to demonstrate that birds undertaking sabbatical years are (i) physiologically capable of breeding but did not and (ii) were not following a rigid programme of sabbatical years determined by single step decision rules based on threshold conditions.

Another way to approach COEs with a view of assessing the role of state-dependent life-history decisions is to manipulate the cost of breeding without directly influencing phenology. By doing so, it is possible to ascertain the response of an animal to its condition since improving condition should not impact the physiological ability to breed (e.g. Bize et al. 2004) and thus any response from enhancement of condition is likely to relate to adaptive plasticity. Here we attempted to reduce the cost of breeding, and thus enhance the condition of breeding birds, without affecting the phenology of the current breeding season in a controlled and quantifiable way by providing a supplementary diet to Manx shearwater chicks for a four-week period during the chick rearing stage before following their parents with miniature light and saltwater immersion loggers. By measuring the attendance of adults to experimental and control nests, we were able to infer that we did not induce any change in the duration of the breeding episode, but did reduce the food-provisioning rate of experimental adults. We therefore measured the phenology of subsequent stages in the annual cycle, activity budgets and the following year's breeding success of parents that were either subject to energetic relief (fed chicks) or were controls.

Materials and Methods

Study system

The study was carried out on Manx shearwaters, *Puffinus puffinus*, a small (400 g) Procellariiform seabird on Skomer Island, Wales, UK (51°44' N, 5°19'W), probably the world's largest colony (Perrins et al., 2012). Manx shearwaters are typical Procellariiformes: being long-lived and laying a single massive egg each year (Brooke, 1990) places them at the extreme slow-end of the slow-fast life-history scale (Shoji et al., 2015). During winter, Manx shearwaters undertake a trans-equatorial migration, spending the winter in the southwest Atlantic off Argentina on the Patagonian shelf, at a latitude of ~40° south (Guilford et al., 2009). The experimental timeline is summarised in Figure 6.1.

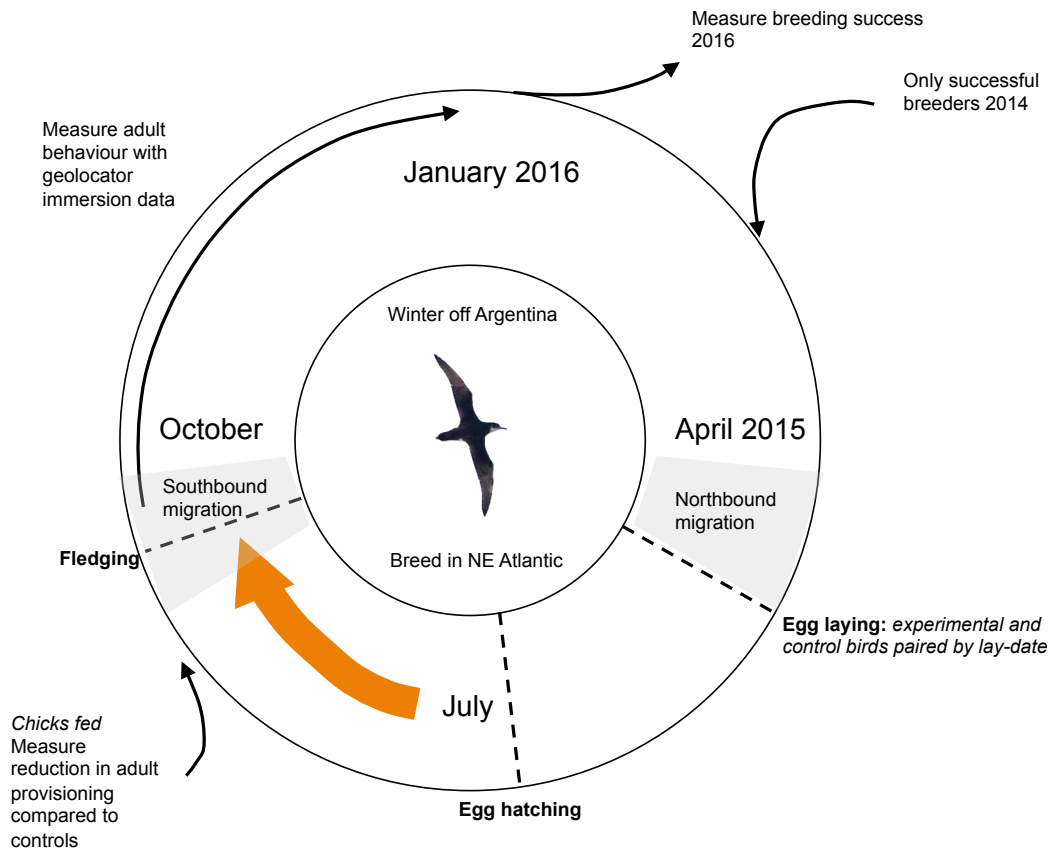


Figure 6.1. Timeline of the supplementary feeding experiment, showing the key parts of the Manx shearwater’s annual cycle that were analysed and/or manipulated. The time line starts in the top right when breeders from 2014 are chosen for the experiment. The orange arrow indicates the period that chicks received supplementary feeding.

Nest monitoring

Manx shearwater nests were monitored as part of an on-going study. Adults and chicks are ringed, nest attendance is monitored and breeding success recorded annually. For this study, we identified 10 pairs of nests each with similar lay date in 2015 and all of which had previously bred successfully in 2014. For each pair, one was assigned to the experimental treatment and one to the control treatment at random.

Chick feeding

Chick feeding was carried out between 16:00 and 20:30 h BST each day for four weeks for each chick between the 21st July and 29th August 2015. Chicks were removed from their nests and fed a high-energy specially prepared fish and oil mix by intubation. The diet was based on that used by Miskelly et al. (2009) for supplementary feeding of relocated Austral *Puffinus* spp. shearwaters, and was the same as that used by Dean (2013, D.Phil. thesis) previously in Manx shearwaters. It consisted of un-drained sardines, *Clupea* spp., in sunflower oil (60% by mass) with additional sunflower oil (10% by mass) and cooled boiled water (30% by mass). The energy content of the supplementary food was approximately 12.9 KJ g⁻¹, considerably higher than 6.6 KJ g⁻¹ of raw Atlantic herring, roughly the presumed energetic payload of parental provisioning (energetic values taken from Dean 2013, D.Phil. Thesis). Food was prepared daily, and stored in a refrigerator. It was warmed in a water bath to ~30°C before being fed by intubation to shearwater chicks. For experimental nests, chicks were initially fed ~10 g of supplementary food, being gradually increased over several days to ~40 g. If the chick would accept more, more was given until the chick stopped accepting food. For controls, chicks were removed from the nest and given the smallest possible measureable amount (~1-2 g) of supplementary food as a sham. All feeding masses were determined by weighing before and after feeding. On all occasions, chick feeding took less than 15 minutes.

GLS deployment

Geolocators (either Migrate Technology C250, 2.5 g or Biotrack mk4083, 1.5 g) were attached to a small plastic ring on the leg of each parent bird using cable ties as in Guilford et al. (2009), either as part of long-term monitoring before the chick feeding or as soon as possible after it started. All were fitted before the end of the chick-feeding period. Handling time was normally less than 5 minutes.

GLS data processing

The two different GLS models gave slightly different saltwater immersion data streams. Biotrack mk4083 (BTs) detected whether the device was immersed in saltwater (0 or 1) every 3 seconds and collated these into a score between 0-200 every 10 minutes. Migrate Technology C65 and C250 (MTs) measured whether the device was immersed in saltwater every 6 seconds (0 or 1) and collected these counts into immersion data every 5 minutes giving a score between 0 and 50. To make the scores comparable, we concatenated adjacent 5-minute bins in the MTs data streams and doubled the score. While theoretically this concatenation should maintain comparability between the two types of data stream (for example the cumulative time spent wet), we anyhow validated that shearwater behaviours did not introduce a bias depending on the data interval recorded by the device using a simplistic simulation approach based on real, high resolution immersion data from a third type of logger (not deployed on birds in this study). To do this, we created realistic time-series datasets of simulated shearwater immersion based on parameters from over-winter deployments of Biotrack Mk19 GLS loggers, which record the time that transitions occur providing they last >6 seconds. The records are not concatenated into bins, but resulting state durations are multiples of six, and thus are a discrete estimation of a continuous variable (time immersed or time dry). State transition time lengths are highly aggregated toward 0, and so we fitted a negative binomial distribution to dry and wet duration lengths in these data and drew values from this continuous distribution to simulate a realistic wet-dry time-series. We then sampled the state of the same simulated data every 3 seconds or every 6 seconds and concatenated them into 10 and 5-minute bins respectively to simulate the logger data streams. We conducted the same transformation of 6s -> 5 minute bins to 3s -> 10 minute bins as was performed on the real data, summed the immersion counts over multiple 1 month periods (any greater was too computationally intensive) and compared the outputs (see supplementary material).

At-sea behaviour

As in previous studies (Shoji et al. 2015 and references contained therein), we classified at-sea behaviour as foraging, resting or directed flight depending on the number of wet events sampled in each 10-minute bin. Each 10-minute bin gives a score of 0-200. Our classifications were: <3 for directed flight 3-197 for foraging and >197 for resting on the water. Because of our transformation of 6 second, 5 minute bins to 3 second, 10 minute bins we did not use $1 < 1-199 < 200$ as our categories as in Shoji *et al.* (2015) since this might bias the attribution of foraging behaviour towards the 6 second binned devices since there is an increased probability over a single 3 second bin that a transition would be missed (though this makes a negligible difference to the behavioural states identified). Intermediate scores in immersion values are thought to accurately relate to foraging behaviour in Manx shearwaters since they are caused by 10 minute bins with transitions between wet and dry associated with alighting and taking off from the water; behaviour atypical during resting and direct flight. These states have previously been shown to confer powerful inference of at-sea behaviour as validated mass gains following foraging trips and satellite telemetry in other seabird species (Lecomte et al., 2010).

Light data processing

Light data from geolocators were processed using the “GeoLight” package in R, using a threshold light level of 10 lux to indicate sunrise and sunset. The software allows sun elevation angle to be varied to reflect idiosyncrasies among loggers caused by age or fouling of the light sensor. For each track, the sun elevation angle that resulted in the fewest over-land points was chosen as a repeatable and objective way of varying elevation angle among tracks (Meier et al., 2017). We then fitted a LOESS filter, implemented in “GeoLight”, which fits a locally weighted least squares path through the latitude and longitude data. Positional fixes were assumed erroneous and removed if they lay more than 2 interquartile ranges from the LOESS estimated position for that time. For latitudinal estimates and for mapping, geolocator data around the equinoxes were

excluded since during this time day length varies only very slightly with latitude and thus small errors in the estimation of sunrise and sunset times result in large error. We excluded data around the equinoxes asymmetrically as in Fayet *et al.* (2016), removing 7 days on the summer side and 21 on the winter side (Linnebjerg *et al.*, 2013), reflecting the asymmetry in variation in day length either side of the equinox. This is conservative and results in the removal of most of the migratory period, but will improve the accuracy of our estimates of over-winter latitude. For the timing of migration, we used unfiltered longitudinal estimates, which are unaffected by the equinoxes.

Chick-feeding visits

We used the frequency distributions of nocturnal immersion events recorded by the geolocator to determine for each geolocator-tracked bird its visits to the colony during and after the experiment. We hypothesised that nights containing visits to the colony would be characterised by having fewer 10 minute bins with immersion logged than nights where no visit occurred since, at sea, Manx shearwaters spend much time at night sitting on the water (Guilford *et al.*, 2009). Correspondingly there was a bimodal distribution in the number of 10 minute bins in a night containing > 1 saltwater detection. We fitted a Gaussian mixture model to these data and assigned nights with greater likelihood of being generated by the lower peak (with fewer wet bins) as nights with a colony visit. As a means of validation, we checked whether the mass gains of the control chicks for which immersion data were retrieved for both parents were correlated with the number of parents (0, 1 or 2) that visited the colony during the intervening night. To compare night visitation among treatments, we quantified the number of night visits out of the total number of nights for which we had geolocator data during the chick-feeding period, giving a proportion of nights for each bird that visited the colony.

Quantifying energetic relief

For control birds for which we had geolocator data, we modelled the chick mass gain as a function of chick age, date of mass gain and the number of parents (0, 1 or 2) that visited the burrow to ascertain predictively the expected mass of a meal provided by controls given known values in those variables. For each visit from experimental birds, we then predicted the chick mass change given these variables that we would expect to be provided to the chick by controls from our model and summed the differences between the actual measured mass change and the expected mass change across the experimental treatment. Thus, each tracked adult differed in the energetic relief provided by the treatment, even for those birds attending the same nest. For birds with incomplete immersion data (where we could not identify nocturnal visits), we assumed that the frequency of visits was the same as controls.

Phenology

The start and end of autumn migration and the start and end of spring migration were determined by analysing breakpoints in the rate of accumulation of 10 minute bins identified as directed flight from deployment, as in Shoji *et al.* (2015). We ran piecewise linear regression using the bootstrap restarting algorithm as implemented in the R package “segmented”, conducted on the immersion data between 20th August 2015 and 15th April 2016 since all birds for which we recovered data were carrying their logger during this period. The same four breakpoint starting values were specified for each track, taken from the mean dates of departure and arrival from the colony in Fayet *et al.* (2016). These breakpoints were validated for the beginning of autumn migration and the end of spring migration by estimates of the birds’ positions based on light data, with start of migration measured as the point that birds in autumn are estimated to have crossed a longitude of -12° as in Fayet *et al.* (2016) before using the breakpoints found for arrival at and departure from the wintering site from the immersion data.

Statistics

We used linear mixed models to test whether chicks gained mass on nights with 0,1 or 2 visits from parents and whether nest visit rate differed between treatments. For the test of chick mass gain, we included chick mass on the day before as an additional fixed effect to account for different possible maximum food deliveries to different sized chicks and bird ID nested within nest ID as random effects to account for repeated measures from the same nest. Nest visit rates were calculated per adult and as such nest was include as a random effect in that model. For the time spent in different behavioural categories, both during the chick feeding period and over winter, we used binomial generalised linear mixed models to test for differences in the proportion of time spent foraging, resting and in directed flight between our treatments, and computed p-values with likelihood-ratio (LR) tests. Each behaviour was coded as a 0 or 1 for each 10-minute bin, and was thus a binomial outcome. For GLMMs, bird ID nested within nest ID was included as a random effect to account for the repeated measures from each bird. P-values for all mixed models were computed by conducting a LR test between a full model and a nested model without the fixed effect of interest. For model estimates, models were fitted using restricted maximum likelihood, for LR tests they were refitted with maximum likelihood. For differences in phenology, over-wintering latitudes and breeding success, which gave highly non-normal residuals in linear models, but were not binomial data, we used a Mann-Whitney U test to test between treatments. While this does not account for repeated measures for birds from the same nest, the non-independence was roughly equal across treatments and thus it offered a pragmatic solution with a relatively small sample size (similarly to Catry et al. 2013). For correlations of dates between consecutive periods in the non-breeding period we used a linear regression and computed p-values with an F test. We did not use paired analyses since, whilst nests were paired on lay date, they were still independent (the balanced design with respect to lay date meant that there was no *a priori* reason to believe that their residuals in fitted models would be correlated). Device model had a significant effect on the proportion of time estimated in different behaviours

(see supplementary material) and so was included as a two level, fixed predictor in all appropriate models. Whilst conceptually device is a random effect, it is not possible to estimate among level variance for predictors with fewer than ~5 levels and as such is not appropriate (Zuur, Leno, Walker, Saveliev, & Smith, 2009).

Ethical statement

All protocols underwent the University of Oxford's local ethical review process and were approved, conditional to any chicks that were abandoned in the study being hand reared to fledging. Previous studies have shown that there is no measurable effect of carrying geolocators or short-term biologging devices on birds' productivity compared to a control plot on Skomer Island (Dean et al., 2015; Fayet et al., 2016; Shoji et al., 2015). Handling time of adults and chicks was kept to a minimum.

Results

Chick feeding

20 nests were monitored which were assigned to either experimental or control treatment groups. Experimental chicks ($n = 10$) were fed an average of 45 ± 10 g and control chicks ($n = 10$) an average of 2 ± 1 g of supplementary food daily for a ~4-week period. The total fed over the chick-feeding period was $1,404 \pm 38$ g for experimental chicks and 56 ± 12 g for controls. There was necessarily some variation in the amount of food delivered and the dates of delivery owing to the size of the chicks (for example, two very late chicks entered the experiment later), but number of days that each experimental chick received food was matched with the chick that had the similar lay date from the control group and the total days fed was the same for all birds. Experimental chicks were the same size at the onset of chick feeding, but grew significantly faster than controls during the treatment (control ($n = 10$): 7 ± 5 g; experimental ($n = 10$): 10.7 ± 2 g; $F = 14.58$, d.f. = 1,18, $p = 0.001$, Figure 6.2a) and reached a significantly higher peak mass before fledging

(control (n = 10): 570 ± 18 g; experimental (n = 10): 712 ± 12 g; $F = 32.49$, d.f. = 1,18, $p < 0.0001$). However, fledging date, estimated by the last date that the chick was seen in its nest after flight feathers were fully developed, did not differ between treatments (control mean (n = 10): 4th September; experimental mean (n = 10): 3rd September; $F = 0.8105$, d.f. = 1,18, $p = 0.4$).

Tracking

Of 38 geolocator-tracked birds, 33 geolocators were retrieved. Of these, 19 (10 control, 9 experimental) recorded the full over-winter period (the remaining devices failed) comprising 3 complete control and 3 complete experimental pairs and a further 4 and 3 single birds from control and experimental treatments respectively. An additional control logger recorded until December 2015, and so was included in analyses of autumn phenology. Our simulation was successful in showing that the different recording schedules of Biotrack and Migrate Technology devices should not theoretically impact on identification of at-sea behaviour since the rate of accumulation of wet events in our simulated data did not differ depending on the recording schedule of the device, as might have been the case if, for example, foraging was characterised by much time resting on water (or in flight) with short time interval transitions that would be more likely to be detected by 3 s Biotrack devices. However, Migrate technology were apparently more sensitive to saltwater immersion and so we conducted a set of supplementary analyses to assess the impact of this on the behavioural classification (supplementary material) and subsequently included device model as a fixed effect in all analyses based on immersion data.

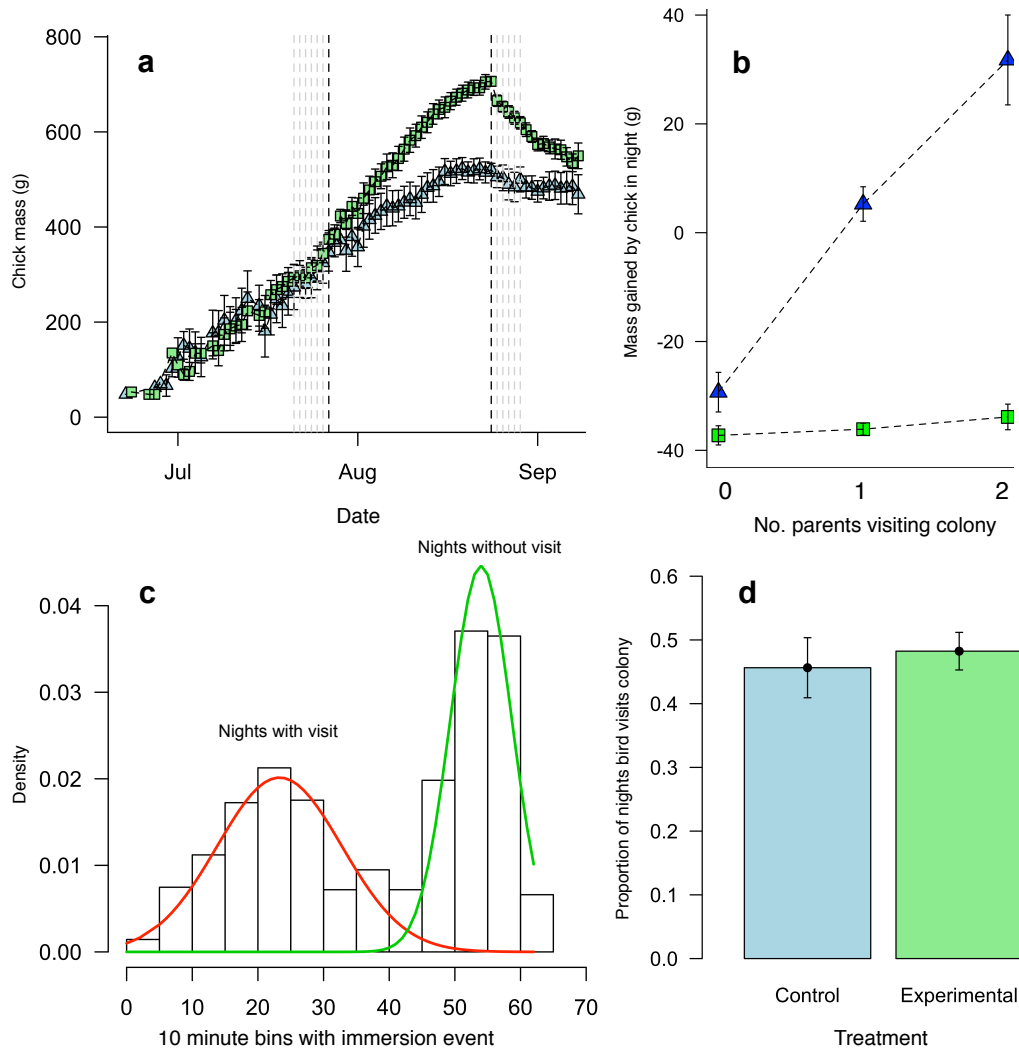


Figure 6.2. a) Chick mass gain over the chick-rearing period from hatching for controls (blue triangles) and experimentals (green squares). All experimental chicks received the full supplementary diet for the period between the bold vertical lines. Some chicks were fed in the periods shown in grey also (see text for details). b) The mass gained by chicks on nights where 0, 1 or 2 adult birds visited the colony as identified in our mixture model (top left), for controls (blue triangles) and experimentals (green squares). c) Probability density of the number of 10 minute binned immersion data with >0 immersion events in a night showing peaks corresponding to nights with a nest visit and nights without. Estimated mixture of two Gaussians is shown labelled for nights interpreted as containing a colony visit (fewer ‘wet’ 10 minute bins, red curve) and nights without (more ‘wet’ 10 minute bins, green curve). d) The proportion of nights during the chick-feeding period that birds carrying geolocators visited the colony. See text for statistics.

Nest visits and mass fed by parents

During the chick-feeding period, the number of parents (0,1 or 2) identified as visiting the colony in a night from our mixture model approach significantly predicted the mass gained (or lost) by control chicks in that night (109 nest-nights, 27 g adult visit⁻¹, LMM: $\chi^2 = 35.1$, d.f. = 1, $p < 0.0001$, Figure 6.2b), suggesting that our mixture model (Figure 6.2c) was correctly identifying night-time visits. Mass gain did not increase with adults from experimental nests visiting the colony (101 nest-nights, 1 g adult visit⁻¹, LMM: $\chi^2 = 2.1$, d.f. = 1, $p = 0.15$, Figure 6.2b), suggesting that experimentally fed chicks were not provisioned by the parents detectably during the chick-feeding period. This difference in provisioning was reflected statistically by the interaction of treatment and number of parents visiting significantly improving our full mixed model analysing both treatments (LMM: $\chi^2 = 58.0$, d.f. = 3, $p < 0.0001$). Furthermore, there was no difference in the magnitude of the mass lost by experimental or control birds for nights that they were not provisioned, controlling for chick mass (LMM: $\chi^2 = 0.8$, d.f. = 2, $p = 0.38$, Figure 6.2a). After the experimental period, the mean mass delivered to all chicks became reduced and all chicks lost mass after chick feeding ended (Figure 6.2a), consistent with the known biology of the species (Brooke, 1990). There was no difference in the visitation rate between our treatments during the chick-feeding period (control (n = 10): 46% of nights; experimental (n = 9): 48% of nights: LMM: $\chi^2 = 0.2$, d.f. = 1, $p = 0.64$, Figure 6.2d). Adult birds attending supplemented chicks were encountered in the nest with the chick during the day more frequently than controls, albeit not significantly (control nests: 1 day visit; experimental nests: 7 day visits, binomial test: $\chi^2 = 3.1$, d.f. = 1, $p = 0.08$).

At-sea behaviour during chick feeding and pre-migration

There was no difference between adults in either treatment in the proportion of time spent foraging (binomial GLMM: control: 0.39 ± 0.01 , experimental: 0.42 ± 0.02 ; $\chi^2 = 0.15$, d.f. = 1, $p = 0.70$, Figure 6.3), resting (control: 0.35 ± 0.02 , experimental: 0.30 ± 0.02 , $\chi^2 = 0.06$, d.f. = 1, $p = 0.80$, Figure 6.3) or in directed flight (control: 0.26 ± 0.03 , experimental: 0.28 ± 0.01 , $\chi^2 = 0.48$,

d.f. = 1, $p = 0.49$, Figure 6.3) whilst chicks were receiving supplementary feeding or in the period between the end of supplementary feeding and the beginning of migration. There was no difference in the date of the last visit to the nest by adults between treatments ($\chi^2 = 0.02$, d.f. = 1, $p = 0.9$).

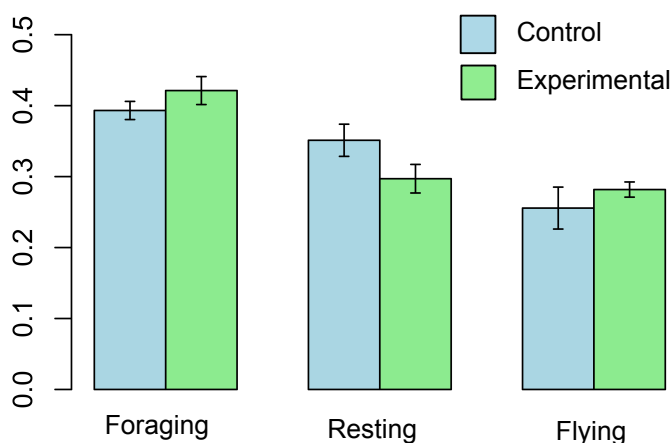


Figure 6.3. Proportion of time spent in each of the three behavioural states of adult birds during chick feeding. The proportions shown are the raw data, before accounting for differences caused by logger model. For modelled estimates accounting for the effect of logger model, see text.

Timing of migration

Our identification of departure times based on the breakpoint analysis (Figure 6.4a) in the immersion data strongly predicted departure times identified by using longitudinal estimates from light data (LM: $r^2 = 0.94$, $F = 280$, d.f. = 1,18, $p < 0.0001$, Figure 6.4c). Since our identified breakpoints in immersion data thus corresponded to migratory decisions, we therefore used breakpoints in immersion data to identify the remaining events in the annual cycle of the shearwaters. From these breakpoints, we could see that arrival date in the wintering grounds was predicted strongly by departure date from the colony (LM: $r^2 = 0.74$, $F = 56.4$, d.f. = 1,18, $p < 0.0001$) and the same was true, but to a lesser extent, for the start and end of return migration (LM: $r^2 = 0.02$, $F = 5.6$, d.f. = 1,17, $p < 0.05$). However, arrival at the wintering grounds was not correlated with southbound departure date from the colony (LM: $r^2 = -0.04$, $F = 0.18$, d.f. = 1,17,

$p = 0.67$) from our breakpoints. There was no difference in phenology of any part of the tracking period between treatments (Figure 6.5), either in southbound migration departure date ($U = 33$, $p = 0.23$) or arrival date ($U = 51$, $p = 0.94$) or northbound migration departure date ($U = 41$, $p = 0.55$) and arrival at the colony ($U = 46$, $p = 0.82$). Departure date was not related to subsequent over-wintering foraging effort (LMM: $\chi^2 = 1.3506$, d.f. = 1, $p = 0.25$).

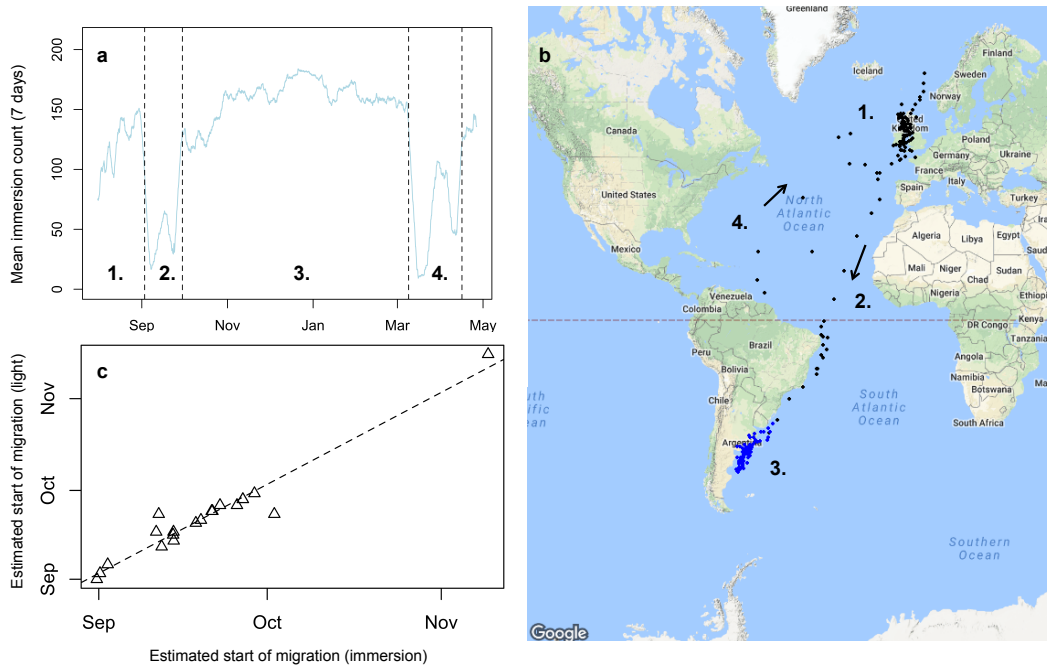


Figure 6.4. a) Example of migratory phenology of one bird identified by our breakpoint analysis. A seven day rolling mean of immersion count is shown with breakpoints corresponding (left to right) to departure from the colony in autumn, arrival at the wintering grounds, departure from the wintering grounds in spring and arrival at the breeding area, overlaid as dashed lines. b) Example migration determined from light data for control bird. Numbers in a and b correspond to: 1. Breeding; 2. Southbound migration; 3. Over-winter residency; 4. Northbound migration. c) The dates identified as the start of southbound migration in Autumn 2015 by the immersion data strongly predicted that identified by the date at which the bird is estimated to have passed -12 degrees longitude, estimated from light data recorded by the geolocators. A single, experimental bird did not begin migration for ~2 months after all other birds.

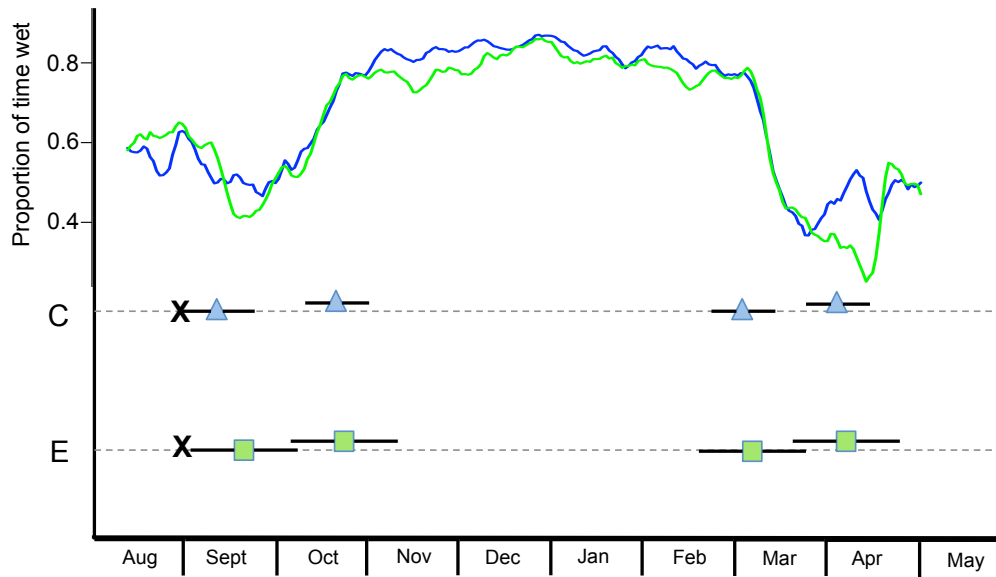


Figure 6.5. Upper panel: Proportion of time for which the logger was immersed for the two treatments over the year for which birds were tracked. Lines show a 7-day rolling mean of all control birds (blue) and experimental birds (green). The increased foraging of the experimental birds is matched with a reduction in the time spent resting and thus the proportion of time that the logger was wet can be seen to be lower throughout the over-winter period in the parents of supplemented chicks. **Lower panel:** Last visit to the nest (X) is indicated, and the start and end of the two migratory periods (mean $\pm$ se) for control (blue triangles) and experimental (green squares).

At-sea behaviour during over-winter residency off Argentina

There was a considerable difference between treatments in the at-sea behaviour during the over-winter period, reflected in the proportion of time that birds spent foraging per day over winter between treatments with control birds spending $31 \pm 1\%$ and experimental birds spending $43 \pm 3\%$ of their time foraging (GLMM: $\chi^2 = 12.454$, d.f. = 1, $p < 0.0001$, Figure 6.5). This was also reflected in time spent resting (control: $61 \pm 1\%$; experimental $49 \pm 3\%$, GLMM: $\chi^2 = 12.2$, d.f. = 1, $p < 0.001$, Figure 6.5) but not in the time spent in directed flight (control: $8 \pm 0.5\%$; experimental: $8 \pm 0.4\%$, GLMM: $\chi^2 = 0.45$, d.f. = 1, $p = 0.50$, Figure 6.5).

Over-wintering latitudes and migration

From our light data, we found that birds from both treatments migrated to the Patagonian shelf, Argentina. There was no difference in over-winter latitudes between the two treatments ($U = 49$, $p = 0.94$, Figure 6.6).

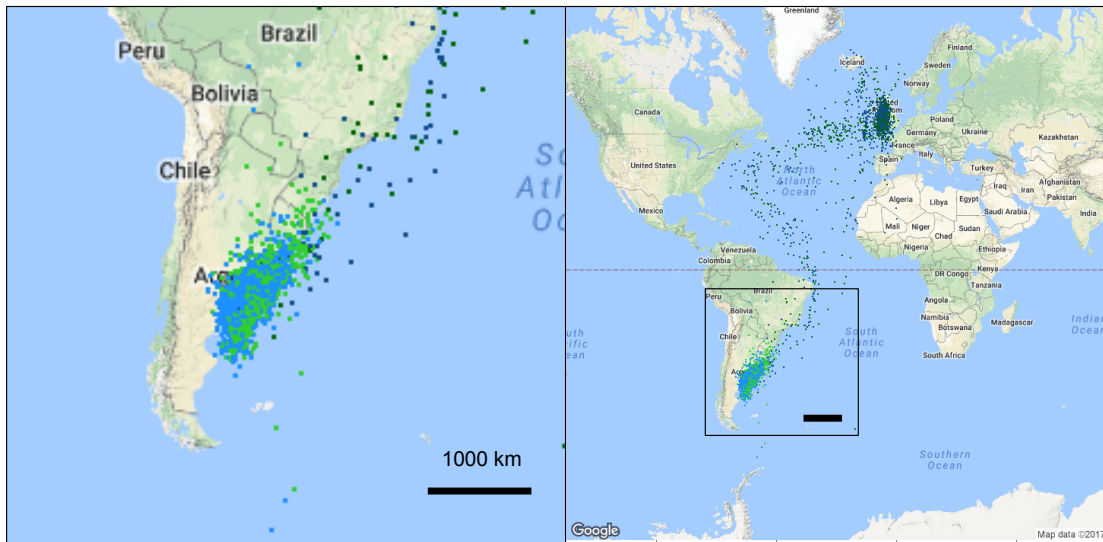


Figure 6.6. Map showing the geolocator positions for all 20 birds. The whole migration is shown on the right. The inset shows the positions during over-winter residency. Over-winter daily locations are shown for control (light blue) and experimental adults (light green) in the non-breeding period following chick feeding. Dark blue and dark green correspond to migration and breeding.

2016 breeding success

Of the 20 pairs, 8 controls and 6 experimentally manipulated pairs were found breeding on the colony the following year of which 7 and 5 fledged a chick respectively. The rest are assumed to have skipped breeding. As a more nuanced measure of breeding success, we measured egg mass and lay date in addition to failure stage or success. Measures of breeding success did not differ between treatments and are summarised for each nest, with test statistics, in Table 6.1.

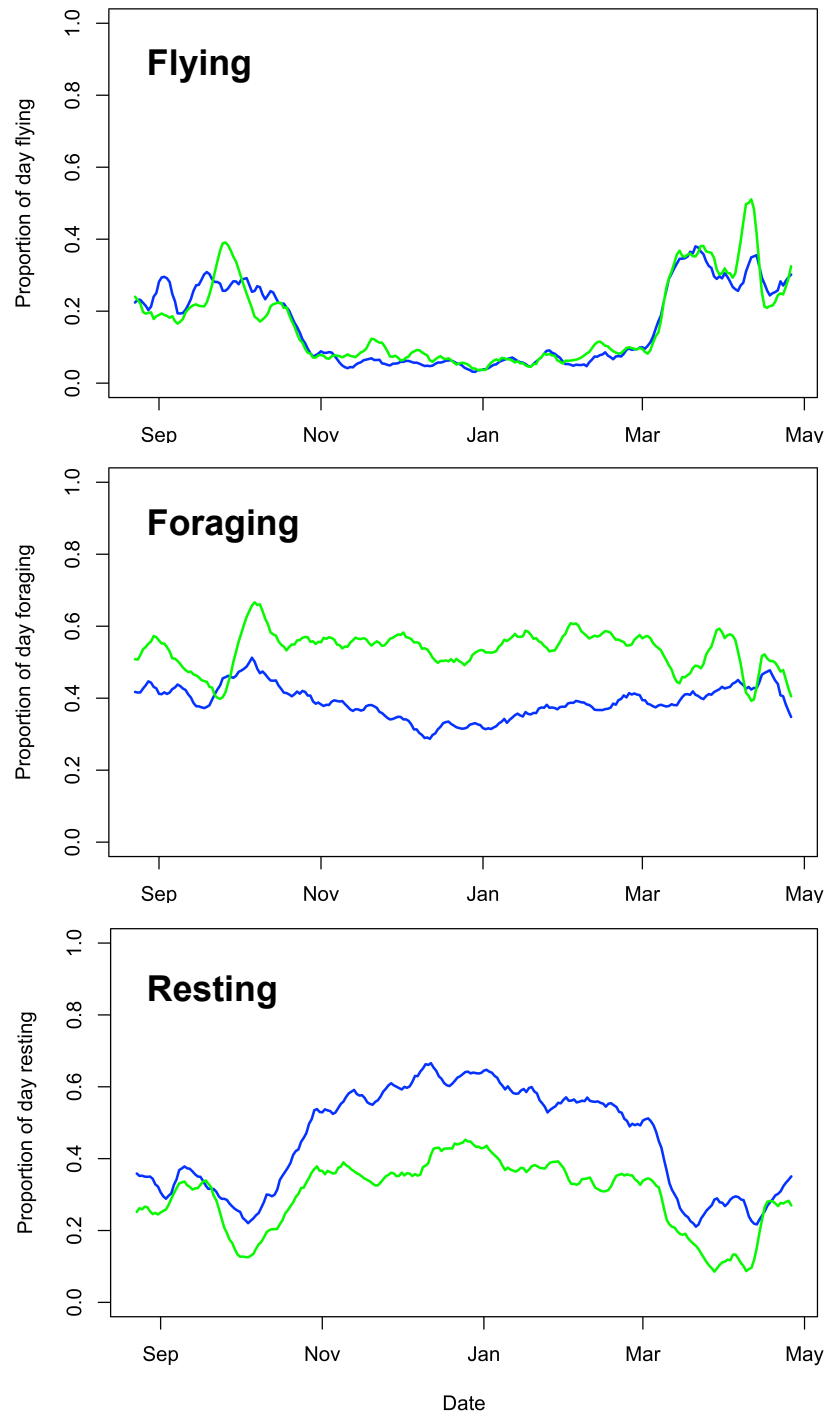


Figure 6.7. Activity budgets for migration and over-winter periods. The proportion of 10 minute bins that were assigned to directed flight (top), foraging (middle) and resting (bottom) are shown for control (blue) and experimental (green) birds as a 7 day moving average.

Table 6.1. Summary of measures of breeding success for 2015 and 2016 for experimental and control birds. There was no difference between treatments in lay date 2016 ($U = 19.5$, $p = 0.60$), egg mass 2016 ($U = 14$, $p = 0.21$), or the differences in measures between years for lay date ($U = 19$, $p\text{-value} = 0.56$) or egg mass ($U = 16$, $p = 0.60$).

Nest	Treatment 2015	Breeding status 2016	Lay date 2015	Lay date 2016	Lay date diff (days)	Egg mass 2015	Egg mass 2016	Egg mass diff
6	Control	Skipped breeding	14-May-15	NA	NA	62	NA	NA
15	Control	Fledged chick	27-May-15	28-May-16	+1	56	56	0
16	Control	Fledged chick	10-May-15	10-May-16	0	62	59	-3
46	Control	Fledged chick	6-May-15	8-May-16	+2	59	59	0
50	Control	Fledged chick	10-May-15	8-May-16	-2	56	61	+5
63	Control	Fledged chick	16-May-15	26-May-16	+10	60	58	-2
117	Control	Fledged chick	24-May-15	31-May-16	+5	61	55	-6
172	Control	Skipped breeding	24-May-15	NA	NA	61	NA	NA
176	Control	Failed at egg	1-May-15	5-May-16	+4	66	58	-8
94R	Control	Fledged chick	21-May-15	7-May-16	-14	62	61	-1
47	Experimental	Skipped breeding	2-May-15	NA	NA	61	NA	NA
73	Experimental	Fledged chick	24-May-15	26-May-16	+2	62	63	+1
107	Experimental	Fledged chick	16-May-15	26-May-16	+10	63	59	-4
142	Experimental	Skipped breeding	12-May-15	NA	NA	54	NA	NA
166	Experimental	Skipped breeding	16-May-15	NA	NA	64	NA	NA
171	Experimental	Skipped breeding	17-May-15	NA	NA	57	NA	NA
174	Experimental	Fledged chick	4-Jun-15	3-Jun-16	-1	61	60	-1
190	Experimental	Fledged chick	6-May-15	7-May-16	+1	55	unknown	NA
DB11	Experimental	Failed at chick	16-May-15	24-May-16	+8	61	59	-1
DB13	Experimental	Fledged chick	6-May-15	8-May-16	+2	57	57	0

Discussion

Here we found that Manx shearwaters that received energetic relief during breeding did not begin migration earlier or have better breeding success in the following year, but did increase the proportion of time spent foraging during over winter residency after migration to the Patagonian Shelf. The supplementary feeding of Manx shearwater chicks in this study resulted in a reduction of the cost of breeding without foreshortening the breeding attempt, since adults attending supplemented chicks reduced drastically the mass of food provisioned but attended the chicks at the nest for the same duration at the end of the season as controls before departing on migration. Despite not provisioning the chicks detectably during nest visits, there was no difference in the frequency of nest attendance between adults visiting supplemented or control chicks and similarly, at-sea behaviour during breeding did not differ between treatments, with similar proportions of time spent foraging, resting and in directed flight. While a single study, which used radio telemetry to identify colony visits found that Manx shearwaters attending supplemented chicks down-regulated slightly their visitation rate compared to controls (Hamer, Quillfeldt, Masello, & Fletcher, 2006), our results are consistent with a GPS tracking study of adults provisioning supplemented chicks that found no difference in the visitation rate (Dean 2013).

Response in adult provisioning behaviour to chick condition in seabirds seems highly variable, most probably in relation to the different dynamics of parent-offspring conflict across systems (Catry, Phillips, Forcada, & Croxall, 2006) depending on species and environment. Both European storm-petrels, *Hydrobates pelagicus*, (Bolton, 1995) and Atlantic puffins, *Fratercula arctica*, (Cook & Hamer, 1997) have been shown to down-regulate both visitation rate and the volume of food provisioned proportionately in response to supplementary feeding of chicks being attended. However, even within species, responses differ and the response of adult provisioning rate to supplementary feeding of Atlantic puffin chicks is known to vary by colony (Harris, 1978). In the Manx shearwaters in the present study, maintenance of visitation rate might reflect good

environmental conditions allowing birds to make visits to the colony for reasons not directly related to chick provisioning, supported perhaps by our finding that over-day visits were more frequent in nests with supplemented chicks, albeit not significantly.

We found no evidence of carry-over effects to migratory phenology, despite birds having considerable time (~2 weeks) between departure on migration and their last visit to the colony during which time migration could have been brought forward. Similarly, breeding success in the year following supplementation was unaffected by energetic relief. Previous studies in Procellariiform seabirds that have experimentally reduced the cost of breeding paint a complex picture of carry-over effects. In Cory's shearwater, shortening the breeding episode brought forward considerably the onset of autumn migration and dramatically enhanced breeding success in the year following (Catry et al., 2013). However, in Manx shearwaters, asymmetrical cross fostering showed that whilst prolonging the breeding period had a large effect on phenology and reduced breeding success in the following year, there was no effect on migratory phenology or breeding success when breeding was shortened (Fayet et al., 2016). The current study suggests that a carry-over effect in Manx shearwaters was not precluded by the already late phenology of the energetically relieved group in that study (Fayet et al., 2016), but rather that enhancement of condition in Manx shearwaters does not cause an immediately evident carry-over effect. Similarly, supplementary feeding of adult kittiwakes had no effect on breeding success in the following year (Gill & Hatch, 2002), though enhanced kittiwake productivity during the supplemented season makes it difficult to infer that adults finished breeding in better condition.

The similar reproductive success of experimental and control birds in this study might be because shearwaters invested surplus condition in future survival. While binary success/failure is a blunt tool for measuring investment in breeding, egg mass and lay date vary considerably among nests and are related to fledging probability (Fayet et al., 2016) and thus if our experiment had affected

investment in the next season similarly to that in Cory's shearwaters this should have been evident even in our relatively small sample size. Previous experiments have shown that investment in future survival is prioritised by Procellariiform seabirds. Navarro and González-Solís (2007) found that condition was unaffected by a cost imposed by reducing the wing surface area of Scopoli's shearwaters, *Calonectris diomedea*, but that breeding performance was poor implying that adult shearwaters diverted the cost of the resulting decreased foraging efficiency on to their chicks. Similarly, in grey-headed albatross, *Thalassarche chrysostoma*, adults did not forego foraging to sufficiently adjust brood guarding duration in response to cross-fostered, differently aged chicks (Fayet et al., 2016). Demonstrating that surplus resource translates to increased survival in Manx shearwaters will require following the experimentally supplemented shearwaters from this study in the long term, but may retrospectively indicate strategic adjustment of life-history decisions if they experience greater longevity or reduce the frequency of skipping breeding. A key component of future studies therefore must also be to measure condition of birds independently of treatments to elucidate condition mediated investment rules and how they change based on previous environmental conditions.

Our results support the idea that there is a 'ceiling' effect preventing adjustment of migratory phenology by Manx shearwaters in response to release from chick rearing (in cross fostering experiments) or increased condition as found in the current study. While early migratory phenology is thought to be linked to birds' condition and/or early release from breeding (Mitchell et al., 2012; Perrins, 1966), the dates over which earlier migration yields an energetic advantage for birds might be bounded closely to the mean migratory date for the population if, for example, weather or regional productivity mean that early migration would not coincide with higher productivity at the wintering grounds. Spring migration seems to be similarly constrained in Manx shearwaters, both in the current study, cross-fostering (Fayet et al. 2016) and correlative studies (Shoji et al., 2015) despite existing variation in egg laying phenology being strongly related to

fledging chicks that successfully recruit to the colony and thus presumably under strong selection (Perrins, 1966). Furthermore, there was no correlation among birds in this study between arrival at and departure from the wintering grounds, consistent with the finding that Manx shearwaters' spring departure date does not predict breeding success (Shoji et al., 2015). Together, these findings suggest that migratory phenology in Manx shearwaters is constrained from being brought forward by seasonal conditions rather than the intrinsic condition of birds. We thus suggest that differences in condition may play out in the pre-laying period in the breeding grounds following spring migration, or be borne of the time taken to secure a nest site at a highly competitive, dense colony. How this will be reflected in immersion data remains to be elucidated, but there is an indication from our analysis that there might be a divergence between the two treatments during April, soon after arrival back in the breeding grounds (Figures 6.5 and 6.7), when there is a peak in the overall time recorded wet by the loggers for control birds that is matched by a trough for experimental birds.

While we found no carry-over effect to breeding success in the following year, we did find a marked carry-over effect to over-winter behaviour, with energetically relieved birds spending much more time in the 'foraging' state. This is in contrast to previous studies. Cory's shearwaters with experimentally reduced wing surface area (Navarro & González-Solís, 2007) spent more time foraging, and in Manx shearwaters, higher foraging effort has been shown to predict breeding failure and thus presumably is linked with poorer condition (Shoji et al., 2015) perhaps itself linked to increased energetic requirements or poor foraging efficiency (Navarro & González-Solís, 2007). However, in Fayet *et al.* (2016) Manx shearwaters with both extended and shortened breeding period foraged less than controls and thus the link between activity budgets and condition is not necessarily easy to interpret. With this in mind, we can think of two potential explanations for an increase in foraging effort related to an increase in condition in the present study. We think that a third explanation, that our treatment inadvertently reduced the condition of

experimental birds, is unlikely, since we observed normal at-sea behaviour, colony visitation during breeding and similar breeding success in the following year. Crucially, the last date that the nest was attended did not vary between treatments.

The first explanation, therefore, questions why our prior expectation would be for a negative relationship between a bird's condition and its foraging effort reflected by its daily activity budget, as shown by Shoji et al. (2015). Indeed, it follows that a bird in poor condition would need to forage for more time to reach the same condition as a bird that started in good condition, and deterministic models from optimal foraging theory predict that an animal in better condition should forage less such that it can allocate time to other activities (Barclay, 1989; Caraco, 1980). This way, costs associated with feeding, such as risk of predation, can be negated by birds in good condition (Bachman, 1993; Godin & Shelley, 1988). However, maintaining the same condition might require higher allocation of time to foraging for birds in good condition because of the energetic costs involved in flight, maintaining fat reserves and muscle mass (Croll, Gaston, & Noble, 1991). Furthermore, birds in better condition might have access to more prey, be better at escaping predators (Lima, 1986), and thus the optimal response to condition depends on unknown factors.

A second explanation relates to foraging in patchy environments. If condition were to influence the decisions to move between patches then state changes (wet to dry) might be more frequently encountered and thus interpreted as increased foraging effort. For example, birds in better condition might be able to undertake 'riskier' foraging behaviour (Caraco, 1980). By risk, we mean take decisions with greater mean pay-off but also greater variance in pay-off that thus require a probabilistic cost to access (Real & Caraco, 1986). If such a cost could be borne more easily by a bird in better condition, then condition-related differences could play out (Krebs, 1980)

in shearwaters if decisions to move between patches follow a similar pay-off structure thus affecting the amount of foraging detected by the immersion loggers.

Our result highlights the need for studies that validate the behavioural inferences from saltwater immersion data. Such validations have previously been conducted on data collected during foraging trips in the breeding season (e.g. Dean et al., 2012), but it is possible that differences between at-sea activity during breeding and over winter caused by the change in foraging ecology (e.g. not being constrained to central place foraging) might limit the validity in extending these behavioural classifications to year-round behaviour. Furthermore, even within the breeding season, few studies have measured the relationship between putative time spent ‘foraging’ and condition (though Fayet et al. (2015) did find that GPS tracked immature Manx shearwaters had to forage more, according to behavioural classifications from GPS data, to gain the same mass as simultaneously tracked adult birds). Future studies combining behavioural classification with measures of condition at the colony might therefore be informative, as might be year-round deployment of new, long-life time-depth recorders.

In this study we found that energetic relief during the breeding season in Manx shearwaters does not carry over to migratory phenology or breeding success in the following year, a finding consistent with a previous cross-fostering study in the same system, and still evident when controlling for birds’ extant phenology in the relieved season. Thus, surprisingly, in the Manx system it seems that migratory phenology is environmentally constrained such that bringing forward migration is not favoured in birds in good condition and/or with the option to do so. This is in contrast to other species studied, where bringing forward migratory phenology is a strong response to relief from the breeding attempt (Bêty, Gauthier, & Jean-François, 2003; Catry et al., 2013). Breeding success was similarly unaffected, but future studies, with observations over multiple years and independent measurements of birds’ condition will be required to ascertain

whether this is the result of adaptive plasticity (Harrison et al., 2011), where shearwaters change each year their investment in breeding in response to their condition strategically, or a fixed investment rule which thus always conserves surplus condition for survival. Manx shearwaters' high breeding success might preclude subtle increases in breeding investment from being found from such studies, but long-term monitoring of experimentally manipulated birds might reveal that effects on decisions to skip breeding in the medium term may well be indicative of life-history responses to condition in the future.

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Supplementary Material

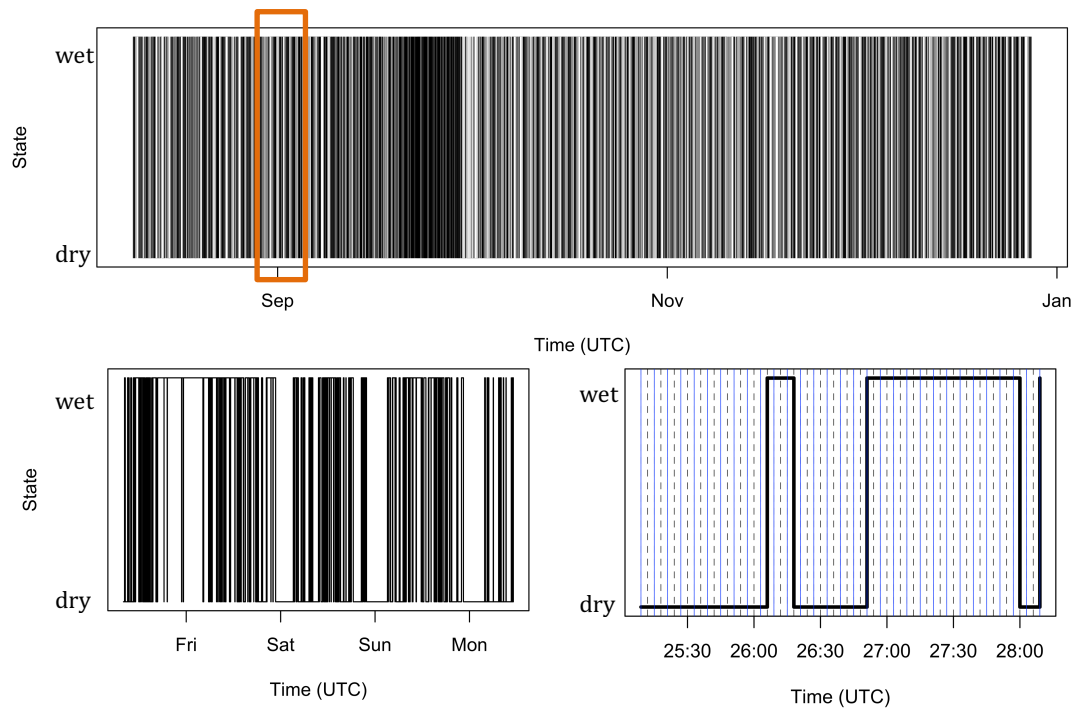


Figure S6.1. Example of high-resolution immersion trace used to simulate data to check for an effect of conversion from 6s->5min to 3s->10 min of coarse GLS immersion data. Top panel: 4 month period of state transitions from wet to dry and dry to wet. Bottom left: magnified section of top panel (indicated by orange box). Bottom right: a ~3 minute section of GLS immersion trace showing five transitions (wet-dry or dry-wet). Hypothetical 3 and 6 second intervals are shown using dashed and solid lines respectively.

Device differences:

We found that there were significant differences in the amount of wet events recorded by our two types of geolocator ($z = -27.584$, $p < 0.00001$). In the main text we accounted for this using device model as a fixed predictor in our mixed models such that it did not influence our assessment of the effect of feeding treatment. We analysed this further since the device type was biased across our feeding treatments. We explored the variation in behaviour assignment resulting from the two different data-streams by modelling the effect of device. Here, we included as many factors other than device as possible to make a model that best predicted the immersion counts of birds and

then tested for the effect of the device model. We then varied the thresholds of behavioural identification to assess the direction that the bias was in. For example, starting with thresholds 0 (directed flight); 1-199 (foraging); 200 (resting) and asymmetrically varying the thresholds for resting and directed flight we could see that Migrate Technology devices were significantly more likely than Biotrack devices to record wet events since when the threshold for resting was 200, the number of 'resting' events recorded by Migrate Technology devices was significantly higher than that recorded by Biotrack devices even when controlling for treatment, chick size, breeding stage and day of the year ($p < 0.00001$). This effect diminished when the threshold was reduced to 198, suggesting that many bins containing 'real immersion values' in the range of 198 - 199 were being recorded as 200 by the MT devices (or immersion was under-recorded by Biotrack devices). The reverse was true at the low-immersion end, where a threshold of 0 for directed flight resulted in Migrate Technology devices recording more foraging (and less directed flight) than Biotrack devices. Increasing the threshold for directed flight from 0 to < 3 , decreased the difference between the devices suggesting that either Migrate Technology devices were recording some 0s as >0 or that Biotrack devices were recording some 1 or 2 event bins as 0. Together, the reverse effects at either end of the immersion thresholds is evidence that Migrate Technology devices recorded significantly more wet events than Biotrack devices, per real wet event. Consequently device model was included as a fixed effect (only two levels) in our models testing for the effect of treatment since there was a bias in the recovered devices across the treatment levels. For plots of activity over the breeding season and winter period, we adjusted the time spent foraging, resting and in directed flight based on the estimated effect of geolocator model from this binomial mixed models.



Figure S6.2. Daily positional fixes of control adults during the non-breeding period following experimental treatment. Blue points represent locations in the over-winter residency period as identified by our breakpoint analysis. Equinox points have been filtered.

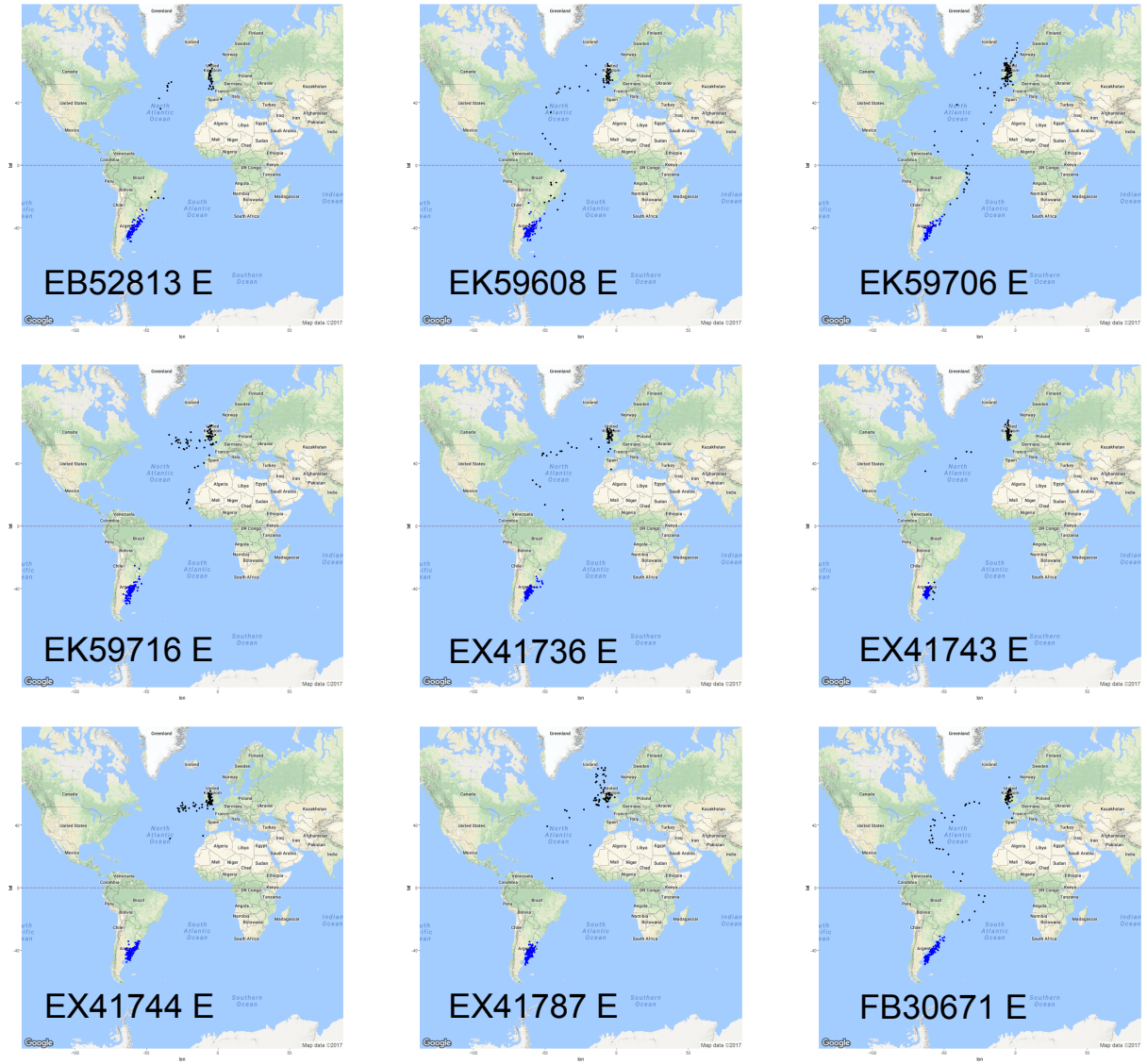


Figure S6.3. Maps for adults from nests that received supplementary feeding. Blue points represent positional fixes that are inside the over-winter residency as identified by our breakpoint analysis. Equinox points have been filtered.

Chapter 7

Future directions

Introduction	227
General discussion and synthesis	227
Future directions	237
References	243

Introduction

The aim of this thesis was to combine field experiments and natural observations with novel analytical methods to investigate some of the mechanisms underpinning oceanic navigation in two species of Procellariiform seabird; the Manx shearwater, *Puffinus puffinus*, and the Scopoli's shearwater, *Calonectris diomedea*. I aimed to cover some of the most fundamental behavioural aspects of avian navigation: the role of map-and-compass navigation in familiar area navigation, the olfactory navigation hypothesis, and the cognitive basis for extrapolated maps. Before writing this thesis, there were many unresolved questions in each of these areas; particularly, how these mechanisms pertain to seabirds and free-ranging movement. After writing this thesis, there are potentially even more. There were also several subsidiary themes to this thesis. First was the quantification of animal movement data such that key decision points and behaviours in trajectories can be analysed. Second was the design and development of carefully controlled experiments with wild seabirds. With this in mind, I also investigated some of the ecological drivers of large-scale movement in Procellariiform seabirds; movements that the navigational mechanisms, that form the main theme of this thesis, have evolved to effect. In this final chapter, I will attempt to synthesise some of the implications of the key results from this thesis, including a more fully worked version of the theory of direction accompanied landmarks from Chapter 2, on our conceptual understanding of seabird navigation. Finally I will suggest some areas that might warrant future study.

General discussion and synthesis

Travelling huge distance over open ocean, the navigational challenge that Procellariiform seabirds face is conceivably different to that faced by any other taxon (Bonadonna et al., 2003). Consequently, there has been long-standing interest in whether the navigation of Procellariiformes could be under the control of similar processes to terrestrial species (Gagliardo, 2013). A further motivation for studying navigational processes in seabirds is that navigational mechanisms that rely on visual landmarks are likely to be redundant at distant locations far from

the coast, but useful close to the coast, permitting studies to investigate not only the sensory basis for true navigation, but also birds' topographic representation of their familiar area (Pollonara et al., 2015). The findings in this thesis consolidate the role of some of the mechanisms discovered in terrestrial species in seabirds, but also give rise to some new ideas that might relate to avian navigation as a whole.

Familiar area navigation – the role of visual cues close to the colony

The role of visual cues in avian navigation has long been debated (Biro et al., 2004; Streng & Wallraff, 1992; Wallraff et al., 1999). The previous finding that anosmic Scopoli's shearwaters displaced in the Mediterranean have reasonable homing ability (Pollonara et al., 2015) compared to Cory's shearwaters, *Calonectris borealis*, released in the mid-Atlantic (Gagliardo et al., 2013), suggests that the use of distant visual landmarks might be important for navigation of pelagic seabirds during homing. The analysis presented in chapter 3 provided further evidence that shearwaters can use landmarks close to home to navigate precisely but also suggested that the resolution of a bird's cognitive representation of topographic features might be relatively low further away from the colony, since shearwaters were unable to compensate fully for anosmia when orienting back to the colony at the end of foraging trips, despite often initiating homing from within sight of the Catalanian coast.

The observation that pigeons adopt stereotyped, recapitulated routes home over the course of a series of releases is evidence that pigeons make use of visual cues in familiar area navigation (Biro et al., 2004) and that this is likely to involve direct guidance from landmarks; a process known as pilotage (Griffin, 1952). Homing within a familiar area by pilotage does not require a compass (Papi, 1992), in contrast to the main theoretic alternative (Holland, 2003), the mosaic map model (Wallraff, 1974), under which movement between familiar landmarks is achieved by effecting remembered directional instructions by reference to a compass. However, while clock-shifted pigeons navigating from highly familiar release sites accurately recapitulate their

stereotyped homing route, implying that guidance is without a compass, they do exhibit small but consistent deflections in the direction expected by sun-compass use throughout the homing journey (Biro, Freeman, Meade, Roberts, & Guilford, 2007). The implication is a mechanism for guiding goal-oriented movement within a familiar area that is both piloted by landmarks and guided by compass cues between landmarks en route (Biro et al., 2007; Guilford & Biro, 2014).

The results presented in Chapter 2 suggest that a similar mechanism might also govern homeward guidance in Manx shearwaters while navigating over the sea, possibly by reference to distant, horizon-limited visual landmarks. The function and mechanistic nature of combining guidance from the sun-compass and familiar landmarks remains unknown. In discussing the role of the sun in animal navigation, Guilford & Taylor (2014) suggest that time-dependent landmark features such as shadows might result in the appearance of a landmark changing sufficiently over the course of a day such that associations made with the same landmark at different times of day are stored as functionally independent entities. A clock-shifted bird might, therefore, not recognise the landmark when its subjective time is offset with the expected appearance of the landmark in objective time. Clock-shift deflections from familiar release sites could perhaps therefore be explained in part by a bird falling back on map-and-compass navigation (based on time-independent positional cues and sun-compass guidance) when a ‘coherent familiar landscape’ is lacking (Guilford & Taylor, 2014), but how that might result in a *partial* deflection under clock-shift remains unclear.

Direction-accompanied landmarks

One possibility is that onward guidance between landmarks is not affected by clock-shift at all, but rather, the evident systematic ‘error’ is somehow introduced at the stage of interpreting location. As a distant and predictable celestial object, a bird might make use of the sun itself as a celestial landmark – inferring the bird’s own position by comparing its distance to a visual landmark and the sun’s position (the sun-compass). If the view of a visual landmark were, for

example, too distant to inform the bird of the direction from which it is viewing the landmark by its visual properties, then reference to a compass would provide valuable positional information much in the same way that humans can fix their location precisely on a map with only a single landmark, but only providing they are carrying a compass and are able to judge the distance to that landmark. While the sun is technically being used as a compass for position finding, in contrast to sun-compass use for onward guidance, the theoretical magnitude of deflection induced by a clock-shift actually remains small while the visual landmark is distant. In Figure 7.1, I demonstrate schematically how these ‘direction-accompanied landmarks’ (DAL) might result in small but consistent deflections using the example of a Manx shearwater as it comes in sight of a familiar coastal island after homing from far out at sea.

Predictions and consequences

Direction-accompanied landmarks could theoretically explain partial deflection with or without the bird having a cognitive representation of the position of the landmark relative to home, if birds could learn to associate the reward of arriving home with the angle taken relative to a landmark at different times of day; a mechanism that may play an important role in pigeon homing (Guilford & Burt de Perera, 2017). For a pigeon, the position of the sun could be integrated during the development of stereotyped homing routes such that the bird can derive directional information at a farther distance from landmarks, or as a way in which to streamline the memory load involved in pilotage (since only the outline of a landmark need be remembered). The theory might yet prove adaptable to explain other observations in clock-shift releases. For example, the magnitude of the clock-shift induced deflection might decrease with familiarity because the bird perceives the range of directions it could approach the landmark from to be limited (thus the bird chooses a location from a subset of the arc in Figure 7.1). The use of direction-accompanied landmarks yields some predictions under clock-shift that vary systematically depending on measurable properties of the homing system (Figure 7.2), and so perhaps re-examination of existing pigeon data might in future provide an indication of whether compass information is used to augment a

map that is either sparse in reality (in shearwaters) or sparse in cognitive representation (possible in pigeons). A limitation of Chapter 2 is that while shearwaters were released out of sight of land, this was by a very small margin and the majority of shearwaters were within sight of land at the point that homing commenced. To consolidate these findings, future studies will therefore need to show that larger clock-shift effects are evident in shearwaters that are released further out of sight of land. The transition during a single trip from areas that are unlikely to provide stable visual landmarks to areas within the visual extent of many topographic features might offer the controlled variation in visual information that proves key to resolving the incorporation of map-and-compass information to a bird's familiar area map.

Familiar area navigation – the role of cues further away from the colony

One way that pilotage is envisaged to guide pigeons is via attraction towards a succession of landmarks, the visual contact made with the next as each is reached (Biro et al., 2004), known as steeple-chasing (Baker, 1984). While visual information could provide piloting information to shearwaters within the vicinity of the colony in a way akin to steeple-chasing, the theory of time-compensated landmarks suggests a way that guidance from the geometric arrangement of landmarks might be facilitated even with very sparse or distant visual information. Quantifying the extent of horizon-limited topographic information available to Manx shearwaters as they initiated homing in Chapter 5 suggested that visual information might also be used much further away from the home colony. The finding that shearwaters beginning journeys from areas with more topographic visual information were more likely to be oriented according to a short-cut route home, that anticipated the advent of intervening obstacles between them and home (Figure 7.3), implies that further from the colony, guidance was probably not toward familiar landmarks themselves since the short-cut routes were, by definition, not oriented toward the closest coast (in these cases the beeline was intercepted by an obstacle). It seems therefore, that landmarks, at least those distant to the colony probably provide either guidance from their geometric relationships,

or guidance from ‘site-specific compass orientation’ from remembered panoramas (Filannino et al., 2014).

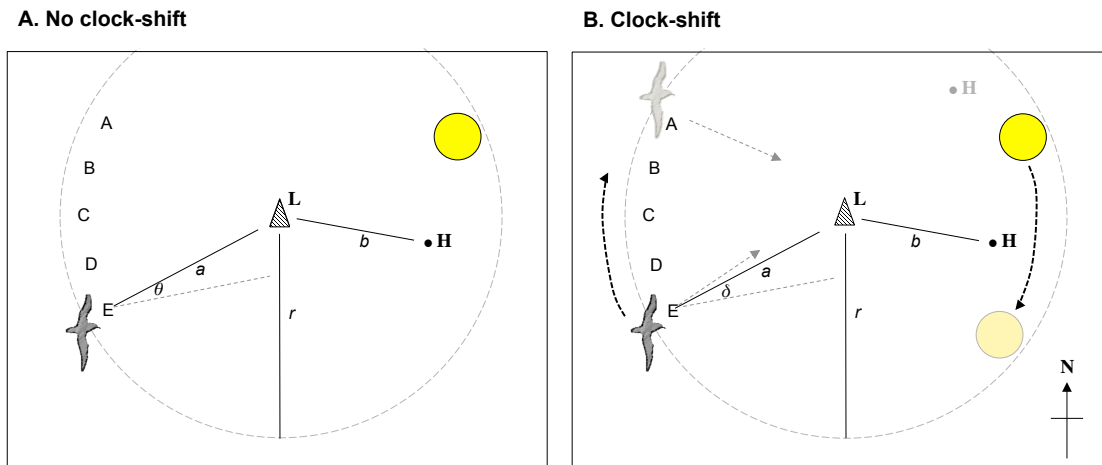


Figure 7.1. Depicted is a shearwater navigating eastwards over sea toward home, **H**, from the point it first can see landmark, **L**, at position **E**. The bird does not have direct sensory contact with **H**, but does have visual contact with landmark, **L**. The visual extent of **L** is shown as a grey dashed arc. Let us assume that the unfamiliar navigational mechanism that has brought a homing bird to its current position has a resolution coarser than the distance of the bird from the landmark, a . **Scheme A**. Thus assuming that a shearwater is navigating without egocentric information, upon seeing the landmark the shearwater can deduce that it is at one of a set of locations that are limited to an arc of a radius, r , that is equal to the bird's distance, a , to the landmark. These potential positions are represented discretely as locations A-E in the schematic, but could be anywhere on the depicted arc. The shearwater cannot know which of these radius limited set of locations it resides at without a second point of reference. I suggest that the sun avails itself to that use and thus confers the bird a (perhaps partly) time-compensated position along the arc. By knowing where on the arc the bird resides, it can thus compute a direction home as a magnitude of deflection from the target by pilotage (angle θ in the scheme). Once a clock-shift ($\sim 50^\circ$ change in sun's azimuth) is applied (**scheme B**), the same shearwater would be misinformed about its position on the arc (faded bird at position A) because it incorrectly references its ephemeris for the sun's location. Thus, the computation of the desired deflection to the target, via the erroneous interpretation of the sun's position would induce, for the depicted fast shift, the anti-clockwise deflection, δ , relative to the beeline. Faded H depicts where the clock-shifted shearwater interprets home is relative to the landmark.

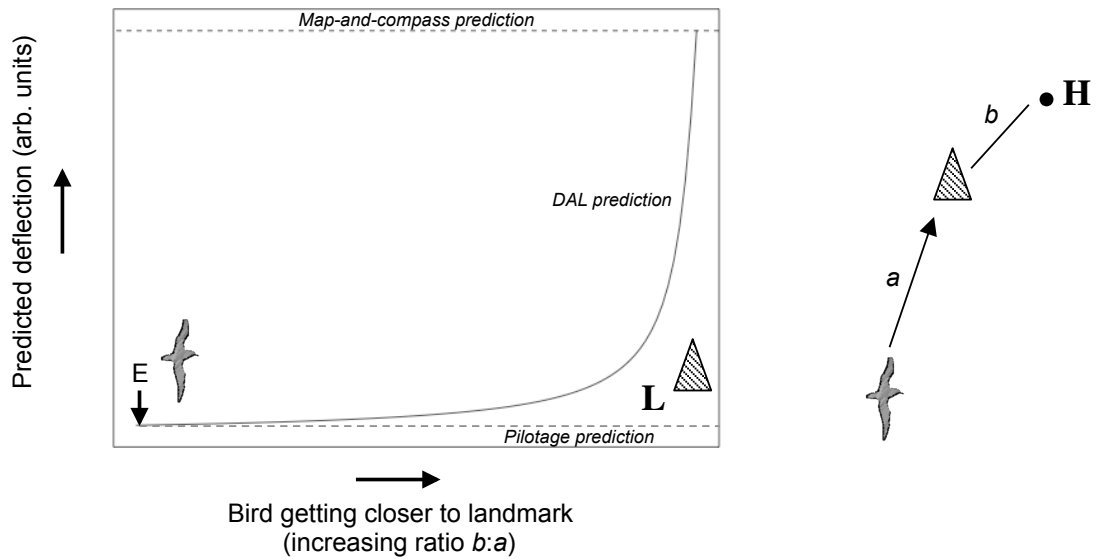


Figure 7.2. Hypothetical predictions for deflections induced by clock-shift for a bird navigating using direction accompanied landmarks (marked *DAL prediction*), pure pilotage or pure map-and-compass. As the bird gets closer to the landmark (ratio $b:a$ increases), the expected magnitude of the deflection caused by a clock-shifted bird making use of the sun to accompany landmark guidance with a direction increases until at the landmark it equals the deflection predicted by pure sun-compass use. The ratio of $b:a$ is the relative distance between the bird and the landmark and the landmark and home as shown (right). The hypothetical scheme presented assumes that, as the bird gets closer to the landmark, the bird does not begin to resolve pilotage information from its visual features. Therefore the prediction, in reality, is probably valid only for the landmark as it remains distant, or without any direction-discerning features.

Implications on true navigation from free-ranging tracking studies

Previously, few studies have investigated navigation in free-ranging birds (though a few examples exist e.g. Bonadonna et al., 2005; Mouritsen, Huyvaert, Frost, & Anderson, 2003; Holland et al., 2009). There are several reasons that this is the case. The first is that in a displacement experiment, there is a clearly defined target and consequently in a well motivated animal, movement from release is navigationally relevant and its orientation measurable with respect to a known goal (Matthews, 1953b). Second, displacement experiments remove outward journey information and as such the resulting navigational phenomena cannot be confounded by an animal's use of egocentric information. Equally, however, there are motivations to trade-in displacement experiments for free-ranging animals. Navigation in a free-ranging animal is always ecologically

relevant. In nature, wild animals will almost never be without any outward journey information and, for most species; navigation will seldom be from places to which the individual has not deliberately navigated.

The role of path integration has been previously suggested as a mechanism by which seabirds might home after free ranging foraging trips (Benhamou et al., 2003), though successful orientation after displacement to unfamiliar sites implies that they are not dependent on path integration to home (e.g. Benhamou et al., 2003; Matthews, 1953a). The results of two of the chapters of this thesis have implications for the role of path integration in homeward guidance at the end of free-ranging foraging trips in Procellariiformes. In Chapter 3, the finding that path integration of the outward journey was not sufficient to restore normal homing orientation in anosmic Scopoli's shearwaters implies that, even from familiar (and coastal) sites, shearwaters incorporate information about their location using true navigation. Furthermore, there is no evidence that the ability to orient homeward declines over time spent at sea (chapter 5) as might be expected due to the propagation of location errors under path integration models (Benhamou & Seguinot, 1995). Theoretically, it is perhaps not that likely that path integration would be sufficient to explain the accurate homing behaviour exhibited by Procellariiformes. While allothetic path integration might actually accumulate error very slowly (Benhamou & Seguinot, 1995), during distant trips at sea, there would conceivably be long periods of time during which only idiothetic cues (the vestibular system) are available to a shearwater as a means for path integration. Idiothetic path integration accumulates error very rapidly even over distances in the order of metres (Benhamou, Sauve, & Bovet, 1990; Seguinot, Cattet, & Benhamou, 1998) and the limited experimental evidence suggests that path integration error in water is likely to be greater still (Benhamou, 1997). From the limited evidence in Chapters 3 and 5 and this reasoning, it seems likely that shearwaters make use of their true navigation ability (Matthews, 1953a) even when endowed with outward journey information. The consequence of this is that studying homing in free-ranging Procellariiformes might be more informative about true navigation than

previously thought. Further still, I suggest that perhaps in addition to the commonly held view that true navigation evolved as a means of dealing with displacement from weather conditions or long distance migration (Bingman & Cheng, 2005), the limitations of path integration in fluid media may actually be the main driver of true navigation.

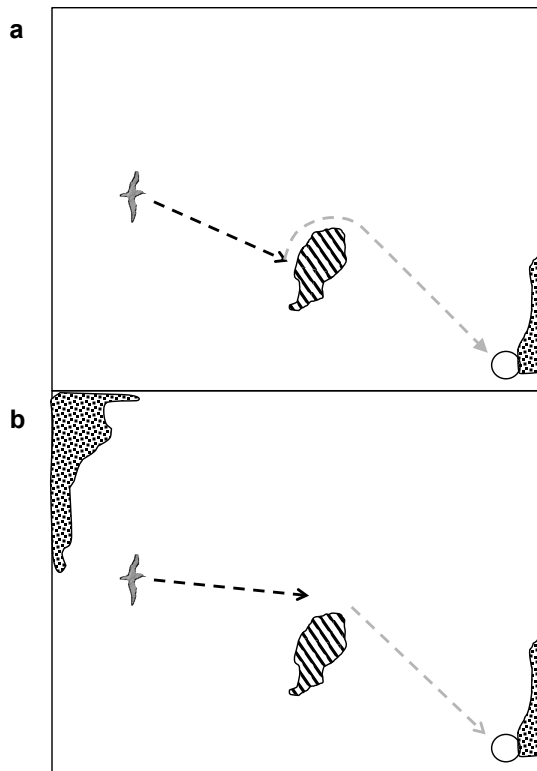


Figure 7.3. The schematic shows two scenarios with a shearwater attempting to navigate back to its colony (circle) from the other side of an obstacle (island), which it cannot yet see. Scenario (a) shows a shearwater's initial orientation (black) with no visual information, resulting in the shearwater ultimately taking a less direct route (grey) home since it has to fly around the obstacle when it arrives within sight of it and (b) the shearwater from an area rich in topographic visual information showing an initial orientation (black) that anticipates the obstacle before consequently completing the most direct route back to the colony (grey). In Chapter 5, the amount of visual topography predicted strongly which of the two routes (a) or (b) a shearwater's initial orientation was consistent with.

One of the arguments in the introductory chapter of this thesis was that behaviour could imply cognitive process. I (very) tentatively suggest that the finding that Manx shearwaters are equipped

with a vector of distance and direction home at the onset of homing after autonomous displacement, presented in chapter 5, constitutes the first direct evidence of true navigation that could not be facilitated by stimulus response learning (see Guilford & Burt de Perera, 2017). Furthermore, if the finding turns out to be robust, this might imply that the mechanisms controlling true navigation in Procellariiform seabirds might be distinct from the mechanisms frequently considered to facilitate true navigation in pigeons (Gagliardo, 2013; Wallraff, 2000). Many authors already consider true navigation to be facilitated by a cognitive module that is tied specifically to navigation (Jacobs, 2003; Jacobs & Schenk, 2003), much like the cognitive map (O'Keefe & Nadel, 1978; Tolman, 1948). However, there has not been an exhaustive attempt to rule out whether homeward orientation from unfamiliar release sites could instead be facilitated by already ubiquitous associative, stimulus-response learning (Guilford & Burt de Perera, 2017). If indeed true navigation cannot be explained by stimulus-response learning, it represents a unique cognitive phenomenon (Jacobs, 2012; Jacobs & Schenk, 2003), since the cognitive module must be capable of predicting the values of cues in space that have not already been experienced such that without outward journey information, a navigating animal can understand its position in space. As pointed out by Jacobs (2003) and Jacobs & Schenk (2003), Tolman's (1948) conceptualisation of the cognitive map cannot facilitate such navigation, since there is no opportunity for a path integration 'gated' learning process that ties specific stimuli to specific places in a cognitive representation (McNaughton et al., 2006). Jacobs and Schenk's (2003) Parallel Map Theory might provide a cognitive framework to explain how a fully extrapolatable map might operate, though the currently proposed neurological basis is at odds with the observation that the role of the hippocampus in the execution of true navigation is at best unclear (Bingman & MacDougall-Shackleton, 2017).

Ecological drivers of long-distance movement

The life-history of avian navigators will probably be a key to understanding the evolutionary drivers behind navigational mechanisms, especially the aspects that differ between taxa (Bingman

& Cheng, 2005). Chapter 6 explored some life-history aspects of the long-distance movements in Procellariiformes that are facilitated by the navigational mechanisms that form the main subject of this thesis. Experimentally manipulating the condition of breeding Manx shearwaters while (in contrast to previous studies) controlling for breeding phenology suggested that constraints on the timing of long distance movements come from the environment (perhaps to do with the temporal pattern of productivity at the breeding and wintering grounds) and the constraints of rearing a chick (Catry, Dias, Phillips, & Granadeiro, 2013; Fayet et al., 2016) rather than being related to the condition of the birds. While the drivers of migratory phenology itself are peripheral to the study of avian navigation, the finding in chapter 6 that phenology is probably driven by environmental constraints rather than the physiological cost of migration itself is consistent with the idea that energetically low-cost flight (Weimerskirch et al., 2000) allows massive space use in Procellariiform seabirds, accompanied perhaps by navigational adaptations to a featureless landscape across which it is perhaps difficult to path integrate.

Future directions

In this thesis, several key areas have emerged that might warrant future investigation. First, a very general aim for the future should be to develop analytical techniques that can be used to expand the study of navigation in free-ranging seabirds. Second, as the principle alternative to true navigation, another rather general aim should be further investigation of the role of outward journey information in free-ranging foraging tips of Procellariiform seabirds. Perhaps the most pertinent question emerging from this thesis is whether pigeons, like free-ranging shearwaters, begin navigation after displacement with a vector of both distance and direction to the loft. In the study of familiar area navigation, the analysis of breakpoints as they correspond to the advent of new visual information might indicate the role of landmarks without the requirement for clock shifting or displacement studies, relating changes in navigational behaviour to available navigational information. In the following section I have expanded on a few of the key analyses

or experiments that might be informative for making navigational inferences from telemetry data in future.

Detailed analysis of clock-shifted and un-manipulated shearwaters as they traverse areas with a variety of quantified visual information. A limitation of the study presented in chapter 2 was that while birds were released out of sight of land, this was by a close margin and consequently many of the shearwaters came within sight of land before homing began preventing a proper analysis of the clock-shift effect as shearwaters traversed from areas with no visual information to areas with increasing visual information. Distinctive breakpoints in seabird trajectories that correspond to underlying mechanistic switches might be expected as shearwaters gain more visual information (the correlation between state-switching identified from state-space models and different types of visual landscape features has already been utilised in familiar area navigation in homing pigeons: e.g. Guilford et al., 2004; Lau et al., 2006; Mann et al., 2014; Roberts et al., 2004). With a slight modification, geometrical methods such as those used to quantify the visual extent of topography in chapter 5 would be able to determine the number and shape of topographic ‘peaks’ (mountains) visible along the homing course.

A variety of methods have been explored to identify breakpoints in bird trajectories that could be used to identify changes in navigational behaviour that correspond to the advent of visual stimuli. In addition to methods invoking latent state-space models (HMMs), one that might be particularly amenable to identifying changes in navigational behaviour with changes in visual topography is breakpoint analysis, which comprises various algorithmic methods for discretising continuous trajectories. There has been recent interest in applying such methods to seabird tracking data to answer ecological questions and its adaptation to navigation (chapters 3, 5) might also prove relatively informative. A problem inherent with breakpoint analyses is identifying a suitable sensitivity that an algorithm will detect changes in behaviour (Thiebault & Tremblay, 2013), since as sensitivity of a method increases, more breakpoints are produced creating a trade-off between

missing real changes in behaviour and retaining segments that are behaviourally distinct. One option is to determine an algorithms sensitivity by *a priori* rules (e.g. “stop increasing sensitivity when n breakpoints are reached”, or “stop increasing sensitivity when the last section is distinctly homeward oriented”, e.g. chapter 5). This is a reasonable approach if there is good *a priori* knowledge about a system to inform n . With identifying the homing phase of a foraging trip this will normally be the case, since we can deduce that a bird will begin homing some point between the time that it reaches the furthest distance from the colony and reaching the colony, and this is likely to correspond to the first breakpoint found in that path. Another approach to sensitivity is an information theoretic approach, looking for increases or decreases in sensitivity that change disproportionately the composition of output segments. This might be a jump in the variance of segment speed, for example, as sensitivity of a breakpoint algorithm is reduced. These methods are well established mathematically, and are similar to those already frequently invoked for choosing the number of ‘behavioural states’ in speed frequency distributions when identifying seabird behaviour from GPS data using statistical machine learning algorithms (see for example: Dean et al., 2012; Fayet et al., 2015; Patterson, Basson, Bravington, & Gunn, 2009). Importantly, these kind of methods might be useful when there is no *a priori* expectation for the number of segments that a track might contain.

The same logic also applies to carefully chosen trajectories that traverse areas with varying amounts of visual information since during homing, there are likely to be few ecological factors that would introduce breakpoints that corresponded statistically to the advent of topographic information.

Experimentally determining whether pigeons have a vector of direction and distance to the loft when released from an unfamiliar site. Some form of extrapolated map to explain true navigation in birds is already a popular idea among ethologists (Gagliardo, 2013; Wallraff, 2000, 2003, 2015). However, the kind of map implied by our results has not been frequently discussed

in the context of olfactory navigation. This is because, as described by Benhamou (2003), an extrapolated map that provides a vector of both direction and distance to home requires a complex learning stage where animals parameterise what can be thought of as a set of geometrically informed linear equations that can thus be extrapolated beyond experience for true navigation. The implications of this map are thus in contrast with the observation that pigeons reared within the confines of a loft still exhibit good orientation in their first release (Gagliardo, 2013). Wallraff's gradient theory (Wallraff, 1990) shows how such limited experience at the home loft might facilitate true navigation, but differs to a true extrapolated map in that it provides only a direction of displacement by consultation of atmospheric odours at the release site (Gagliardo, 2013). The resolution of this might be ecological. Shearwaters are afforded ample opportunity to parameterise such a map, and the imperative to do so may be considerably greater. However, it remains unknown whether pigeons too resolve a vector of both distance and direction when navigating from unfamiliar sites. If they do, it might be that motivation to home varies with distance and as such, an indication of pigeons' ability to judge their distance of displacement might already exist in extant datasets if leaving times were quicker at sites that pigeons perceived to be further away from the loft. One possibility to examine this directly might be to train pigeons from different unfamiliar release sites, rewarding pigeons for arriving back at the loft at a certain time. Simultaneously, rewarding them also for staying at the release site. One could balance the relative value of these rewards such that it is worth remaining at the release site until the reward will be obtained at the loft – thus, the pigeon would be expected to optimise its time at both sites by planning its journey home accounting for the distance that it must travel, if it is able to do so.

Analysis of free-ranging anosmic seabirds that forage along continental shelves, out of sight of land, in comparison with those that forage coastally. Since in displacement experiments, anosmia confers a complete inability to home to displaced shearwaters in the mid Atlantic (Gagliardo et al., 2013), but not in areas rich with topographic information even at similarly distant sites (Pollonara et al., 2015), comparing the homing ability and orientation of anosmic

shearwaters that attempt to home after autonomous displacement to pelagic rather than coastal foraging areas might indicate whether they are capable of homing by path integration. In the case of a shearwater foraging in pelagic waters, there is unlikely to be any stable visual information and so homeward orientation would, without olfaction, necessarily be by path integration. Such experiments might be possible with the morphologically constrained *Calonectris* shearwaters since there are colonies in the Mediterranean (where there is much topographic information) which could be compared with colonies in the Atlantic (where there are regions with none). In particular, colonies breeding in Cape Verde (Cape Verde shearwater, *Celonectris edwardsii*) appear to have more pelagic foraging distributions (Ramos et al., 2013) and so might be a candidate system for this experiment.

Looking for a back-up compass in Procellariiform seabirds. In many songbirds, and other animals, there is an important role in migration of non-solar compasses based on stars and/or the geomagnetic field (Emlen, 1975; W. Wiltschko & Wiltschko, 1972). Full dependence on a sun compass also seems unlikely for seabirds, since the sun's azimuth will vary not only across each day but across seasons as well. While reference to the geometric arrangements of the loft or landscape might theoretically provide pigeons a means for continual updating of an ephemeris without reference to a second compass, this is even less plausible at sea during long foraging trips far from the coast, or during long periods during over-winter residency in the wintering grounds when presumably a shearwater has few geometrically stable landmarks with which to reference its direction. Furthermore, for trans-equatorial migrants, such as many Procellariiformes, the sun's azimuthal progression over the course of a day will become mirrored as they cross the equator, raising a mechanistic problem that might be calibrated by references to other types of compass information (Akesson et al., 2015). The most likely second compass is probably the magnetic compass. Interestingly, however, there is no experimental evidence for magnetic involvement in any stage of navigation for any seabird (Bonadonna et al., 2005; Gagliardo et al., 2013; Massa, Benvenuti, Ioalè, & Lo Valvo, 1991; Mouritsen et al., 2003; Pollonara et al., 2015).

It is worth bearing in mind, however, that experiments investigating the magnetic compass in seabirds have thus far been limited to the attachment of jostling magnets to the heads of displaced (Gagliardo et al., 2013; Massa et al., 1991; Pollonara et al., 2015) or free-ranging shearwaters and albatrosses (Bonadonna et al., 2005; Mouritsen et al., 2003). Similarly, while the role of the magnetic compass in homing pigeons has been evidenced by the carrying of rare earth magnets in some situations (Wiltschko, Nohr, & Wiltschko, 1981), there is no effect of carrying magnets in many others (Gagliardo, 2013). The effectiveness of head mounted rare earth magnets interfering with a bird's use of the geomagnetic field has not been independently tested. In future, testing the effectiveness of jostling magnets at interfering with compass orientation in songbirds known to use the magnetic compass might yield an indication as to whether the method can work theoretically. Alternatively, investigating magnetic sensitivity in seabirds using choice experiments (Kirschvink & Kirschvink, 1991), might give an idea of whether seabirds are magnetosensitive and furthermore whether jostling magnets can interfere with this sensitivity.

Concluding remarks

Despite navigating over open ocean, and over many thousands of kilometres, the aspects of seabird navigation that have thus far been studied seem to conform to similar rules as navigation in terrestrial birds though many aspects of avian navigation remain poorly understood. Perhaps surprisingly, olfaction seems capable of providing seabirds with positional information many hundreds of kilometres away, and is important in free-ranging navigation as well as after displacements. In Manx shearwaters, as in pigeons, this appears to operate as an extrapolated map as evidenced by its constraints in anticipating landmarks, though the cognitive nature of this map might prove to be distinct. Within a familiar area, seabird navigation appears to be under control of a visual familiar area map, with birds probably guided by some form of pilotage close to home, but possibly a mosaic representation of visual landmarks further away. Sun-compass information is important, and it seems probable given its near ubiquity among diurnal animals, that it operates as the principle compass in seabirds, though searches for a magnetic compass might ultimately

reveal how trans-equatorial migrants deal with a changing ephemeris. As in the homing pigeon, information from the sun compass appears to be incorporated into a familiar area representation of landmarks, and the seabird system might provide a realistic approach to quantify the mechanistic basis for this in the near future since in both systems the precise reasons for *partial* deflections under clock-shift remain unknown.

Since Matthews and Lockley's famous release experiments in the 1950s (Brooke, 1990; Matthews, 1953a), the navigational ability of Procellariiform seabirds has been evident. In 2017, with the elevation of seabirds to a model taxon for avian navigation, there is scope to explore previously uncharted territory. No other collection of species use space to the same extent. The 6,000 km-long path lengths of Manx shearwater foraging trips to the east Atlantic continental shelf exemplify the different scales of movement that these birds can traverse in hours or days. In the last 20 years, tracking technology has revolutionised the study of seabirds, though until recently the focus has been almost exclusively ecological (Guilford et al., 2011). The prospect of identifying decision points to determine periods of oriented movement in free-ranging Procellariiform seabirds and relating this to quantified navigational cues potentially makes available vast quantities of extant data to the study of seabird navigation. This might be especially convenient since Procellariiform seabirds are also the most threatened avian taxon (Croxall et al., 2012) and so experimental intervention is not always possible or appropriate. Studies aimed at uncovering the sensory and cognitive control of natural movement such as distal food searching behaviours, control of migratory behaviour and its development in young seabirds, possibly through exploration (Guilford et al., 2011), and the role of memory, will certainly have conservation relevance as environmental change accelerates in decades to come.

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