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Scale-dependent approaches in conservation biogeography of a cosmopolitan raptor: the Osprey.

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- *Morocco = Inchallah !*

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- *Ferrara = "A corar e cagàr a s' immerda i garìt"* ovvero ... a fare troppe cose insieme si rischia di fare delle cavolate (traduzione rivisitata per un piú vasto pubblico).

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- *Maremma = "Siena mi fé, disfecemi Maremma" – Dante Alighieri – Divina Commedia, Purgatorio V, 134.*

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ABSTRACT

Being a cosmopolitan and migratory species, the osprey *Pandion haliaetus* provides a good opportunity to explore how behavioural adaptations in different populations, that evolved under different ecological conditions and are widely spaced, can be the proximate causes of geographical distribution, genetic divergence, population connectivity, migratory strategies and foraging ecology. According to this, a multi-scale integrated approach has been adopted for the osprey: through an interdisciplinary framework made by molecular ecology, trophic ecology as assessed via stable isotopic analyses, spatial ecology through the use of novel biotelemetry tools, as well as population dynamics, fish censuses and assessments of levels of human disturbance, we: a) evidenced the existence of four different lineages at global scale that should be treated as Evolutionary Significant Units (ESUs) and deserve specific management; b) revealed population connectivity in the Western Palearctic; c) disentangled the migratory behaviour and winter ecology of Mediterranean ospreys and compared it with northern European populations, in an evolutionary context; d) stressed the need of adequate management measures to be adopted for three local populations of the Mediterranean basin (Corsica, Morocco and Italy). Overall, this work has led to some important advances with respect to the conservation biogeography of ospreys at different scales. Those insights are particularly valuable with respect to the effective management of this emblematic species.

Keywords: phylogeography, population connectivity, migration, wintering, marine protected areas, human disturbance, reintroduction, Palearctic, Mediterranean.

RIASSUNTO

Essendo una specie cosmopolita e migratrice, il falco pescatore *Pandion haliaetus* rappresenta un buon modello di studio per investigare come adattamenti comportamentali evolutisi in popolazioni geograficamente distanti e in diverse condizioni ecologiche, possano aver condizionato l'attuale distribuzione geografica, la divergenza genetica, la connettività e le strategie migratorie e di alimentazione delle singole popolazioni. Per meglio indagare questi aspetti, questo studio ha adottato un approccio multidisciplinare, articolato su diverse scale spaziali e temporali. Le discipline di cui ci si è avvalsi spaziano dall'ecologia molecolare, l'ecologia trofica valutata attraverso l'analisi degli isotopi stabili, l'ecologia spaziale attraverso l'uso di innovativi strumenti di biotelemetria, così come l'analisi di dinamica di popolazione, i censimenti di specie ittiche e le valutazioni dei livelli di disturbo antropico. Questa impostazione ha consentito di: a) dimostrare l'esistenza di quattro diversi lignaggi a scala globale, che dovrebbero essere trattati come quattro Unità Evolutive Significative (ESUs) e meritano specifiche strategie di gestione; b) rivelare la connettività tra le diverse popolazioni del Paleartico occidentale; c) far luce sulle strategie migratorie e l'ecologia invernale dei falchi pescatori del Mediterraneo, confrontandole con le popolazioni del Nord Europa, in un contesto evolutivo; d) sottolineare la necessità di adeguate misure di gestione da adottare per tre popolazioni locali del bacino del Mediterraneo (Corsica, Marocco e Italia). Questo lavoro ha portato, in generale, ad alcuni importanti passi in avanti per la conservazione del falco pescatore a diverse scale biogeografiche; risultati particolarmente importanti per la messa a punto di più efficaci azioni di gestione di questa specie emblematica.

Parole chiave: filogeografia, connettivitá delle popolazioni, migrazione, svernamento, reintroduzione, Paleartico, Mediterraneo.

Résumé

Le balbuzard pêcheur *Pandion haliaetus* est une espèce de rapace cosmopolite et migratrice mais menacée. C'est un modèle intéressant pour explorer comment les adaptations comportementales au sein de populations géographiquement distantes, ayant évoluées dans conditions écologiques différentes, pourraient être une cause proximale de leur distribution géographique et de différences génétiques et comportementales (stratégies migratrices et comportement de recherche alimentaire). Ainsi, j'ai mené une étude intégrative à différentes échelles afin de répondre à plusieurs questions fondamentales et appliquées.

Grâce à une approche phylogéographique (phylogénie moléculaire) j'ai mis en évidence l'existence de quatre lignées différentes à l'échelle mondiale qui peuvent être considérées comme des Unités Evolutives Significatives (ESUs) et qui méritent des mesures de gestion spécifiques. En combinant des méthodes d'écologie moléculaire et d'observations d'oiseaux marqués, j'ai pu estimer le taux de connexion populationnelle dans le Paléarctique occidental. En combinant des outils d'écologie trophique en utilisant les isotopes stables, et d'écologie spatiale en utilisant de nouveaux outils en bio télémétrie, j'ai étudié les comportements migratoires et de l'écologie hivernale des balbuzards pêcheurs méditerranéens, que j'ai pu comparer avec ceux des populations nord européennes. Finalement, j'ai étudié la nécessité d'adopter des mesures de gestions adéquates pour trois population du bassin méditerranéen (Corse, Maroc, Italie), en comparant la dynamique des populations, en lien avec leurs ressources alimentaires (recensement des espèces piscicoles) et les perturbations d'origine anthropique. Dans l'ensemble, ce travail a conduit à des avancées significatives par rapport à la conservation des balbuzards pêcheurs à différentes échelles biogéographiques. Ces connaissances sont particulièrement utiles à l'égard d'une gestion efficace de cette espèce emblématique.

Mots-clés: phylogéographie, connectivité des populations, migration, hivernage, aires marines protégées, perturbations humaines, réintroduction, Paléarctique, Méditerranée.

1. INTRODUCTION

1.1. SPATIAL AND TEMPORAL SCALE ISSUES IN CONSERVATION BIOLOGY

Biodiversity can be considered at three levels: genetic diversity among individuals within the same species, the diversity of organisms expressed as different species, and ecosystem diversity through the variety of habitats and ecosystem processes that occur within a territory. Biodiversity is currently seriously threatened by natural and human-made processes (Primack, 2000; Rodrigues *et al.*, 2014). While natural processes usually occur in the longer term, human processes occur rapidly, causing abrupt changes. Major threats to biodiversity derive from the impact of human activities leading to rapid habitat loss and fragmentation, species overexploitation, and enhanced extinction rates (Ladle & Whittaker, 2011). Specifically, human activities are fragmenting habitats into small, poorly connected biogeographical islands, increasing the occurrence and intensity of human/wildlife conflicts. Many such isolated populations are suffering fast population declines, while others are driven to extinction as a result of environmental and demographic stochasticity, loss of genetic diversity and inbreeding depression (Höglund, 2009). However, a series of natural processes have the capacity to remedy these population-level issues. Dispersal, for example, may alleviate competition for resources when local density-dependent processes occur (Sutherland *et al.*, 2002; Ims & Andreassen, 2005), help replenish lost genes (decreasing inbreeding depression) and even allow the recolonization of patches vacated by extinct populations (Hanski & Gilpin, 1997; Ims & Yoccoz, 1997; Bowler & Benton, 2005). Nevertheless, natural and/or anthropogenic circumstances may not always allow natural dispersal between habitat patches. For example, both anthropogenic and natural landscape features can play a significant barrier effect and impede movements, as shown in forest songbirds (Tremblay & St Clair, 2009) or in populations of mountain caribou *Rangifer tarandus caribou* (Apps & McLellan, 2006). In such cases, dispersal may have to rely upon effective management (e.g. ecological corridors; Crispo *et al.*, 2011) or artificial translocations (e.g. reintroduction of *Castor fiber* in Europe; Halley & Rosell, 2002) that increase existing populations and promote gene flow between them (Festa-Bianchet & Apollonio, 2003; Tremblay & St Clair, 2009). Importantly, the management of vulnerable species must be based upon a good understanding of spatio-temporal functioning in animal populations. Indeed, unfortunate management decisions have often been associated to limited scientific/technical information and insufficient knowledge. This is particularly the case when managing wildlife in remote locations where extensive scientific studies are impracticable (Gilchrist *et al.*, 2005). In these cases, such as in the Arctic or in wide deserts, information on species distribution and abundance were evaluated by using local ecological knowledge "LEK", the knowledge provided by human indigenous population on the local fauna (Gadgil *et al.*, 1993), as reported for populations of arctic tundra caribou *Rangifer tarandus* (Ferguson *et al.*, 1998), cetaceans (Huntington, 2000) or seabirds (Gilchrist *et al.*, 2005).

Because of the impossibility to preserve all natural areas and species, conservation plans often focus on high-priority sites, such as biodiversity hotspots (Myers *et al.*, 2000; Neel, 2008). Alternatively, focal species (e.g. umbrella and/or flagship species; sensu Simberloff, 1998) have been used extensively, as an efficient way to garner public support and obtain funding, and ultimately to take broader conservation-related decisions (Raphael & Molina, 2007). However, while in the past decades the concept of biodiversity was restricted to species richness and referred to a static and predictable system of reference (Poiani *et al.*, 2000), more recently the concept of biodiversity has been extended to include evolutionary processes across genes, populations, species and ecosystems (Ladle & Whittaker, 2011; Mace *et al.*, 2012). As a result, current recommendations for biodiversity conservation focus on the need to preserve dynamic, multiscale ecological patterns and processes that sustain the full spectrum of biota in their supporting natural systems (e.g. Richardson & Whittaker, 2010; Mace *et al.*, 2012).

The need to choose an adequate spatio-temporal scale has largely been dictated by practical issues related to habitat and biodiversity conservation, yet scale-dependent approaches still fuel intense academic debates on the best model to follow (Dungan *et al.*, 2002).

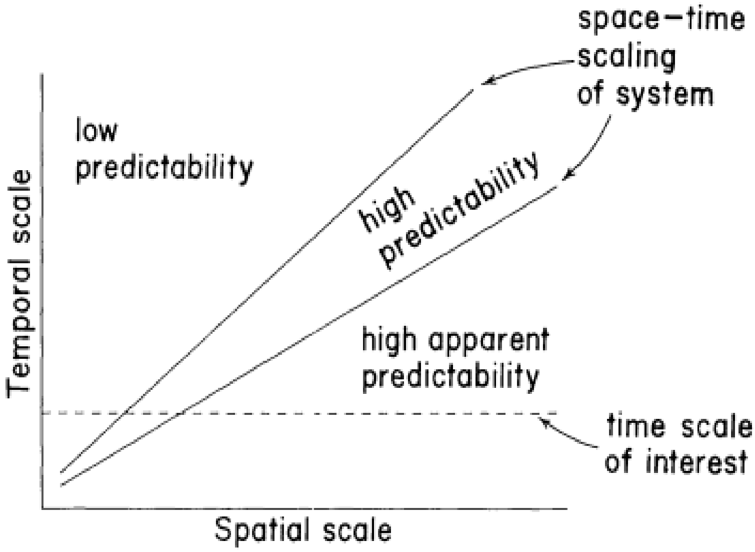


Figure 1: As the spatial scaling of a system increases, so also does its temporal scaling, although these space-time scalings differ for different systems. Studies conducted over a long time at fine spatial scales have low predictive capacity. Investigations which fit tightly with space-time scaling functions have high predictive power. Short- term studies conducted at broad spatial scales generally have high apparent predictability (pseudo-predictability). Adapted from: (Wiens, 1989).

From a formal point of view, our ability to predict ecological phenomena depend on relationships between spatial and temporal scales of variation (Wiens, 1989; Fig. 1), since the dynamics of different ecological phenomena in natural systems follow different trajectories in space and time. For example, studies conducted over a long time at fine spatial scales have low predictive capacity because they fail to consider the open-structure of natural systems, where several variables interact at the same time at very large scales (e.g. climatic phenomena). In this sense, plant physiologists have concluded that stomatal mechanisms regulate transpiration, whereas meterologists working at the broader scale of vegetation have concluded that climate is the principal control (Wiens, 1989). Conversely, short-term studies conducted at broad spatial scales generally have high apparent predictability (pseudo-predictability) because the natural dynamics of the system overshoot the period of study, so that important effects are not assessed. It is as if one was to take two snapshots of a prey-predator model a few moments apart and use the first to predict the second, without considering instead that it works on a longer-term (e.g. cyclical demographic fluctuations of hare *Lepus americanus* populations affecting lynx *Lynx canadensis* populations at high latitudes; (Akcakaya, 1992)). In this context, studies that were too constrained in time or space diminished the predictive power of the investigation, and provided inaccurate conclusions. For example, studies dealing with presence and absence data for small and/or elusive mammals resulted in declaring false absences that biased model parameter estimates (Mortelliti *et al.*, 2010). Only occasionally, reliable deductions about specific questions were provided by studies that appropriately matched the time-space reference scale (e.g. Schmitz, 2005). A resource patch suitable to one species may be not so for another: the lifetime movements of a passerine bird may occur within an area of a few km^2, whereas a raptor may move over an area of hundreds or thousands of km^2.

Proper conservation planning therefore requires management of the habitat mosaic itself, rather than of selected patches within the mosaic (Harris, 1984; Hobbs, 1993). Since the creation of national parks in 1850 in the USA, direct management actions have relied mostly on the establishment of nature reserves (Ladle & Whittaker, 2011). Those are certainly important, but habitat fragments are strongly influenced by forces from other habitats within the landscape mosaic (Wiens, 1995). Thus, conservation of key species or habitats may not target particular patches or landscape fragments for management, but conservation programmes should rather emphasize species richness or complexes of communities, and focus on preserving broader-scale landscape mosaics (Noss, 1987; Andrén, 1994; Mace *et al.*, 2012).

As it is impossible to take into consideration the totality of natural variables and effects simultaneously acting on ecological systems in space and time, a robust conservation program should point the goal of choosing variables which yield maximum predictability, giving priority to those scales that match with this prerequisite.

But, what is an **'appropriate scale'**, depends in part on the questions tackled. Difficulties in advising managers stem from the fact that many species include migratory populations relying on different spatial and temporal scales during their life cycle. Traveling through continental flyways, migratory individuals experience multiple habitats within a landscape system (Newton, 2010). Protecting only part of this complex life-scheme would compromise conservation plans, because of the possibility that threats occur at other spatial and temporal scales. Red knots (*Calidris canutus),* for instance are (relatively) protected at their arctic breeding sites, but encounter threats during migration and wintering, especially through the loss of key staging sites (Baker *et al.*, 2004; Rogers *et al.*, 2010). Further issues arise in the case of fragmented and exploited habitats, where the landscape system consists in poorly connected, perturbed patches. Within such a landscape, species spatial dynamics are particularly difficult to predict, because of scale-dependence in habitat patterns and in contrasting individual and populational responses to fragmentation effects (Tscharntke *et al.*, 2002). For instance, Tscharntke *et al.* (2002) found that the percentage of polyphagous butterfly species and their abundance were higher in small than in large grassland fragments, showing the relative importance of small habitat patches to conservation of insect communities of grassland-cropland landscapes.

All those considerations have been the subject of intense scientific debates, which are still ongoing. Notably, scale-dependent issues have been identified in three major ecological sub-disciplines:

1) *Population and Evolutionary Genetics*: genetic diversity exists at multiple spatial and temporal scales, and naturally affects spatial genetic structure of populations as well as our ability to infer gene flow consistency. Spatial and temporal considerations figure prominently in sample design; the efficient choices of molecular marker types and selection of appropriate analytical tools (Storfer *et al.*, 2007) are fundamental to analyse spatial genetic structures, the time line of landscape features, and all aspects concerning a species' life history (reviewed in Anderson *et al.*, 2010). In this context, should we refer to individuals, breeding units, geographic groups or to all populations under study? Ideally, the choice should be made so that data reflect the spatial and temporal scales of the ecological and evolutionary processes under consideration (Palumbi, 2003; Anderson *et al.*, 2010).

2) *Movement Ecology*: movement is a vital process linking organisms to their ecosystems in space and time (Nathan *et al.*, 2008); thus, changes in movements may have important ecosystem-level consequences (Lundberg & Moberg, 2003). Variations in movement patterns can occur at different levels: a) *community level* = different animal species interact with each other in a continuous spatial-temporal ecological system, sharing abiotic and biotic components of the landscape; b) *species level* = a species may be mobile during some stages of its life cycle but much more sedentary during other stages; many marine invertebrates present pelagic larvae and sessile, benthic adult stages (Eckman, 1996), and such contrasting life-phases also occur in migratory mammals and birds (Berger, 2004; Newton, 2010); c) *population level* = in the case of metapopulation, assemblages of local populations living in discrete habitat patches are connected by individuals which move between patches in different life periods (Hanski & Gilpin, 1997). Three main different phases occur in a metapopulation system: emigration, inter-patch movements and immigration (Bowler & Benton, 2005); d) *individual level* = some organisms can decide to move or rest, shifting their behaviour according to environmental factors during years, seasons, days (e.g. daily and seasonal vertical migrations (Afonso *et al.*, 2014) or lunar rhythms (Cruz *et al.*, 2013)).

3) *Landscape and Population dynamics:* landscapes, and the populations they contain, are not stable through time (Wiens, 1995) but change as a consequence of small- and large-scale disturbances, some natural (e.g. fires, insect outbreaks), others anthropogenic (e.g. forest cutting, agricultural practices). As a result, fluctuation of landscape structure is a dynamic process, with profound effects on population persistence (Fahrig, 1992). Further, the frequency and spatial distribution of habitats and resources determine species distribution patterns (Pearson, 1993). At finer scales, populations may be separated within patches of habitat, by less suitable patches acting as ecological barriers (e.g. Opdam, 1991). Furthermore, spatio-temporal fluctuations of habitat components (space, ecological niches, breeding sites, competitors, predators) may strongly affect population trends (e.g. prey-predator system as described by the Lotka-Volterra model; Lotka, 1925).

Understanding the ecology of animal populations and planning adequate conservation measures therefore requires knowledge of mechanisms acting at different spatial and temporal scales, as well as integrative methods and analytical approaches. In this context, a sound approach should strive to: **(1)** target selected species that may serve as models for a larger suite of species that share ecological, life-history or distributional features, **(2)** develop a multi-scale dependent approach (“mosaic theory” sensu Wiens, 1995) that has a specified and restricted domain of application and **(3)** integrate these two approaches (Wiens *et al.*, 1993; Collins *et al.*, 1993).

In this thesis, I followed this approach driven by the necessity to answer specific questions, which nonetheless require extensive knowledge of the phenomena investigated. I built a multi-scale dependent approach to deal with each topic in a “step-by-step” process across this thesis. The primary goal was to explain these phenomena with respect to the evolutionary history and the current ecology of the model species under study.

In the following, I first introduce general aspects of the ecology and behaviour of the selected model species, explaining why it is suitable and exemplary for a multi-scale approach. Then, I explain the structure of this multi-scale approach in relation to the scientific questions addressed.


A. Troisi

1.2. THE MODEL SPECIES: A COSMOPOLITAN MIGRATORY RAPTOR, THE OSPREY.

Part of the informations reported in this chapter (and in chapter 2.1: Study sites) have been the subject of a monograph published in the framework of the « Albatros project » as part of the "Mediterranean Small Islands Initiative" (PIM) coordinated by the French Conservatoire du Littoral and presented at the international workshop "3rd MEDITERRANEAN SMALL ISLANDS MEETING" organised in Bizerte (Tunisia), in April 2012 (Monti, 2012). A network of >30 persons from 10 different countries produced a state of knowledge concerning 7 target species of Mediterranean seabirds (including Osprey). All these activities were aimed at a better sharing of information and data between all the actors of seabird conservation in the Mediterranean.

With a worldwide distribution between 49° S and 70° N of latitude, the osprey *Pandion haliaetus* is considered as one of the six landbird species, together with the Great Egret *Ardea alba*, the Cattle Egret *Bubulcus ibis*, the Glossy Ibis *Plegadis falcinellus* the Barn Owl *Tyto alba* and the Peregrine Falcon *Falco peregrinus*, which is fully cosmopolitan (Newton, 2003a).

The Osprey is a medium-size raptor (body: 55-58 cm, tail length: 14-21 cm, wing-span: 145-170 cm, weight: 1.5-2.0 kg). Its plumage presents dark brown upperparts, while it is ventrally pale with contrasting black carpal patches, blackish bands on greater coverts and long black tips to primaries. The tail is short and square-cut. Diagnostic is the black stripe through eye to hindneck. Sexes look similar, but females tend to be 5-10% larger than males and show on average prominent brown breast-band. Juveniles have feathers of upperparts clearly tipped of whitish, being distinguishable from adults if observed at close distance. Average life span in the wild is of ca. 30 years (Poole, 1989). The species has evolved specialised physical characteristics and exhibits unique behaviour to assist in catching prey, consisting of live fish only (Cramp & Simmons, 1980; Poole, 1989). Adaptations to its specialized manner of feeding are particularly manifest in the structure of the tarsus, that is provided, as well as the lower surface of toes, of sharp spicules essential to catch and manipulate slippery fishes. In addition, the outer toe is reversible and allows the bird to grip the prey with two toes forward and two toes backwards (Cramp & Simmons, 1980; Poole, 1989).

Scientific classification

Kingdom: Animalia
Phylum: Chordata
Class: Aves
Order: Accipitriformes
Family: Pandionidae
Genus: *Pandion*
Species: *Pandion haliaetus*

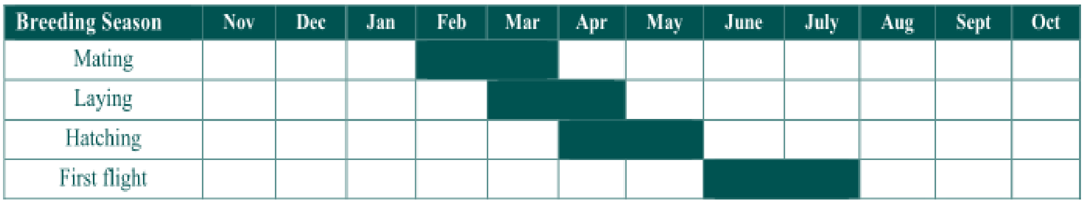
Figure 2: Taxonomic classification (left) and image of a juvenile osprey (right).

Conservation status: Since the osprey has an extremely large range and considering that global population trend and size appear to be increasing (e.g. Schmidt-Rothmund *et al.*, 2014), the species does not approach the thresholds for being considered as "Vulnerable" under the IUCN criterions; hence it has been evaluated as Least Concern, at global scale (Birdlife International, 2014). However, it is included in Annex I of the European Directive (2009/147/EC) on the conservation of wild birds, in Annex II of the Bern Convention (79/409/EEC) as a strictly protected species, in Annex II of the Convention of Bonn (CMS) as a migratory species with a unfavourable status and in Annex II of Barcelona Convention; thus the osprey is considered a priority species for conservation along its whole distributional range. In Europe, is ranked as "rare" since (category SPEC3 - Species of European Conservation Concern) the whole breeding nucleus is inferior to 10,000 pairs. At the regional scale of the Mediterranean, the species is considered as "endangered". Further, it is mentioned as "critically endangered" in the red book of the birds of Spain (Triay & Siverio, 2008) and, up to 2011, "exctinct as a breeding species" in Italy (Brichetti & Fracasso, 2003).

Habitat: Because of a wide climatic tolerance, especially in range of temperature and humidity, osprey's habitat varies in different parts of its extensive range. Although almost exclusively tree-nester in the vicinity of rivers and lakes in northern parts of its Palearctic range, the osprey chooses rocky cliffs for nesting and marine or brackish water environments for fishing at southern latitudes such as in the Mediterranean area, Atlantic islands, Red Sea, Caribbean (Cramp & Simmons, 1980). In some cases, osprey nests can be found on power lines, bridges and other artefacts (e.g. artificial nest-sites or platforms erected on poles) (Poole, 1989), but always in strict association to water bodies where fishing is possible. Ospreys also visit estuaries, marshes and other coastal waters as well as inland-located lakes and pools.

Diet: Being opportunistic, focusing on fish that are the most available, the species shows a wide diet that can shift during the year, depending on region and season (e.g. most marine fish migrate seasonally). The diversity of fish species leads to a variety of diving techniques that can be performed at different heights, in flight (hovering) or from a perch. The diet includes both freshwater and marine species. It concentrates on fish weighing 150-300 grams (about 25-35 centimetres in length) although larger and smaller fish can be taken (Poole, 1989; Francour & Thibault, 1996). Within the Mediterranean basin, ospreys commonly eat mullets (e.g. *Mugil* spp.) and other euryaline species of medium-sized fish caught near the sea surface as: *Liza* spp., *Diplodus sargus*, *Dicentrarchus labrax* (Thibault & Patrimonio, 1992). For example in Corsica, the diet of breeding ospreys has been studied by identifying fish remains at nests; the identified species mainly belonged to mullets (e.g. *Liza ramada*, *Liza aurata*, *Chelon labrosus* and *Mugil cephalus*) and breams (e.g. *Diplodus sargus* and *Diplodus vulgaris*) and to other less representative species (Francour & Thibault, 1996). In the Balearic Islands the most representative species detected by direct observation and remains of fish at nests are mullets (*Mugil* spp), salema (*Sarpa salpa*), saddled bream (*Oblada melanura*) and gild-heat bream (*Sparus auratus*) (R.Triay, unpublished data).

Breeding: The breeding season in the Mediterranean starts between February and April, when pairs gradually return to their own nests that are generally used for successive years (Thibault & Patrimonio, 1991). The nest consists of a large structure of branches and twigs stuffed with grass or other soft material (e.g. *Posidonia oceanica*) for lining. Materials are taken from the ground or snatched from trees or plants provided of dead sticks at their top. Generally built by both sexes, nests are placed on the top of trees (e.g. continental Europe) or on rock pinnacles close to the sea (e.g. in the Mediterranean area). Laying mainly occurs in March and April (Fig. 3). One to four (3 on average) eggs, creamy-white coloured with brown-red spots, are normally laid in a clutch at an interval of 1-3 days (Alerstam *et al.*, 2006; Thibault & Patrimonio, 1992; Bretagnolle & Thibault, 1993). Replacement clutches are possible (Cramp & Simmons, 1980). Eggs measure 47x60 mm and weigh about 72 grams (Cramp & Simmons, 1980). The incubation phase, performed by both parents but mostly by the female, lasts 34-40 days (37 days on average; Green, 1976; Cramp & Simmons, 1980). Fledging occurs between June and July, generally after about 50 days from the hatching date (Stinson, 1977; Bretagnolle & Thibault, 1993). For the Corsican population, first juvenile flights were mainly recorded during the first week of July (Thibault & Patrimonio, 1991). Parents feed juveniles even after fledging, with fish left at the nest. Broods start to follow males to the fishing place after ca. 10 days; juveniles mature fishing techniques at about 7 weeks after the first flight (Stinson, 1977; Cramp & Simmons, 1980).



Breeding Season	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct
Mating				■	■							
Laying					■	■						
Hatching						■	■					
First flight								■	■			

Figure 3: Breeding phenology of osprey in Corsica (data reported refers to: Thibault & Patrimonio, 1991; Bretagnolle & Thibault, 1993).

Movements: Osprey populations of northern latitudes are known to perform long-distance migration to wintering grounds located at lower latitudes (Alerstam *et al.*, 2006). In contrast, osprey populations living at southern latitudes of the breeding range seem to carry out only reduced movements, although basic knowledge of movement ecology of these populations is still fragmentary.

Because of high plasticity in habitat requirements of this cosmopolitan and migratory species (Cramps & Simmons, 1980), the osprey provides a good opportunity to explore how behavioural adaptations in different populations, that evolved under different ecological conditions and are widely spaced, can be the proximate causes of geographical distribution, genetic divergence, population connectivity, migratory strategies and foraging ecology. At the same time, the osprey is considered an umbrella species which indirectly protects many other species that make up the ecological community within its habitat: protecting ospreys means securing their breeding habitats, that can vary from lakes in coniferous forests in the Northern Hemisphere to coastal or marine environments at southern latitudes. Moreover, ospreys can be used as boundary objects (sensu Star & Griesemer, 1989), to promote adequate management of aquatic environments (i.e. osprey feeding sites), thereby providing protection for entire fish communities. Being a charismatic and spectacular raptor, it plays also the role as a flagship species that stimulate broad conservation awareness and action (and fund-raising) in the public (Mackrill *et al.*, 2013). Thanks to its characteristics, it well serves as model for a larger suite of species that share ecological, life-history or distributional features such as large migratory birds that have a wide distributional range and can potentially fill the role of umbrella/flagship species (e.g. hen harriers *Circus cyaneus* for Holarctic agricultural landscapes (Trierweiler, 2010), white-tailed eagle *Haliaeetus albicilla* and black storks *Ciconia nigra* for freshwater and forested habitat (Rosenvald & Lõhmus, 2003; Lõhmus *et al.*, 2005) and bearded vulture *Gypaetus barbatus* (Schaub *et al.*, 2009) for mountain ecosystems in Europe and Asia)).

Within this thesis, special emphasis has been placed on the study of the Mediterranean population for the following reasons: a) the Mediterranean breeding nucleus of this population is scattered in a fragmented and human-exploited coastal habitat; b) the Mediterranean population is considered as vulnerable in the long-term; c) several reintroductions projects of the species have been launched in the region, using different source populations and d) multiple gaps in knowledge are present for this population.

1.3. THESIS OUTLINE: THE MULTI-SCALE INTEGRATED APPROACH

Following a multi-scale approach (Tab. 1), I addressed the following questions:

Population and Evolutionary Genetics

- What is the genetic divergence between osprey populations and what is the evolutionary history of the species on a worldwide scale?
- What is the degree of connectivity among populations? What is the intensity of exchanges between populations in the Mediterranean basin?

Movement ecology

- Do different migration strategies exist along ecological gradients for ospreys of the Western Palearctic?
- Where do Mediterranean ospreys spend the winter?

Population dynamics

- Are population trends affected by environmental and/or anthropic variables?

Answering this wide range of research questions required developing an interdisciplinary framework, drawing expertise from molecular ecology, trophic ecology as assessed through stable isotopic analyses and prey availability (i.e. fish census), spatial ecology through the use of novel biotelemetry tools, as well as population dynamics, and assessments of levels of human disturbance.

Moreover, these questions were tackled using an eco-regional approach along 3 different spatial scales: local, regional and global. Simultaneously, this project was also stratified along 3 temporal scales, with different levels of resolution: present, historic and evolutionary time. These aspects are summarized in Tab.1.

GLOBAL SCALE and EVOLUTIONARY TIME:

At a worldwide scale, I explored and compared the evolutionary history of osprey populations and their rate of divergence by means of **phylogeographic genetic analysis** using genetic markers from **mitochondrial DNA**. By sequencing particular genes in the mitochondria, that evolves very slowly and at a constant rate in all species, it was possible to recreate phylogenetic trees with all osprey populations from the whole distributional range of the species. Such analysis allowed evaluating relatedness between populations, determining if it matched with the current recognized taxonomic status (on the basis of morphological classification) and informing about any historical and evolutionary genetic divergence.

In a second step, I made use of indirect methods to study connectivity between osprey populations by means of tools from molecular biology, especially **genotyping techniques using microsatellites**. Microsatellites are small sequences of genes repeated in the nuclear genomes that can serve to identify a particular individual or a population. From both fresh and historical samples from museums I assessed genetic distances between individuals and populations, and the rate of exchanges of individuals between populations. Such analysis aimed to clarify the level of connectivity of osprey populations across their distributional range and within different habitat matrices (e.g. continuous forested habitat in the north vs fragmented marine islands in the south of the distribution) to ascertain effects of eventual population declines, isolation and/or inbreeding due to small population sizes.

REGIONAL SCALE and HISTORIC TIME:

At the scale of the Western Palearctic, I was interested in studying the migratory strategies of different osprey populations over a latitudinal gradient, to understand to what extent both geographical and environmental constrains favoured the evolution of different strategies, and how they shaped migratory behaviour. The use of inland marshes, rivers and lakes in tropical Africa by ospreys from northern Europe has already been well described (Prevost, 1982; Saurola, 2005) but virtually nothing is known about migration and over-wintering of Mediterranean ospreys. I therefore compared migration routes of Swedish ospreys, which have been studied continuously by satellite telemetry since 2000 (Hake *et al.*, 2001; Kjellén *et al.*, 2001; Alerstam *et al.*, 2006), with those of Mediterranean ospreys which I tracked using novel GPS-GSM tags. This comparison gave a full understanding of migratory and dispersal processes across populations living at different latitudes. This study of osprey movement ecology was also useful for testing the existence of a metapopulation system at the regional scale of the Mediterranean basin. Outcomes from GPS-tracking were combined with results from the population genetics study (see above), and therefore considered in the light of connectivity and gene flow at a larger scale.

Further, I investigated the winter ecology of Mediterranean ospreys through an additional indirect complementary method. **Stable isotopes analyses** based on samples of feathers collected on adult birds during ringing actions were performed on carbon isotopes to detect the latitudinal gradient of the wintering grounds, especially to know if birds wintered at *tropical or at temperate latitudes*. Nitrogen isotopes were also dosed, to ascertain the trophic level at which adult ospreys foraged (this was compared to values found in osprey chicks during the breeding season at known locations). Finally sulphur isotopes were dosed, to reveal if birds used *freshwater or marine environments* in winter (Bearhop *et al.*, 1999).

LOCAL SCALE and PRESENT TIME:

At the local scale, we used both individual monitoring such as ringing and GPS tracking and census of several environmental variables (included human disturbance) potentially affecting breeding osprey populations in three different sites of the Mediterranean. At these sites, important management actions have been put in place in the last decades, but human presence seems to affect population dynamics and trends of local breeding populations in different manner. Specific studies allowed me to individuate the best conservation strategy for the species at the local level.

Tab. 1 Summary of research topics on osprey within different spatial and temporal scales.

			Spatial Scale		
		Tools/Methods	***Local***	***Regional***	***Global***
Temporal Scale		Ringing	Residency	Dispersal	
		GPS-tracking	Predispersal Wintering	Migration Metapopulation	
	Recent	Stable Isotope analysis	Diet	Freshwater vs Marine	Wintering Grounds
		Trophic Resources analysis	Reserve Effect		
	Historic	Genetics (microsatellites)	Local Exchanges	Metapopulations	
	Evolutionary	Genetics (mtDNA)			Phylogeography

2. METHODS

2.1. STUDY SITES

Fieldwork was conducted in 4 study sites of the Mediterranean basin, for which a brief description is given below. The Mediterranean osprey's population is estimated to count less than 80 breeding pairs and consists of small and isolated groups of breeders distributed between Corsica, Balearics, Morocco and Algeria (Fig. 4; Monti, 2012). At this regional scale, the species is considered as "endangered" and it shows traits of weakness and instabilities within a long time span. In the last years, three reintroduction projects started in the region: Spain in 2003 (Muriel *et al.*, 2006), Italy in 2006 (Monti *et al.*, 2012; Monti *et al.*, 2014) and Portugal in 2011 (CIBIO, 2011).

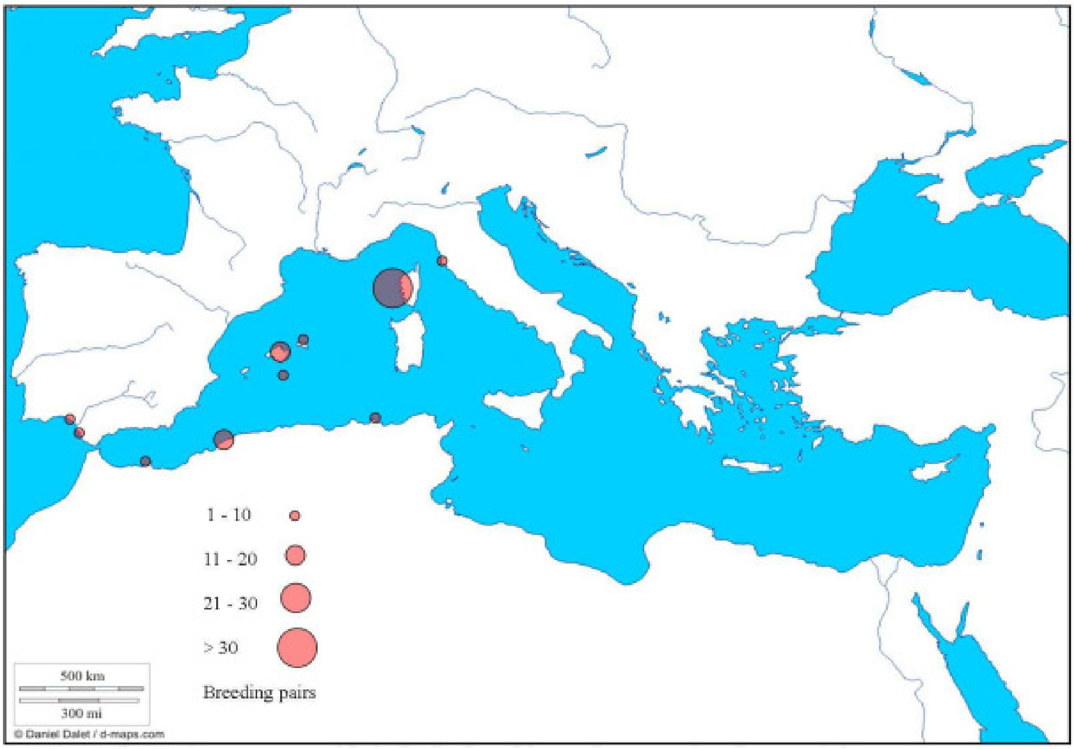


Figure 4: Geographical distribution of the Mediterranean osprey breeding population (Monti, 2012).

Corsica:

At the beginning of the 20th century a swinging number of 40-100 ospreys' breeding pairs occupied the majority of Corsican rocky coasts. In 1974, because of the strong direct persecution only three pairs remained (Thibault *et al.*, 2001; Thibault & Bretagnolle, 2001; Bretagnolle *et al.*, 2008). In 1975, the Natural Reserve of Scandola, a dual marine and terrestrial protected area located along the north-western coast of Corsica, was created. In the same years a new law about the protection of nature and birds of prey (1976) was voted and direct management actions were adopted: a rigorous watch on osprey's last nest sites, and the building of nine artificial nests on the rocky cliffs in order to recover the ancient suitable habitat nesting sites and to facilitate the recolonization phase. Thanks to these actions, osprey population gradually recovered, but it has never recolonized its former range from the early 20th century (most of rocky coasts of Corsica except the east coast). Two main phases can be detected: 1) a first period of rapid increase in population size between 1974 and 1990 (up to ca. 20 pairs); 2) a period of relative stability with fluctuations in population size after 1990 (Bretagnolle *et al.*, 2008). Due to the high philopatry of the species and to the local shortage of available nest sites, birds returned to breed in a relatively small area. Consequently, the mean distances between breeding territories reduced drastically (from 12 km during 1970's to only 2 km since 1980's); an increase in neighbours' numbers was recorded and the competition for nest sites became intense. In fact, an increase in the number of floaters and a greater frequency of interactions between conspecifics were observed. This leads to important changes in the demography. Both, population growth rate and number of young fledged per pair have decreased with the population increase. In particular, a reduction in hatching and fledging success were recorded (Bretagnolle *et al.*, 2008). The latter authors suggested that attracting non-breeders to other areas would reduce interference in the original area. Hence between 1991 and 1998, nine artificial nests were built in Corsica, outside the original breeding area. In 1995, five new territories were established (Bretagnolle *et al.*, 2008). Nowadays, Corsican osprey population counts 32 breeding pairs (Fig. 5), but it is considered still threatened (Bretagnolle *et al.*, 2008). So in order to accelerate the return of the species, actions aiming at the recovery of the historical osprey's breeding sites like those in Corsica, Tuscany and Sardinia were considered fundamental to re-establish the ancient range of distribution in the Central Mediterranean area.

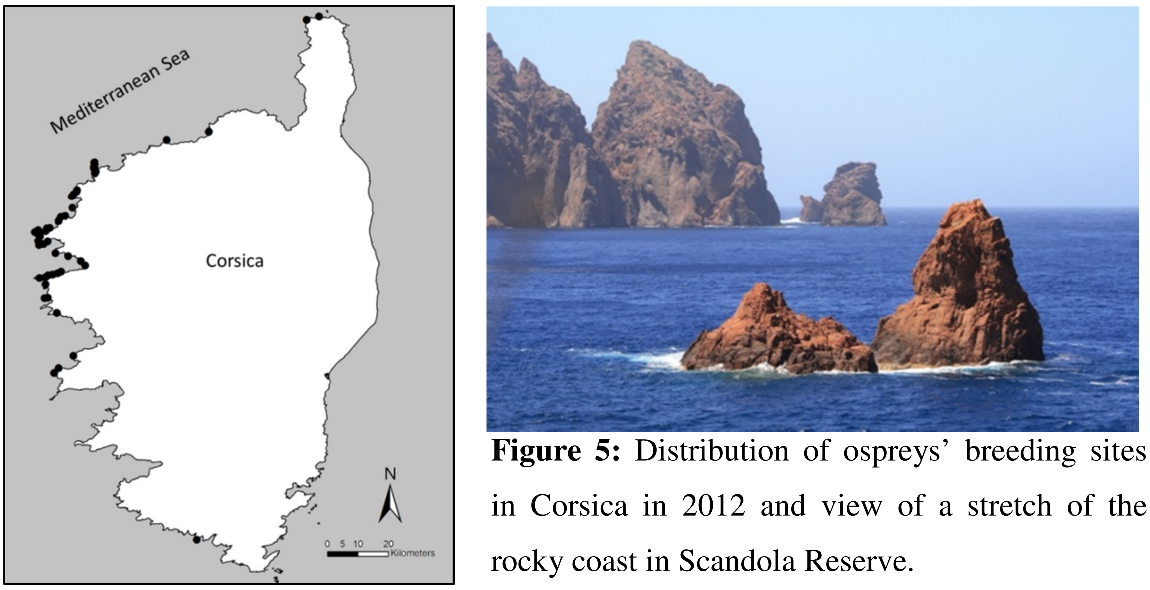


Figure 5: Distribution of ospreys' breeding sites in Corsica in 2012 and view of a stretch of the rocky coast in Scandola Reserve.

Balearic Islands:

During the 20th century, about 35-40 pairs inhabited all the main islands of the archipelago, before disappearing from Ibiza and Formentera (Terrasse & Terrasse, 1977) in the 1970's. Because of the strong direct persecution only 8 pairs remained in the 1980s. Successively, thanks to new laws about the protection of nature and birds and to direct management actions, the osprey population gradually recovered, reaching rapidly a total of 16-18 breeding pairs in 1999 (Triay & Siverio, 2008). Nevertheless, the positive trend stopped and a decrease phase occurred between 2001 and 2007 with a reduced population of only 13 pairs. In 2008, two more pairs added to the population that hence counted 15 territorial pairs (Fig. 6). A sound monitoring highlighted the high mortality rate of adult birds, especially recorded in Cabrera and Menorca, as the main problem affecting the population's health and threatening its stability in a long-time span. Electrocution, due to the presence of power lines pole running close to sea shores, was identified as the major cause of mortality, with 10 cases recorded in Minorca of 15 cases of mortality (66,67 %) between 1993 and 2010 (R.Triay unpublished data).

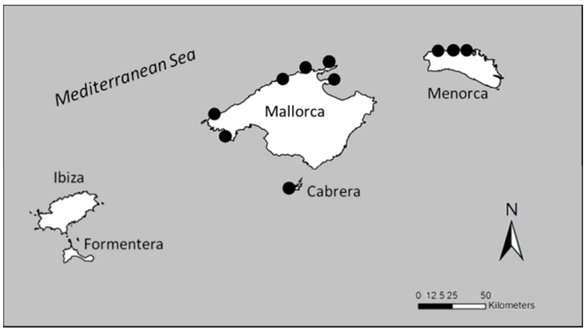


Figure 6: Distribution of ospreys' breeding sites in Balearic Islands (modified from: Triay & Siverio, 2008) and a typical view from rocky cliffs in Menorca Island.

Morocco:

The first exhaustive survey on the coast of Morocco occurred in 1983 (Berthon & Berthon, 1984). The osprey population was estimated in 10-15 pairs scattered along the rocky coast from Cabo Negro to Al Hoceima, maintaining a stable trend during the period 1983-1993 (Thibault *et al.*, 1996). More recent data showed few variations in numbers: 19-21 pairs in 1993 (Thibault *et al.*, 1996) and 15-20 pairs mentioned by Franchimont (1998). In 2008 the local AGIR association reported a total number of 14-18 pairs within the territory included in the Park National d'Al Hoceima (PNAH; Fig. 7) (Orueta & Cherckaoui, 2010). In the Chafarinas Islands, belonging to the Spanish territories, osprey was present with two breeding pairs in the 1950 (Terrasse & Terrasse, 1997). Since 1994, only one pair inhabits the archipelago; in particular breeding in the island of Congreso (Triay & Siverio, 2008).

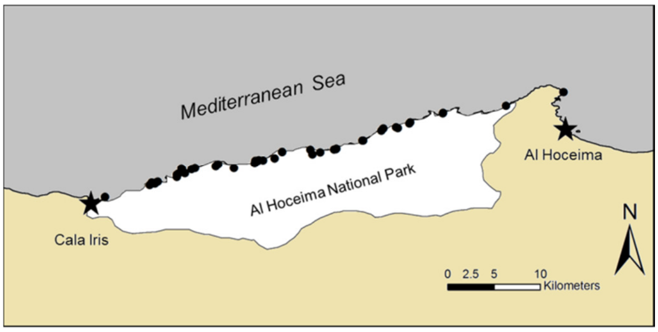


Figure 7: The coastal area included in the Al Hoceima National Park and distribution of osprey nest structures (both ancient and new sites); image of the cliffs where ospreys breed.

Continental Italy:

In Italy, a strong human persecution (especially made by shooting and egg-collecting) together with the loss of suitable nesting sites are considered as the main causes that drove the species to extinction (Spina & Volponi, 2008). The species is protected in Italy since 1977. The last breeding sites for the species were attested in Sardinia, Sicily and Tuscany Archipelago (Montecristo Island) (Thibault & Patrimonio, 1992). Since 2006, a common project started between the Parc Naturel Régional de Corse (France) and the Maremma Regional Park (Tuscany-Italy), both to re-establish an osprey breeding population and to secure the future of the Corsican population. The management consisted in translocating chicks from Corsica to Maremma RP and releasing them by the hacking technique (Monti *et al.*, 2012). In 2011, after >40 years from its extinction, the osprey returned to breed in Italy, in the Maremma Regional Park (Tuscany; Fig. 8). Nowadays (2014), two breeding pairs are actually present in the region (Monti *et al.*, 2014). At the same time, a spread of the Corsican population through the connection with the Italian one is expected and favoured by the creation of artificial nests in the islands of the Tuscany Archipelago that, operating as "stepping stones", could allow easier exchanges between the two populations. These seven islands located in the Mediterranean Sea, between the west coast of central Italy and Corsica, were officially recognized as National Park and Marine Protected Areas (MPA), in 1996. The final aim is to create a new self-sustaining population in the long term.

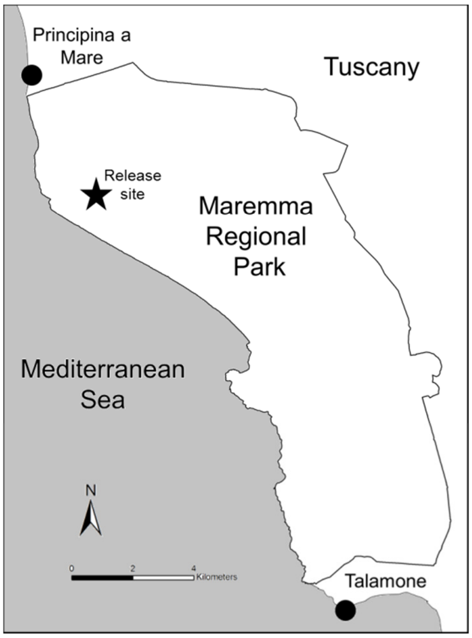


Figure 8: Map of the Maremma Regional Park, Tuscany. The localization of the release site is reported with a black star. View of a typical wetland in the Maremma region.

Algeria:

The feasibility of the field mission foreseen in Algeria at the beginning of the project was impeded by delicate political aspects that made difficult the planning of such mission, especially for obtaining visa and special permissions to work properly. Considering that the majority of Algerian osprey population breeds on rocky cliffs partially included in a military zone and in view of the last political events occurred in 2012-2013, we decided to avoid any hazardous mission in this country. In spite of this, a brief description of the osprey population in this site is reported.

Data referring to the past situation in Algeria are very scarce. During the 1960's, information available only refers to the fact that osprey reproduced along the rocky coast. A survey conducted in 1978, allowed to detect two breeding areas. The first located west of Oran and the second near El-Kala, as reported by Jacob *et al.* (1980) and after confirmed by Boukhalfa (1990) and Thibault *et al.* (1996). During the period 1989-1993 the population was estimated at 9-15 pairs (Thibault *et al.*, 1996), similarly to data previously collected by Jacob *et al.* (1980). Repeated surveys carried out in the framework of the Mediterranean Small Island Initiative (PIM) co-ordinated by the Conservatoire du Littoral, allowed to detect the presence of the species between 2004 and 2006, in the Habibas Islands. Both in 2007 and 2008, a breeding pair was found in the island of Grande île (nest located in the Baie de la Morte). In 2011, no breeding pair was observed.

2.2. DATA COLLECTION PROTOCOLS

a. Catching and sampling adult birds

To refer to Figure 9:

The **trapping** method consisted in deploying a noose carpet (i.e. a metal grid provided by nylon loops) on osprey nest after have climbed the rocky cliffs. Then, operators waited at distance (usually on a boat at sea) for a successful trapping (i.e. osprey feet clamped into loops). Once trapped, each osprey was ringed (with both metal ring and a coloured darvic ring with an alpha-numeric code (for at distance identification)), measured and bio-sampled (blood and feather samples were collected for genetic and isotopic analyses). Finally, ospreys were equipped with a 24 g GPS-GSM tag which was attached as backpacks with a harness made of 7-mm-wide Teflon ribbon (Kenward, 2001). Birds were therefore released on the vicinity of their nests.

b. Catching and sampling juvenile birds

Juvenile ospreys (from Balearic Islands, Corsica, Morocco and Italy) were tagged during ringing actions at their respective nesting sites, in summer. As for adults, each individual was measured (i.e. wings, beak, tarsus, weight, etc.), marked with both a metal ring and a coloured darvic ring with an alpha-numeric code (for at distance identification) and equipped with a 24 g GPS-GSM tag. In addition, for each individual about 0.5 ml of blood was taken by venepuncture from the wing and stored either on filter blotting papers or in 70 % ethanol in Eppendorf tubes. In some cases, growing body feathers containing traces of blood within the calamus were collected and stored in envelopes.

c. Additional samples for genetics and isotopic analyses

The majority of osprey samples were kindly provided by colleagues during ringing actions carried out around Europe and Atlantic Islands (fresh samples). However, to fill some gaps in the distribution of our samples (to cover the entire species' distributional range) we relied also on **museum specimens**. During the PhD I had the opportunity to visit the Naturalis Biodiversity Center, Leiden (the Netherlands) to collect 58 samples from specimens stored in the museum collection. I also received other samples from the collection of the Natural History Museum of London-UK, the National Museum of Nature and Science of Amakubo (Japan), the Zoological Museum of Moscow (Russia), the Museo de la Naturaleza y el Hombre of Tenerife Island (Spain) and from the Museo Civico di Storia Naturale "G. Doria", Genova (Italy). Samples were obtained collecting small pieces of skin (about 2 mm from the toepad) from naturalized specimen ospreys following the procedure described by *(Mundy et al.*, 1997). Mitochondrial and nuclear DNA have been extracted and amplified by PCR in the laboratories of the CEFE-CNRS in Montpellier. Historical (museum) samples were manipulated in the special platform for degraded DNA of the ISEM lab in Montpellier.

d. Estimating food resource availability and osprey behaviour

To refer to Figure 10:

To collect data on the abundance and distribution of fishes considered as potential prey for osprey (i.e. fishes living <1m below the sea surface) we set up **a new specific protocol**: using a camera fixed below the bow of a kayak we performed transect at sea to obtain underwater video recordings. A Secchi disk was used to control water turbidity and to assess for good visibility conditions before performing each transect.

Focal observations at osprey nests were carried out from distant vantage-points using binoculars and telescopes to record osprey behaviour (time budget) and anthropogenic disturbance (i.e. boat passages).

Figure 9: Trapping: a) placing a noose-carpet on a osprey nest; b) an osprey landing on the trap c) moment of the trapping; d) and e) ringing; f) and g) blood sampling and centrifugation; h) wing measurement; i) feather sampling for stable isotope analyses; l) attaching the harness and GPS tag to an osprey; m) a juvenile osprey equipped with GPS; n) release; Genetic sampling: o) museum collection of naturalized osprey specimens; p) toepad sampling using a scalpel; q) preparation of PCR; r) verification of PCR products on an Agarose gel.

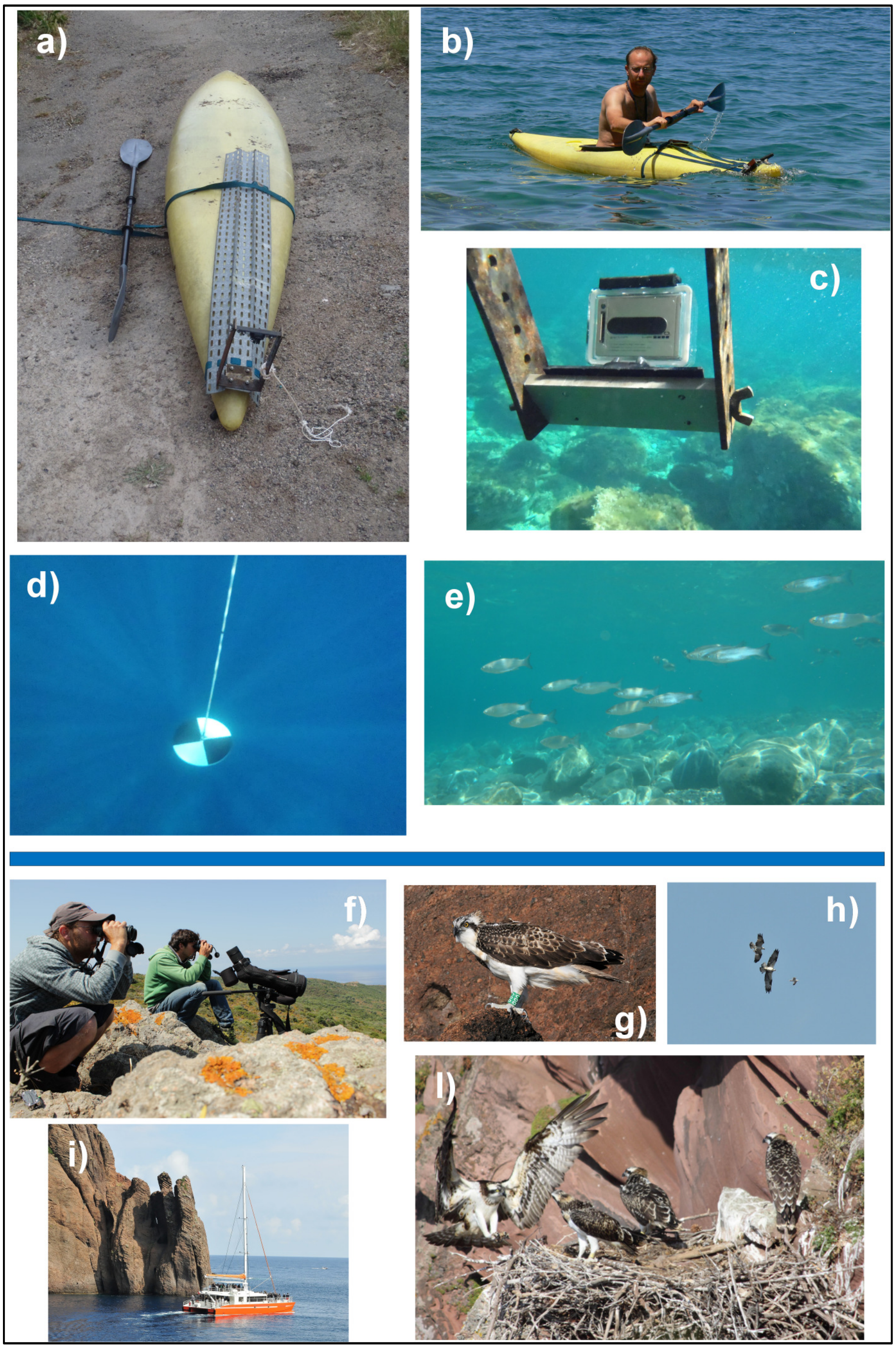


Figure 10: Fish census protocol: a) kayak with its fixed support to attach the camera; b) operator on the kayak during video recording c) GoPro camera filming along the transect; d) Secchi disk to estimate water torbidity; e) snapshot showing a shoal of mullets. Observation protocol: f) observation at distance by means binocoulars and telescope; g) a juvenile osprey perched on a rock (photo © Olivier Duriez); h) conspecific interaction in flight (photo © Olivier Duriez); i) a catamaran passing close to an osprey nest in the Scandola Reserve; l) three chicks attending a female returning at nest (photo © Rafel B. Triay).

3. SECTION I: GLOBAL SCALE AND EVOLUTIONARY TIME

3.1. BACKGROUND

The criteria adopted for the classification of living organisms and the reconstruction of their evolutionary histories was traditionally based on morphological and anatomical characters which were used by taxonomists to define species and subspecies. However, referring to morphology alone for describing diversity and interrelationships between different taxa, have often lead to interpret controversially the ancestral relationships of organisms and the group's evolutionary history (Wink, 2007). Examples of cryptic biodiversity can be found in mammals (Mayer *et al.*, 2007), birds (Johnsen *et al.*, 2010), and even reptiles or insects (Hebert *et al.*, 2004; Welton *et al.*, 2010). Similarities in anatomical characters (e.g muscles and skeletal structures) can be the result of past parallel ecological forces of natural selection, that rapidly modified such characters on unrelated species looking similar nowadays, as a consequence of convergent evolution processes (Avise, 2000). As a result, the understanding of the evolution and distribution of species was limited by the lack of a well-supported systematic analysis of their evolutionary history.

Faced with the growing challenge of deriving strategies for conserving diminishing flora and fauna, conservation biologists and ecologists continue to search for methods that can distinguish unambiguous units for conservation purposes (Fraser & Bernatchez, 2001). In this context, molecular methods and biochemical techniques have become a useful tool for phylogenetic studies, complementary to morphological characters. The genetic structure of an organism tends to evolve at a fixed rate and is less affected by the pressure of natural selection.

For example, the mitochondrial genome (mtDNA) is a small circular molecule in which genes evolve very slowly and at a rather constant rate in all species. This allows measuring the relatedness between populations and determining their taxonomic status and even informing about any historical and evolutionary genetic divergence. Sequencing mtDNA helps to identify the mitochondrial lineages of groups (so-called haplotypes).

On these bases, the analysis of nucleotide sequences of marker genes has been used as a powerful method for reconstructing the phylogeny of organisms and for defining the evolutionary significant units (ESUs) (Moritz, 1994). The process of establishing ESUs is a crucial step for conservation purposes, to define lineages that evolved separately in the past and that will eventually lead to further speciation in the future. The concept of ESU takes into account the fact that populations from a species, still interbreeding but with different evolutionary past, may lead to different species in the future (Moritz, 1999; but see Johnson *et al.*, 2005)). Despite difficulties, recognition of species is essential and should be based on repeatable scientific analyses. Uncertainties on species or subspecies classification could not allow to adequately planning management strategies at the right scale of resolution. So, an understanding of the genetic diversity and the spatial structure of populations is important for establishing the appropriate scale and subunits for conservation management and minimizing genetic erosion (Moritz, 1999).

In this context, raptors, as top predators, represent one of the main avian groups which have been mostly threatened by anthropogenic factors (e.g. shooting, pesticide contamination, ecc.) and which suffered important population declines, during the 20th century. For that reason, many raptor species have been protected by the law being included under different international conventions and listed as top priority species for conservation.

Phylogeny for raptors based on morphological traits has been difficult to resolve, so in the last decades many studies addressed to molecular methods to identify phylogenetic relationships within raptors families (Helbig *et al.*, 2005; Lerner & Mindell, 2005; Griffiths *et al.*, 2007). Despite this, contradictory conclusions resulted from different studies and altered taxonomic arrangements were proposed by various authors (Sibley & Monroe, 1990; Wink & Sauer-Gurth, 2004; Helbig *et al.*, 2005; Lerner & Mindell, 2005; Hackett *et al.*, 2008). Del Hoyo *et al.* (1994) have been grouped diurnal raptors into five families (Accipitridae, Pandionidae, Sagittaridae, Falconidae and Catharthidae). However this traditional classification is currently debated and, for instance, Falcons are now separated in another order than all other raptor families, making the order Falconiforms obsolete (Hackett *et al.*, 2008).

Here we present an exhaustive study on a particular raptor which has been included in the unique family of Pandionidae composed by only one Genera *Pandion* and one single species: the osprey, *Pandion haliaetus*.

Furthermore, I present also a study about the connectivity between osprey populations by means of tools from molecular biology, especially genotyping techniques using micro-satellites. This work was part of the subject of Florie Delfour which I co-supervised together with Oliver Duriez and Claudine Montgelard, for her master II in “Environnement et Gestion de la Biodiversité - (EGB)” at the University of Montpellier 2.

4. BEING COSMOPOLITAN: EVOLUTIONARY HISTORY AND PHYLOGEOGRAPHY OF A SPECIALIZED RAPTOR, THE OSPREY.

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4.1. ABSTRACT

Only few bird species exist with an almost world-wide distribution. We investigated the geographical pattern of mitochondrial DNA diversity in the Osprey (*Pandion haliaetus*), a cosmopolitan raptor, aiming at clarifying its phylogeographic structure and elucidating its taxonomic status. We suggest a hypothetical evolutionary scenario explaining how the species' distribution and differentiation took place in the past and how such a specialized raptor was able to colonize most of the globe. The osprey appeared structured into four main genetic groups representing quasi non-overlapping geographical regions (Americas, Indo-Australasia, Europe-Africa and Asia). Each lineage, though including birds from well-distant geographic areas, showed slight internal genetic variability. Historical demographic reconstructions suggested that three out of the four lineages experienced stable trends or slight increases. Contrary to the low within group variation, a larger number of nucleotide differences were recorded between the four clades. Molecular dating estimates the initial split between lineages at about 3.1 Ma ago, in the Late Pliocene. Our study supports a pattern of colonization from the American continent (where the species originated) towards the Old World, possibly via the Bering Strait. Populations of the Palearctic represent the last outcomes of such colonization history. At a global scale the osprey complex is composed of four different evolutionary significant units that should be treated as specific management units. Our study brought essential genetic clarifications, which have implications for conservation strategies in identifying distinct lineages across which birds should not be artificially moved through exchange/reintroduction schemes.

Keywords: Accipitriformes, Aves, cytochrome *b*, Evolutionary Significant Unit, mitochondrial markers, molecular dating, subspecies.

4.2. INTRODUCTION

The modern distribution of living organisms has been shaped by multiple processes that had profound effects on the dispersal, genetic structure and evolutionary histories of plant and animal populations. Movements of land-masses and successive multiple glacial events that occurred during the Pleistocene caused severe habitat changes which confined many species to warmer refugia and led other taxa to experience demographic reductions or complete extinction (Hewitt, 2000). Favourable periods during climatic fluctuations allowed successive population expansions, together with the recolonization of portions of the ancient ranges (Hewitt, 2004). Despite the high potential dispersive power of flying birds, it is striking that only few taxa did colonize most of the world. Excluding seabirds, for which the distribution pattern depends more on ocean basins than on the major land-masses (Newton, 2003a), only six landbird species (out of ca. 9,500 species) are known to be cosmopolitan, breeding in each biogeographical region of the world, except Antarctica. This group includes the Great Egret *Ardea alba*, the Cattle Egret *Bubulcus ibis*, the Glossy Ibis *Plegadis falcinellus* the Barn Owl *Tyto alba*, the Peregrine Falcon *Falco peregrinus* and the Osprey *Pandion haliaetus*.

The osprey is a medium-sized raptor with flexible breeding habitat requirements across its range. Despite its high specialization in catching fish, it is an opportunistic forager that can feed in both freshwater and marine environment. Also, its northern populations are known to engage in long-distance migrations (Poole, 1989; Alerstam *et al.*, 2006), whereas individuals from lower latitudes (e.g. Caribbean, Atlantic islands and Mediterranean basin) seem to be sedentary, or to perform small-scale interbreeding movements (Poole, 1989; Thibault *et al.*, 1996). One could therefore predict that broad habitat requirements and high mobility capabilities at the basis of such a wide distribution may have resulted in limited genetic variability across populations at a continental level, as described in other widespread raptors (e.g. *Haliaeetus albicilla:* Hailer *et al.*, 2007; *Falco peregrinus:* Bell *et al.*, 2014). However, adult ospreys tend to return to their natal area to breed (Martell *et al.*, 2002). Such strong philopatry may have played in favour of genetic structuring across the extensive range.

On the basis of comparative non-molecular characters such as osteology, pelvic musculature and the distribution of feather tracts, the osprey is considered sufficiently distinct from other raptor species (from Accipitridae family) to merit a monotypic family (*Pandionidae*), including only one species, the Osprey (Lerner & Mindell, 2005; Griffiths *et al.*, 2007). The most widely accepted taxonomic arrangement recognises four subspecies: *P. h. haliaetus* (Linnaeus, 1758) in the Palearctic from Europe, northwest Africa, and Asia north of the Himalaya, *P. h. carolinensis* (Gmelin, 1788) in North America, *P. h. ridgwayi* (Maynard, 1887) in Caribbean, and *P. h. cristatus* (Vieillot, 1816) in the Indo-Pacific and

Oceania (Fig. 1). The four subspecies were traditionally split on the basis of morphometry and plumage characteristics, but the differences are not straightforward (Poole, 1989; Strandberg, 2013). Therefore, referring only to morphology for describing diversity and interrelationships between subspecies has lead to controversies in taxonomy.

In this context, using molecular markers is a powerful method for inferring the evolutionary history of the osprey. Only two genetic studies have been carried out on this species (Wink *et al.*, 2004; Helbig *et al.*, 1998), but they did not investigate all subspecies, in the entire distributional range. Here, we carried out an extensive phylogeographic study based on mitochondrial DNA sequences (cytochrome *b* and ND2 genes) from samples covering the worldwide distribution of the osprey. This exhaustive dataset allowed addressing specific questions. a) Does the osprey show any phylogeographic structure in mitochondrial DNA at the continental level? b) How was such a specialized raptor able to colonize the entire world during the geological epochs? We propose a hypothetical evolutionary scenario explaining how the species' distribution and differentiation took place in the past. In the light of these new genetic clarifications, we discuss the potential implications for revisions of the taxonomy and for conservation (e.g. defining Evolutionary Significant Units (ESUs; Moritz, 1994)) to design adequate conservation strategies at the adequate spatial scale).

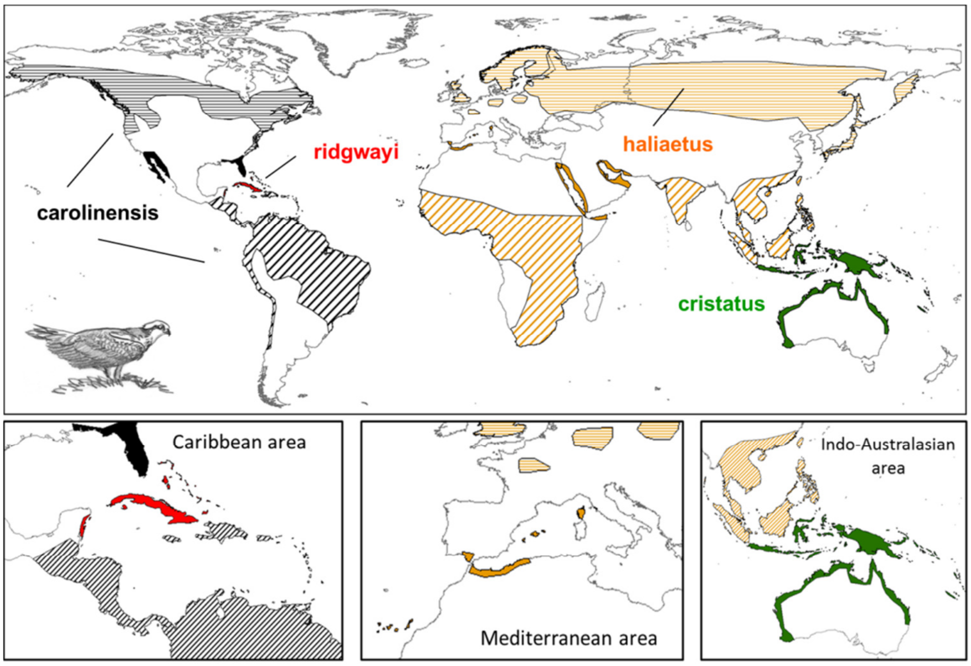

Figure 1: Geographical distribution of *Pandion haliaetus*. Ranges for the four recognized subspecies are in different colors: black for *carolinensis*, red for *ridgwayi*, orange for *haliaetus* and green for *cristatus*. Horizontal stripes are for breeding areas, skew lines for wintering areas and color-filled zones represent areas with sedentary populations. In the small

boxes (from left to right) three zones are zoomed in: Caribbean, Mediterranean and Indo-Australasian areas.

4.3. MATERIAL AND METHODS

a. Sampling, DNA sequencing and alignment

Sampling covered the whole species' distributional range, with 225 individuals from 31 countries across five continents (Fig. 1; Annex 1: Additional file 1). Fresh samples (n = 118; both blood and feather) were obtained from wild ospreys at the nest during ringing activities in different breeding sites. For each individual, about 0.5 ml of blood was taken by venepuncture from the wing and stored either on filter blotting papers or in 70 % ethanol in Eppendorf tubes. In some cases, growing body feathers containing traces of blood within the calamus were collected and stored in envelopes. For remote areas, where it was not possible to collect fresh samples, we completed our sampling using 107 museum specimens. We collected small pieces of skin (about 2 mm from the toepad) from museum study osprey-skins following the procedure described by (Mundy *et al.*, 1997). From each museum, we mainly choose museum study skins of certain origin and collected during the breeding season, so excluding possible vagrants or dispersing animals.

DNA was extracted using both fresh and museum specimens which were amplified by PCR for the mitochondrial Cytochrome *b* (cyt *b*). We discarded 21 samples that did not provide good amplification because of DNA degradation. Five cyt *b* sequences from Genebank were also included, leading to a total of 209 sequences (Annex 1: Additional file 1). For a subset of 38 individuals which returned high-quality PCR products, we also sequenced the mitochondrial NADH dehydrogenase subunit 2 (ND2), paying attention to choose samples distributed worldwide. We also included one sequence available in Genbank (Annex 1: Additional file 1). Finally, four other raptor species from the Accipitridae family for which cyt *b* and ND2 sequences were available in Genebank (Lerner & Mindell, 2005) were used as outgroups: White-tailed eagle (*Haliaeetus albicilla*), Tawny eagle (*Aquila rapax*), Black-breasted buzzard (*Hamirostra melanosternon*) and Gray-headed kite (*Leptodon cayanensis*).

Total genomic DNA was extracted from fresh and historical samples using a Qiagen DNeasy Tissue kit, following the manufacturer's instructions (Qiagen, Hilden, Germany). To avoid contamination with contemporary DNA (Bantock *et al.*, 2008), all extractions from museum specimens were performed using the facilities of the platform "ADN dégradé" (Labex CeMEB, Montpellier, France) dedicated to degraded DNA experiments, where we adopted the following specific safety measures. Equipment, consumables and Qiagen DNeasy Tissue kits used in the platform were purchased new, while the room was regularly cleaned and exposed to UV overnight after each DNA extraction cycle, in order to destroy possible traces of DNA between successive extractions. Experimentators wore protective clothing and footwear. As a further precaution and following Bantock *et al.* (2008), we worked with a maximum of 12 samples during each series of extraction to reduce the risks of cross-contamination and possible handling errors with tubes. We only used half of each foot-skin sample (about 10 mg of tissue) from which total DNA was extracted. Samples were incubated at least for one night at 56°C to be digested during the lysis. The tissue was digested in 180μL buffer ATL/20μL proteinase K solution for 20-hours at 55 °C; other reagents and the spin column were used according to the manufacturer's instructions ("Tissue protocol"), and final DNA elution was performed with 2 x 100μL of 10 mM Tris, 0.5 mM HC1 pH 9.0 preheated to 70°C. Multiple negative extraction and amplification controls were carried out simultaneously, using the same instruments and reagents, to detect possible contamination.

Portions of the mitochondrial cyt *b* and ND2 were amplified by PCR. Specific external and internal mitochondrial cyt *b* and ND2 primers were designed in this study for *Pandion haliaetus* (Annex 1: Additional file 2). PCR was performed using 1-µl (fresh samples) to 3-µl (museum specimens) of total DNA extracted, 5-µl of Multiplex Qiagen (containing HotStarTaq DNA polymerase, DNTPs and buffer) 1-µl for each primer at 2 pm and 2-µl of purified water. PCR reaction was performed using a MasterCycler Eppendorf thermocycler and began with an initial denaturation of 15 min at 95 °C, followed by 30 cycles of 30 s denaturation at 94 °C, 90 s annealing at 54 °C, 1 min extension at 72 °C and a 30 min final extension at 60 °C. A mitochondrial cytochrome b 1040 nucleotides fragment was amplified with PANHF1 and PANHR5 primers; F13 and PHND2-R1 primers were used to amplify a ND2 nucleotide fragment of 1100 bp (Annex 1: Additional file 2). In case of degraded DNA, we used internal primers to amplify cyt *b* and ND2 in 300 to 500 nucleotides overlapping fragments. Screening of the PCR products was performed by running on a 1% agarose gel using GelRed TM nucleic Acid gel stain (Biotium). Size products have been compared to long fragments ladder from Eurogentec, Smart LadderTM. After band sizes were determined, PCR products were sequenced at the Genoscope thanks to the “Bibliothèque du Vivant” sequencing project (CNRS-INRA-MNHN).

Electrophoregrams were read using CODONCODE ALIGNER 4.0.4 software and sequences were aligned by eye using SEAVIEW 4 software (Galtier *et al.*, 1996). Sequences were also translated into amino acids to check for any stop codons and possible amplification of pseudo-genes. Consensus sequences obtained for cyt *b* and ND2 from both fresh and museum samples were deposited in EMBL under accession numbers given in Annex 1: Additional file 1.

b. Partitioning and phylogenetic analyses

Phylogenetic relationships were inferred from the cyt *b* alone or from the concatenated cyt *b* + ND2 datasets. We determined both the best-fit partitioning scheme and the best models of sequence evolution using PARTITIONFINDER 1.0.1 (Lanfear *et al.*, 2012).

Phylogenetic trees were reconstructed using two probabilistic methods: Bayesian inference (BI) and maximum likelihood (ML). Bayesian analyses were performed with MRBAYES 3.1.2 (Ronquist *et al.*, 2003) using partitioning strategy as previously described (see also Results). Two separate runs of five million generations (sampled every 250 generations) were conducted simultaneously. TRACER 1.5 (Rambaut & Drummond, 2007) was used to check the convergence between the two runs and to determine the burn-in period. On this basis, the first 2000 phylogenetic trees were discarded (10%), and the remaining 18000 trees were used to estimate posterior parameters and probability distributions. ML tree was constructed with RAXML 8.0.17 (Stamatakis, 2006). As GTR is the only nucleotide substitution model available in RAXML, GTR+G was applied to all partitions previously determined by PARTITIONFINDER. The robustness of nodes was evaluated with 1000 bootstrap replicates with the option –b. The consensus tree was obtained using the program CONSENSE of the PHYLIP 3.69 package (Felsenstein, 2005).

Relationships between haplotypes were also visualized as a minimum spanning network, using the Median-Joining (MJ) network algorithm implemented in the program NETWORK 4.1.1.0 (Bandelt *et al.*, 1999).

c. Genetic diversity, demographic history and molecular dating

DNASP 5.10 (Rozas *et al.*, 2003) was used to compute the number of haplotype (nH), haplotype diversity (H), nucleotide diversity (π) as well as the average number of nucleotide differences (k). Mean genetic distances within and between groups were computed using the *p*-distance and a pairwise deletion for the gaps/missing data treatment, as implemented in the MEGA 5.10 software (Tamura *et al.*, 2011).

Demographic history of the haplogroups and the whole dataset was determined with different methods. Firstly, R2 (Ramos-Onsins & Rozas, 2002), Fu's Fs (Fu, 1997) statistics and their significance were calculated with DNASP. Ramos-Onsins & Rozas (2002) recommended using R2 when population sizes are small (~10) and Fs when sample sizes are large (~50). Demographic changes (e.g. bottlenecks or expansions) were also tested based on pairwise mismatch distributions of substitution differences using DnaSP. Finally, the historical demography of the main haplogroups was also estimated on the cyt *b* dataset using the skyline plot method (BSP; Drummond *et al.*, 2005; Drummond & Rambaut, 2007) implemented in BEAST 1.8.0. BSP analyses were performed on each group separately with the cyt *b* partitioned according to codon position and using the HKY+G model as sequence evolution. The likelihood-ratio test performed with TREE-PUZZLE 5.2 (Schmidt *et al.*, 2002) rejected the strict molecular clock hypothesis ($p<0.05$). BSP analyses were thus conducted using a lognormal-relaxed molecular clock with a substitution rate of 0.01973 per lineage per million years as estimated by Nabholz *et al.* (2009) for the osprey cytochrome *b*. Analyses were run for 50 million generations, sampled every 1000 generations, after discarding the first 10% as burn-in. We used TRACER 1.5 to analyse the results and draw the BSPs.

Time of the most recent common ancestor (TMRCA) was estimated with BEAST 1.8.0 based on 43 sequences (39 ospreys and 4 outgroups) of the two concatenated genes (cyt *b* + ND2; 2079-bp). The whole alignment was partitioned according to the three codon positions using a HKY+G model of sequence evolution. Four runs were performed, each of 50 million generations, sampled every 1000 generations, and a 10% burn-in was applied. The resulting tree files were combined with LOGCOMBINER 1.8.0 and the maximum clade credibility tree (mean height) was obtained with TREE-ANNOTATOR 1.8.0.

4.4. RESULTS

a. Phylogenetic relationships

For the mitochondrial cyt *b* gene, the best-fit scheme was a partitioning according to codon position with the models HKY+I for position 1, TrN for position 2 and K80+G for position 3. As the TrN substitution model was not available in MRBAYES, the parameter Nst was set to 2 as the closest model. The partitioned ML analysis was performed with 1000 bootstrap replicates using a GTR+G substitution model for each codon position in RAXML software. The average Bayesian posterior probabilities (pp) and bootstrap values (BP) for supported clades are shown on the tree in Fig. 2. The cyt *b* phylogenetic tree revealed the existence of four well-supported groups which represent quasi non-overlapping geographical lineages. A first clade (AMER; pp=0.71, BP=84) includes all 26 ospreys from the New World: 2 samples from the Pacific coast of USA (Oregon), 11 from the Atlantic coast of USA (Massachusetts, Virginia and Florida), 8 from the Caribbean (5 from the Dutch Antilles and 3 from Bahamas) and 5 of unknown origins (two of them collected in Suriname, South America). No genetic structure was evidenced and it can be noticed that the Caribbean samples (supposed to belong to *ridgwayi* ssp) are scattered in this group.

The second cluster (IND-AUS; pp=0.61, BP=58) is composed of 37 individuals retrieved from the Indo-Australasian area: 10 from western Indonesia (Sumatra, Java and Borneo), 14 from various islands of Central Indonesia, 1 in New Guinea, 1 in New Caledonia and 10 from Australia. One sample coming from India also belonged to this haplogroup. In this group, basal branches were only composed of individuals collected on Indonesian islands, whereas Australian birds emerged as terminal branches in the clade.

A third group was composed of 15 ospreys from Asia (ASIA; pp=0.76, BP=83): six from the pacific coast of Siberia (e.g. regions of Magadan, Khabarovsky, Primorskii and the Kurile islands) and five from Japan. In addition four samples which belonged to this haplogroup were collected in other geographic areas: one from continental Asia (Mongolia), while the three others from the Indo-Australasian region (New Guinea, Pulau Batjan Island in Central Indonesia and Java Island). In the ASIA group, Siberian samples were mainly located within the basal branches (together with those coming from Indonesia) while the five samples from Japan constituted a well-supported subclade (pp=0.98, BP=98).

Finally, the largest clade (EUR-AFR; pp=1, BP=91) was formed by 131 individuals, mainly belonging to the Western Palearctic area, with a few exceptions. Ninety-five of these samples were collected in Europe along a latitudinal gradient scattered from northern Europe (Fenno-Scandia and western Russia), central Europe (Germany, France), to southern localities in the Mediterranean area (Corsica, Balearics, Italy, Portugal). Samples from North African coasts (e.g. Morocco) and Atlantic islands (e.g. Canary and Cape Verde) were included in this haplogroup, together with ospreys from the Red Sea and Persian Gulf areas. Interestingly, four geographical exceptions were recorded in this group: two individuals from central Siberia (Tuva and Baikal regions), one from India and one in western Indonesia (Sumatra Island). In the EUR-AFR group, no evident genetic structure was detected between the most distant populations (e.g. from Finland to the Mediterranean). Despite this, basal branches were represented by individuals mainly collected in the Middle East (Persian Gulf and Red Sea); a well-supported subclade arose for the Canary Islands (pp=0.94, BP=63), but not for the other Atlantic archipelago of Cape Verde.

In total, three potential mixing areas were detected between phylogenetic lineages: a) one in central Siberia between EUR-AFR and ASIA; b) one in Indonesia between ASIA and IND-AUS and c) a third one between west Indonesia and India between EUR-AFR and IND-AUS.

Despite the different sample sizes, mean genetic *p*-distances within groups (Tab. 1) were very low and showed comparable values (p = 0.001- 0.002), indicating slight genetic variability internal to each lineage. On the other hand, the greatest genetic differences between groups (Tab. 1) were recorded between AMER and EUR-AFR (p = 0.026), whereas lowest values were obtained between IND-AUS and ASIA (p = 0.015) and between IND-AUS and EUR-AFR (p = 0.017). At the same time, distance between AMER and IND-AUS was smaller (p = 0.020) than those between AMER and ASIA (p = 0.025).

Table 1: Uncorrected pairwise (p)-distance expressed as percentage (%) for cyt b within (in bold) and between clades in *Pandion haliaetus*.

	IND-AUS	EUR-AFR	AMER	ASIA
IND-AUS	**0.1**			
EUR-AFR	1.7	**0.1**		
AMER	2.0	2.6	**0.1**	
ASIA	1.5	2.1	2.5	**0.2**

Relationships between the four haplogroups did not appear as well resolved. The structure of the phylogenetic tree (Fig. 2a) revealed that the AMER group should be the basal clade but this relationships is not supported (pp=0.56, BP=49), just as the sister group relationships between EUR-AFR and ASIA (pp=0.22, BP=43) among the three Old World lineages. In order to improve resolution between groups we sequenced the ND2 gene for a subsample of individuals. Thirty-nine osprey sequences (Annex 1: Additional file 1) were used for a total alignment of 2079-bp in the concatenation of the two mitochondrial fragments (1067 bp cyt *b* + 1012 bp ND2). Four partitions were obtained for the best-fit scheme: one for the position 1 of cyt *b* (with the model K80+G), one for the cyt *b*-position 2 and ND2-position 3 (model HKY+I), one for the cyt b-position 3 and the ND2-position1 (model TrN) and one for the ND2-position 2 (model K81uf+G). As previously stated, the TrN model was approximated with Nst = 2 in MrBayes.

The analysis carried out on the two genes (Fig. 2b) reinforced the support for the AMER, IND-AUS and EUR-AFR lineages. The ASIA group is the exception, in being paraphyletic because the individual from Khabarovsky region in Siberia (sample code: 171_Russia) appears as the sister taxon of EUR-AFR. However, this result could be due to the low number of ASIA samples that have been reduced to six among which the sample 171_Russia is incomplete because of problems of PCR amplification. If the support increased for the node EUR-AFR/ASIA (pp=0.87, BP=49), there was no improvement concerning the support for the AMER clade as the first emergence in the *Pandion* phylogeny. Consequently, and despite the fact that the number of nucleotides has been doubled, the position of the root is still unresolved.

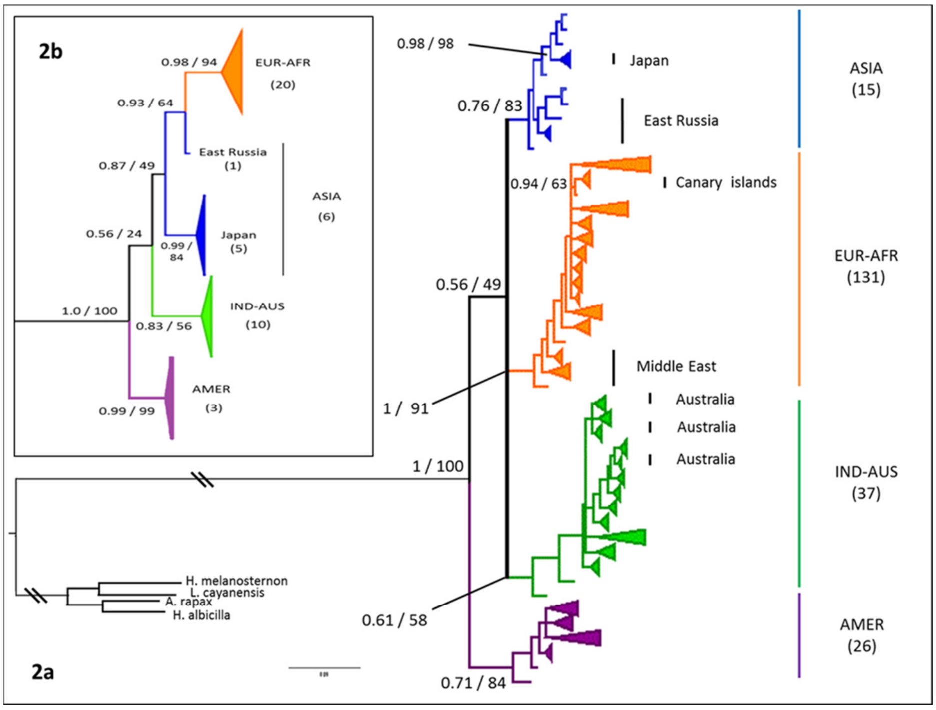

Figure 2: Phylogenetic trees of ospreys: a) bayesian phylogenetic tree constructed from 209 cytochrome *b* sequences (1103 nucleotides) showing the four supported clades as well as the geographic origin of the samples; // means that the branch length leading to the outgroups has been reduced but remains proportional to the number of substitutions per site. (b) bayesian tree representing phylogenetic relationships obtained with the concatenated genes (cyt *b*+ND2; 2079 nucleotides, 39 sequences). In both trees, four species belonging to the Accipitridae family were used as outgroups. For supported clades, bayesian posterior probabilities and ML bootstrap are indicated at nodes, respectively.

b. Network, genetic variability and demography

In order to avoid artefactual groupings due to missing data, the MJ network was built considering the most complete dataset in terms of nucleotides and individuals, which is a fragment of 661 bp of the cyt *b* on 146 samples. The network (Fig. 3) confirmed four major groups which were included in 19 unique mtDNA haplotypes. The EUR-AFR clade (*n* = 102) resulted in nine haplotypes differing by only one nucleotide change. Two out of the nine haplotypes were frequent, and shared by the majority of the individuals (58 and 25 individuals, respectively). Despite remarkable differences in breeding and movement ecology, osprey populations of lower latitudes within the EUR-AFR did not show notable haplotypic differences when compared to the northern and central European birds. The four samples from the Canary Islands shared a single haplotype. Within the IND-AUS group (*n* = 16) only three haplotypes were found, differing by only one nucleotide position. Five haplotypes were observed within the AMER group and 13 samples out of 17 showed the same haplotype, which was shared by ospreys from both western and eastern USA and from the Caribbean. Finally, within the ASIA group (*n* = 11) two haplotypes were recorded.

Despite a slight variation within each group (haplotypes were mainly distant by only one or two positions), a larger number of nucleotide differences were recorded between clades. The AMER group recorded the greatest genetic distance with EUR-AFR (a minimum of 15 nucleotides changed), whereas the minimum number of changes is 11 positions between IND-AUS and ASIA (Fig. 3).

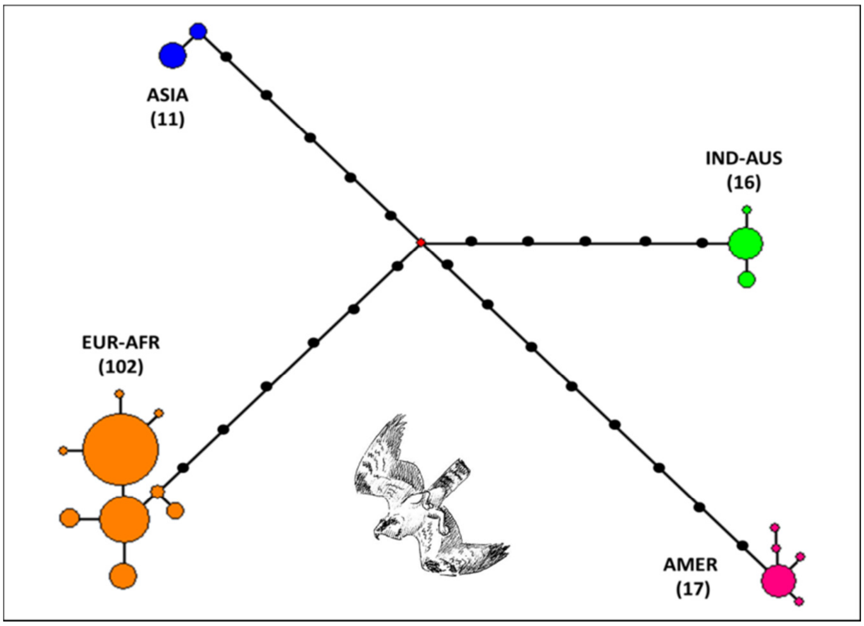

Figure 3: Phylogenetic network of osprey based on 146 individuals and 661 pb. Coloured circles indicate different haplotypes with size proportional to the number of individuals. Black dots represent missing haplotypes. The median vector is reported with a red dot. The number of individuals is indicated in parentheses for each group.

In the subset of 146 ospreys, 34 polymorphic segregating sites were discovered within the 661 bp cyt *b* fragment. The haplotype diversity (H), nucleotide diversity (π) and other statistics were computed for the four recognized haplogroups and the whole dataset combined (Tab. 2). Haplotype diversity was higher in the largest group of EUR-AFR (H = 0.615) and lower for the three other groups (range: 0.425-0.436). The nucleotide diversity showed similar patterns between groups, being very weak in each lineage (range: 0.00066-0.00138). Overall, H was 0.795 and π was 0.01064 for all *Pandion* samples. Demographic history of the four phylogroups, as inferred on the basis of Fu's F_S and R2 statistics (Tab. 2), indicate that only the AMER group yielded significant values for these indices, whereas the EUR-AFR lineage showed a significant value for the Fu's FS only.

Table 2: Estimates of across and within-population variability of cyt b sequences of osprey mtDNA. Sample size (n), number of haplotypes (nH), haplotype diversity (H), nucleotide diversity (π), and the average number of pairwise differences (k).The value of the Fu's Fs test and R2 of Ramos-Onsins & Rozas (2002) are also reported; stars indicate significant values (*: p<0.05 and ***: p<0.001).

Phylogroups	n	n_H	H (s.d.)	π (s.d.)	k	*Fs*	R_2
Overall	146	19	0.795 (0.026)	0.01064 (0.00087)	6.872	1.413	0.100
AMER	17	5	0.426 (0.147)	0.00087 (0.00035)	0.574	-2.826***	0.099***
EUR-AFR	102	9	0.615 (0.043)	0.00138 (0.00015)	0.901	-3.44*	0.056
ASIA	11	2	0.436 (0.133)	0.00066 (0.00020)	0.436	0.779	0.218
IND-AUS	16	3	0.425 (0.133)	0.00068 (0.00023)	0.450	-0.571	0.145

Based on these indices, population expansion can be assumed for the American and possibly the Western Palearctic. Mismatch distributions for the total dataset yielded a four-modal pattern reflecting the four lineages (Annex 1: Additional file 3). However, when each clade was analysed separately, the shape of distribution showed a similar unimodal pattern. Based on the Bayesian skyline plots (Fig. 4), the two haplogroups AMER and IND-AUS remained demographically stable or underwent a slight constant expansion. The EUR-AFR haplogroup also showed a clear trend of demographic expansion starting >10000 years ago. Conversely, the ASIA haplogroup was the only one showing a slight continuous demographic decrease (Fig. 4).

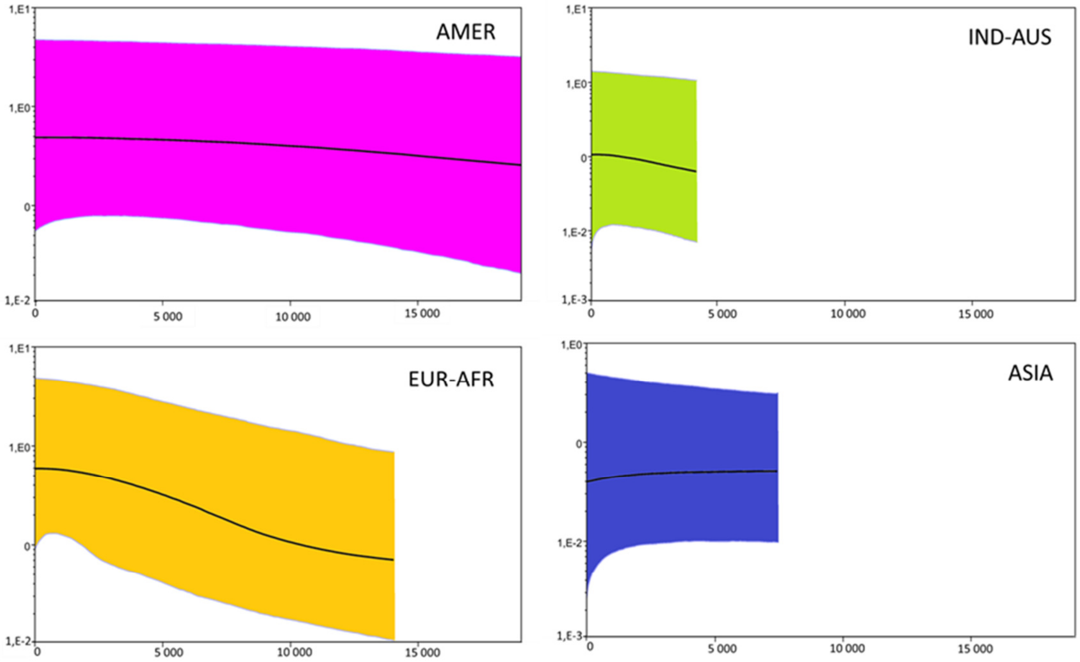

Figure 4: Bayesian skyline plots showing the demographic histories of the four main haplogroups identified in the *Pandion haliaetus* sequences. On the x-axis the time is

expressed in years. The population size (expressed in units of Neτ, the product of the effective population size per generation length) is reported on the y-axis.

c. Molecular dating

Diversification for each group was dated between 0.3 and 0.5 Ma within the Middle Pleistocene (intervals were partially overlapping), while the first divergence event which generated ASIA and EUR-AFR occurred earlier, about 1.1 Ma, during the Early Pleistocene. The values indicate that the initial split between lineages occurred about 3.1 Ma in the Late Pliocene (Fig. 5). This first event of divergence separated the AMER and IND-AUS birds from the remaining individuals (EUR-AFR/ASIA). However, the cluster consisting of these samples was not robustly supported, making branching patterns unclear. This is congruent with the results of the Bayesian analyses where the position of the root clade was not strongly resolved; though disjunction between clades was clearly supported.

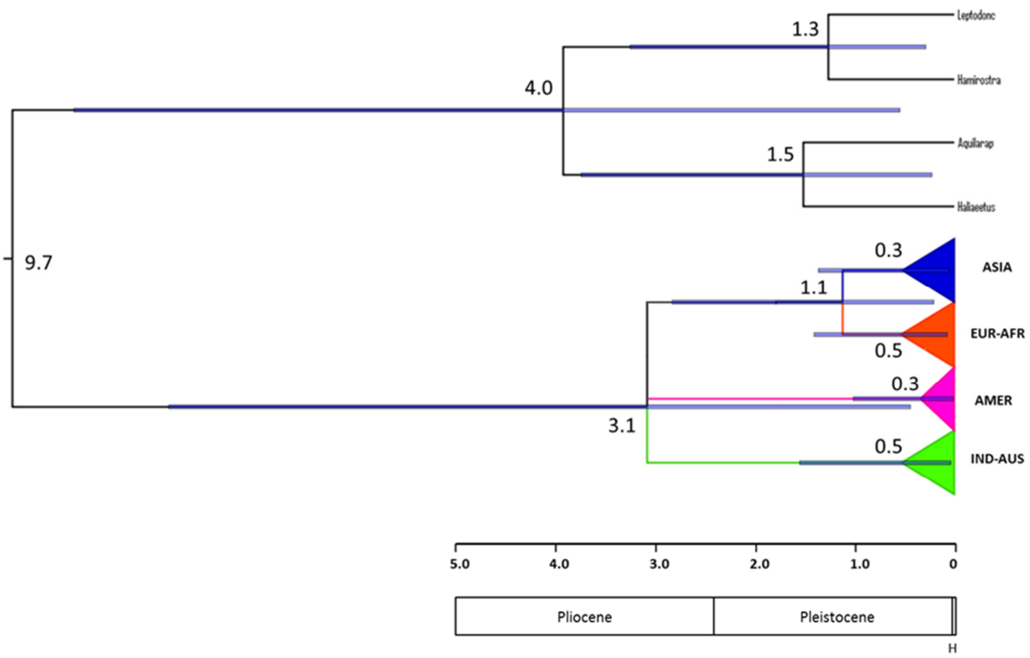

Figure 5: Chronogram obtained with BEAST 1.8.0 showing the time of divergence for the diverse splits in *Pandion haliaetus* using a substitution rate of 0.01973 per lineage per million years. Blue bars at nodes indicate 95% highest posterior densities. The colour code used for each haplogroup is the same as in previous figures. A partial geological time scale is reported below the chronogram (H = Holocene).

4.5. DISCUSSION

a. Species diversity and demography

Our study revealed that the osprey is structured into four main genetic groups, representing quasi non-overlapping geographical regions. Each lineage, though including birds from distant geographic areas, showed slight internal genetic variability. The mean genetic distance yielded very low values (range: 0.1-0.2%). Haplotypic and nucleotide diversities were also very weak, with only 9 haplotypes observed for the largest groups including 102 ospreys (EUR-AFR), and values were even smaller for the other three lineages. These values are comparable or lower than those recorded in single populations of other large raptors. For instance, similar low genetic diversity was found in the mitochondria of various populations of Red kites *Milvus milvus* (Roques & Negro, 2005) and White-Bellied Sea-Eagles *Haliaeetus leucogaster* (Shephard *et al.*, 2005) that were geographically closer than some of our osprey populations within the same clade. Such low values are usually related to populations that experienced demographic crashes or remained isolated in fragmented habitats (Lifjeld *et al.*, 2002; Martínez-Cruz *et al.*, 2004).

Populations within each group were poorly differentiated, suggesting that they might have experienced a reduction of genetic variation. However, the historical demographic reconstruction for each lineage suggested that populations within each clade did not experience any strong bottleneck phases, but rather underwent stable trends or slight increases. Moreover, mismatch distributions (Annex 1: Additional file 3) as well as significant values for Fs and R2 are also compatible with the hypothesis of demographic expansion for the AMER and IND-AUS clades. This is in agreement with census data showing that both American and Australian osprey populations have historically increased in size (Poole, 1989).

Then, why does each clade show such low genetic variability? Low levels of genetic variation can be the consequence of population declines, or represent an ancestral state (Wandeler *et al.*, 2007). Several studies have reported stable genetic diversity despite declines in population size (e.g. Pertoldi *et al.*, 2001). For example, no obvious loss of genetic diversity was detected among Canadian peregrine falcons (*Falco peregrinus*) despite a population bottleneck (Brown *et al.*, 2007). In our case, one possible explanation is the colonization of new areas by few individuals carrying only a few mitochondrial lineages of the genetic pool (founder effect). Furthermore, source populations could have experienced a reduction in genetic variability due to climate fluctuations during the Quaternary; remnant populations in refugia represented the genetic source for the following recolonization (see below).

For EUR-AFR, bayesian skyline curves (Fig. 4) suggest that populations encountered a recent expansion, which probably started about 10000 years ago. This fits well with a recent review of the Holocene fossils of osprey in central Europe from 10000 years BP (Zachos & Schmölcke, 2006). In Europe, the osprey expanded its distribution area in the following centuries until the beginning of the 20th century. Then, despite declines during the 1960-70s, populations were able to recover, showing positive demographic trends (Poole, 1989).

On the other hand, the ASIA clade seems to be the only one which has suffered a slight continuous demographic decrease (Fig. 4). The same trend is also suggested by very low nucleotide diversity and a positive Fs value. However, such values might also be related to the small sample size of this group (15 samples). This result needs hence to be confirmed by further samples from Asia.

Despite the low variation within each group, the network (Fig. 3) revealed a high number of nucleotide differences between the four distinct clades. Overall, the mean distance across all populations (the entire *Pandion* mix) is 1.2%, which is higher than the values recorded for the red kite (0.75%; Roques & Negro, 2005) or the white tailed eagle *Haliaeetus albicilla* (0.7%; Hailer *et al.*, 2007). The mean genetic distance between groups (range: 1.5-2.6%) is comparable to, or even greater than, those observed for several members of closely related sister eagle species from the genera *Aquila*, *Hieraaetus* (range: 1.7-2.1%; Wink & Sauer-Gürth, 2004) and *Haliaeetus* (range: 0.3-9.8%; Wink *et al.*, 1996).

b. Hypothetical Evolutionary scenario

The cyt *b* phylogenetic tree (Fig. 2a) suggests that the AMER haplogroup constitutes the first genetic group that emerged among the four found for *Pandion haliaetus*. Unfortunately, this relationship was not supported by the cyt *b* alone (1103 bp) nor by the combination of cyt *b* and ND2 (2079 bp). We can assume that this irresolution partly arose as a misplacement of the root due to the use of too divergent outgroups. Indeed, this is not surprising because there are no optimal outgroups available for the phylogeny rooting of *Pandion*, since it constitutes a long branch in the phylogeny of raptors, distant from its sister Accipitridae family (Helbig *et al.*, 2005; Lerner & Mindell, 2005). Similar problems of rooting have been reported in other phylography studies (Godoy *et al.*, 2004; Igea *et al.*, 2013).

Nevertheless, other arguments can be advocated to reinforce the hypothesis that the osprey originated in the New World (1 in Fig. 6). First, the oldest recognized osprey specimen is a *Pandion homalopteron* of the mid-Miocene of California dated 13 Ma (Poole, 1989). This is in accordance with our molecular dating which estimated at ca. 9.7 Ma the origin of a first ancestor for *Pandion sp* (Fig. 5). In addition, as far as we know no fossil was found in Australia (where the species is frequent today), whereas seven fossils from late Pleistocene have been found in Florida (Zachos & Schmölcke, 2006). Second, calculation of the *p*-distances between groups (Tab. 1) indicates that the AMER group is the most divergent compared to the other three groups, suggesting its more ancient origin (and a closer relationship with IND-AUS group). Our molecular dating estimated at 3 Ma (Pliocene) the origin of *Pandion haliaetus*. Further, the phylogenetic trees did not support a sister group relationships between AMER and EUR-AFR, as it would be expected in the case of a direct colonization from America to Europe (across the Atlantic Ocean). On the contrary, a pronounced phylogenetically old separation between American and Western Palearctic populations emerged, in accordance with previous studies (Helbig *et al.*, 1998; Wink & Sauer-Gürth, 2004).

Thus, the second phase of the evolutionary scenario (2 in Fig. 6) should have involved the colonization of the Indo-Australasian region. We hypothesize an early passage via the Bering Strait, which allowed the colonization of the pacific coast of Asia until Indonesian islands and, in a second step, of Australasia. This hypothesis is corroborated by the fact that Indonesian samples occupy a basal position in the IND-AUS clade, whereas Australian samples occupy the uppermost branches (Fig. 2a). This first colonization might have occurred during the Pleistocene when sea levels were at their lowest, i.e. ca. 150 m below current levels, shortening distances between islands and certainly facilitating the movement of birds from Indonesia to Australia (Hewitt, 2000; Shephard *et al.*, 2005). We then need to postulate the extinction of first established northern populations in Asia, most likely because of unfavourable climatic conditions, during the glaciations of the Quaternary (from 2.58 Ma to present). Between 3.1 and 1.1 Ma (Fig. 5), large ice sheets in North America and Siberia might have pushed animal populations to find refugia at lower latitudes (e.g. in the Caribbean for American ospreys and Indonesia-Australia for populations of Asia). As a result, Indo-Australasian ospreys may have remained isolated in the Southern Hemisphere until subsequent more favourable interglacial periods.

From refugia located in Indonesia-Oceania, two routes of re-colonization can be inferred. The first one (3 in Fig. 6) involves an expansion towards north with settlement the eastern Asia. The Japanese population likely arose from just one colonizing event, as suggested by a single, strongly supported Japanese subclade. The second route (4 in Fig. 6) followed a westward direction to India and Middle East and eventually reached Europe where the population rapidly expanded (in accordance with BSP; Fig. 4). This hypothesis is corroborated by the *p*-distances which displayed the lowest values between IND-AUS and both EUR-AFR and ASIA groups (0.017 and 0.015, respectively). Conversely, a greater distance recorded between ASIA and EUR-AFR (0.021) indicates that neither of these groups originated directly from one another. In this scenario, the overlap between EUR-AFR and ASIA zones in the Tuva and Baikal regions can be interpreted as a secondary suture zone (Taberlet *et al.*, 1998). However, more samples from Russia and Siberia are required to better understand this secondary suture zone and explain differences. These two main colonization pathways (3 and 4 in Fig. 6) were probably influenced by environmental and geographical barriers such as the Himalaya, which might have prevented a direct passage from Indonesia to Central Russia. The split between EUR-AFR and ASIA groups is dated at 1.1 Ma during the Early Pleistocene.

Climatic changes during Pleistocene glaciations possibly facilitated secondary contacts between North America and Eastern Palearctic via the Bering Strait (Prevost, 1982), accounting for the morphological similarities between the subspecies *carolinensis* and *haliaetus*. Although such hypothesis cannot be rejected we found differences sufficient enough to distinguish these groups, at least at the mitochondrial genes level.

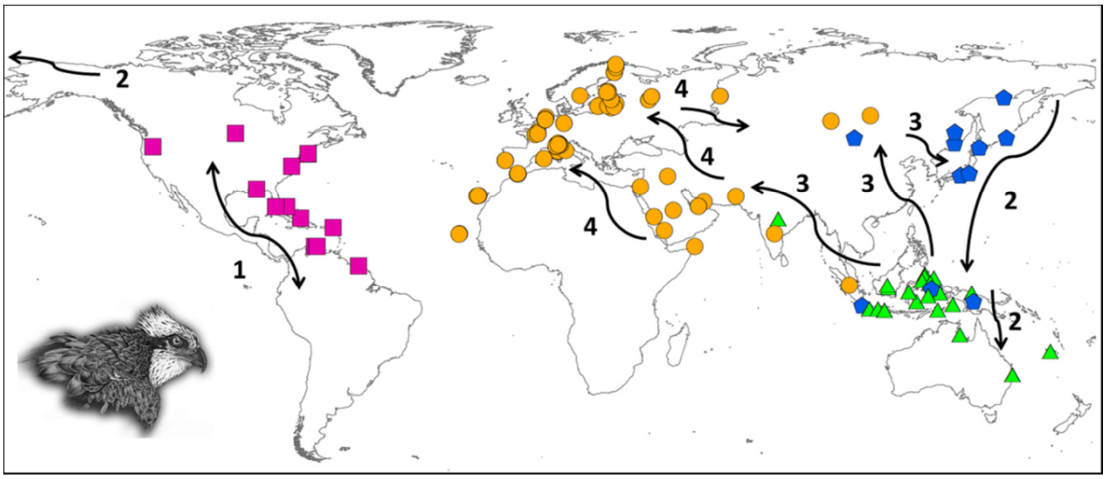

Figure 6: Geographical distribution of the four haplogroups of *Pandion haliaetus*. Symbols and colours indicate both sample locations and genetic group: violet squares for AMER, orange circles for EUR-AFR, blue stars for ASIA and green triangles for IND-AUS (see results). Numbers with their corresponding arrows describe the different phases of the hypothetical colonization scenario.

c. Implications for taxonomy and conservation

Our four genetic groups do not enterely correspond to the four subspecies based on morphological characters (Poole, 1989; Strandberg, 2013). The IND-AUS lineage matched geographically with the subspecies *cristatus* (Christidis & Boles, 2008). Contrary to current taxonomy, North American *carolinensis* ospreys do not differ from Caribbean *ridgwayi* birds as there is no evident structure in the phylogeographic tree and in the network. On the other hand, in Eurasia, we found that the subspecies *haliaetus* was actually composed of 2 clades (EUR-AFR and ASIA). This can reflect the poor knowledge of the species in Asia where detailed information about biology and distribution are needed (Shoji *et al.*, 2011). Further samples should be collected to clarify the geographical limits of these lineages, especially in the sectors where we found a zone of overlap.

Overall, genetic distances between osprey clades are in a range which has already been used by taxonomists for designating distinct raptor species (e.g. Wink *et al.*, 1996). However, we detected a relatively low overall nucleotide diversity (1.0%) compared to another large raptor species with a similar wide distribution (e.g. *Gypaetus barbatus*, 2.9%; Godoy *et al.*, 2004); but see Hailer *et al.*, 2007 for *Haliaetus albicilla*, 0.7%). The decision for splitting ospreys into different species (e.g. Christidis & Boles, 2008) should integrate also other factors besides morphology and mtDNA differences; e.g. behavioural aspects could have an important role as reproductive barriers between distant populations (Helbig *et al.*, 2002).

The first step towards a sound global management and conservation plan is to define Management Units (MUs) and Evolutionary Significant Units (ESUs; Moritz, 1994). As a matter of fact, subspecies have often been used as proxies for units of conservation in absence of a genetic data indicating distinct evolutionary units (Zink, 2004). Our results evidenced four different ESUs that deserve specific management. A better knowledge of the range of each ESU is strongly needed in the near future. In particular, the ASIA clade should be a priority target for multiple reasons: a) this lineage has never been described before; 2) it relied on a limited number of samples (n = 15) from only a few areas; 3) the majority of these samples were museums specimens, so the current presence of this clade in East Russia and Indonesia needs to be confirmed; 4) it is the only clade to show clear signals of a slight continuous demographic decrease; and 5) it has no clear morphological characteristics which help identification.

Despite the osprey is currently globally listed as of Least Concern (BirdLife International, 2014), it is considered a priority species for conservation across its distributional range. Indeed, the osprey has experienced a severe decline during the 19th and 20th century that led to important demographic declines or local extinctions (Cramp & Simmons, 1980; Dennis & Dixon, 2001; Bierregaard *et al.*, 2014). Overall, the osprey is considered an important flagship species and during the last decades, the species has been involved in 25 reintroduction projects across 14 states in USA (Martell *et al.*, 2002; Bierregaard *et al.*, 2014) and also in Europe (Dennis & Dixon, 2001; Monti *et al.*, 2012). Our results suggest that reintroduction projects foreseen e.g. within Europe could be conducted using source populations within the entire Western Palearctic but not using birds belonging to the other ESUs. However, before concluding that no restriction needs to be adopted for translocations between populations within the Western Paleartic, other variables must be considered. Further genetic study using microsatellite markers could reveal more recent differences and the occurrence of gene flow between populations. Besides differences at mtDNA level, local populations (e.g. Mediterranean, Canary Islands, Cape Verde, Red Sea) could in fact show recent differences contained at the level of nuclear genes (e.g. microsatellites loci) and also in migratory or reproductive behaviours.

5. GENOTYPING TECHNIQUES REVEAL CONNECTIVITY IN OSPREY POPULATIONS: A MULTI-SCALE APPROACH USING MICROSATELLITES.

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5.1. ABSTRACT

Genetic variability and population structure in osprey were studied using DNA microsatellite markers. Special emphasis was placed on the subspecies living in the Afro-Palearctic (*Pandion haliaetus haliaetus*). For comparative purposes, North American osprey subspecies (*P. h. carolinensis*, *P. h. ridgway*) and Indo/Australian subspecies (*P. h. cristatus*) were included in this analysis. 20 DNA microsatellite loci were analyzed across a total of 200 individuals. Cluster analysis of genetic distances generally grouped populations of osprey in accordance with their subspecific designation and with previous results from mtDNA analysis. Ospreys from America and Australia were clearly separated from *P. h. haliaetus* suggesting a more ancient isolation which prevented recent gene flow across these groups. Within *P. h. haliaetus*, significant genetic differentiation was found between populations in northern and southern Europe, suggesting that the Afro-Palearctic group is structured into two interconnected entities (Mediterranean and continental Europe). Population structuring was supported by an assignment test and by analysis of allele-sharing among individuals. At the Mediterranean scale, no significant differences of allelic information were found between populations. Behaviours such as dispersal, migration and philopatry seem to have played simultaneously and in contrary directions in shaping the genetic structure and diversity of populations. A better understanding of these behaviours is therefore needed since it could help in reconstructing population dynamics providing essential information for management and conservation of the species, namely in the Mediterranean area.

Keywords: genetic connectivity, Osprey, nuclear molecular markers, dispersal, migration, philopatry, Palearctic, Mediterranean.

5.2. INTRODUCTION

Acquiring a good knowledge of ecology, life history and demography of animal populations is essential to establish reliable management measures in the light of long-term conservation purposes (Primack *et al.*, 2000). One important component affecting population dynamics is genetic diversity (Avise, 1989; 1995), which includes variability both at the individual and population level. Such variability, resulting from both neutral and adaptive processes, evolved in response to biotic and abiotic conditions. The loss of a portion of genetic diversity is one of the main processes which concur to impact future chances of persistence of a species. This is true especially for small and isolated populations which, because of inbreeding and genetic drift, can be trapped in an extinction vortex (Höglund, 2009). In this context, understanding the genetic structure and connectivity of populations is of fundamental importance to establish appropriate conservation plans, especially for those populations which are threatened and so require specific management measures (Agudo *et al.*, 2011). An accurate management of local populations may indeed prevent the loss of genetic variation resulting from population declines (Draheim *et al.*, 2010). The genetic structure of populations is clearly determined by evolutionary forces (e.g. natural selection, mutations, genetic drift), but is also influenced by behavior (Nesje *et al.*, 2000; Agudo *et al.*, 2011). Intensity of gene flow may differ between species according to several behavioural factors which often act antagonistically. One factor is dispersal that is defined as the permanent movements an individual makes from its place of birth to its first breeding site (natal dispersal) or between successive breeding sites (breeding dispersal) (Clobert *et al.*, 2001). It may allow colonization or recolonization of favorable habitats/vacant sites for the species (Hanski & Gilpin, 1997), thus resulting in homogenization of the gene pool between populations by balancing potential differences in Hardy Weinberg equilibrium (deficit or excess heterozygotes; Salanti *et al.*, 2005). At the same time migration, the widespread behaviour by which an animal periodically moves from one region to another in order to better satisfies its requirements for a phase of its life cycle (Berthold, 2001), can interact synergistically with dispersal in the regulation of allelic frequencies between the different populations. Indeed, individuals may choose to go to reproduce on a site they have previously visited during migratory journeys. On the other hand, philopatry (the behavior by which individuals tend to return to their natal area to reproduce once they reached sexual maturity) is an antagonist factor to dispersal and migration, since it tends to favor a local sub-structuring by preventing gene flow between populations. Therefore it is expected that strict philopatric species will show strong population genetic structure, characterized by many private alleles and heterozygote deficiency. For example, in the migratory Egyptian vulture (*Neophron* *percnopterus*) individual GPS tracking showed that geographical barriers such as the Mediterranean sea do not operate as obstacle to gene flow between populations, but rather that genetic isolation might be due to a strong philopatric behaviour of the species (Agudo *et al.*, 2011). In this sense, species with a high philopatric behavior may have populations with a genetic structure similar to species living in islands (so following the model of island biogeography; MacArthur & Wilson, 1967). Therefore, behaviours operating as barriers (e.g. high territoriality, philopatry) may have an important role in explaining the genetic structure of populations. Furthermore, these antagonistic behaviours (e.g. dispersal and migration vs philopatry) can often act concurrently, being more or less prominent but affecting the ultimate genetic aspect of populations. Understanding the role of such behaviours and their concomitant effects on population dynamics is crucial to guide and develop effective actions of conservation, especially for threatened species that have experienced strong decline after centuries of persecution by humans (e.g. raptors).

In this context the osprey, *Pandion haliaetus*, is of notable interest. This highly specialized fish-eating raptor is the only representative of the family Pandionidae (order Accipitriformes) and four subspecies have been described on the basis of morphological criteria: *P. h. carolinensis*, *P. h. ridgwayi*, *P. h. haliaetus* and *P. h. cristatus* (see chapter 1; Poole, 1989). Across its distributional range, both resident and long-distance migratory populations are evidenced (Poole, 1989). Moreover, it has been shown that this raptor has a large capacity of natal dispersal that varies according to sex (sex-biased dispersal with females dispersing over a greater distance than males; Martell *et al.*, 2002) and at the same time has a strong philopatric behavior (Poole, 1989). Thus the osprey represents a good biological model for investigating how genetic pools were structured among different populations by these antagonistic factors. At regional scales, dispersal movements, migration and genetics of this species are still poorly known, preventing the full understanding of their ecology and in turn arising many questions about the long-term maintenance of populations. For example, the absence of connectivity (thus the absence of gene flow) between Mediterranean populations living on islands and those from mainland (continental Europe), could lead to a significant loss of genetic diversity in the former because of their low numbers and related risks of extinction (Höglund, 2009).

In this context we investigated the existence of connectivity between osprey populations at different scale of resolution (from global to local extent) using genotyping techniques such as microsatellites. Starting from a global level, we estimated the degree of genetic divergence between populations over the world and then, at a more precise scale, we evaluated the degree of connectivity between populations in the Western Palearctic, with a special attention to the Mediterranean area. In particular, we postulated that both geographic (e.g. sea, islands) and behavioral (e.g. philopatry, territoriality) factors could act as barriers to gene flow between populations, thus affecting the genetic structure of populations.

5.3. MATERIAL AND METHODS

a. Microsatellites genotyping

A microsatellite library was specifically developed for *Pandion haliaetus* by the biotechnology company Genoscreen, using the method of high-throughput pyrosequencing (GS FLX ®, Roche Diagnostics ®) (Malausa *et al.*, 2011). 411 loci were obtained showing different repeated units: di-nucleotides (272 loci), trinucleotides (112 loci), tetra-nucleotides (19 loci), penta nucleotides (6 loci) and hexa-nucleotides (2 loci). Considering that markers with more complex patterns and multiple repetitions are likely to have a higher polymorphism (Frankham *et al.*, 2002), we selected 40 loci according to this criteria for genetic analysis. A correct amplification (a single band at the expected size) of these loci was firstly checked by PCR using non-labeled primers (cold primers). This step allows keeping 28 out of the 40 loci tested. Subsequent tests performed with fluorochrome-labeled primers (hot primers) confirmed the validity of 27 loci after reading their electrophoretic profile. Amplification reactions contained 5 µl of QIAGEN Mix ®, 1 µl of forward and reverse primers, 1 µl natif DNA and 2 µl H_2O.

b. Sampling and DNA extraction

A total of 200 individuals were collected from many localities heterogeneously distributed over the world (Fig. 1; Tab. 1), allowing to consider three spatial scales: global, Afro-Palearctic and Mediterranean. Samples were grouped in 14 groups according to their geographic provenance (populations were set as different at a minimum of 500 km) and from results yielded by the STRUCTURE software (see below in methods). Each population was coded with a letter (Tab. 1). DNA was extracted from blood (preserved in alcohol in the Queen buffer or dried on filter paper), feathers, muscles or toe-pad fragments using the Qiagen "DNeasy ® kit Blood and tissue "(Ref. 69506, Qiagen Inc.). The extraction protocol of DNA was adapted according to the type of sample. Once extracted, the quality and quantity of DNA samples were checked by electrophoresis using an aliquot of 5 µl DNA of each individual and of a size reference marker (Thermo scientific GeneRuler ™ DNA Ladder, Fermentas), upon an agarose gel containing 1% ethidium bromide (a intercalating fluorescent DNA).

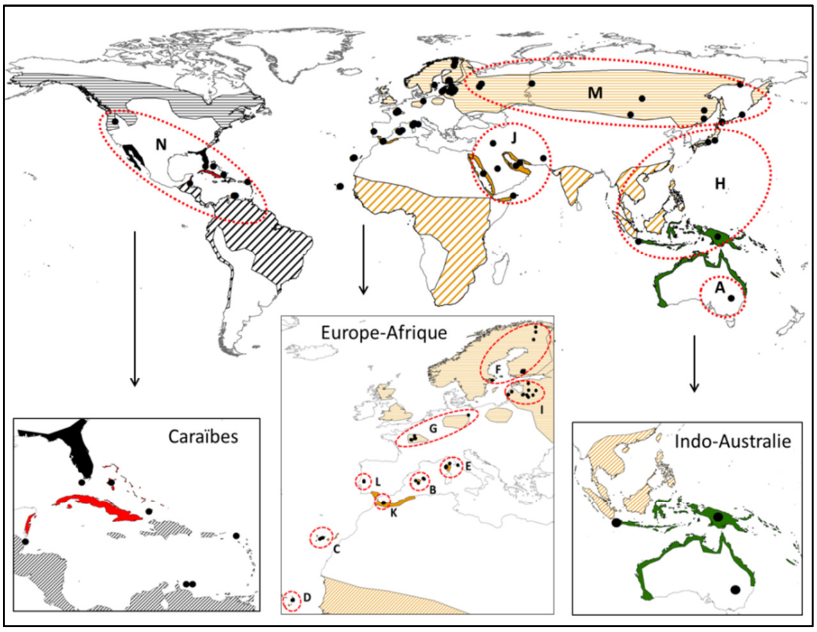

Figure 1: Geographical distribution of *Pandion haliaetus*. Ranges for the four recognized subspecies are in different colors: black for *carolinensis*, red for *ridgwayi*, orange for *haliaetus* and green for *cristatus*. Horizontal stripes are for breeding areas, skew lines for wintering areas and color-filled zones represent areas with sedentary populations. Circles symbolize sample locations. In the small boxes (from left to right) three zones are zoomed in: Caribbean, Mediterranean and Indo-Australasian areas. Red dotted circles include different populations as defined in this study (see methods and Table 1) and represented by a letter code (A: Australia, B: Balearic Islands, C: Canary Islands, D: Cape Verde Islands, E: Corsica and Italia, F: Finland and Sweden, G: continental France and Germany, H: Japan, Indonesia and New-Guinea, I: Estonia and Latvia, J: Middle East and Persian Gulf, K: Morocco, L: Portugal, M: Russia and N: America and Caribbean).

Table 1: Description of the 200 samples of *Pandion haliaetus* used in this study. Code, locality, tissue origins, sample type and number (N) for each population are reported.

Code pop	*Locality (N)*	*Origin*	*Type*
A	Australia (9)	fresh	Blood (alcool)
B	Balearic Islands (23)	fresh	Blood (alcool + filter paper)
C	Canary Islands (10)	fresh	Blood (filter paper) + feathers (dry)
D	Cape Verde Islands (8)	fresh	Blood (filter paper) + feathers (dry)
E	Corsica (29), Italia (3)	fresh	Blood (filter paper) + feathers (dry)
F	Finland (13), Sweden (1)	fresh	Feathers
G	France (17), Germany (1)	ancient/fresh	Feathers (dry + alcool) + eggs shell
H	Japan (5), Indonesia (1), New-Guinea (1)	ancient/fresh	Toe pad + muscle (alcool)
I	Latvia (15), Estonia (8)	fresh	Feathers (dry)
J	Middle East (7), Persian Gulf (3)	ancient/fresh	Toe pad + blood (alcool)
K	Morocco (6)	fresh	Blood (filter paper)
L	Portugal (7)	ancient/fresh	Blood (alcool) + eggs shell
M	Russia (21)	ancient/fresh	Toe pad + blood (filter paper)
N	USA (3), Caribbean (9)	ancient/fresh	Toe pad + blood (alcool)

c. Genetic analyses: population structuring method

Principal Component Analysis (PCA) is a multivariate statistical procedure allowing representing the general organization of the global genetic variability of the sampling considered. PCA places each individual within a hyper-space with X dimensions (X referring to the total number of alleles contained in the totality of loci) and that is projected on a 2 dimension space explained by two axes representing the best dispersion of points. The percentage of inertia of each axis represents the proportion of variance explained by the axis that was calculated. This analysis was conducted by using the software R v.2.15.1 (package ade4) to rank individuals in function to their genetic proximity.

The number of genetic units within *Pandion haliaetus* has been evaluated with the Bayesian method implemented in STRUCTURE v. 2.1 (Pritchard *et al.*, 2000; Falush *et al.*, 2003). This software estimates the number of populations (K), maximizing the likelihood L (K) of the data observed from the likelihood values of the model parameters. Each individual is thus assigned to a population with a certain probability, without *a priori* on its geographical location. The simulations under the admixture model were performed with a Monte Carlo Markov Chain (MCMC) runs of $2x10^6$ iterations with a burn-in period of $3.5x10^5$. The number of cluster (K) tested ranged from 1 to 14 and for each value of K, 10 simulations were performed for each K to test for the stability of the results. All the simulations were convergent and yielded the same results. The determination of the most likely number of genetic group was estimated by the value of maximum likelihood as well as by the Evanno's method which is based on the calculation of the Delta K function (Evanno *et al.*, 2005).

5.4. RESULTS

The PCA (Fig. 2) carried out on the 20 polymorphic microsatellite loci counted 76 variables (total number of alleles expressed in the set of loci). The two axes explained the 18.8% and 10% of the variance, respectively. The PCA distinguished three main distinct genetic groups: Australia (A), America + Caribbean (N) and all the others populations of the Palearctic. Outcomes from the STRUCTURE program gave further information. First analysis was run on the totality of the dataset (200 individuals from all over the world) with a K variable value shifting from 1 to 15. The likelihood curve L (K) and those of delta (K) gathered with Evanno's method returned a maximum value of 2 clusters (Fig. 3A). The graph of genetic assignation with K = 2 (Fig. 3a) showed a marked structuring distinguishing individuals from Americas (together with only one individual from Portugal) and Australia (red cluster) within a group and the remaining others from the rest of the world in another group (green cluster). To have a more detailed view of this structuration, we repeated the analysis for each of the two groups obtained. The analysis on the group containing Australians, Americans and one Portuguese individual (N = 22) was run with a K values shifting from 1 to 8, while the analysis of the second group (rest of the world: N = 178) with a K value from 1 to 13. In both cases, the likelihood curve L (K) and the delta (K) gave a maximum value of K = 2 (Fig. 3B, 3C). First group (Fig. 3b) was hence split in two sub-groups with Americas + Portugal from one side (green cluster) and Australian on another side (red cluster). The second group (Fig. 3c) was equally split into two sub-units: individuals from the Mediterranean basin (*sensu largo*: see red cluster = MEDIT) and individuals coming from Continental Europe, Cape Verde Islands and Arabic peninsula (green cluster = CONT). Hybrid individuals (HYB) between these two groups have been identified in different places (Fig. 4): Finland-Sweden (F), continental France-Germany (G), Estonia-Latvia (I), Portugal (L) and Corsica (E) and Cape Verde Islands (D).

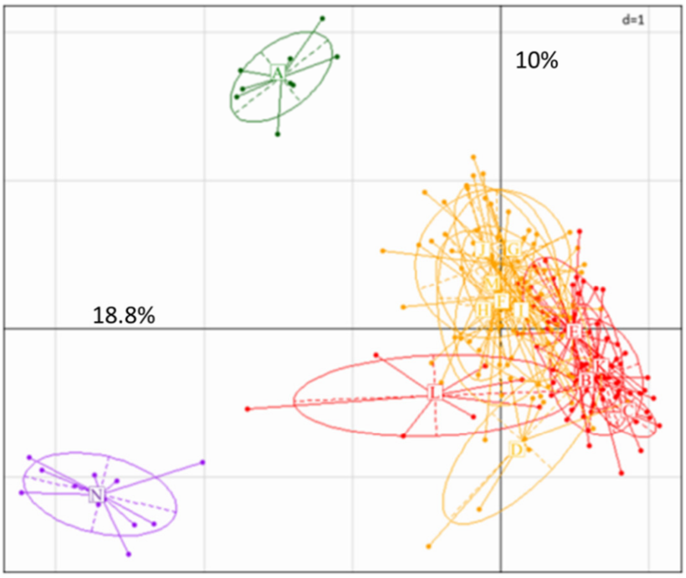

Figure 2: PCA based on the polymorphic character of 20 microsatellites loci (76 alleles) of osprey from 14 populations (N=200). Genetic groups were green for Australia (A), violet for America + Caribbean (N) and red/orange for all the others populations of the Palearctic. Letters identify different populations as defined in Table 1.

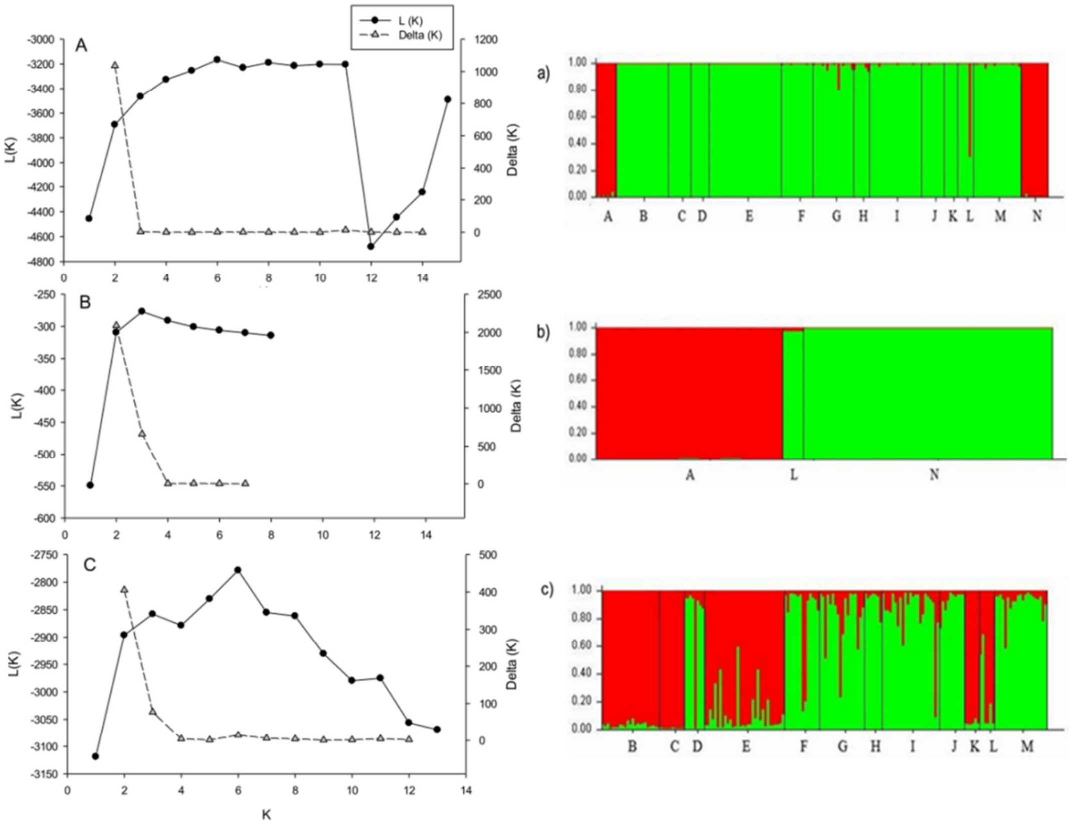

Figure 3: Graphic representation of genetic assignation using STRUCTURE. On the left-hand graph (A, B, C), mean of the natural logarithm of the likelihood L (K) (left y-axis) and of delta K (right y-axis) computed following the Evanno's method (2005) using 10 simulations for each K (x-axis). On the right-hand graphs (a, b, c), the y-axis represent the probability to belong to a certain cluster, while on the x-axes is reported a letter code for each population (see Fig. 1 and Tab. 1). Each colour represents a cluster and each vertical bar a single

individual. Different populations are divided by a black solid vertical line. A and a) analysis carried out on the whole dataset with ospreys from all around the world (N=200; K = 1 to K = 15); B and b) analysis carried out only on the red cluster of the analysis a (N=22; K = 1 to K = 18); C and c) analysis carried out only on the green cluster of analysis a) (N=178; K = 1 to K = 18). For each analysis, the best number of groups determined was K=2.

5.5. DISCUSSION

At the global scale the osprey appeared to be genetically split in three main groups: Australasia, America and Palearctic. Isolation has been probably promoted by the presence of huge geographical barriers, such as oceans, which might have prevented connectivity and gene flow between these groups. These outcomes are only in partial accordance with the taxonomic classification of osprey subspecies based on morphological characters (Cramp & Simmons, 1980). We did not find any difference between North Americans and Caribbean's ospreys (which belong to *P. h. carolinensis* and *P.h. ridgway* subspecies). These results were in accordance with those found in the previous analyses carried out with mtDNA (see chapter 1). Similarly, the Palearctic group (from west to east) matches with the supported clade which includes EUR-AFR and ASIA (see clade 0.87/49 in Fig. 2b of article 1). Two sub-entities interconnected by gene flow were found in this group. The internal splitting was geographically featured with sub-groups from the Japan and north-east Europe (CONT group) from one side and the others included in the Mediterranean area (MEDIT group). In the Palearctic, the existence of two sub-entities (despite mitochondrial DNA analyses identified only one clade) could be due to the different mutation rate beween microsatellites and mtDNA that respond to diverse temporal evolutive scales, to different types of genetic transmission (i.e. mtDNA transmitted exclusively via maternal), and/or related to the different sample size adopted for analyses. On these bases, is difficult to exactly infer the origin and the current distribution of this group.

Moreover, individuals presenting both colours on Fig. 3, show signs of introgression between the two clusters ("hybrid" individuals: HYB), thus suggesting the presence of gene flow between populations of the two groups. These individuals were found especially in central continental Europe (Fig. 4). Factors influencing such genetic diversity and structuration are therefore probably linked to migratory habits and wintering fidelity on the one hand and to the phylopatric behaviour on the other hand. The presence of hybrid individuals would suggest connectivity between groups maintained by a certain number of dispersing individuals which contribute to maintain this flow. In this sense, a more focused analysis is compulsory to quantify the rate of dispersal occurring between populations. Another possible explanation for this genetic structuration at the scale of the Palearctic could be due to the migratory behaviour of different populations. For example, three main flyways are known in the Palearctic for ospreys (Fig. 4): a western flyway passing through the strait of Gibraltar, the Sahara desert until sub-Saharan wintering grounds followed by north-western ospreys (Alerstam *et al.*, 2006; flyway “w” in Fig. 4), a central flyway through Corsica-Sardinia and Italy (Bai & Schmidt, 2011; flyway "c" in Fig. 4) and another eastern one through Middle East and Red Sea (Newton, 2010; flyway "e" in Fig. 4). Accordingly, northern ospreys migrating to wintering sites in tropical Africa (flyway “w”) could have probably colonized Cape Verde Islands (from Senegal), assisted by easterly trade winds, and thus would explain the dominance of CONT-like genotypes in Cape-Verde. On the other hand, the Canary Islands share the same MEDIT genotype with the populations from Balearics, Morocco and Corsica. A similar process could have been happened for populations residing in the Red Sea area (that are predominantly with CONT genotype) across the eastern flyway. Within the Mediterranean, no genetic differentiation was found between the different populations. The absence of structuration at this level might be due to the fact that movements of individuals are probably not much affected by the sea crossing (which seems not to operate as a physical barrier). In this sense, osprey populations living in the Mediterranean still seem to be connected by gene flow. Despite strong philopatric behaviour, ospreys show a certain degree of dispersal which allows populations to maintain genetic variability and admixture. Dispersal in ospreys is known to be sex-biased in favour of females that cover greater distance, contrary to males (Martell *et al.*, 2012; Monti *et al.*, 2014). These antagonistic behaviours seem to play concurrently in shaping genetic structure and diversity at different scales. At the time of writing, analyses for genetic determination of sex have been launched in order to check at any eventual relationship with dispersal and genetic diversity between groups. To better understand how genetic structuring can be influenced by evolutionary behaviours (dispersal and philopatry) which act simultaneously, it is mandatory to investigate migratory strategies and dispersal patterns of individuals from different populations and along different migratory flyways. A better understanding of these behaviours is therefore needed since it could help in reconstructing population dynamics providing essential information for management and conservation of the species, namely in the Mediterranean area. To achieve this goal and to answer these questions, I here introduce the next chapter concerning migration of Palearctic ospreys.

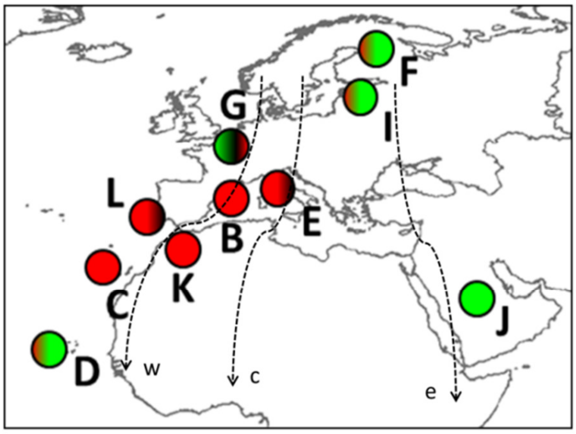

Figure 4: Graphic representation of genetic structuration of osprey populations in the Western Palearctic. Each circle indicates a population, defined by a letter as reported in Tab. 1. Green colour is for the CONT genetic group (continental Europe), red for the MEDIT group (Mediterranean) and black for HYB (hybrids) individuals sharing both of the allelic information. Dotted lines represent main migration flyways in the Western Palearctic: w = western flyway, c = central flyway and e = eastern flyway.

6. SECTION II: REGIONAL SCALE AND HISTORICAL TIME

6.1. BACKGROUND

The spatial pattern of distribution of organisms is generally affected by movements (Begon *et al.*, 2006), that are motivated by a variety of needs: finding food or mates, avoiding predation, seeking nesting sites or shelter from rigorous weather (Festa-Bianchet & Apollonio, 2003).

Recent studies highlighted the importance of understanding the role of the factors affecting behavioural schedules of the individuals: feeding ecology, dispersal and habitat selection represent key features to investigate the important consequences in population dynamics and changes linked to the global climate changes (Goss-Custard & Sutherland, 1997). Animal movements can range from short-distance displacements to long-distance journeys that, for example, can occur during migratory movements. Migration is a widespread behaviour by which an animal periodically moves from one region to another that better satisfies its requirements for a phase of its life cycle (Dingle, 1996; Berthold, 2001).

In the course of the evolutionary history of birds, migratory behaviour evolved repeatedly in a number of independent lineages from sedentary ancestors (Rolland *et al.*, 2014). During past glacial cycles, natural selection favoured those species that, being able to escape changing environmental conditions, avoided extinction by long distance seasonal movements. Simultaneously, movement capabilities of migratory species promoted the colonization of new areas where adapted founders turned sedentary, diverging from their ancestor migratory species (Rolland *et al.*, 2014). The origin and the recurring evolution of seasonal migration highly impacted and shaped the avian distribution and speciation around the globe. As a result nowadays, a huge variation in migratory behaviour is detectable with migratory strategies that vary greatly between families, species, or populations within a species (Pérez-Tris & Tellería, 2002; Netwon, 2007).

Seasonal migratory movements occur as response to seasonal changes, with the non-breeding period generally spent at lower latitudes than the breeding one (a part of existing cases of migration over longitude, such as the intra-Amazonian migration of the Orinoco Goose *Neochen jubata*; Davenport *et al.*, 2012; for other examples see: Newton, 2007). Normally moving towards lower latitudes in autumn, birds leave behind their breeding habitats (highly productive in spring-summer) to avoid food shortage, increase in thermoregulation costs due to decreasing air temperatures and day length shortening typical of winter periods at high latitudes. In this way, they improve their chances to survive in winter and to reproduce again in the subsequent season (Newton, 2010). However, travelling long-distances exposes individuals to high energy demands and the possibility to encounter unfavourable conditions on the way (e.g. poor resource availability at stopovers, harsh weather events, hazards, etc.) that may increase the risk of mortality as well as originate demographic fluctuations at the population-level (Netwon, 2008). As a result, dispersal and migration are some of the factors that much influence the animal survival (Sillett & Holmes, 2002; Klaassen *et al.*, 2014) and consequently can control the dynamics and the demographic trends of any population. To face with these restrictions, birds developed a high capability in modulating their migratory performances in relation to environmental factors and obstacles encountered on the way.

Here, we investigate the movement ecology of the osprey in the Western Palearctic. Although migratory strategies and movement patterns of larger osprey populations in northern Europe and North America were well described (Hake *et al.*, 2001; Kjellén *et al.*, 2001; Alerstam *et al.*, 2006, Dennis, 2008; Martell *et al.*, 2014; Rodríguez-Santana *et al.*, 2014), the dispersal dynamics (Berthold, 2001) and the areas used outside from the breeding season (wintering sites) were not yet investigated in a detailed manner for the Mediterranean basin. Therefore, basic information on spatial ecology of osprey in the Mediterranean basin is still lacking. Two mutually-dependent specific studies are presented: a first one concerning osprey migratory strategies and the way they cope with crossing sea barriers, and a second provisional manuscript about wintering ecology and habitat selection. These studies are fundamental not only to ascertain proximate causes of movement ecology, but also to plan sound conservation measures.

The second study was part of the subject of Aloïs Robert which I co-supervised together with Oliver Duriez and Ilham Bentaleb, for his master I in “Environnement et Gestion de la Biodiversité - (EGB)” at the University of Montpellier 2.

7. SCALE-DEPENDENT MIGRATION: CONTRASTING SPATIO-TEMPORAL EFFECTS ACROSS POPULATIONS AND AGE-CLASSES IN OSPREYS.

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7.1. ABSTRACT

Through scale-dependent analyses of migration strategies, we studied migratory plasticity between age-classes and populations of a large migratory raptor, the Osprey *Pandion haliaetus*, in the Western Palearctic. Fifty-four ospreys from Scandinavian and Mediterranean populations were GPS-tracked across 70 migratory trips to investigate variation in migratory traits across a latitudinal divide, among individuals of different age classes and in relation to a broad set of ecological factors (e.g. geographical barriers, wind conditions, etc.). Scandinavian ospreys performed homogeneous long-distance migrations (6000 km range), crossing the Strait of Gibraltar before reaching sub-Saharan wintering grounds in West Africa. In contrast, Mediterranean ospreys showed a heterogeneous migratory behaviour typical of partially migrating populations, with individuals remaining resident in 46% of cases, 39% performing long-distance migration, and 15% travelling short-distances. Mediterranean migratory ospreys also performed long non-stop flights over the open sea, which were not observed in Scandinavian birds. Higher levels of variability in the choice of migratory routes, timing and wintering grounds, revealed higher plasticity in migratory behaviour in the Mediterranean region, potentially due to more favourable ecological conditions on a year-round basis. Across populations, adult birds travelled longer distances per day and displayed less sinuous migratory paths than juveniles, suggesting that migratory capabilities improve over time. Juveniles also had lower abilities to use favourable winds, and to cope with sea-crossings. Overall, our study demonstrates that detailed knowledge of migratory patterns at multiple spatio-temporal scales is of fundamental importance for the design of conservation and management plans of vulnerable migratory bird populations.

Keywords: migratory strategy, raptor, barrier crossing, wind assistance, leapfrog migration.

7.2. INTRODUCTION

A migration strategy is the product of a set of rules that determine the overall process of migration (Alerstam *et al.*, 2006) and that results from the interaction of multiple factors simultaneously operating at varying spatial and temporal scales. Major migratory traits, such as timing (i.e. departures and arrivals) and routes (i.e. direction of migration axis, distances covered and destination), are thought to be mostly controlled by an inherited genetic component and driven primarily by an endogenous clock-and-compass system (Berthold, 1996; Thorup & Rabøl, 2001). Conversely, minor traits such as daily speeds, flight performances and straightness of the tracks rather seem to be predominantly shaped by the environment (weather conditions and habitat matrix) and the experience of each bird.

Specifically, birds seem to be able to adjust the spatial and temporal schedules of their migratory journeys (e.g. daily distance covered, time spent at stopovers) to avoid detours triggered by adverse weather, to minimize energy consumption, and thus to enhance their survival chances (Sergio *et al.*, 2014; Vansteelant *et al.*, 2014; but see: Lok *et al.*, 2013). Such seasonal modulation of migratory parameters has been recorded in relation to windscapes (Sinelschikova *et al.*, 2007; Klaassen *et al.*, 2011; Yamaguchi *et al.*, 2011), food peak resources availability (van der Graaf *et al.*, 2006; Duriez *et al.*, 2009; Tøttrup *et al.*, 2012a) and exceptional storm events (Tøttrup *et al.*, 2012b; Vansteelant *et al.*, 2014). Such ability to regulate migratory parameters plays in favour of intraspecific variation in migratory behaviour, showing that multiple external and internal factors concur to shape migration traits (Alerstam, 1990; Berthold, 2001).

In this context, it is essential to assess migratory plasticity across age-classes and populations of a same species. Until now, most studies aimed at clarifying migratory behaviour at the population-level, and very few performed scale-dependent analyses of migration strategies (e.g. Mandel *et al.*, 2011; Singh *et al.*, 2012).

Here we explored the migration strategies of a large migratory raptor, the osprey *Pandion haliaetus haliaetus*, in the Western Palearctic. Because of the high plasticity in habitat requirements typical of a cosmopolitan species (Cramps & Simmons, 1980), this species provides a good opportunity to explore behavioural adaptation to migration in populations that evolved under different ecological conditions. In the Western Palearctic, osprey populations from northern and central Europe commonly breed on the tree-top of forested habitats, being strictly associated to freshwater lakes or rivers for feeding (Poole, 1989; Wahl & Barbraud, 2014). Previous studies in these populations revealed long-distance migratory journeys conducted towards sub-Saharan wintering grounds (Hake *et al.*, 2001; Alerstam *et al.*, 2006; Klaassen *et al.*, 2008; Bai & Schmidt, 2011), using a combined strategy made up by fly-and-forage bouts and stop-over (Strandberg & Alerstam, 2007). In contrast, osprey populations living in southern Europe, like around the Mediterranean basin, breed mostly on rocky pinnacles within a fragmented coastal habitat, being tightly linked to marine environments for fishing (Cramp & Simmons, 1980; Monti, 2012). Classically, information on the spatial ecology of osprey populations at this latitude only consisted in sporadic ring recoveries and anecdotal information (Thibault & Patrimonio, 1992; Thibault *et al.*, 1996; Thibault *et al.*, 2001), limiting the understanding of their migratory ecology. In order to investigate migration strategies along a Western-European latitudinal divide we investigated the behaviour of osprey populations from Sweden (representative of northern and central European populations), and from the Western Mediterranean.

We hypothesized that a wide gradient of environmental and habitat variables might have accounted for the evolution of different migratory strategies across populations. First, we predicted that more favourable ecological conditions, present all year round in the Mediterranean, could have favoured the presence of resident or nomadic individuals at this latitude. Further, we postulated that southern individuals living on marine islands might be constrained in their migratory behaviour by the absence of suitable feeding habitat on the move. Indeed, osprey cannot feed offshore because they need to perch to bite-feed on their prey.

According to this, we tested the following hypotheses: a) northern birds, that are supposed to carry out longer distance migrations in comparison to Mediterranean birds, should leave earlier both in autumn and spring, to arrive on time for wintering and reproduction, respectively; b) northern birds should also cover greater daily and maximum distances, to compensate for long refuelling at stopover sites in Europe (Klaassen *et al.*, 2011); c) a latitudinal divide should occur with respect to the preferred direction of movements (abbreviated PDM; Kemp *et al.*, 2012a), on the assumption that different climatic conditions exerted constrating selection pressure across populations; (d) furthermore, we expected passages at favourable points and/or highly detoured trips along Mediterranean coasts to minimize the crossing of large water-tracts (as observed in other large soaring birds; Kerlinger, 1989; Strandberg *et al.*, 2008; Chevallier *et al.*, 2010). During such sea-crossings, because thermal soaring is unlikely, we expected birds to perform mostly flapping flight, and thus to record more constant and higher airspeeds and a less sinuous track than on land.

In addition, at the population-level, we compared migratory strategies between age-classes, starting from the assumption that juveniles are naive travellers and this can lead to differences in the straightness of routes travelled (Péron & Grémillet, 2013; Cresswell, 2014). We therefore expected more sinuous routes and longer migration bouts for younger birds.

Finally, as wind is known to be the most important factor affecting flight performance (Kemp *et al.*, 2012b; Vansteelant *et al.*, 2014), we investigated how weather affected individuals (Shamoun-Baranes *et al.*, 2003; 2011; Nilsson *et al.*, 2014; Vansteelant *et al.*, 2014) especially according to their life stage (Shillinger *et al.*, 2012; Péron & Grémillet, 2013), taking into account the role of the wind assistance during migration. We thereby hypothesized that experienced adults would perform better at selecting favourable winds and thermal currents, and therefore would fly at greater groundspeeds.

Through this cross-scale study design, we aim to provide essential evidences that clarify the spatial ecology and migratory strategy in response to a broad set of ecological components, and identify drivers of migratory movements in an evolutionary context.

7.3. MATERIAL AND METHODS

a. Tracking technologies

Overall, 54 adult and juvenile ospreys from the Western Palearctic were tagged during our study. Birds come from Scandinavian (18 birds from Sweden) and Mediterranean (15 from Corsica, 14 from Balearic Islands and 7 from mainland Italy) populations. Details are provided in Appendix 2. All tracking devices were attached as backpacks with a harness made of 7-mm-wide Teflon ribbon (Kenward *et al.*, 2001). Details on trapping and tagging methods are available in Klaassen *et al.* (2008 and 2011). The mass of the equipement never exceeded 3% of bird body masses. All birds were color ringed, measured, and sexed (based on the size and on plumage and/or using molecular sexing, following Griffiths *et al.*, 1998). Bird handling lasted 30-50 minutes.

Adult and juvenile Swedish birds were fitted with 45-g Solar Argos/GPS PTT-100s (Microwave Telemetry Inc., Columbia, USA) at their breeding site. Transmitters contained a GPS receiver that logged the position at 1-h intervals, operating between 0400 and 1800 GMT. These loggers provided data on latitude, longitude, altitude, instantaneous groundspeed and heading. For our study, we re-analysed adult osprey tracks previously published by Klaassen *et al.* (2008), and integrated new tracks of both adult and juvenile individuals tagged during the 2006-2011 period. The complete Swedish dataset hence included 39 tracks (8 incomplete) from 7 adults (3 males, 3 females and one undetermined) and 10 juveniles (undetermined sex).

In the Mediterranean, 13 adult ospreys were caught between 2009 and 2013 in three different areas. Five adults (3 males, one female and one undetermined) were trapped using a perch-trap in wetlands on the NE of Mallorca Island (Balearics). Birds were fitted with 30-g Solar Argos/GPS PTT-100s (Microwave Telemetry Inc., Columbia, USA) that were programmed to on a cycle of 12 h ON and 12h OFF in autum-winter and 16 h ON and 8 h OFF in spring-summer, whereby positions were sent at hourly intervals when the device was ON. Yielded locations were firstly filtered: for analyses we only used those with a good measure of GPS accuracy according to ARGOS-class (LC; ARGOS, 2011).

Other birds were equipped with a 24-g solar powered GPS/GSM device (model Duck-4, Ecotone Company, PL). These loggers were programmed to collect GPS fixes at hourly intervals but only contained data of latitude and longitude (not altitude and speed). Further, 7 adults (5 females and 2 males) were caught in Corsica, France, before the onset of the breeding season in March-April 2013, using a noose carpet laid on the nest. One additional adult was trapped by the same method in Italy (southern Tuscany) at the end of the reproduction. Finally, 23 juvenile ospreys (9 from Balearics islands, 8 from Corsica and 6 from Italy) were fitted with GPS/GSM during ringing actions before or shortly after fledging at their respective nesting sites in June-July 2013 and 2014.

b. Tracking data processing

In order to compare migratory parameters and flight performance across habitat types, migratory tracks were filtered in ArcGis 9.3, distinguishing segments travelled over the sea to those over the land. We computed analyses only on fixes equally spaced at 1 h intervals to avoid misinterpretation in migration estimates (Tanferna *et al.*, 2012). On land, we first eliminated movements close to stopover sites and selected those corresponding to effective travel movements. We defined a migratory movement only when locations were spaced by a minimum of 10 km (Sergio *et al.*, 2014), to avoid the inclusion of local movements between nocturnal roosts, and to exclude possible prospections for feeding places along the way.

To compare flight performance of individuals across the same habitat matrix, we selected a geographic area between N25° and N45° of latitude and W25° and E20° of longitude, including the Mediterranean area from southern France to northern Sahara in Morocco. Consequently, average groundspeeds (the flying speeds in relation to the ground) for adults and juveniles of both populations were computed for tracts over the land and at sea.

We estimated wind-assistance by interpolating tracks with the package 'RNCEP' (Kemp *et al.*, 2012a) using weather data from the NCEP/NCAR Reanalysis project (Kalnay *et al.*, 1996) and the NCEP/DOE Reanalysis II dataset (Kanamitsu *et al.*, 2002; http://www.cdc.noaa.gov). For each point of the track we downloaded the -u (West-East) and -v (South-North) wind components, which were combined in a single wind vector incorporating the strength and the direction of the wind, from which we obtained a tailwind component (Kemp *et al.*, 2012b). For tracts over land, wind data were extracted for a pressure level of 925 hPa, which corresponds to an altitude of ca. 750 m a.s.l., i.e. the altitude at which ospreys have usually been measured to migrate (Klaassen *et al.*, 2011). For locations over the sea a pressure level of 1000 hPa (corresponding to 110 m a.s.l.) was set, corresponding to mean flight altitudes that were recorded using Argos/GPS PTT-100s). Airspeed was calculated by subtraction of the wind vector from the track vector (track direction, ground speed) of the bird (following Kemp *et al.*, 2012b and Nilsson *et al.*, 2014).

Since tagged birds showed different migration strategies and complex movement patterns, we first categorized these movements. We distinguished individuals as follows: a) residents (RES): individuals that remained within 200 km from the nest during the whole year, b) short-distance migrators (SDM): individuals that moved 200-500 km away from the nest, and c) long distance migrator (LDM): individuals that migrated >500 km away from the breeding site (Fig. 1).

For short and long distance migrators, the onset of autumn and spring migration was defined as the last day in which the bird was present at the breeding site and wintering ground, respectively. On the other hand, the end of migration was defined as the arrival day of a bird at the wintering ground (for autumn migration) or at the nesting site (for spring migration). A stopover site was defined as an area where a bird spent more than 24 h during the migration period (Strandberg *et al.*, 2008; Limiñana *et al.*, 2012). Pre- and postmigratory round trips were defined as movements carried out before and after migratory journeys, towards a secondary feeding site (see below for details). In the case of SDM individuals, we distinguished short-distance migratory journeys from repeated pre- and postmigratory round trips during which birds did not return to the previous secondary feeding site (used also in winter), but rather started to breed (e.g. Strandberg *et al.*, 2009a). For resident birds only, seasons were arbitrarily distinguished as: interbreeding season (from October to February) and breeding season (from March to September).

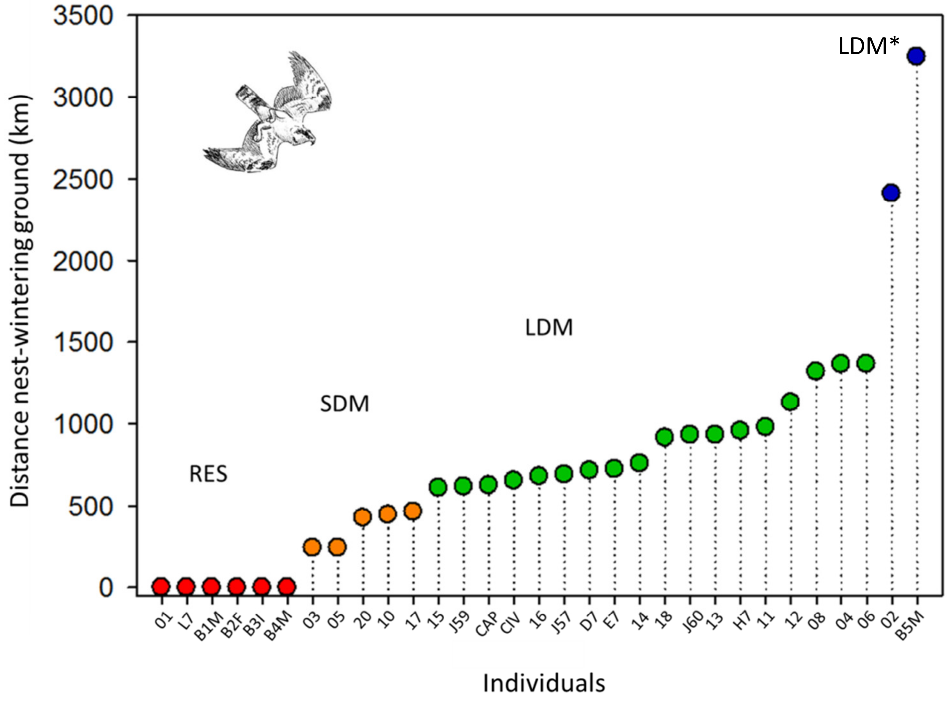

Figure 1: Migratory categories for Mediterranean individuals: RES (in red) = resident birds (maximum movement range of 200 km from the nest); SDM (in orange) = birds performing short distance movements betwwen 200 and 500 km; LDM (in green) = long distance migratory birds who moved more than 500 km during migration; Two long distance migratory ospreys who moved for > 2000 km (but < 5000 km) were reported as LDM* (in blue). All Swedish birds were long distance migrants (> 5000 km) and are not reported in the graph.

c. Movement Data Analyses

For migratory periods we calculated: a) migration duration (days) ; b) the total distance travelled (km), as the sum of total daily distances during travel days, excluding movements at stopover sites and both pre- and postmigratory movements (following Strandberg *et al.*, 2008); c) the direct distance (km) between nest and wintering site; d) the average and the maximum distances (km) covered per day and e) migration path straightness calculated as the ratio of the total distance covered to the straight distance between the nest and the wintering site; f) stopover duration (days); g) preferred direction of movements (PDM) expressed as degrees from the North, calculated according to the rhumb line (or loxodrome).

d. Statistical analyses

For autumn migration we used Generalized Linear Mixed Models (GLMMs). We treated as response variables: a) distances covered, b) duration of the migratory journeys, c) departure and arrival dates, d) stopover duration, e) daily distances and f) straightness index, while ‘individual’ and ‘year’ were included as random factors. Differences were tested considering ‘population’, ‘season’, ‘age’, and ‘sex’ as fixed factors in the models. To compare model fit for each response variable we adopted the Aikake’s Information Criterion (AIC).

For spring migration we only had 16 tracks of 10 birds and therefore used parametric and non-parametric tests. To avoid misinterpretations related to repeated journeys, we arbitrarily selected the first migratory track per individual. Results are reported as mean ± standard deviations.

To compare flight performances, differences in groundspeeds, tailwinds and airspeeds were tested with GLMMs, with ‘individual’ and ‘year’ included as random factors, and ‘population’, ‘season’, ‘age’, and ‘habitat’ as fixed factors. All statistical analyses were conducted in R 2.15.0 (R Core Development Team).

7.4. RESULTS

The entire dataset includes 70 migratory tracks (88.6% of which were complete): 54 in autumn and 16 in spring, carried out by 38 individuals (Annex 2: Additional file 1). Six adults out of the remaining 16 tagged ospreys did not migrate, while other 10 juveniles disappeared/died during the post-fledging dependence period or in the first phases of migration (Annex 2: Additional file 2). Tracks from more than one migratory journey were available for 12 individuals, only adults. Ten birds which survived one migration cycle and were hence tracked for more than one year, showed a high fidelity by returning to the same breeding and wintering ground during successive years. For Mediterranean birds, 14 juveniles out of 20 died/disappeared in the first year (70% loss, whereby 40% disappeared and 30% perished mainly due to both direct - e.g. illegal shooting- or indirect - e.g. electrocution, wind turbines collision, net-trapping in fish farms - human threats). In nine cases transmission stopped due to devices’ malfunctioning and/or data transmitting failures, resulting in migratory data being partially available. According to the available parameters these incomplete tracks were only partially included in the analyses (e.g. we used time data of departure or flight performance (Annex 2: Additional file 1)).

a. Migratory patterns: effects across populations, sexes and age-classes

Swedish and Mediterranean ospreys showed a clear different migratory behaviour. Adults of the former group showed a homogeneous migration pattern, performing long-distance journeys across Europe, towards sub-Saharan wintering grounds mainly located in West Africa between 7°N and 16°N latitude (latitude width = 9°) and 16°W and 0° longitude (longitude width = 16°; Fig. 2a). They followed a narrow migratory corridor, crossing the Strait of Gibraltar. In clear contrast, Mediterranean birds showed a heterogeneous migratory pattern. Six adults (46%) never migrated, occupying breeding areas all year round. This behaviour was observed in adults of all three populations. Further, two adults (15.5%) migrated only for short-distances (less than 500 km; SDM category), and the remaining five (38.5%) travelled greater distances (> 500 km; LDM category; Fig. 1). Mediterranean adult ospreys did not congregate to the same area over winter, and rather spread between 28°N and 42°N latitude (latitude width = 14°) and 12°W and 8°E longitude (longitude width = 20°) within the Mediterranean basin (e.g. Spain, Morocco, Algeria, Sardinia), usually avoiding crossing the Sahara (except for 2 individuals that went to southern Morocco and Mauritania) (Fig. 2c).

In autumn, migration distances were about five times greater for the Swedish than for the Mediterranean ospreys (mean distances from nest: 5252.9 ± 773.9 km and 938.7 ± 656.9 km, respectively; mean distances effectively covered: 6231.8 ± 1007.7 km and 1296.5 ± 740.9 km, respectively) (Tab. 1: model a.; Fig. 3a). Males migrated shorter distances than females (Tab. 1: model a.). This difference was more prominent for Swedish birds (females: 6935.2 ± 879.8 km; males: 5868.2 ± 154.9 km) than for Mediterranean (females: 1554.1 ± 584.8 km; males: 1347.8 ± 1885.9 km). Swedish ospreys travelled for more days than Mediterranean ones (Tab. 1: model b.; Fig. 3b), and spent more time at stopover sites (Tab. 1: model c.; Fig. 3c). Mediterranean ospreys travelled only for 5.1 ± 2.5 days and stopped occasionally (mean: 0.33 ± 0.8 days) during the journey. Despite these differences, timing of departures was similar between populations in autumn (mean dates of departures: 73.5 ± 22.2 days after the 01/06; range SWE: 11/07-14/09; range MED: 24/06-09/11). Arrivals were significantly different between populations (Tab. 1: model e.) with Mediterranean birds arriving at wintering sites about 50 days before the Swedish ones (arrivals after the 01/06: SWE = 132.9 ± 22.2 days; MED = 81.6 ± 28.5) (Fig. 3d).

Average daily distances covered did not differ between populations (207.2 ± 69.3 km/day). Along the migratory routes, the straightness value varied slightly between populations (SWE = 0.85 ± 0.1; MED = 0.73 ± 0.17) and sexes (males = 0.83 ± 0.3; females =

0.67 ± 0.3), but this was not significant (Tab. 1: model g.). Finally, despite a shared mean value around 200° from the North (i.e south-south-west), the preferred direction of movements (PDM) was widely spanned for Mediterranean individuals (mean: 199.9 ± 42.6°, ranging from 94.7° to 250.78°) compared to Swedish birds, which showed restricted values and low variance (mean: 207.11± 7.01°, ranging from 192.8° to 219.44°; Fig. 3h). Magnitude of variances in PDM was significatively different between populations (F_{24}=36.89; $p < 0.001$).

Swedish juveniles showed a broader migration front compared to that of adults: three individuals travelled along the same routes of adults (via Iberia and Gibraltar), whilst four others travelled towards south through the Mediterranean (e.g. passing through Italy and Sicily) before reaching Africa (Fig. 2b). While adult returned to their breeding areas in spring in Sweden, juveniles (< 2 years) mostly remained in Africa until their sexual maturity. Mediterranean juveniles migrated different distances (mean: 721.74 ± 202.7 km; combining birds of SDM and LDM categories) and towards different directions (e.g. Spain, Morocco, Algeria, Italy; Fig. 2d). No differences were found between adults and juveniles in migration distance (Tab. 1: models a1.-a2.), duration of migration and stopover (Tab. 1: models b1.-b2.-c1.-c2.) and timing of departures (Tab. 1: model d.). However, adults travelled 33% faster than juveniles (Tab. 1: model f1.): this difference in age was prominent especially for Mediterranean birds, with adults travelling for 260.4 ± 107.9 km/day vs only 176.4 ± 48.7 km/day for juveniles (Fig. 3e). Further, adults travelled straighter than juveniles (0.87 ± 0.1 vs 0.69 ± 0.1) (Fig. 3f; Tab. 1: model g1.). PDM varied between age classes ($F_{23} = 0.205$; $p = 0.00039$), especially in the Mediterranean population, where the variance for juveniles (± 48.2) was twice than that for adults (± 26.9).

In spring, comparisons were possible only for adults. Significative differences between populations were found for migration distance ($t_8 = 5.46$, $p = 0.01$), duration ($t_8 = 5.82$, $p < 0.001$) and time spent at stopover sites (Mann-Whitney *U*-Test: U = 0.0; p = 0.04). Interestingly, departures ($t_8 = 2.356$, $p = 0.046$) and arrivals ($t_8 = 4.623$, $p = 0.002$) were also different (Fig. 3g). Swedish adult ospreys left wintering grounds one month after the Mediterranean ones (SWE: 49.3 ± 19.8 days after 01/02, range: 04/03-10/05; MED: 20.3 ± 16.3 days after 01/02, range: 06/02-25/03). Mediterranean birds arrived about 50 days before at breeding sites than Swedish ones (SWE: 76.7 ± 17.9; MED: 26 ± 19.01 days after 01/02). No significant differences between populations were found in the daily distances travelled ($t_8 = -1.20$, $p = 0.264$; mean = 258 ± 55 km/day) and for straightness values ($t_8 = 0.36$, $p = 0.722$).

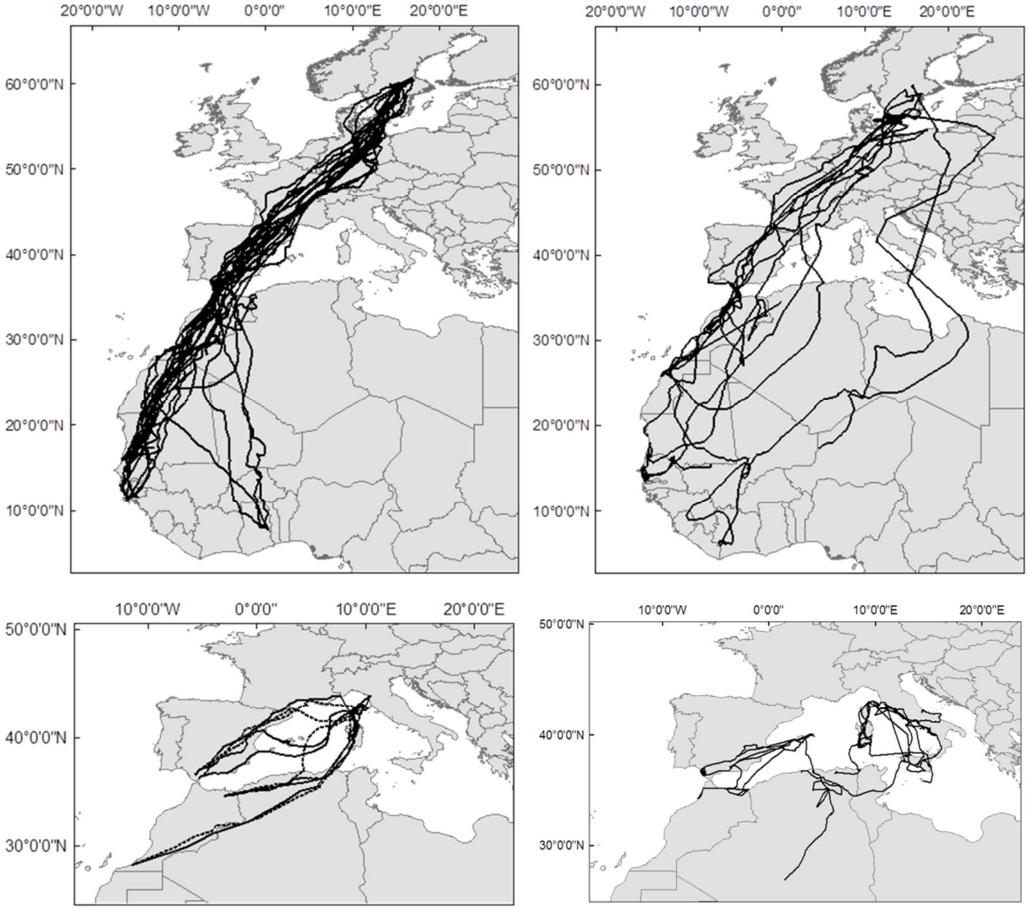

Figure 2: Migratory journeys of Western Palearctic ospreys. 13 individuals from Sweden (5 adults, top left, 8 juveniles, top right panel) wintering in sub-Saharan Africa. Four Corsican adults migrated (LDM) crossing the Mediterranean Sea and reaching wintering grounds located in southern Spain (2) and Morocco (2) (down left panel: solid and dotted lines for fall and spring migration, respectively). Exceptionally, a male from Balearics #B5M migrated to Mauritania in 2009, but was resident next years (not showed). Short-distance migration journeys (SDM) were recorded for two Corsican birds which moved to Sardinia to spend the winter. Such movements spaced less than 300 km from the nest and required only one or two days of flight. 15 Mediterranean juveniles migrated in different locations (down right panel).

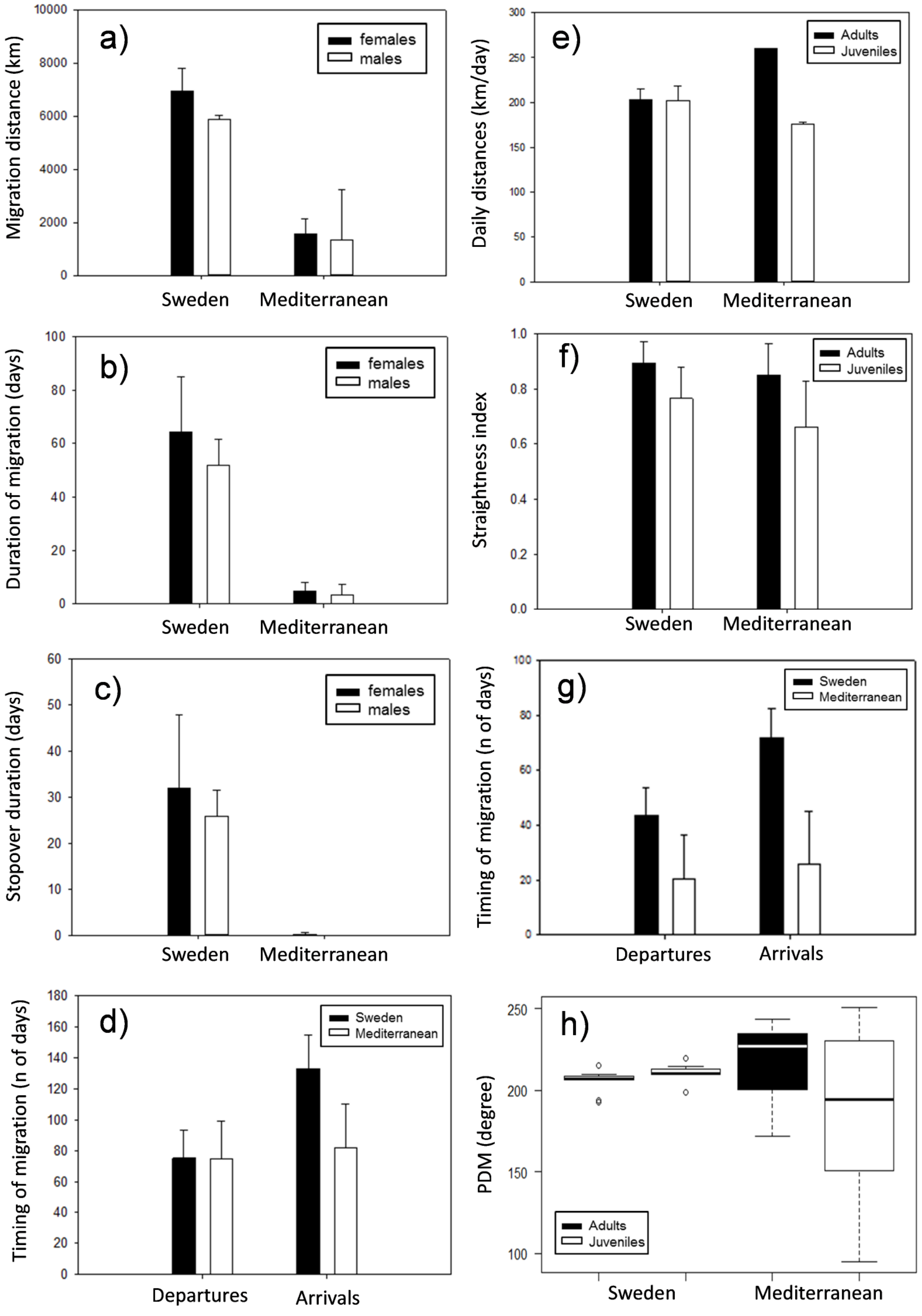

Figure 3: Migration parameters between populations, sexes and ages in autumn: a) migration distance, b) duration of migration, c) stopover duration, d) departures and arrivals, e) daily distances and f) straightness index. In spring: (g) departures and arrivals. Preferred direction of movements (PDM) between populations and ages (h) is also showed.

Table 1: Effects of population, sex and age on autumn migratory components of Swedish and Mediterranean migratory ospreys.

Dependent Variable (n)	Selected Model	Population	Sex	Age	Variable	Parameter estimates	AICC
a. Migration distance (23)	Population + Sex	*	*	NA	Intercept	1696.4 ± 355.2	387.73
					Population_Sweden	6555.2 ± 417.8	
					Sex_M	-344.7 ± 514.0	
a1. Migration distance for MED (26)	no effect	NA	NA		Intercept	1696.4 ± 387.9	415.31
a2. Migration distance for SWE (21)	no effect	NA	NA		Intercept	6216.2 ± 280.6	356.32
b. Duration (23)	Population + Sex	*	*	NA	Intercept	6.8 ± 4.1	196.21
					Population_Sweden	55.3 ± 5.2	
					Sex_M	- 7.9 ± 5.4	
b1. Duration for MED (26)	no effect	NA	NA		Intercept	5.4 ± 0.6	126.17
b2. Duration for SWE (21)	no effect	NA	NA		Intercept	60.4 ± 4.1	356.32
c. Stopover Duration (23)	Population + Sex	*	*		Intercept	1.2 ± 2.9	181.75
					Population_Sweden	29.6 ± 3.6	
					Sex_M	- 3.8 ± 3.7	
c1. Stopover Duration for MED (24)	no effect	NA	NA		Intercept	0.4 ± 0.2	69.6
c2. Stopover Duration for SWE (21)	no effect	NA	NA		Intercept	31.5 ± 3.2	177.8
d. Departures (24)	Sex		*		Intercept	80.5 ± 12.9	229.5
					Sex_M	10.7 ± 19.8	
e. Arrivals (48)	Population	*			Intercept	82.5 ± 5.0	457.64
					Population_Sweden	52.4 ± 8.2	
f. Daily distances (23)	Sex	*	*	NA	Intercept	249.5 ± 29.2	276.44
					Sex_M	-11.8 ± 50.3	
f1. Daily distance for age classes (45)	Age	*	NA	*	Intercept	234.3 ± 16.4	515.00
					Age_juvenile	- 46.8 ± 21.6	
g. Straightness (47)	no effect	*	*	NA	Intercept	0.7 ± 0.0	-46.38
g1. Straightness for age classes (47)	Age	*	NA	*	Intercept	0.8 ± 0.0	-54.62
					Age_juvenile	- 0.15 ± 0.04	

b. Flight performances across habitat types: effects in populations and age classes

Within the considered region (between N25°-45° of latitude and W25°-E20° of longitude), the average groundspeed of migrating ospreys was similar between populations and seasons (mean: 31.6 ± 11.8 km.h^{-1}), but significatively differed in relation to the habitat. Groundspeed was in average 10 km.h^{-1} greater during sea-crossing tracts compared to land (Fig. 4a). However, GLMM returned additive factors for each of the three variables (Tab. 2: model a.). In general, both populations experienced tailwinds that were of analogous speed and directions in both habitats, but wind assistance was lower during spring (Fig. 4b). Mediterranean birds benefitted from highly favourable tailwinds in autumn (Tab. 2: model b.), but often travelled with headwinds in spring (especially on land; Fig. 4b). Airspeeds were generally greater for sea-crossing tracts in both populations and similar in seasons, with except for Mediterranean birds in spring that flew faster despite the presence of headwinds (Fig. 4c; Tab. 2: model c.).

Comparative data for looking at age classes were available only for autumn migration journeys. No differences in groundspeeds were detected between Mediterranean adults and Swedish adults and juveniles, but smaller values were associated to Mediterranean juveniles (Fig. 4d; Tab. 2: model d.). Mediterranean juveniles experienced worse tailwinds, in contrast to Swedish juveniles who benefitted from better wind assistance (Fig. 4e; Tab. 2: model e.), and had slower airspeeds at sea (only 4 km/h difference; Fig. 4f; Tab. 2: model f.).

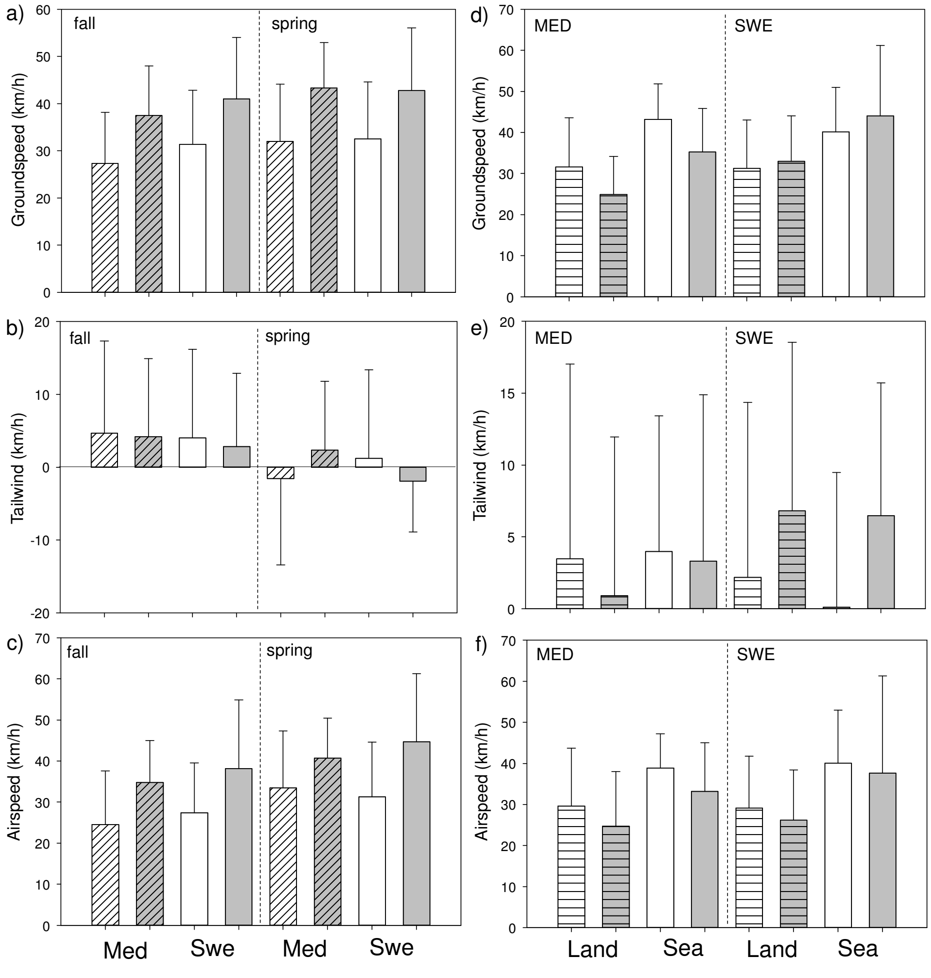

Figure 4: Flight parameters (groundspeed, tailwind and airspeed) between seasons, populations and habitats (panels a, b, c; white and grey bars represent respectively tracts over land and at sea; Mediterranean population is indicated by skewed lines on bars; bars with no lines are for Swedish population); and between populations, habitats and ages (panels d, e, f; white and grey bars represent adults and juveniles respectively, tracts over land are indicated by horizontal lines on bars).

Table 2: Results of model selection of GLMM on the effects of population, season, habitat and age on flight performances (groundspeed, tailwind and airspeed, all expressed in km.h^{-1}) of migrating ospreys crossing the Mediterranean region.

	Dependent Variable	Selected Model	Population	Season	Habitat	Age	Variable	Parameter estimates	AIC$_C$
a.	Ground speed (2473)	Population+Habitat+Season	*	*	*	NA	Intercept	27.8 ± 1.4	19017.94
							Population_Sweden	3.9 ± 1.9	
							Habitat_Sea	10.4 ± 0.8	
							Season_Spring	1.8 ± 0.6	
b.	Tailwind (2209)	Population*Season	*	*	*	NA	Intercept	12.1 ± 3.1	17139.51
							Population_Sweden	- 8.0 ± 3.2	
							Season_Spring	- 21.0 ± 2.3	
							Population_Sweden:Season_Spring	19.6 ± 2.4	
c.	Air speed (2209)	Population*Season+Habitat	*	*	*	NA	Intercept	21.8 ± 2.2	17430.06
							Population_Sweden	5.7 ± 2.5	
							Habitat_Sea	10.0 ± 1.0	
							Season_Spring	13.9 ± 2.2	
							Population_Sweden:Season_Spring	- 10.4 ± 2.4	
d.	Ground speed in autumn(1849)	Population*Age+Habitat	*	NA	*	*	Intercept	32.8 ± 2.2	14083.01
							Population_Sweden	- 2.7 ± 2.6	
							Age_juvenile	- 7.4 ± 1.8	
							Habitat_Sea	10.4 ± 0.9	
							Population_Sweden:Age_juvenile	9.7 ± 2.6	
e.	Tailwind in autumn (1585)	Population*Age	*	NA	*	*	Intercept	9.4 ± 3.8	12157.92
							Population_Sweden	- 10.0 ± 4.0	
							Age_juvenile	- 7.0 ± 2.7	
							Population_Sweden:Age_juvenile	12.5 ± 3.7	
f.	Airspeed in autumn (1585)	Population+Habitat+Age	*	NA	*	*	Intercept	24.7 ± 2.7	12381.87
							Population_Sweden	5.3 ± 2.8	
							Habitat_Sea	10.0 ± 1.1	
							Age_juvenile	- 2.4 ± 1.5	

c. Pre- and post-migratory movements

Before the autumn migration 90% of Mediterranean adults (both SDM and LDM individuals) performed pre-migratory trips. These movements were aimed to reach a distant feeding site (mean: 103.02 ± 65.41 km) where birds spent a variable number of days (range: 5-60 days; mean = 23.2 ± 22.6) before returning to the nesting site (pre-migratory round trips; Fig. 5); they differed in both distance and duration from the foraging trips performed during the breeding season. In the same way, we observed repeated post-migratory round trips performed by 3 MED adults after the spring migration and before the onset of the breeding season, to the same secondary feeding site. The duration of these staying ranged between one and two weeks. Only for a SDM male, two post-migratory round trips were recorded during the winter season: it came back to visit the breeding site in Corsica from Sardinia (239 km). On the contrary, we did not observe round trips through distant secondary feeding sites in winter, despite one exceptional exploration. In 3 cases, autumn migration started from the secondary feeding site. One Corsican bird moved first to a northern secondary feeding site in Tuscany (Massaciuccoli Lake; where it remained between May and July) then travelled south to Sardinia.

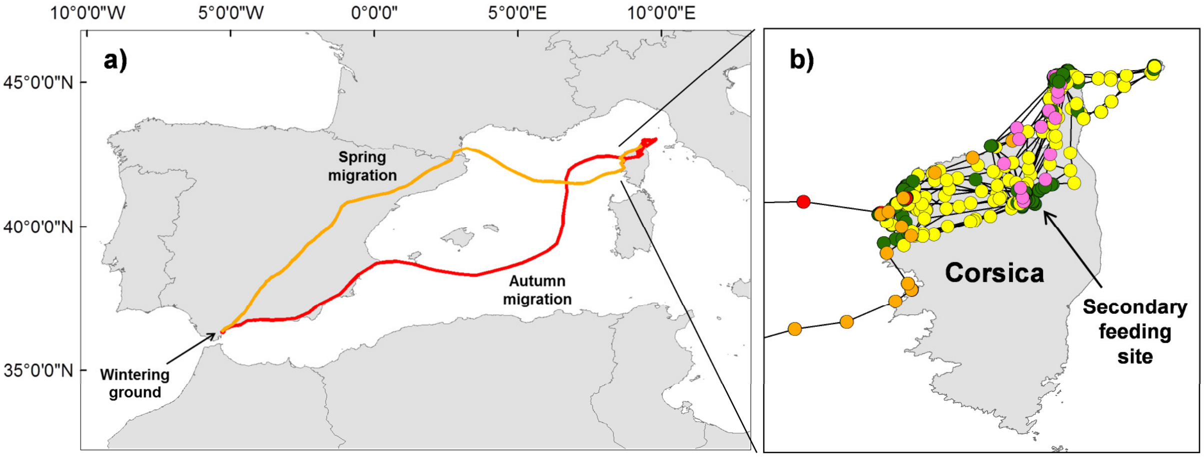

Figure 5: a) example of loop-migration of a Mediterranean adult during a complete migratory cicle: in red the autumn migration, in orange the spring migration; b) zoom of Corsica Island: in green the movements during the breeding season and in yellow and pink the pre-migratory and post-migratory movements, respectively.

d. Loop migration

Only two out of the adult birds which migrated from Corsica performed a loop-migration, taking different routes in autumn (crossing the Mediterranean Sea directly from Corsica to reach Spain) and spring (crossing Spain over land until the Pyrenees, then reaching Corsica from the Continental French shores; Fig. 2 down left panel and Fig. 5); in these cases, spring journey caused a detour of 93.07 km and 2 days in one case and of 298.9 and 5 days in the second one. The Balearic bird wintering to Mauritania in 2009-2010 (and then being resident in Mallorca during successive years; B5M) also carried out a loop-migration across the Sahara desert.

e. Resident individuals

Six Mediterranean adults did not migrate at all (Appendix 2; but see exception of B5M). They were hence considered as resident individuals (RES), frequenting inland sites for feeding, but also regularly moved to the coast where nests were present. Such movements were regular during the year as nesting and feeding sites were within a distance of about 10-20 km in line of sight. In the Balearics, the wetland of Albufera and Ses Salines, respectively on the north and on the south of Mallorca Island, represented the main feeding areas frequented. Only two exceptional exploratory movements (of 65 and 77 km respectively) to Menorca Island were recorded for 2 males during spring 2009 and 2011, respectively. The former bird stayed principally in Cabrera Island (15 km from Ses Salines site). Similarly, a Corsican female and an Italian male stayed all year round along the coast, fishing in marine coves and in the Ombrone River mouth (Maremma Regional Park, Italy), respectively.

7.5. DISCUSSION

a. Migratory strategies over a latitudinal divide

Our cross-population comparison validated our first working hypothesis that ospreys living at different latitudes in the Western Palearctic migrate in different ways. Such contrasting migratory strategies probably evolved as a response to different ecological conditions over the latitudinal divide (Cresswell, 2014). These results are in line with the general pattern of a gradual increase in the proportion of long-distance migrants in breeding species and populations when going towards the high latitudes (e.g. Newton & Dale, 1996; Newton, 2010).

Specifically, all Swedish ospreys showed a homogeneous behaviour to cope with long-distance journeys, combining fly-and-forage and stop-over migratory phases (sensu Strandberg & Alerstam, 2007; Klaassen *et al.*, 2008). In contrast to previous studies (e.g. Strandberg *et al.*, 2009b, using Argos PTT and not precised GPS tracks), our tracking data also pinpointed strong funnelling of adult migratory movements towards the Strait of Gibraltar (Fig. 2). Swedish ospreys showed a low variance in the PDM, which was constant among individuals; probably because the migratory direction is genetically inherited and scheduled for long-distances paths and favoured by the change of evident cues at land across very different regions (e.g. continental Europe, Mediterranean Sea, Sahara desert).

On the other hand, the Mediterranean population showed a heterogeneous migratory behaviour, typical of a partially migratory population (sensu Chapman *et al.*, 2011; Shaw & Levin, 2011) with 38.5% of individuals which migrated long distances >500 km, while 15.5%

carrying out short-distance displacements from the breeding areas and 46% being sedentary. High levels of variability in the choice of migratory routes (and preferred direction of movements), timing and wintering grounds, revealed a more relaxed system and consequently a higher plasticity in behaviour (facultative migrants sensu Newton, 2012), probably promoted by a low variability of the ecological conditions during the year in the Mediterranean region.

However, contrary to our specific predictions, we found that: 1) Swedish ospreys did not anticipate dates of departures and 2) they did not cover greater distances/day compared to Mediterranean ospreys, to account for longer distance migrations. In autumn, at the end of the reproduction, individuals of both popualtions left breeding sites almost simoultaneously (synchronous departures). Conversely, in spring Swedish ospreys left wintering grounds one month after (March-April) the Mediterranean ospreys (February). Indeed it is certainly useless for Swedish birds to start migrating so early, since individuals would arrive too early in spring and they would encounter severe weather and environmental constrains (e.g. low temperatures and frozen lakes) at breeding sites. Alternatively, they could start migration in February and travel at slower speeds to arrive at the good time at breeding sites. However we did not observe that, maybe because it is safer to stay one additional month at wintering site than to spend more time in migration, and rather delay time of departures. Migration is indeed a risky period as shown by Klaassen *et al.* (2014); then flight conditions in temperate and northern Europe are certainly better for soaring migrants later in spring than at the end of the winter. At the same time, arriving too late may imply reduced possibilities to choose best territories for reproduction, already occupied by other conspecifics. Both these factors might shape migratory choices and affect flight performances, especially in spring, when adults are likely to select an early return for reproduction (Berthold, 2001; Alerstam *et al.*, 2006). Indeed, spring migration was not just the simple reversal of autumn migration: autumn journeys lasted twice (60.6 ± 17.3 days) than spring ones (27.4 ± 7.0 days). Time spent on stop-over sites accounted for the half of the autumnal migration duration (31.5 ± 13.3 days on average), whereas it represented only 20% of the time spent during the springtime journey (5.4 ± 3.4 days). That way, Swedish ospreys reduced the timing component of the spring migration rather than modify routes and mean distances travelled per day (that did not differ significatively between seasons).

Different migratory strategies were also recorded in North American ospreys, whereby birds coming from different populations across a longitudinal gradient (east coast, mid-western and western USA) were studied (Martell *et al.*, 2014). American ospreys followed different flyways and adapted their journeys (i.e. dates of departures and arrivals, time spent at stopovers) according to the season. The geographic location of the breeding areas has been proved to influence migratory patterns and wintering locations (Martell *et al.*, 2001; Martell *et al.*, 2014; Washburn *et al.*, 2014).

Overall, a leap-frog migration system (Alerstam & Hogstedt, 1980; Boland, 1990) was hence detected in Western Palearctic ospreys, with populations breeding at higher latitude crossing over the latitudes where southern osprey populations exist. Ospreys of northern latitudes migrated across entire Europe, over-flying the Mediterranean population to winter beyond them in sub-Saharan tropical Africa, thus reversing their latitudinal sequence of distribution between summer and winter. As has been already postulated for other bird species (e.g. Drent & Piersma, 1990; Kelly *et al.*, 2002), this system suggests that northern ospreys, migrating over southern Europe, probably find suitable wintering habitats with good feeding opportunities that are already occupied by their Mediterranean conspecifics, so they are forced to avoid these latitudes and search for alternative vacant sites, more southward. That way, they encounter the desert barrier necessary to cross before reaching wintering grounds in sub-Saharan Africa.

b. Flight performance at barrier crossing

Birds can use different methods to cope with the crossing of ecological barriers. Since crossing barriers requires high energy consumption, due to harsh conditions or absence of feeding and resting opportunities over long distances, prior physiological adaptations are of great relevance, such as shrinkage of body organs to reduce weight and fuel storage at stopover sites (e.g. Red knot *Calidris canutus*; Piersma & van Gils, 2010). When migration starts with the need to cross a barrier, different strategies can be adopted, with specific physiological or behavioural adjustments. That’s the case of populations leaving on the edge of ecological barriers, such as those breeding on marine islands. The tendency to avoid the crossing of large water bodies is well known in large soaring bird such as storks and raptors which rely on thermals generated only over land during the daylight (Kerlinger, 1989; Strandberg *et al.*, 2008; Chevallier *et al.*, 2010). Flapping flight is an energy demanding activity for protracted time, so that to accomplish a long sea-crossing birds need to rely on a good tailwind. In many cases, unfavourable conditions of the habitat matrix surrounding the breeding range of a species lead individuals refrain to leave, promoting sedentary behaviour (Ferrer *et al.*, 2011). That way risks related to migrations are avoided. However, in other cases migration is mandatory as the only solution to face with seasonal changes in food supply and weather conditions.

Osprey is classed among soaring migrants and it is expected to select convenient migratory routes favouring the avoidance of water crossing (Alerstam *et al.*, 2006). In line with this assumption, GPS tracking revealed that journeys of Swedish ospreys were mainly conducted over lands, whereas the water-crossing was reduced by funnelling through the shortest passages both in the Baltic and Mediterranean Sea (Klaassen *et al.*, 2008). The crossing of the 14 km of the Gibraltar Strait required only about two hours flying. Swedish ospreys faced with the passage of this ecological barrier travelling at a greater speed than on land to ensure a rapid crossing of the barrier (Klaassen *et al.*, 2008 and this study). They probably searched for thermals generated at land (both in Spain and Morocco) to start crossing over the 14-km wide sea channel, as described in other species in this area (e.g. Griffon Vultures *Gyps fulvus*; Bildstein *et al.*, 2009).

Unexpectedly, Mediterranean ospreys were able to cover long distances at open sea, performing non-stop long movements (since they cannot land at sea like seabirds), sometimes overnight. They rarely carried out long detours following the coasts, as observed in other raptor species relying on thermals (e.g. Short-toed eagle *Circaetus gallicus*; Mellone *et al.*, 2011; Panuccio *et al.*, 2012; Oriental honey-buzzards *Pernis ptilorhyncus*; Yamaguchi *et al.*, 2008). By leaving breeding grounds (mostly located on islands) individuals were forced to cope with the crossing of the Mediterranean Sea, which was supposed to play the role of an ecological barrier. Before leaving, most of the individuals performed pre-migratory movements to a secondary feeding site. This behaviour was also observed in other raptor species (e.g. Marsh harrier *Circus aeroginosus*; Strandberg *et al.*, 2008) and birds living in close contact with sea environments (Lesser black-backed gull *Larus fuscus*; Klaassen *et al.*, 2012). Time spent in these sites ranged between few days to several weeks and visits were repeated several times before the onset of migration. The function of such movements might be probably related to the necessity to store body reserves before crossing the barrier and/or to gather information on the surroundings before choosing the right direction at the onset of migratory movements.

Secondly, to be able to face with the crossing of broad water tracts in absence of thermals, Mediterranean ospreys probably adopted a mixed strategy: they used an active flight, as suggested by higher airspeeds than on land (even when encountered headwinds, like in spring) and they partially benefited of the use of tailwinds. In autumn Mediterranean ospreys crossed the sea (Fig. 2a-2b), but in spring two individuals preferred to travel over land, reducing oversea passages at specific spots. In such cases, we recorded a loop-migration system probably dictated by adverse weather conditions in spring (as described for Oriental honey-buzzards *Pernis ptilorhyncus*, Yamaguchi *et al.*, 2011). We in fact detected lower tailwinds assistance during spring. Though, this strategy was not strictly adopted by all the individuals (FOSP02 and FOSP08 used same routes in both seasons). In one case (FOSP06) a bird preferred a detoured route by land (passing through Spain and southern France), rather crossing the sea as it did in the previous autumn.

These results showed that the migratory behaviour of Mediterranean ospreys is highly flexible and can be adapted to local circumstances. Since distances to reach wintering sites are short and a little amount of time is required, individuals can choose to invest part of their energies to cross the sea in case of favourable winds, otherwise select a safer route over land. In general, Mediterranean ospreys flew faster in spring to return to breeding grounds, even in case of headwinds. Considering a short migration, they can probably decide to concentrate efforts in flying at sea for a reduced time, also if energetically demanding. Swedish birds instead, which must engage in a longer migration, probably did not choose to venture at sea or against unfavourable winds but rather to wait for better conditions (they rather diminish time at stopover to accelerate the return to breeding sites).

c. Differences between age classes

The development of migratory behaviour is a gradual process being promoted by individual improvements related to age (Sergio *et al.*, 2014). Previous studies highlighted how differences in age classes and experience play an important role in shaping migratory decisions and flight performances (Thorup *et al.*, 2013; Cresswell, 2014; Péron & Grémillet, 2013; Sergio *et al.*, 2014). Accordingly, we found that adults travelled faster and showed straighter migratory paths than juveniles. The latter had also a greater variance in the PDM. As we hypothesized, this suggests that in juveniles migrating for the first time and searching for a place to settle for the winter is more a fact of random combination of factors. On the other hand adults can rely on the experience matured in previous years to reach a precise goal. This would accounts also for the high mortality rate (70%) recorded for juveniles. Similar differences between adults and juveniles were also observed in flight performances during the sea-crossing: juveniles showed reduced speeds compared to adults (i.e. Swedish) and seemed to experience worse tailwinds (i.e. Mediterranean). Mediterranean juveniles in particular, were probably affected by the fact that their first long movements after fledging entail an immediate sea-crossing (compared with Swedish juveniles who started migration in a suitable habitat over land). In general, worse flight performances in juveniles appeared to be related to a limited experience in getting thermals (on land), in choosing favourable wind currents or in compensating for eventual drifts (Klaassen *et al.*, 2011).

7.6. CONCLUSIONS

The strategy adopted by southern ospreys include several advantages in terms of energy and risks saving that should account for a higher adult survival and a greater breeding success, compared to long distances migratory populations.

Mediterranean ospreys migrating for reduced distance have minor energy expenditures related to the journey. At the same time, remaining close to breeding sites allows a faster return to nests for an early start of the breeding season, which contributes in increasing chances of a good settlement (favourable sites) and breeding success. Mediterranean ospreys, in fact, have potentially one additional month, compared to Swedish ospreys, to be dedicated at the reproduction (e.g. for choosing a territory and a mate, building the nest, ecc.).

Klaassen *et al.* (2014) demonstrated how hazardous might be performing long distance migratory journeys in different raptor species, identifying the causes of death encountered on the way. They explained how such threats have an important role in controlling the demographic trends of populations, in turn reflecting the health of the population and its fate. In this sense, avoiding the crossing of large ecological barriers such as the Sahara desert has obvious benefits (to ensure a rapid crossing flight speeds are higher when crossing desert; Klaassen *et al.*, 2008; Mellone *et al.*, 2012). Hazards might occur also in sub-Saharan wintering grounds where poaching and illegal shooting is a still widely diffused practice (Zwarts *et al.*, 2009). Despite all this, residing in the Mediterranean basin is not completely safe neither. The Mediterranean is known to be an important crossroad for migratory birds, were illegal shooting and killing of protected species is still high (see cases of mortality described in results). The Mediterranean osprey population experienced important historical decreases and nowadays shows few breeding nucleus characterized by a limited number of reproductive pairs (Monti, 2012). These populations live in a fragmented habitat at sea, which is highly exploited by humans; available nesting sites are scarce compared to habitats of northern Europe, where continuous forests provide potentially unlimited opportunities for nesting (Saurola, 2005). In this area, reintroduction programs have been launched by using source populations from north and central Europe (Muriel *et al.*, 2010; CIBIO, 2011; except in Italy; Monti *et al.*, 2014): translocating migratory birds from source populations with different migratory strategies (or even resident) might have ecological consequences and promote new behaviours in newly established populations, as described in other studies on birds (e.g. Little Bustard *Tetrax tetrax*; Villers *et al.*, 2010). For these reasons, understanding variation in population and individual level of migratory patterns is of fundamental importance to prepare management actions and corrected conservation strategies in migratory bird populations (Nathan *et al.*, 2008; Mandel *et al.*, 2011).

8. BEHAVIOURAL PLASTICITY IN WINTERING MEDITERRANEAN OSPREYS REVEALED BY STABLE ISOTOPES ANALYSES AND GPS TRACKING.

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8.1. ABSTRACT

To infer wintering ecology in Mediterranean ospreys (*Pandion haliaetus*) we relied on a dual and complementary approach, using both GPS tracking and multi stable isotope tracer approaches. A control sample of feathers from 98 individuals (mostly chicks) was collected over a large latitudinal gradient (from Lapland to Africa) to assess the variability of carbon, nitrogen and sulphur stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, respectively) between breeding sites and habitat types across the Western Palearctic. Then, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ from an experimental set of 18 Mediterranean adults were examined to infer wintering ground locations and habitat types used during the inter-breeding period. Additionally, 12 adult ospreys were fitted with GPS devices and tracked during migration and the wintering season. By combining the two techniques we evidenced a partial migratory population with 41.7% of tagged individuals being resident and 58.3% that actually migrated. Ospreys spent the winter at temperate latitudes and showed a high plasticity in habitat selection. They made use of marine bays, coastal lagoons/marshland and inland freshwater sites. Movements and home range areas were reduced during the season. Wintering grounds were largely spread over the coasts of different countries of the basin, rather than concentrated in one single area. Such behavioural plasticity in the choice of location and habitat type suggests the implementing of broad-scale approaches for the protection of important areas for ospreys in winter. To contribute at assuring a right level of conservation of the osprey populations in the

Mediterranean basin, a harmonization of the management protocols of wetland sites among countries is necessary.

Keywords: feather isotope ratios, raptor, foraging ecology, habitat choice, wintering grounds.

8.2. INTRODUCTION

Animal population dynamics are dependent on a series of processes which occur during different periods of the annual cycle (Newton, 2003b). To study migratory birds, it is therefore mandatory to understand the ecology of the species at each biological phase (e.g. breeding, migration and wintering). Assessing migratory connectivity (individuals from the same breeding site that migrate to the same wintering site; Webster *et al.*, 2002; Trierweiler *et al.*, 2014) is becoming an important factor to understand how population processes may affect breeding success, influencing survival and reproduction (Holmes, 2007; Drent *et al.*, 2007; Trierweiler *et al.*, 2014). For decades, bird migratory connectivity has been studied by means of ring-recovery data (Baillie *et al.*, 2009). Despite the huge amount of knowledge gathered with the use of ringing, implicit limitations of this tool (e.g. low probability of recovery resulting in overall low sample size and lacks of detailed tracks; Guillemain *et al.*, 2013) did not allow to determine in detail ecological traits related to each life periods, especially when birds were distributed over large and low-populated areas in winter. Recent advances in technology and chemistry provided tools for gathering precious information on individual movements and connectivity between breeding, wintering and stopover sites (Webster *et al.*, 2002). Satellite (GPS) tracking revealed wintering grounds and migratory routes (tracks) followed to get there from the breeding areas (Newton, 2010). Stable isotopes analyses (SIA), collected from feathers or other tissues, allowed further determination of habitat use and diet in seasons during the tissue growth (Hobson, 1999; Blight *et al.*, 2014). Such information are of fundamental importance to plan adequate management measures at the right scale of resolution, especially for isolated populations living in fragmented habitats.

In this framework, the osprey *Pandion haliaetus* breeding in the Mediterranean area is a case of particular interest. It is a migratory piscivorous raptor that lives in strict association with aquatic environments for fishing and accomplishing its life cycle (Cramp & Simmons, 1980). In the Western Mediterranean area, the breeding population is fragmented in 4 main breeding sites (Corsica, Balearics, Morocco and Algeria) located on islands and marine coasts, and is considered at a vulnerable status (Muriel *et al.*, 2010; Monti, 2012; Monti *et al.*, 2014). While the migratory and wintering strategies of osprey breeding in North Amrica and Northern Europe have been studied (Martell *et al.*, 2014; Washburn *et al.*, 2014; Alerstam *et* *al.*, 2006), these aspects remain poorly known for the Mediterranean basin (Thibault *et al.*, 2001). In the last decades, sporadic data about wintering areas across the Mediterranean basin (e.g. North African coasts, Italy) have been collected by means of ring recovery (Thibault *et al.*, 1996). Some ospreys were regularly observed wintering close to the breeding territories (J.-M. Dominici and R. Triay, unpublished data), while others were detected hundreds of km away at different sites within the Mediterranean area (Thibault & Patrimonio, 1992; Thibault *et al.*, 1996; Thibault *et al.*, 2001). Moreover, information about habitat selection and detailed movement patterns during winter remains blurred, preventing a full understanding of year round ecology of this population.

In this study, we aimed at understanding movements and feeding areas of Mediterranean ospreys during winter by a dual and complementary approach. We used GPS technology to track Ospreys' movements from the breeding grounds to the wintering sites. However, the expensive costs of GPS devices limited the sample size available. To infer wintering areas for a larger sample of individuals, we used in addition stable isotope analysis (SIA), analysing the carbon, nitrogen and sulphur stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, respectively). Once feathers are formed, their composition does not change, but integrates the elements ingested through the diet during the growing period (Hobson, 1999; Ramos *et al.*, 2009). This technique has the advantage to be non-intrusive (using osprey feathers collected during ringing actions), relatively cheap and integrative of diet studies (Hobson, 1999). Because Ospreys moult their body and flight feathers in winter (Prevost, 1983), we expect that the isotopic chemical composition of the feathers depends on the isotopic chemical composition of the habitat used in winter. Therefore, differences in fraction of stable isotopes present in feathers would help determining if individuals wintered at different latitudes and foraged in different habitats (Bearhop *et al.*, 1999; Inger & Bearhop, 2008).

By combining these two techniques, we aimed at estimating the proportion of sedentary and migratory osprey breeding in the Mediterranean area. To achieve this goal, we needed to answer to the following questions: a) Are there differences in stable isotope ratios of osprey feathers between breeding sites and habitat types (from European Arctic to tropical Africa)? b) Do ospreys use one or several wintering areas during winter? Answering these two questions is compulsory to validate the use of SIA for the other following ones. c) Do Mediterranean ospreys spend the winter at tropical or temperate (Mediterranean) latitudes? d) Do they use freshwater or marine environments during winter? For these last two questions, the GPS tracks of a few individuals, whose feathers were also sampled for stable isotopes, serve as a calibration for a study on a larger sample of individuals using SIA. Our integrative analyses will have important implications for planning management actions and the conservation of ospreys in the Mediterranean area.

8.3. MATERIALS AND METHODS

a. Osprey moult scheme

Osprey has an irregular moult sequence of the primary feathers which occurs in successive waves (Fig. 1), each starting at primary 1 and moving outwards to primary 10 (descendant) (Prevost, 1983). If interrupted in one season, it resumes the next year from the points where it let off: in one wing, there can be primary feathers moulted across two, or even 3 years. Moult of the secondaries progresses towards the body (ascendant) and is completed after 17-19 months (Cramp & Simmons, 1980). Moult mainly occurs from June-July to August-September and from October-November to February-March, resulting in an interruption during migratory periods (Prevost, 1983). During breeding, males are provisioning the nest. Thus males are forced to postpone moult until the end of the breeding season, whereas females can start moulting flight feathers while incubating (Hake *et al.*, 2001).

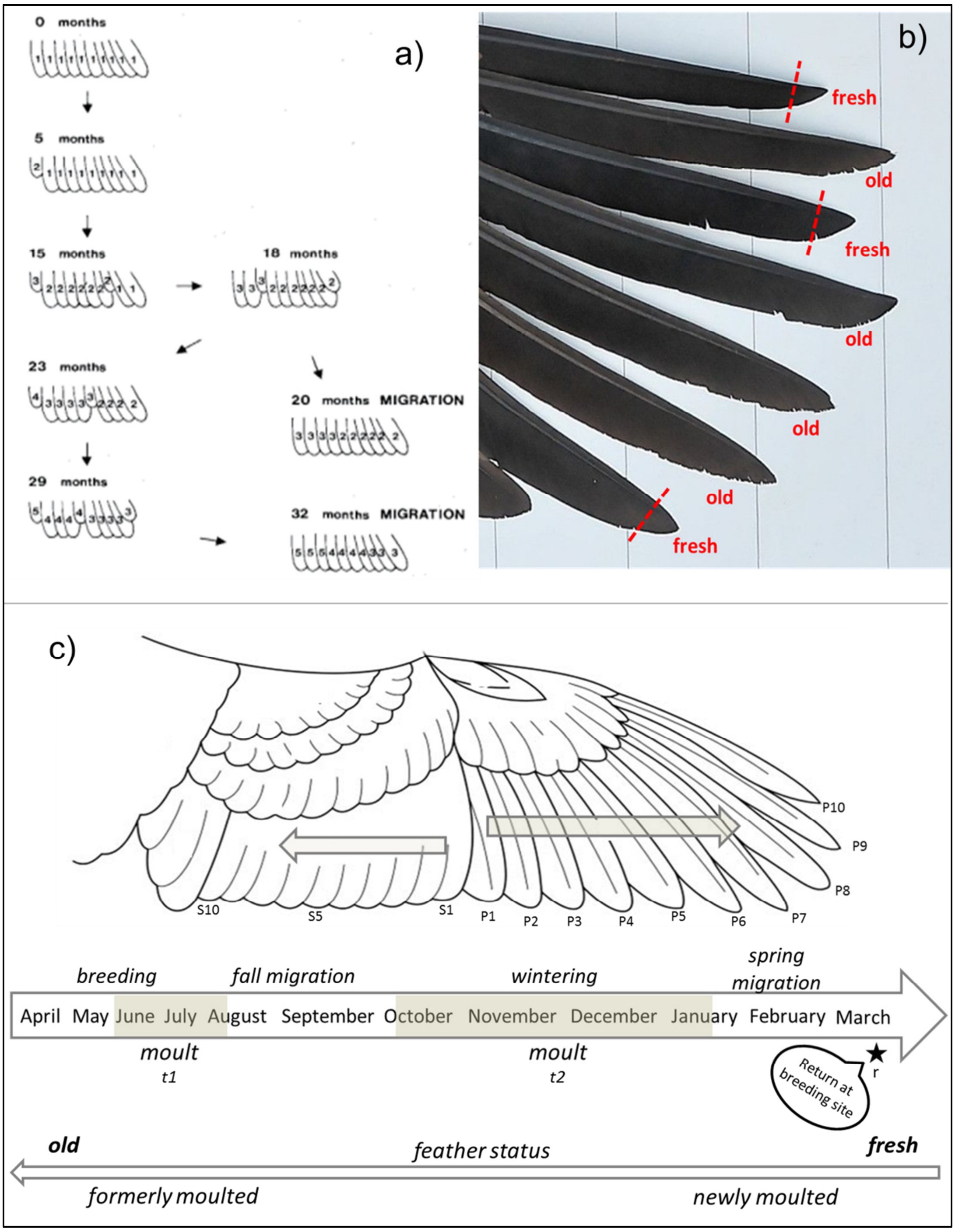

Figure 1: a) Osprey's moult scheme (figure extracted from: Prevost, 1982); b) image showing fresh and old primaries according to moult pattern; 2 cm at the tip of fresh moulted feathers were collected (red dotted lines); c) moult pattern showed by grey arrows (descendent for primaries and ascendant for secondaries) and phenology of ospreys and moult periods during the year: a feather moulted during breeding season (*t1*) will appear partially used at time *r* (when birds return at breeding sites) and thus will not selected for sampling; conversely a feather moulted in winter (*t2*) will appear as newly formed (darker colour and border more regular) at time *r* and thus selected for sampling (black star). Small arrow represents the feather status during time.

b. Feather sampling

A total of 244 feather samples were collected from 98 individuals from 11 locations throughout the Western Palearctic and West Africa, whereby four main areas were included: a) north and central Europe; b) Mediterranean basin; c) Atlantic sub-tropical Islands and d) sub-Saharan Africa (Tab. 1, Fig. 2). Such approach was designed to have a better resolution of the isotopic variation in osprey feathers over a latitudinal gradient and between different types of habitat (e.g. freshwater vs saltwater). Sampling included both control and experimental manipulations. Chick's feathers, collected during ringing actions at the nest in summer, were used as control because they reflected the isotopic signature of the environment around breeding sites and allowed us to obtain baseline isotopes values in a strict marine or freshwater environment. Hence, we sampled body feathers of 30 chicks coming from Continental Europe (e.g. Finland, Estonia, Latvia and France; Tab. 1, Fig. 2), where ospreys nest close to freshwater and chicks are fed with stenohaline fish species (Poole, 1989). We also sampled the body feathers of 29 chicks from nests located in the Mediterranean basin (Tab. 1), where ospreys nest on rocky pinnacles at sea, feeding their chicks with euryaline species (Francour & Thibault, 1996; Thibault *et al.*, 2001). To achieve a high level of resolution for the Mediterranean basin, chicks from three osprey populations breeding at sea were collected (Corsica, Morocco and Balearics Islands). We also added samples from Italy, where ospreys have been reintroduced, breed in a coastal marsh (Monti *et al.*, 2014) and can feed on marine, brackish or freshwater fishes. We also included additional samples from chicks of Atlantic islands (Canary and Cape Vert islands) in order to gather other marine isotopic signatures from southern latitudes to be compared with those of the Mediterranean area. Finally, we added samples from West Africa (Senegal), used as wintering ground from northern European ospreys (Alerstam *et al.*, 2006; Bai & Schmidt, 2011). We also collected 2 cm of vexillum at the tip of primaries (actively moulted) from 2 specimens of adult osprey belonging to the collection of the IRD (Institut de la Recherche et du Développement of Dakar, Senegal); these two individuals were killed during the winter and so their moulting feathers should reflect the isotopic signature of wintering grounds in West Africa. To increase our sample size at this latitude, we added 6 samples of a surrogate raptor species, the African fish eagle *Haliaeetus vocifer*, which is known to be sedentary all year round and to have a similar ecological niche to the osprey in winter (Whitfield & Blaber, 1978; Prevost, 1982).

Finally, for inferring whether adults' breeding in Corsica had wintering grounds different from breeding areas we sampled 18 adult ospreys trapped in March 2012 and 2013 on their nests in Corsica, before the onset of the breeding season. From these birds, only recently-moulted feathers (identified as such on the basis of darker colour and border more regular) were selected, and we used for the analyses only the first 2 cm of the tip of primaries and secondaries which very likely had grown during the previous winter and, thus, contained the isotopic signature of wintering grounds (e.g. Zelanko *et al*, 2011).

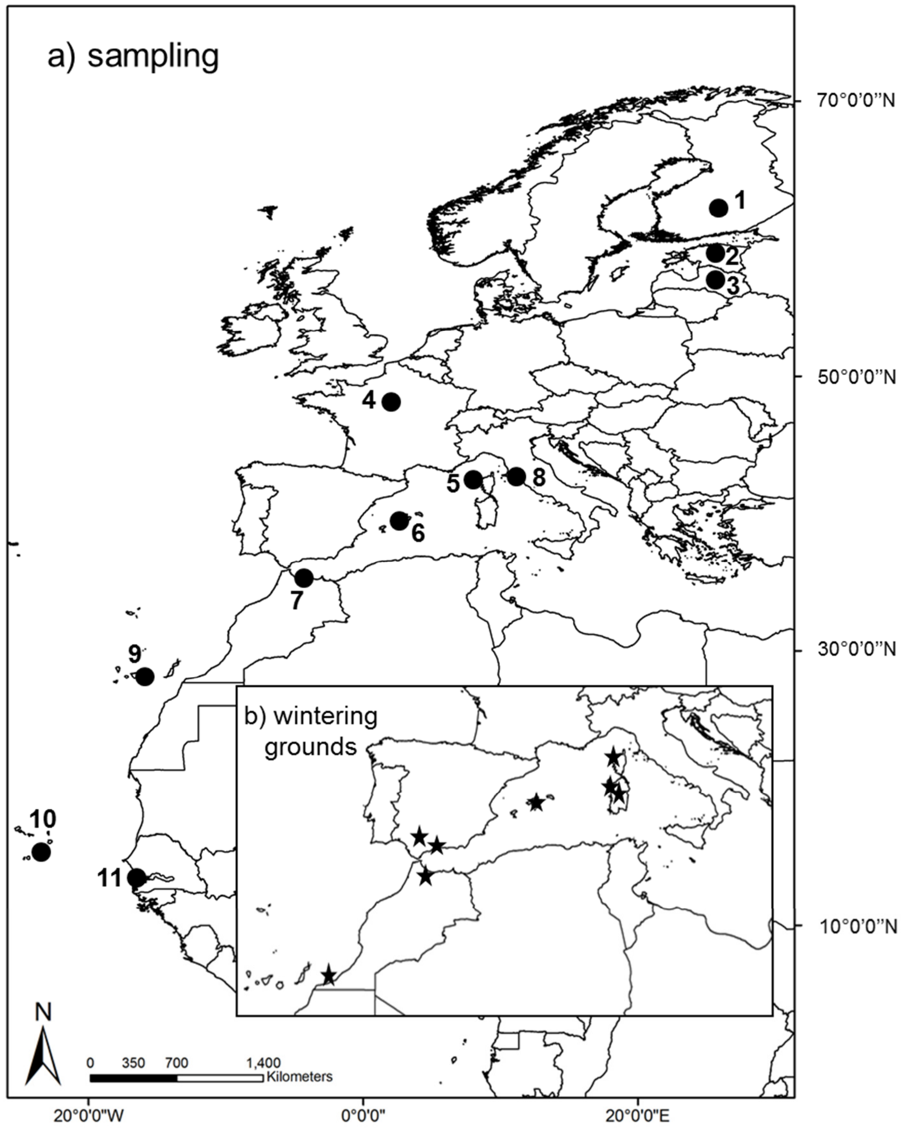

Figure 2: Maps with location of a) sampled feathers (black dots and numbers that refer to countries are reported in Tab. 1) and b) wintering grounds of GPS tagged ospreys (black stars; see Tab. 2).

Table 1: Region, country and age of osprey feathers sampled across Western Palearctic. For each stable isotope is reported the number of samples used. *six out of the eight samples concerned adult individuals of African fish eagle *Haliaeetus vocifer*. Values in brackets indicate the number of countries (see Fig. 2).

Region	Country	Habitat	Age	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Continental Europe	Finland (1)	Freshwater	chicks	10	10	1
	Estonia (2)	Freshwater	chicks	4	4	1
	Latvia (3)	Freshwater	chicks	5	5	1
	France cont. (4)	Freshwater	chicks	11	11	3
Mediterranean		?	adults	18	18	18
	Corsica (5)	Saltwater	chicks	12	12	5
	Balearic Islands (6)	Saltwater	chicks	7	7	1
	Morocco (7)	Saltwater	chicks	7	7	2
	Italy (8)	Brackish	chicks	3	3	3
Subtropical Atlantic islands	Canary Islands (9)	Saltwater	chicks	4	4	3
			adults	7	7	2
	Cape Verde Islands (10)	Saltwater	chicks	2	2	0
West Africa	Senegal (11)	Saltwater	adults*	8	8	8
Total				98	98	48

c. Stable isotope analyses

We run preliminary analyses on 15 individuals (15 primaries / 15 secondaries) to assess whether isotopic signatures of C and N vary in relation to the type of feather (e.g. body feather, primaries, secondaries; Zelanko *et al.*, 2011). We used stable isotopes of Carbon ($\delta^{13}C$), Nitrogen ($\delta^{15}N$) and Sulphur ($\delta^{34}S$) ratios to compare the signature of feathers of Corsican adults ospreys with the signature found in feathers from control birds from temperate to tropical latitudes. The $\delta^{13}C$ and $\delta^{15}N$ of primary producers vary predictably among ocean basins. High-latitude pelagic ecosystems as observed in the Southern Ocean have much lower $\delta^{13}C$ and $\delta^{15}N$ than at lower latitudes (Goericke & Fry, 1994; Bentaleb *et al.*, 1998; Trull & Armand, 2001; Cherel & Hobson, 2007). We included also the $\delta^{34}S$ isotope that is an efficient tool to discriminate between coastal and marine habitats (Hobson, 1999; Caccamise *et al.*, 2000; Knoff & Richmond, 2000; Lott *et al.*, 2003).

C, N, S isotopic ratios are good indicators of the foraging ecology patterns (Hobson, 1999). More specifically, we expect the $\delta^{13}C$, $\delta^{15}N$ and δS^{34} values in feathers to be potential proxies 1) to investigate latitudinal variation (Kelly *et al.*, 2002) and locate ospreys wintering grounds at a broad geographical scale; 2) to track trophic levels and food chains (DeNiro & Epstein, 1978; Deniro & Epstein, 1981; Thompson & Furness, 1995; Hobson, 1999; Romanek *et al.*, 2000). Indeed the isotopic ratios of carbon, nitrogen and sulphur in animal tissues reflect the corresponding ratios in ingested foods. However, trophic level effect induces offsets that vary with the tissue types. According to Hobson (1999), the isotopic fractionations between diet and C, N isotopes of feathers of falcons and piscivorous birds vary in the range of 2.1-3.8 ‰ and 2.7-3.7 ‰, respectively, while trophic level enrichment for sulphur is close to 0‰. Differences in sulphur isotopic ratios between terrestrial and marine biota make this isotope extremely useful in tracing contributions of terrestrial and marine diet sources.

Stable isotope ratios were reported as deviations from a standard in per mil (‰) using the δ notation:

$$\delta^{m}X = 1000 * \left(\frac{Rsample}{Rstandard} - 1\right)$$

where δ is the isotope ratio of the sample relative to a standard, R_{sample} and $R_{standard}$ are the fractions of heavy to light isotopes in the sample and standard, respectively. Feathers were thoroughly washed in distilled water using an ultra sonic bath and then dried in an oven at 50°C over night (Guillemain *et al.*, 2014). Fragments of appropriate weight of feathers (for C and N = 0.3 mg, for S = 1 mg) were then cut and placed in 8x5 EuroVector tin capsules. Carbon and Nitrogen of Paleartic samples were analysed at the isotope platform of Institut des Sciences de l'Evolution de Montpellier (France) by means of a mass spectrometer Micromass Optima-AC117-coupled to an elemental analyser EuroVector 3000. Carbon and Nitrogen of samples collected in tropical feathers were analysed at the isotopic platform LIENSs of University of La Rochelle using the isotope ratio mass spectrometer in continuous flow (CF-IRMS) Delta V Advantage, coupled with a Flash EA 1112 elemental analyser. The precision for C and N isotopic ratios are better than 0.1 ‰ for both ISEM and LIENSs mass spectrometer devices. The C (-23.7‰) and N (-0.5‰) isotopes of the alanine standards of the ISEM laboratory were measured on both ISEM and LIENSs machines showing no difference for the carbon but a significant difference for Nitrogen of +0.34‰ at the LIENSs. The results have been corrected considering this value. All the sulphur analyses were run at LIENSs laboratory. The precision was better than 0.33‰.

d. Statistical analysis

Statistical analyses were performed with the R software (package ade4; Dray *et al.*, 2007). We used One-way ANOVA to examine effects of sites on three isotopes ratios. We started from the null hypothesis that isotopic ratios in feathers of Corsican adult ospreys were not significantly different from those of Corsican chicks (supporting a residency hypothesis). Even if we tested for differences in the mean, our main interest was in the variance between age classes because if a portion of the population is migratory, the range of isotopic values would increase the variance of that population. We used Student-Fischer test of equality of variance to compare values of Corsican adult ospreys and Corsican chicks and then, because the variance were not equal, we compared means with a Kruskall-Wallis test. Relationships between three isotopes variations were assessed using piecewise regression. Correlation was shown by Pearson correlation test. Isotopic signatures of adults from Corsica have been linked to chicks' signature and so to different habitats thanks to discriminant analysis by reassigning each adult to a chicks sample batch.

e. Tagging and tracking

Wintering ecology was studied in 12 adult ospreys from the Mediterranean population, including five birds from Balearic Islands (39°30′N, 3°00′E) and seven from the Corsica (42°06N, 9°07'E) (Tab. 2). Since three birds were tracked for more than 2 consecutive years, our dataset was composed of a total of 16 complete wintering events (Tab. 2). Five adults were trapped during the winter season in the Albufera wetland site (Mallorca Island, Balearics), using a perch-trap triggered with a remote control. These birds were fitted with 30-gr Solar Argos/GPS PTT-100s (Microwave Telemetry Inc., Columbia, USA). Further 7 adults (5 females and 2 males) were caught in Corsica, France, before the onset of the breeding season in March-April 2013, using a noose carpet laid on the nest. These individuals were equipped with a 24-g solar powered GPS/GSM device (model Duck-4, Ecotone Company, PL). All tags were programmed to collect data at hourly intervals during the whole winter season. For migratory birds, the wintering season was defined as the period between the arrival on the wintering grounds, after the post-breeding migration, and the onset of the next pre-breeding migration (Strandberg *et al.*, 2008; Mellone *et al.*, 2012). For resident birds, winter was considered as spanning from October to February, according to the biology of osprey at these latitudes (Poole, 1989; Triay, 2007). Locations were retrieved in geographical coordinates and converted to UTM coordinates using the software ArcGis 9.3, for metric calculations. To map the wintering areas in detail, we estimated the individuals' home ranges (95% fixed kernel) and core areas (50% fixed kernel) of every wintering event through fixed kernel density contours (sensu Worton, 1989), using the Hawth's tool Extension (Hooge & Eichenlaub, 2000). Since osprey is strictly associated to the presence of water bodies to catch fish (Cramp & Simmons, 1980), habitat type composition during winter concerned only aquatic environments. In order to ascertain water bodies composition of wintering areas, we considered these areas as marine waters, brackish waters or freshwater. Then, we calculated the percentage of every habitat type within the core areas for each one of the 16 wintering events separately.

8.4. RESULTS

a. Differences in isotopic ratios between breeding sites in Western Palearctic

As no significant differences in isotopic signatures of C and N between feather types (primaries vs secondaries) were found (Wilcoxon signed-rank test: N: $p = 0.4$; C: $p = 0 .24$), we used both primary and secondary feathers for our analyses. Values of the three isotopes found in chicks' feathers were highly different between breeding sites across the Western Palearctic (δ^{13}C: $t_{10} = 64.49$, $p < 0.001$; δ^{15}N: $t_{10} = 6.61$, $p < 0.001$; δ^{34}S: $t_{10} = 56.86$, $p < 0.001$; samples from Senegal included). In particular, δ^{13}C values, and to a lesser extent values of δ^{34}S, were high in African and Mediterranean areas, while they dropped sharply in Continental Europe (Fig. 3). No pattern was noticeable for δ^{15}N (δ^{15}N: $t_{10} = 6.61$, $p < 0.001$), which varied greatly from one site to another and within each site regardless of its latitude (Fig. 3).

Values of δ^{13}C and δ^{34}S of marine environment were significantly greater than those of brackish and freshwater habitats (δ^{13}C: $t_2 = 205.59$, $p < 0.001$; δ^{15}N: $t_2 = 10.79$, $p < 0.001$; δ^{34}S: $t_2 = 141.11$, $p < 0.001$; Fig. 4). Values of δ^{13}C and δ^{15}N were strictly correlated to those of δ^{34}S (δ^{13}C: $p < 0.001$, $r^2 = 0.77$, δ^{15}N: $p < 0.001$, $r^2 = -0.72$). A relationship between habitat type (marine vs freshwater) and isotope values was observed.

Table 2: Winter home ranges (fixed kernel 95%), core areas (fixed kernel 50%) and mean cumulative distances of daily movements of Mediterranean adult ospreys. Arrivals, departures and time spent (days) at wintering sites is reported for migratory individuals. For resident birds, the winter period has been considered between October and February (see methods). Locality and place of wintering grounds are reported as well as the percentage of habitat type of core areas for each of the wintering event. ID stands for tagging reference of each bird.

ID	*Breeding origin*	*Winter*	*Arrival date*	*Departure date*	*Time elapsed (days)*	*Core area (km^2)*	*Home range (km^2)*	*Distance per day (km)*	*Wintering Country*	*Place*	*Marine %*	*Saltwater %*	*Freshwater %*
FOSP01	Corsica	2013	Oct	Feb	NA	5.56	28.16	###	Corsica	Scandola	100	0	0
FOSP02	Corsica	2013	05/10	25/03	171	2.92	15.74	###	Morocco	Oued Drai River mouth	0	100	0
FOSP03	Corsica	2013	21/08	05/01	137*	9.09	49.80	10.76 ± 15.15	Sardinia	Sale Porcus marsh and Is Benas lagoon	0	100	0
FOSP04	Corsica	2013	15/09	16/03	182	3.45	25.98	7.8 ± 8.3	Spain	Guadiaro River	0	0	100
FOSP05	Corsica	2013	25/06	06/02	226	18.22	159.63	22.8 ± 32.5	Sardinia	Omodeo Lake, Tirso River, Cabras and Mistras marshes	0	14.2	85.8
FOSP06	Corsica	2013	15/08	19/03	216	4.22	18.93	4.3 ± 8.3	Spain	Guadalcacín dam	0	0	100
FOSP08	Corsica	2013	17/08	21/02	188	6.88	58.31	12.7 ± 9.11	Morocco	Mohamed V dam	0	0	100
BAL1M	Balearics	2010	Oct	Feb	NA	8.75	65.09	11.48 ± 11.8	Balearics	north coast of Mallorca and Albufera marsh	44.5	55.5	0
BAL2F	Balearics	2009	Oct	Feb	NA	7.50	79.91	3.35 ± 4.7	Balearics	Alcudia marshes and coasts	29.3	70.7	0
BAL3IND	Balearics	2010	Oct	Feb	NA	3.40	22.93	7.54 ± 8.3	Balearics	Ses Salines marsh	0	100	0
BAL3IND	Balearics	2011	Oct	Feb	NA	4.01	49.11	13.15 ± 18.7	Balearics	Ses Salines marsh	0	100	0
BAL3IND	Balearics	2012	Oct	Feb	NA	2.73	18.75	6.94 ± 14.0	Balearics	Ses Salines marsh	0	100	0
BAL4M	Balearics	2009	Oct	Feb	NA	13.74	121.57	22.17 ± 21.9	Balearics	Albufereta marsh and Cabrera coasts	42.2	57.8	0
BAL4M	Balearics	2010	Oct	Feb	NA	12.96	123.42	23.8 ± 20.7	Balearics	Cabrera coasts, Colonia St Jordi, Ses gambes marsh	43.8	56.2	0
BAL5M*	Balearics	2009	17/11	25/03	128	4.14	47.82	8.67 ± 6.8	Mauritania	Senegal River delta	0	100	0
BAL5M	Balearics	2010	Oct	Feb	NA	8.13	74.14	15.4 ± 13.9	Balearics	north coast of Mallorca	100	0	0
Mean					*178.28 ± 5.9*	*7.23 ± 4.5*	*59.95 ± 42.9*	*13.23 ± 6.6*		***mean % habitat***	*22.50*	*53.40*	*24.10*

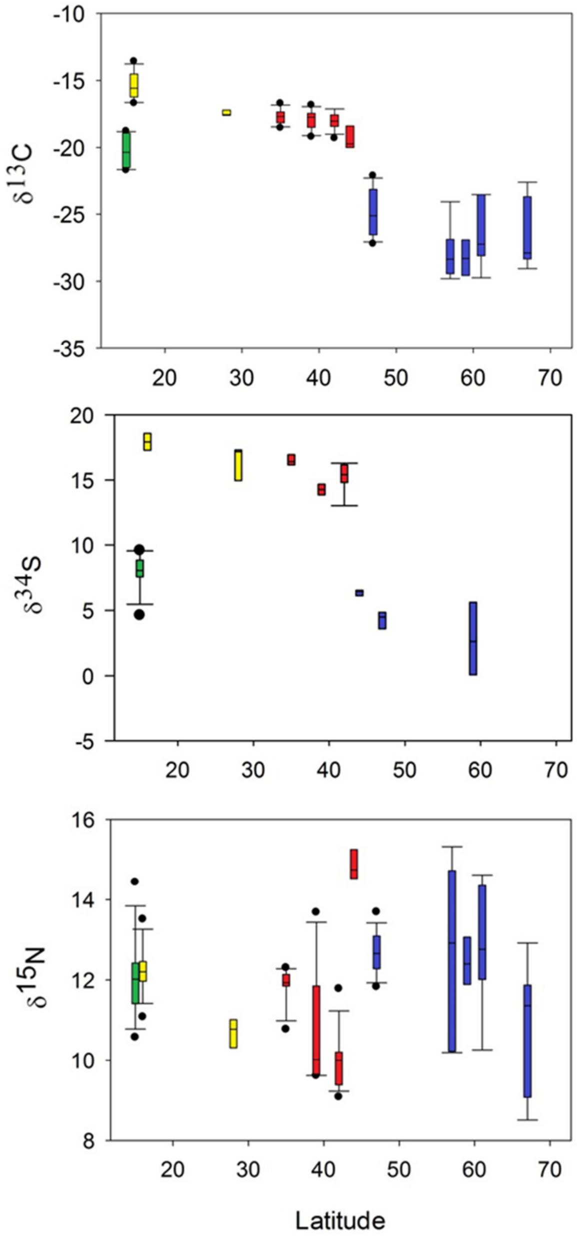

Figure 3: Differences in isotopic ratio for $\delta^{13}C$, $\delta^{34}S$, $\delta^{15}N$ in chicks' feathers (used as control) over the latitudinal gradient in the Western Palearctic and West Africa (blue = continental Europe, red = Mediterranean, yellow = Atlantic islands and green = Senegal).

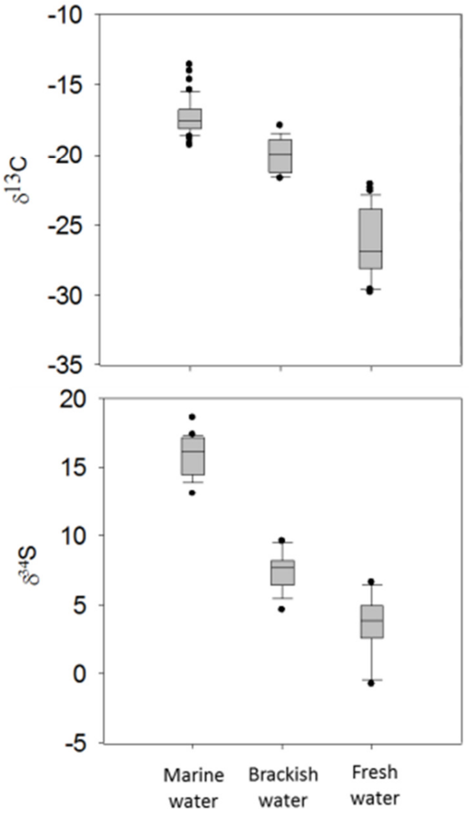

Figure 4: $\delta^{13}C$ and $\delta^{34}S$ values in chicks' feathers according to habitat type classification across breeding sites in the Western Palearctic.

b. Corsican ospreys wintering grounds

Mean isotopic values of Corsican adult and chicks (Fig. 5) were significantly different for $\delta^{15}N$ and $\delta^{34}S$ (respectively: $t_1 = 16.73$, $p < 0.001$; $t_1 = 6.42$, $p < 0.001$) but not for $\delta^{13}C$ ($t_1 = 0.03$, $p = 0.857$). Moreover concerning the three isotopes, the variance was higher in adults than in chicks ($\delta^{13}C$: $t_{17} = 15.87$, $p < 0.001$; $\delta^{15}N$: $t_{17} = 16.44$, $p < 0.001$; $\delta\ ^{34}S$: $t_{17} = 11.04$, $p = 0.0316$; Fig. 4). Discriminant analysis for adults did not allow us to assign precise latitudes of wintering areas for every adult, but rather a habitat type. Six Corsican adults out of 18 (33%) showed an isotopic signature for the three isotopes typical of a Mediterranean marine environment; two out of 18 (11%) presented equivalent values to freshwater habitat; the remaining 10 (56%) corresponded to intermediate values, i.e. possibly brackish water habitat or an alternation between saltwater and freshwater.

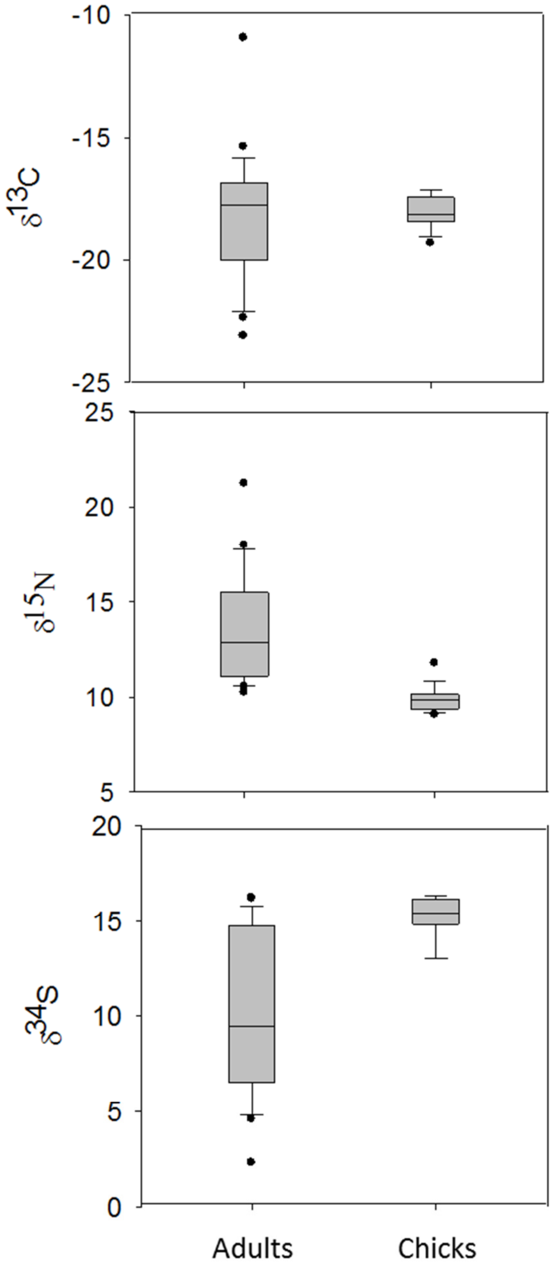

Figure 5: Difference in isotopic ratios of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ in feathers of both Corsican adults and chicks ospreys.

c. Movements and habitat selection

All the 12 experimental birds tracked with GPS spent the winter at temperate latitudes, avoiding crossing the Sahara desert (apart from two individuals that went to southern Morocco and Mauritania; Tab. 2). 41.7% of tagged individuals were residents (5 individuals) and 58.3% (9 individuals) migrated (Tab. 2). They did not concentrate in the same area for winter. The wintering grounds were located between 28°N and 42°N latitude within the Mediterranean basin (e.g. Spain, Morocco, Algeria, Sardinia-Corsica complex, Balearics and Italy). Exploratory movements were only occasional (e.g. FOSP03 moved for ca. 50 km and then come back to its wintering site); basically ospreys tended to make use of the same area throughout the whole winter. Daily movements were restricted (mean = 13.23 ± 6.6 km per day) and home ranges were very small during winter (core area = 7.23 ± 4.5 km^2; home range = 59.9 ± 42.9 km^2; Tab 2). Home range and core areas sizes did not differ between resident and migratory birds (Mann-Whitney U-Test: home range: $U = 12$, $p = 0.37$, $N = 12$; core areas: $U = 14$, $p = 0.57$, $N = 12$). Migratory ospreys spent about six months (mean: 178.3 ± 36.7 days, $N = 7$; Tab. 2) at wintering grounds before pre-nuptial migration. A high site-fidelity was shown by the 3 birds tracked during consecutive wintering seasons (Tab. 2); they used the same areas that they had frequented in the previous years (mean overlap home ranges: 58.9 ± 13.1; mean overlap core areas: 37.9 ± 22.9; $N = 2$). The only exception was for bird (BAL5M), which wintered in the delta of the Senegal River (Mauritania) in 2009, but was resident in Balearic Islands in 2010. Interestingly, birds resident in Mallorca Island, partially shared home ranges and core areas, visiting the same sites during winter (e.g. Albufera marsh).

Individuals used marine and coastal saltwater habitats as well as freshwater sites in both coastal and inland areas (e.g. bays and coastal waters, river mouths, marshes, dams and artificial ponds), demonstrating no specific preferences for one of the habitats considered. Inter-individual plasticity in habitat choice was high since 16.7% of the birds used only marine coastal habitats, 25% only freshwater sites and 25% used brackish habitats such as marshland or coastal lagoons. The remaining 33.3% frequented different habitats during the same season, so being opportunistic and eating both euryaline and stenhoaline species (Tab. 1 & 2).

8.5. DISCUSSION

a. Residency/migratory hypothesis

We performed a multi-isotopic analysis (including three specific isotope markers: Carbon, Nitrogen and Sulphur) to determine the isotopic ratios and improve resolution in assessing osprey wintering ecology. First, the stable isotope ratios of osprey chicks' feathers varied greatly between different breeding sites over the latitudinal gradient in the Western Palearctic. Such variation was concomitant to different values recorded also in relation to the habitat type. Marked differences in isotopic ratios allowed us to make a clear distinction for chicks that were fed at freshwater, brackish or marine environments over the latitudinal gradient. Results were concordant with the ecology of osprey populations: we found that northern populations which live in strict association with freshwater habitats showed values of $\delta^{13}C$ between -29.8 and -22.1 ‰ and of $\delta^{34}S$ between 0.0 and +6.6 ‰, while $\delta^{13}C$ values were between -19.3 and -13.6 ‰ and $\delta^{34}S$ between +13.4 and +18.6 ‰ for populations of marine environments at mean latitudes of the Mediterranean and Atlantic islands. Accordingly, intermediate values were found for samples from West Africa (Senegal) collected in a brackish water system. These trends were fundamental to test our residence hypothesis of Corsican adult ospreys and to compare isotopic ratios of their feathers with those of Corsican chicks fed in a marine habitat in the Mediterranean.

New insights from both SIA and GPS tracking revealed a partial migratory population (*sensu* Chapman *et al.*, 2011), with part of the sampled individuals that showed a resident behaviour and others that migrated.

This plasticity in migratory and wintering strategies was already hypothesized on the basis of ring-resightings of Corsican birds returning to different places of the Mediterranean basin (Thibault *et al.*, 1996). This Mediterranean population behaves rather differently than those breeding in continental Europe, in which all individuals migrate to West Africa (Prevost, 1982; Alerstam *et al.*, 2006). Even if it was impossible to determine exactly the latitude of each wintering area, stable isotopes analysis revealed that the majority of Corsican ospreys overwinter in a habitat different than the breeding habitat (marine environment in Corsica). Only 33% of Corsican adults showed similar isotopic signature than Corsican chicks, suggesting at least the presence of few residents in this population. Such resident behaviour in the Mediterranean osprey population was confirmed by GPS tracking (41.7% of tagged birds). The remaining individuals showing different isotopic values had most likely spent the winter in different habitats such as brackish and freshwater sites, located away from Corsica. Such sites are rare in Corsica and rarely if ever used by ospreys in winter. There are indeed very few records of Ospreys wintering in wetland inland sites in Corsica (Thibault *et al.*, 1996).

Furthermore, low variances in $\delta^{13}C$ and in $\delta^{34}S$ within each breeding site suggested that the source of organic matter in the food web was similar for all sampled chicks (e.g. marine prey during breeding season). This is in accordance with the strict piscivorous feeding habits of the species and the limited movement recorded during the breeding period (Monti, unpublished data). In Cape Verde, where we sampled both sedentary adults and chicks, values found in the former were substantially equivalent to those found in chicks, even if sampling was not sufficient to realize statistical tests (n = 7 adults: $\delta^{13}C$ mean = -15.16 ± 1.12 ; $\delta^{15}N$ mean = 12.46 ± 0.55 and chicks n=2 ; $\delta^{13}C$ mean = -16.15 ± 0.56; $\delta^{15}N$ mean = 11.53 ± 0.63). The absence of a significant difference in isotopic ratios between adults and chicks of Cape Verde islands confirms a resident behaviour of the adults of this population, already suspected (Poole, 1989). Further tracking studies should be carried out on this population to check for such preliminary outcomes (low sample size to be statistically tested) from SIA.

b. Tool limitations: SIA vs GPS tracking

Although largely used as good indicator of geographic origins in birds (Hobson, 2005), we did not make use of deuterium isotope because of high possibilities that a mixed diet made by both marine and freshwater prey may influence deuterium ratios and create a bias, as already evidenced in other studies (e.g. Lott *et al.*, 2003). At the same time, some intrinsic limitations of stable isotopes methodology prevented us to discern the exact latitude of wintering grounds from values of carbon in this specific case; this is because carbon was correlated to the latitude of the sampling site but also to the habitat used for fishing. For instances, both Carbon and Sulphur values for samples from Senegal were equivalent to those recorded for Italy: in both these regions ospreys are known to fish mostly in brackish water habitats (or to regularly shift from marine to brackish water to freshwater). The osprey is a specialized raptor in catching fish living near the water surface, but its diet can rely on different fish species according to the season and the location, so that it may result in an opportunistic feeding behaviour (Cramp & Simmons, 1980; Poole, 1989; Francour & Thibault, 1996). Thus the overall large variance found in C and N ratios may be due to a high plasticity in latitude and trophic level selection (both herbivorous and carnivorous fish species).

If on the one hand GPS tracking allowed to exactly identify migratory routes and location of wintering grounds of tagged ospreys (solving issues with intrinsic limitations of SIA), on the other hand it did not provide information about trophic level and shifts in diet composition (especially in saltwater habitats where both stenhoaline and euryaline fish species can be present). Then, further constrains mostly due to the expensive price of these devices which limited the sample size, made the choice of stable isotopic methodology a valid integrative solution in this case.

c. Behavioural plasticity in winter

Our osprey tracked in winter mostly used temperate areas, but using different habitat types (from marine bays to marshlands and/or freshwater sites). If from one side a high inter-individual plasticity was detected within the Mediterranean population, on the other hand each individual tended to use only one site (or two) during the winter. This general lack of mobility in winter supported our assumptions for isotopes analyses. Once arrived at the wintering ground, birds rarely moved around, but rather exploited a small area associated to a specific water body. This behaviour can suggest a strategy aiming at reducing energy consumption and minimize efforts in movements and fishing activities during this season. This accounts for the strict values of C, N and S found in some of the individuals that were faithful to the same spot, feeding on local available species.

Mediterranean ospreys did not show preferences for a specific habitat type during winter. In our opinion, the choice of the wintering ground for adult birds was much more related to the experience an individual matured in previous years (adults returning to sites that assured survival during previous winters) and probably a matter of fate for juveniles migrating for the first time (that decide to settle where they find good fishing opportunities). However our dataset of juveniles tracked in winter is still too limited in time to confirm these hypotheses: none of the juvenile birds tracked since 2013 have reached sexual maturity yet. Similar findings on adults were recorded for North American ospreys (Washburn *et al.*, 2014). Beyond their origins (birds from 5 different populations/regions were studied), North American ospreys spent ca. 5-6 months at tropical latitudes for wintering, using a diversity of aquatic habitats and foraging on a large variety of fishes; during the winter period they also moved infrequently, showing limited home-ranges and core areas (12.7 km^2 and 1.4 km^2, respectively). These results show how the wintering ecology of this raptor can be surprisingly similar though across different continents and regions within the wide distributional range.

Indeed, ospreys tend to be opportunistic, adapting their behaviour in function of the location and water bodies availability, with fishing opportunities possibly being the most important requirement selected. This study proved that the integration of multiple SIA and tracking techniques was useful to overcome the intrinsic limits of each method and achieve greater information of ecological aspects of the population under study.

d. Implications for conservation

The behavioural plasticity of Mediterranean ospreys in the choice of location and habitat type does not require the securing of a single key staging site (e.g. like in some species of waders such as the red knot *Calidris canutus*; Rogers *et al.*, 2010). However, osprey wintering grounds lie in one of the most exploited marine environments (i.e. the Mediterranean Sea) where intense human activities occur, including the reclaim of coastal habitats (e.g. marshland) into productive lands for building. In the Mediterranean region, habitat loss and fragmentation are severely affecting coastal wetlands (Tomaselli *et al.*, 2012), ultimately influencing the population dynamics of many birds associated to such environments (e.g. Greater flamingo, *Phoenicopterus roseus*; Bechet & Johnson, 2008). This suggests the implementing of broad-scale approaches for the protection of wintering areas for ospreys. To contribute at assuring a right level of conservation of the osprey populations in the Mediterranean basin, a harmonization of the management protocols of wetland sites among countries is necessary.

9. SECTION III: LOCAL SCALE AND RECENT TIME

9.1. BACKGROUND

At a global scale, "*biodiversity hotspots*" (areas with the most important level of biodiversity) are also the most threatened regions (Mittermeier *et al.*, 1999; Myers *et al.*, 2000). The Mediterranean basin is considered as one of these hotspots for its richness in term of species (often endemic) and ecosystems (Médail & Quézel, 1999). Unfortunately, current threats are especially hitting such habitats and populations that show features of endemism, that are isolated and/or small and that have few possibilities of rescue, being unable to recover themselves in a natural time span.

In this framework, of notable interest is the case of the **Osprey in the Mediterranean basin**. While it is a relatively common species in freshwater ecosystems of northern Europe and other places in the world (North America, Asia, Australia), the Mediterranean population is particular, being tightly linked to marine habitats. With less than 80 breeding pairs distributed within Corsica, Balearics, Morocco and Algeria, the Mediterranean osprey's population has suffered important demographic decreases and currently shows traits of weakness and instabilities in a long time span.

Generally, conservation strategies aim at protecting existing populations in their own habitat (in-situ conservation plans) and/or at integrating ex-situ conservation plans, when needed, by creating new wild populations away from their native habitat; Gipps, 1991; Bowles & Whelan, 1996). An increase in number and size of the threatened populations can reduce the risk of extinction both at the local and global scale (Primack, 2000).

Here local examples of conservation management approach for osprey are presented for three different sites of the Mediterranean basin.

Case study I and II: Corsica and Morocco

Mediterranean Sea represents one of the most exploited marine environments, where intense commercial and touristic activities produce continuous disturbances that are affecting the biological diversity at different scales (Charton *et al.*, 2000; Lloret & Planes, 2003). In order to achieve the aims of conservation and management of coastal and marine habitats a network of terrestrial and marine reserves has been established. Altought reserves are widely accepted as an effective tool for species conservation, the extraction of some natural resources and/or the human disturbance to wildlife is still abundant and it is seriously affecting biodiversity whithin each level (Ward *et al.*, 2001). These kinds of threats are discussed for the osprey populations of Corsica and Morocco, where human disturbance seems to jeopardise these vulnerable breeding nucleus. Sound in-situ management actions should be adopted at these sites for safeguarding species' persistence in the future.

Case III: Italy

In the background of conservation actions, reintroductions programmes represent the ultimate to re-establish species or populations in the places where they disappeared and from which the past causes of extinction are known and eliminated (Griffith *et al.*, 1989; Seddon *et al.*, 2007). Any reintroduction programme is considered successful when the new population becomes self-sustaining (Schaub *et al.*, 2009), satisfying simultaneously the following conditions: (1) the population had to reach a good stability or an evident increase during time; (2) human operations are no more necessary; (3) the species/population has recovered its former genetic diversity and expresses meta-population dynamics (when dispersal and genetic flow with other populations occurred). Generally, the reintroduced populations that have successfully reached their carrying capacity are those with the highest and more stable growth rates (Robert *et al.*, 2015). These characteristics would be well structured in any wild population, especially for those that, being small and/or isolated, risk entering in an extinction vortex due to the inbreeding depression, to the casual demographic trends, to environment stochasticity and/or catastrophes, at any time (Höglund, 2009). Then, reintroductions must be considered within a more integrated approach that works at different resolution scales (population, metapopulation and ecosystem level; Armstrong & Seddon, 2008). In fact, starting from the population level, pre- and post-release management affect post-release survival and dispersal of translocated individuals, ultimately influencing their successful establishment and a possible persistence during a greater time span. Hence, both positive and stable trend of a population can directly lead it to be regulated by metapopulation dynamics and by several ecosystem factors (Armstrong & Seddon, 2008). That's true especially for the first phases of population establishment. Here, I report results from the osprey reintroduction project in central Italy, paying attention to estimate apparent survival rate of translocated individuals and describe patterns of initial dispersal. Finally, as important milestone of the reintroduction program, first breeding occurrences have been reported.

10. THE PRICE OF SUCCESS: NEGATIVE IMPACT OF TOURISM UPON AN EMBLEMATIC MEDITERRANEAN RAPTOR IN A MARINE PROTECTED AREA.

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10.1. ABSTRACT

Disturbance of charismatic wildlife by eco-tourism has become a major concern in the last decades. In the Mediterranean, sea-based tourism and related recreational activities increased rapidly, especially within marine protected areas (MPAs) hosting emblematic biodiversity. Here we investigated the impact of the Scandola MPA (Corsica, Western Mediterranean) on the population of a conservation flagship, the Osprey *Pandion haliaetus*. Over the 37-years study tourists flow increased rapidly. Osprey breeding performance initially increased, but then dropped for pairs nesting within the MPA compared to those breeding elsewhere in Corsica. Recent osprey breeding failures in the reserve are not caused by food scarcity, since 98 underwater transects and GPS-tracking of nine breeding adults which we conducted in 2012-2013 showed that fish consumed by ospreys were more numerous inside the MPA. Further, we performed focal observation at nests in 2013 and 2014, which revealed that the overall number of boat passages and of boat approaches within <250 m from osprey nests were significantly higher within the MPA than in a control area. Further observations at osprey nests conducted across 2012-2014 demonstrated that boat traffic modified osprey time-budgets significantly, by decreasing the number of prey items brought to the nest by males, and increasing time spent alarming and flying off the nest by females. Finally, corticosterone levels in chick feathers from Scandola were 3 times higher than in places with lower tourist flow in Corsica, the Balearic Islands and Italy. This strongly suggests high stress levels in chick within the Scandola MPA. Overall, our integrative assessment combining raptor demography, movement ecology, behavioural ecology, with fish and boat surveys clearly demonstrates the impact of the Scandola MPA on the Corsican osprey population.

This case study stresses the worldwide importance of rigorously implementing sustainable ecotourism.

Keywords: corticosterone, human-wildlife conflict, flagship species, osprey, Marine Protected Area, nautical traffic, population dynamics, GPS tracking, fish census.

10.2. INTRODUCTION

There is a wide consensus upon the importance of protected areas for preserving biodiversity (Primack, 2000; Fraser & Bernatchez, 2001). The designation of conservation units is often motivated by the protection of rare flagship species, which is an efficient way to gain support from the public and to attract funding. Such charisma is often ecologically justified (Sergio *et al.*, 2006), whereby large predators serve as umbrella-species allowing the conservation of entire communities (Crooks & Sanjayan, 2006). Yet reserves also attract ecotourists, which may become a threat for local biodiversity, including the flagship species they are interested to observe in the wild (Tapper, 2006). This is the case for grizzly bears *Ursus arctos* in North America (e.g. Hood & Parker, 2001), Amur tigers *Panthera tigris altaica* in Russia (Kerley *et al.*, 2002) and Imperial Eagles *Aquila aldalberti* in Spain (González *et al.*, 2006).

In the marine environment, marine protected areas (MPA) are an essential conservation tool (Leenhardt *et al.*, 2013); more than 6500 MPAs have already been implemented worldwide, and countries of the European Union are planning to establish marine protected areas over 10% of their national waters by 2020 (Lubchenco *et al.*, 2003; McCook *et al.*, 2010; Velando & Munilla, 2011). MPAs proved to be efficient tools for the preservation of benthic communities (Selig & Bruno, 2010) and of the pelagic realm and its associated top-predators (Pichegru *et al.*, 2010; Aburto-Oropeza *et al.*, 2011; Péron *et al.*, 2013). Similarly to terrestrial reserves, MPAs are being marketed for eco-tourism, and therefore tend to attract more visitors than ‘unprotected’ areas. This leads to a potential direct disturbance by recreational activities, which have been already reported for populations of fish (e.g. Bracciali *et al.*, 2012), seabirds (McClung *et al.*, 2004; Velando & Munilla, 2011), marine mammals (Hodgson & Marsh, 2007; Vermeulen *et al.*, 2012), sea turtles (Hazel *et al.*, 2007) and related marine habitats (e.g. Lloret *et al.*, 2008).

Herein, we present an integrative study of the impact of touristic-associated activities on the Scandola MPA and its emblematic raptor, the Osprey *Pandion haliaetus*. The Osprey is a flagship species for conservation across its whole distributional range. It is often seen as a symbol of nature comeback, saved from extinction due to DDT usage and direct persecution by means of successful direct management actions and reintroductions programmes (Ames, 1966; Martell *et al.*, 2002; Dennis & Dixon, 2001). In North America and Europe, regional socio-economic marketing strategies (e.g. ecotourism) are often tightly linked to the presence of ospreys (e.g. Loch Garden and Rutland Water in UK; Mackrill *et al.*, 2013). They also serve as boundary objects (*sensu* Star & Griesemer, 1989) enhancing the awareness of the public, and that of policy makers, with respect to environmental issues. This is very much the case in the Mediterranean region, where the presence of ospreys is rare but essential e.g. to facilitate the establishment and adequate management of reserves in Morocco (Al Hoceima National Park), Italy (Maremma Regional Park), Spain (embalse Guadalcacin, Barbate reservoir in Andalucia), and France (Scandola reserve, Corsica) (Monti, 2012; Monti *et al.*, 2013; 2014).

In the Mediterranean, the osprey is the only raptor entirely dependent on the marine environment across its life cycle, as it feeds exclusively on live, epipelagic fish. Ospreys mainly nest on rocky pinnacles in sea-cliffs, at heights between 5-30 m (Cramp & Simmons, 1980). The island of Corsica currently hosts the largest osprey population in the Mediterranean, with a breeding nucleus of ca. 30 pairs (37.5% of the entire estimated Mediterranean population; Monti *et al.*, 2012). At the beginning of the 20th century 40-100 breeding pairs occupied most of the Corsican coastline, but in 1974 this population had shrunk to three pairs, mainly due to direct persecutions (Thibault *et al.*, 2001; Thibault & Bretagnolle, 2001). In 1975, the Natural Reserve of Scandola, a marine and terrestrial protected area located along the northwestern coast of Corsica (Fig. 1b) was created with the main objective to protect the last breeding pairs of ospreys (Thibault *et al.*, 2001). The osprey population then gradually recovered, and it is currently present along most of the west coast of the island (Bretagnolle *et al.*, 2008). However, population growth rate started diminishing since the 1990s, and breeding success seemed to decline from 2000 (Bretagnolle *et al.*, 2008).

In this study, we aimed at understanding to what extent the management of the MPA of Scandola affected the Corsican osprey population, and tested two competing hypotheses: (1) the reserve, since its creation, had a positive incidence on osprey population dynamics. In particular, we postulated that the establishment of the reserve might have played an important role in producing multiple indirect benefits such as: a) better protection of birds because of reduced human disturbance; and b) greater food availability in terms of fish abundance, fostered by the fishing ban within the reserve. As an alternative hypothesis, we postulated that (2) the Scandola MPA generated additional constrains, due to sea-based tourism and recreational activities, called for by the existence of the MPA, unique landscape features and the presence of emblematic ospreys. In this context, our specific goals were: a) to reconstruct historical trends of the sea-based tourism expansion in Corsica and to connect it with historical osprey population trends and breeding parameters, in areas of either intense or low boat traffic; b) to quantify marine traffic within the Scandola MPA during summer (the highly sensitive chick-rearing period for ospreys); c) to quantify the abundance of prey (sub-surface coastal fish) along the Corsican coast and d) to assess the effect of boat traffic on osprey adult behaviour and chick corticosterone levels (following Bortolotti *et al.*, 2008).

Our integrative analyses have important implications for MPA design and management, and the conservation of Mediterranean biodiversity.

10.3. MATERIAL AND METHODS

a. Study area

This study was conducted along the 250 km of the west coast of Corsica (France), from Cape Corse in the North, to Ajaccio in the South, where the entire Corsican osprey population is known to breed (Thibault *et al.*, 2001; Fig. 1a). The study area includes the Scandola MPA (42° 358’N, 8° 560’E), which is both a terrestrial and a marine protected area of ca. 2,000 ha and, since 1983, a UNESCO World Heritage Site (Fig. 1b). Due to its rugged terrain Scandola is almost exclusively visited by sea, with ca. 300,000 visitors concentrated between June and August (Richez & Richez Battesti, 2007; Tavernier, 2010). This sea-based tourism increased rapidly during the last decades, along with the total number of visitors to the whole island (Fig. 2). Fishing within the reserve is limited to 12 professional local fishermen, but no limit is set upon the number of visiting vessels: ca. 400 boat passages occurs each day between June and August (Morvan, 2010); approaching the coastline for visiting geologic formations, marine caves and osprey nests, is a common practice (Thibault *et al.*, 2001).

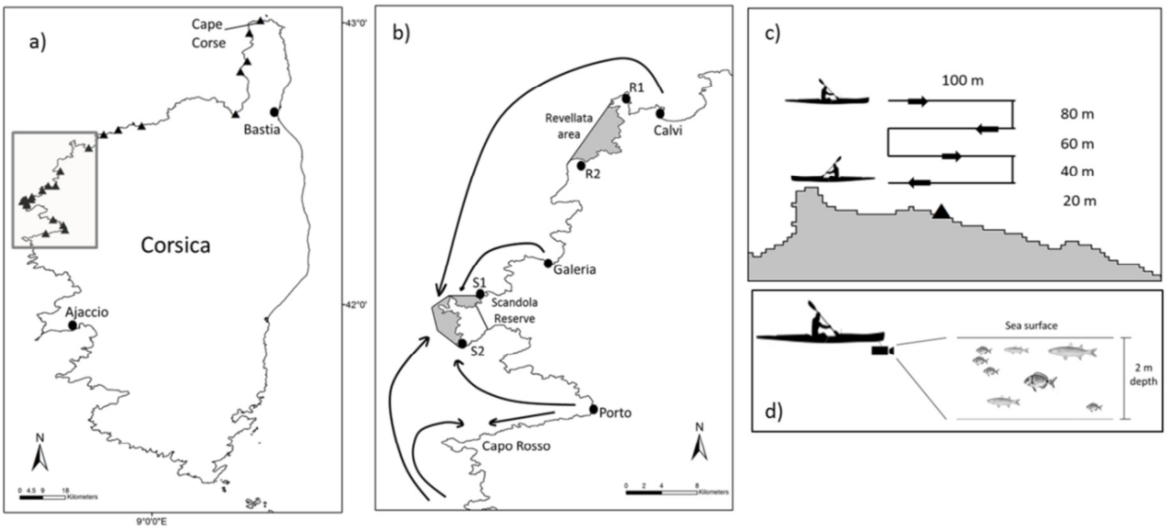

Figure 1: a) position of the 24 transects spread along the west coast of Corsica (transects were determined according to the position of osprey territories); b) zoom on the two areas of the Scandola Reserve and of the Revellata area (coloured in grey); for each area land-based vantage-points are reported as: R1, R2, S1 and S2; harbours and main touristic boat circuits are also included, according to: Richez & Richez Battesti, 2007; Tavernier, 2010); c) structure of the transect for fish video recording from a kayak; d) simplified view of the water column recorded by the camera attached to the bow of the kayak.

b. Historical osprey population dynamics

The Corsican osprey population has been monitored since 1977 (Thibault *et al.*, 2011; Bretagnolle *et al.*, 2008). Available historical breeding data used for our analyses covered a 37-years period (1977 to 2014). For each nest site and each year the following parameters were recorded: number of eggs laid, number of eggs hatched and number of chicks fledged. From these, we calculated an annual breeding success (young fledged/eggs laid), hatching success (young hatched/eggs laid) and fledging success (young fledged/eggs hatched). Nests were grouped (1) with respect to their position inside/outside of the Scandola MPA and 2) regarding their vicinity to touristic boat circuits. We considered the time variable as the number of years elapsed after the reserve's creation in 1975 (variable "time_since_reserve"). Since Bretagnolle *et al.* (2008) found density-dependent processes in breeding parameters occurring after 1990, we also analysed processes before and after this threshold year. We fitted generalized linear mixed-effect models (GLMMs) using the above cited breeding parameters as response variables. The binary variable (0="out of the reserve" or 1="inside reserve") and "time_since_reserve" (or alternatively the threshold of 1990) were used as fixed factors. Nest and "time_since_reserve" were also included as random effects, to avoid pseudoreplication at the level of territories, and to account for between-year variation. A Poisson error distribution was set for discrete categories such as number of eggs laid, young hatched and fledged, while the binomial error distribution was fixed for hatching, fledging and breeding success. We used AICc values to compare model fit; all modelling was conducted in R 2.15.0 (R Core Development Team). Unless stated, all average values are given with standard deviation.

a. Feeding areas of breeders

Foraging home ranges of 9 breeding adult ospreys (2 males and 7 females) were determined by GPS tracking (see details in chapter 2 of the thesis). Birds were trapped at nests before the beginning of the breeding season (early March 2012 and 2013) and fitted with a GPS/GSM tag (Duck-4 model, ECOTONE, Poland, 35 x 55 x 15 mm, 24 g ~ 1.5% of body mass). Devices recorded one fix every 30 minutes across the entire breeding season (March-July). Since parental care and nest attendance is performed by both parents during incubation and chick rearing (Poole, 1989), we defined as failures any abrupt abandonment of the nesting site. In case of breeding failure, atypical ranging movements performed by birds were excluded from home range analyses. Thus, home ranges were calculated only during breeding attempts. We used a fixed kernel density estimator (Worton, 1989), under the Hawth's Tool extension as implemented in ArcGis v 9.3.2 (Hooge & Eichenlaub 2000; www.esri.com), and the Animal Movement Extension to calculate 95% foraging home ranges (UD95%) and 50% core foraging areas (UD50%).

b. Prey distribution and accessibility

We assessed prey availability to ospreys at 24 sites hosting osprey nests along the west coast of Corsica (Fig. 1a). Eight sites were located within the Scandola MPA, while the remaining 16 sites were located outside the MPA. Surveys were performed twice for each site, i.e. a total of 48 transects per year. The monitoring protocol was repeated in 2012 and 2013, yielding a total of 96 transects.

The subsurface area (0-2m depth), which corresponds to the osprey feeding horizon, was filmed with a HD-Hero 2 GoPro camera (USA) attached below the bow of a kayak, set with a wide angle of 170° to scan a field of approximately 3 m left/right. Transects were composed by 4 stretches of 100 m parallel to the coastline, set 20, 40, 60 and 80 m away from the shoreline (Fig. 1c-1d). Each transect was pre-recorded on a GPS, which allowed the paddler to maintain headings and speed (ca. 5 $km.h^{-1}$). Transects were performed during the osprey breeding season (in June and July), during daylight and on calm days, to optimize viewing conditions and mimic osprey foraging conditions (as ospreys do not hunt at sea when conditions are harsh; F. Monti unpublished results). We also used a Secchi disc to control water turbidity and to ascertain good visibility conditions before each transect.

Video recordings were inspected by two different persons to minimize errors in fish species identification and counting. Each fish was identified following Louisy & Trainito (2010). Since objects appear 4/3 larger in water than in the air (Kinney, 1985; Ross & Nawaz, 2003), we performed preliminary tests using fish models of different sizes to calibrate fish sizes estimates. To further limit such errors, we used five size classes (1 = <10 cm; 2 = 10-20 cm; 3 = 20-30 cm; 4 = 30-40 cm; 5 = > 40 cm). To estimate biomass from underwater length observations we used the following formula: $W = aL^b$, where W is mass in grams; L is the standard length in centimetres and a and b are constants, following (Ricker, 1973; 1975) and Morey *et al.* (2003). For each transect we calculated the following parameters: a) total number of fish per transect; b) total fish biomass (g) along the transect; c) density index (total number of fish per m transect); and d) the total number of fish >20 cm per transect. For data analyses all parameters were log+1 transformed to account for normality; sites were ranked as 0 (outside reserve) and 1 (inside reserve). We used general linear models (GLM) to test between-year effects (2012 vs 2013). We then ran GLMM including ’year' and ‘transect’ as random effects and log of biomass, log of number of fish and log of density index as dependent variables.

c. Tourism and boat traffic

The number of tourist shuttles operating within the protected area of Scandola and their transport capacity in terms of passengers increased from only 3 ships transporting ca. 200 persons per day in 1977 to 32 ships transporting ca. 2,200 persons per day in 2010 (Richez & Richez Battesti, 2007; Tavernier, 2010). However, data were not available for each year during the study period. Therefore, we extracted the total annual number of tourists visiting Corsica (by airline or ferry companies) between 1986-2014 using data from the Observatoire régional des transports de la Corse (www.ortc.info; Fig. 2). A strong positive correlation was found between the annual number of tourists visiting Corsica and the number of shuttles working within Scandola (Spearman rank correlation: $r_{S(12)} = 0.963, p < 0.001$; Fig. 2). Accordingly, we used the total annual number of tourists visiting Corsica as a proxy for the annual and daily tourist inflow into the Scandola MPA.

We carried out two further censuses in 2013 and 2014. In 2013 we assessed the at-sea distribution and frequency of boat passages within the reserve, as well as boat distance to the shore (a = 0-250 m; b > 250 m), since under 250 m ospreys are systematically disturbed by approaching boats (Bretagnolle & Thibault, 1993). Two land-based vantage-points, located at the northern and southern limits of the reserve were used to monitor boat entrances and exits. The same observations were performed within a control area (Revellata) with a similar density of osprey nests located outside of the reserve. Both of these areas are located between two harbours from which tourist boats depart (Fig. 1b). Two observers worked simultaneously in each area between 9:00-17:00 during 4 observation-days (two days during the second half of June and two during the first half of July 2013). We selected this period because it corresponds to osprey chick-rearing, during which disturbance is critical in this species (Poole, 1989), and in birds in general (e.g. Leseberg *et al.*, 2000; McClung *et al.*, 2004; González *et al.*, 2006; Martínez-Abraín *et al.*, 2010). In 2014 the number of boat passages at osprey nests was recorded while studying the behaviour of breeding pairs (see details below). In this case, distance categories considered for boat passages were a) 0-100 m and b) 100-250 m, to focus on boats that were more likely to disturb ospreys.

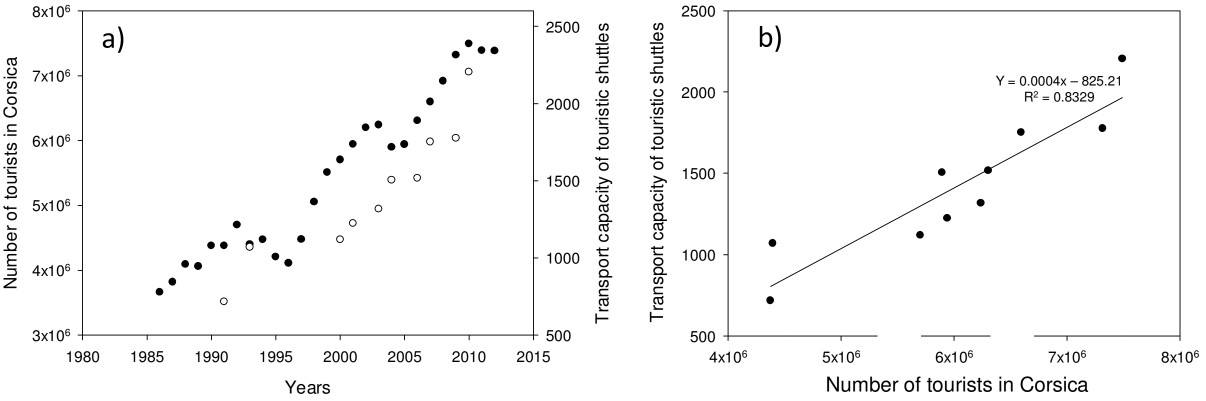

Figure 2: a) historical trends of total annual numbers of tourists (millions) visiting Corsica during 1986-2012 (black dots; data extracted from: http://www.ortc.info) and of the transport capacity of tourist shuttles operating in the Scandola MPAs (open dots; data extracted from Richez & Richez Battesti, 2007; Tavernier, 2010); b) linear regression between annual estimates of number of tourists in Corsica and transport capacity of tourist shuttles operating in the reserve.

d. Behavioural observations

Between 2012 and 2014, focal observations at osprey nests were carried out from vantage-points located at a minimum distance of 300 m from occupied nests. Each nest was monitored at least once from 6:00 to 20:00 and, when possible, we performed 2 or 3 full-day repetitions.

Behaviour of the focal animal was observed with binoculars, and a 60x telescope to confirm specific behavioural events (e.g. successful fishing, chick feeding). Following standard criteria for osprey behaviour classification (Bretagnolle & Thibault, 1993), we considered the following variables: a) time spent by the pair at the nest, and alternatively away from it, as a proxy for parental care (e.g. McClung *et al.*, 2004); b) number of prey items brought per hour; c) total amount of time alarming for an approaching boat. We also recorded the occurrence of alarm calls and of flights triggered by boat passages. We ran a Friedman test (for k related samples) to test whether daily differences in behaviour occurred for nests monitored for more than one day during the same year. For each behavioural variable, we tested if birds from a nest being placed along tourist shuttle circuit behaved differently than birds nesting in low traffic sites; the binary variable 0="low traffic" or 1="high traffic" was used as fixed factor in GLMMs.

e. Stress level of chicks

Since corticosterone in feathers is stable over time and indicates the overall exposure of the individual to this hormone over a time of days and weeks (Bortolotti *et al.*, 2009), we sampled body feathers of osprey chicks to have indicative values of corticosterone levels, and hence of stress during the chick-rearing period. Following the previous classification for tourism and boat traffic (see methods), we distinguished samples collected at nests in high ($n = 4$) and low ($n = 5$) traffic areas. As a control, we also included samples from undisturbed chicks from Italy ($n = 4$) and the Balearic islands ($n = 5$).

Feathers were stored in paper envelopes before analyses, during which we extracted corticosterone following Bortolotti *et al.* (2008). Before removing the calamus we measured the length of the feather. Feathers were then cut into pieces < 5 mm^2 and placed in 16 x 100 mm glass tubes. Three glass beads and 10 ml methanol (HPLC grade) were added and the tubes were placed into an ultrasonic waterbath for 30 min and then at 50° C overnight. The methanol mixture was filtered through filter paper placed on a glass funnel. The methanol extracts were collected in extraction tubes placed in a 50° C waterbath until dry. Feather extracts were then redissolved in 200ul steroid dilution of the ICN I^{125}radioimmunoassay kit (Cat. #07-120102; ICN Biomedicals/MP Biomedicals, Solon, Ohio; USA) for measurements. We followed the protocol of the company with modifications as described in Washburn *et al.* (2002): the volume of all reagents was halved; the dilution of the samples was performed at 1:50 instead of 1:200. The standard curve was extended by 2 points.

10.4. RESULTS

a. Historical trends in population and breeding parameters

We analysed a total of 745 nest-data occurrences across a 37-years period (from 1977 to 2014). The Corsican osprey population increased from 3 to a maximum of 34 breeding pairs (in 2011). Numbers of pairs and chicks fledged as well as reproductive parameters (hatching, fledging and breeding success) varied substantially over time (Fig. 3).

We found no significant differences in the average number of eggs laid per nest (2.84 ± 0.39) for pairs breeding inside or outside the reserve (Appendix 3: Additional file 1; Fig. 4a). Conversely, a significant decrease in the average number of eggs hatched was recorded in the reserve (Appendix 3: Additional file 1; Fig. 4b). Further, the number of chicks fledged did not change over time for territories outside the reserve, while a strong reduction occurred inside the reserve (Appendix 3: Additional file 1; outside the reserve = 1.36 ± 0.51 chicks fledged; inside the reserve = 1.21 ± 0.73 chicks fledged; Fig. 4c). Similarly, hatching success decreased in the reserve and increased outside of the reserve with time (Fig. 4d). Fledging success showed a general decrease over time in Corsica as a whole (both inside and outside the reserve; outside the reserve = 0.67 ± 0.20; inside the reserve = 0.65 ± 0.27), but the decline was stronger inside the reserve (Fig. 4e). Finally, breeding success decreased strongly over time for pairs breeding in the reserve, compared to those nesting outside (outside of the reserve = 0.50 ± 0.19; inside of the reserve = 0.39 ± 0.23; Fig. 4f). When testing for a density dependent effect (using 1990 as a threshold year), all breeding parameters significantly declined after 1990 (Appendix 3: Additional file 1). Finally, annual trends for breeding success (outside/inside the reserve) were significantly correlated to the annual number of visitors to Corsica (Fig. 5).

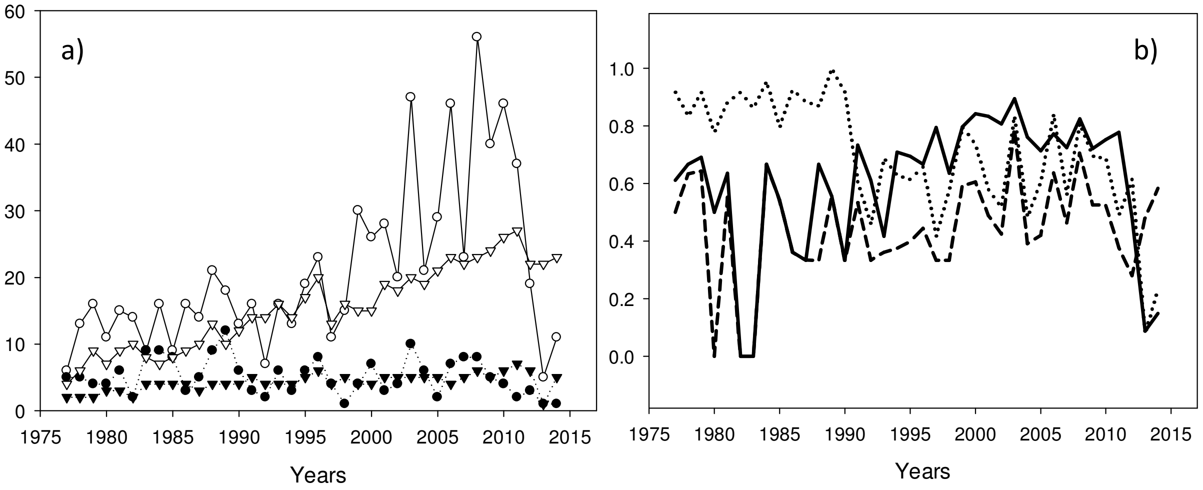

Figure 3: Historical trend of the Corsican osprey population in 1977-2014: a) number of breeding pairs and chicks fledged outside of the reserve (white triangles and dots,

respectively, and solid lines) and inside the reserve (black triangles and dots, respectively, and dotted lines); b) hatching (dashed line), fledging (dotted line) and breeding success (solid line) in the whole Corsica, over time.

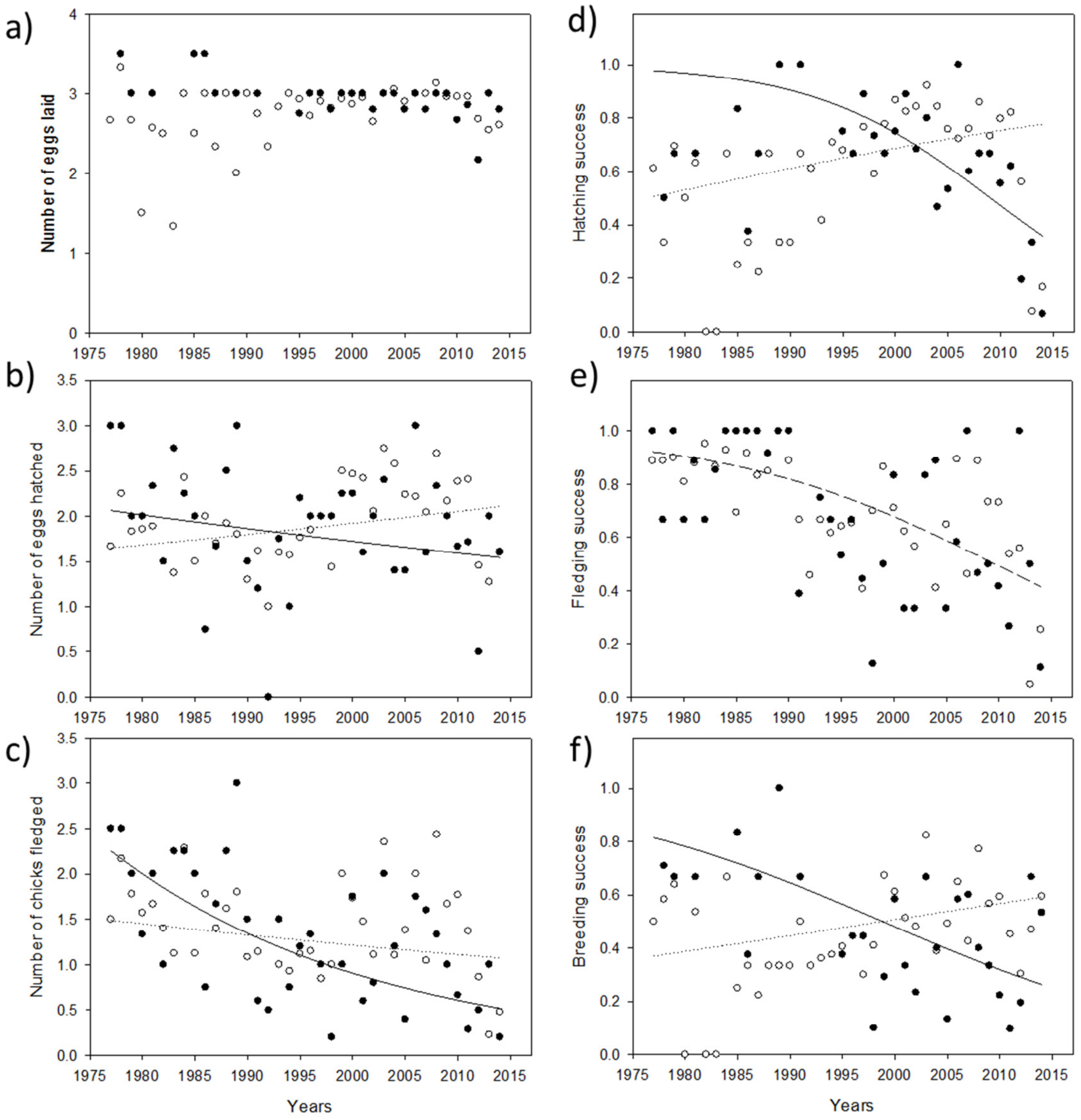

Figure 4: Historical trend in Corsican osprey breeding parameters within the Scandola MPA (black dots and solid line) and outside of it (white dots and dotted line): a) number of eggs laid; b) number of eggs hatched; c) number of chicks fledged; d) hatching success; e) fledging success (dashed line for the whole Corsica) and f) breeding success. Dots represent raw data and lines estimates of the selected model from GLMM (see Annex 3: Additional file 1).

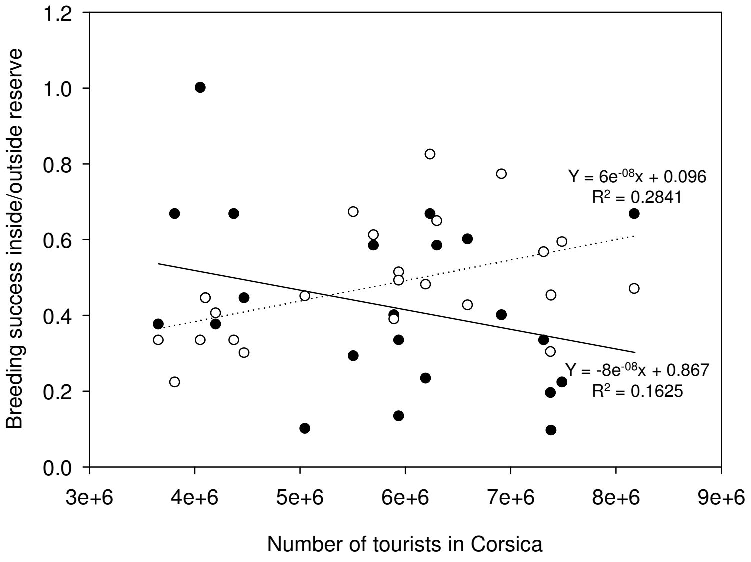

Figure 5: Linear regressions between breeding success inside (black dots and solid line; $F_{1,20}$ = 5.076, p= 0.035, r^2 = 0.16) and outside (white dots and dotted line; $F_{1,25}$ = 11.32, p= 0.0024, r^2 = 0.28) the reserve and number of tourists in Corsica.

b. Home ranges and feeding areas of breeding ospreys

Home ranges estimated during the breeding season showed that feeding areas of adult ospreys were concentrated along the coast (Fig. 6; Appendix 3: Additional file 2). Ospreys never ventured offshore to fish (median distance from the coast = 0.012 km, range: 0-3.2 km), but rather remained in the surroundings of the nesting sites, fishing in marine coves. Mean individual foraging home range was 64.05 ± 59.54 km^2 and mean core feeding area 5.5 ± 3.57 km^2. Exploratory foraging trips were performed by ospreys along rivers when sea conditions were exceptionally harsh for an extended period (Fig. 6).

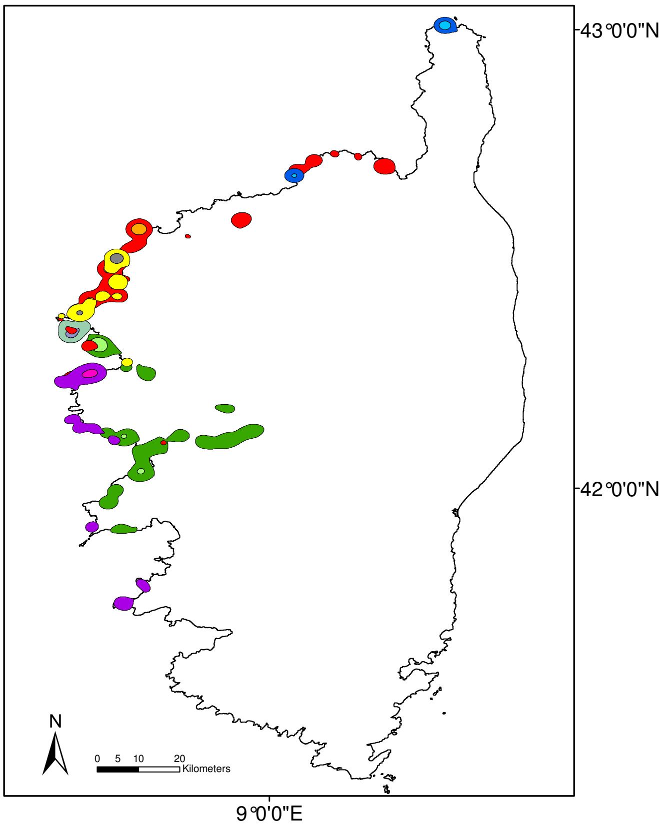

Figure 6: Foraging home ranges (fixed kernel at 95%) and core foraging areas (fixed kernel at 50%) with darker and lighter colours respectively: each colour represents one of the 9 adult ospreys monitored during the breeding season in Corsica.

c. Prey distribution and accessibility

Fish biomass, fish numbers and density followed a Gaussian distribution after a logarithmic transformation (Shapiro-Wilk normality test: Log_Biomass, W=0.94 $p<0.0001$; Log_Number of fish, W=0.96 $p<0.0001$; Log_Density Index, W=0.69 $p<0.0001$). There were no significant differences between 2012 and 2013 for the three parameters: Log_Biomass (GLM: $F_{1,93}$ =0.426, p=0.515), Log_Number of fish (GLM: $F_{1,93}$=0.0, p=0.991), Log_Density Index (GLM: $F_{1,93}$=1.17, p=0.281). We therefore pooled data across years. Our models showed a strong reserve effect, and the three parameters considered were not affected by random effects such as transect and year repetitions. The reserve hosted a larger number of fish (Log_Number of fish: $F_{1,96}$ = 0.38 p = 0.016) and a higher total biomass (Log_Biomass: $F_{1,96}$ = 0.90 p = 0.001) compared to sites located outside of the reserve (Fig. 7), although the density index was not significantly higher (Log_Density Index: $F_{1,96}$ = 0.005 p = 0.617). Furthermore, within the reserve, large fish (> 20 cm) tended to be more abundant (reserve = 6.12 ± 11.2; outside = 1.9 ± 8.9).

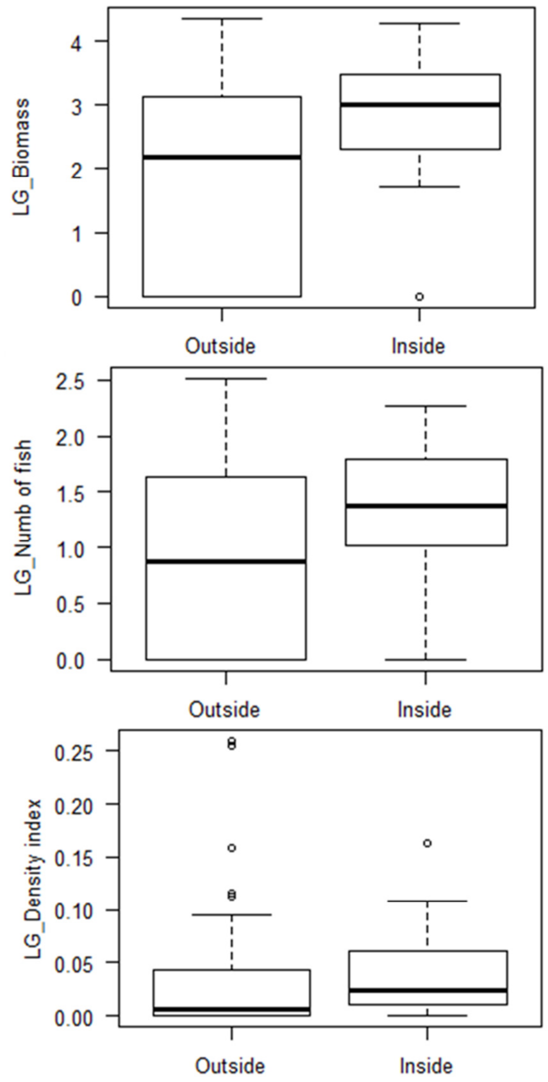

Figure 7: Mean values of biomass, number and density index of fish (expressed as Log normal function) for transects located inside and outside of the reserve.

d. Evaluation of boat traffic

The total annual number of tourists visiting Corsica increased consistently, from ca. 3.6 millions in 1986 to ca. 7.5 millions in 2013 (Fig. 2; source www.ortc.info). Our census conducted in 2013 showed that the number of boats visiting the reserve each day (June: 221 ± 29.69; July: 388 ± 43.84) was twice that recorded within the control area outside of the reserve (June: 100 ± 46.66; July: 192 ± 43.84). In both cases, numbers practically doubled in July compared to June (Fig. 8). Further, more boats approached the coastline <250 m within the reserve (June: 252 ± 79.2; July: 288 ± 28.28) than within the control area (June: 35.5 ± 27.57; July: 106.5 ± 10.60). The number of boats passing at a distance of >250 m from the coast was similar between the two areas in both months (Reserve = June: 54.5 ± 12.02; July: 100 ± 15.55; Control area = June: 64.5 ± 19.09; July: 85.5 ± 33.23).

In 2014, the number of boat passing close to osprey nests (<250 m) was significantly higher for nests located inside the reserve than for those outside of the reserve (GLMM: $\chi^2_{1,147}$ =10.484; p = 0.001), especially when considering those passing at <100 m (GLMM: $\chi^2_{1,147}$ =15.95; p = 0.000).

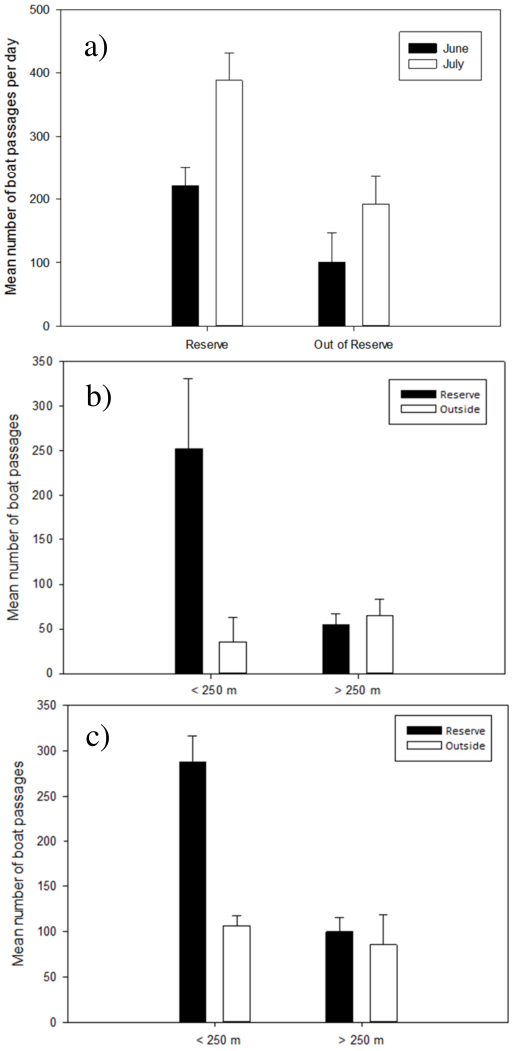

Figure 8: Boat traffic during summer in Corsica: a) mean number of boat passages per day in June and July for sites inside and outside of the reserve. b) and c) mean number of boat passages per day < 250 m and > 250 m from the coast in June and July, respectively.

e. Bird behaviour at nest and level of stress

Overall, 41 days of observations (ca. 570 hours) were carried out over the 3 years at 13 nest sites, 10 outside of the reserve and 3 inside. Because samples were unbalanced between these 2 categories, we used a classification based on the intensity of tourist boat traffic, whereby 6 nests were located in ‘high traffic’ areas and 7 in ‘low traffic’ areas (see methods). All nests except one were observed for a minimum of 2 days and six of them also for 3 days. We found no significant differences among 2- or 3-days repetitions in any of the behavioural patterns considered for each nest (Friedman test for each behaviour: all $p > 0.05$). Data were therefore pooled across day-repetitions.

The number of prey items brought to the nest per hour was 50% lower (GLMM: $\chi^2_{1,41} = 6.26$; $p = 0.012$) for nests located in high traffic areas (Fig. 9). At these nests the occurrence of disturbing events was also six times greater than at low traffic areas (GLMM: $\chi^2_{1,41} = 7.229$; $p = 0.007$). The number of occasions in which parents left the nest after a disturbance tended to be higher for nest located within high traffic areas (GLMM: $\chi^2_{1,41} = 3.691$; $p = 0.054$). Females rearing chicks at high traffic sites spent more time alarming for an approaching boat (GLMM: $\chi^2_{1,41} = 4.559$; $p = 0.032$). All the other behaviours considered did not vary significantly between nests belonging to the two categories.

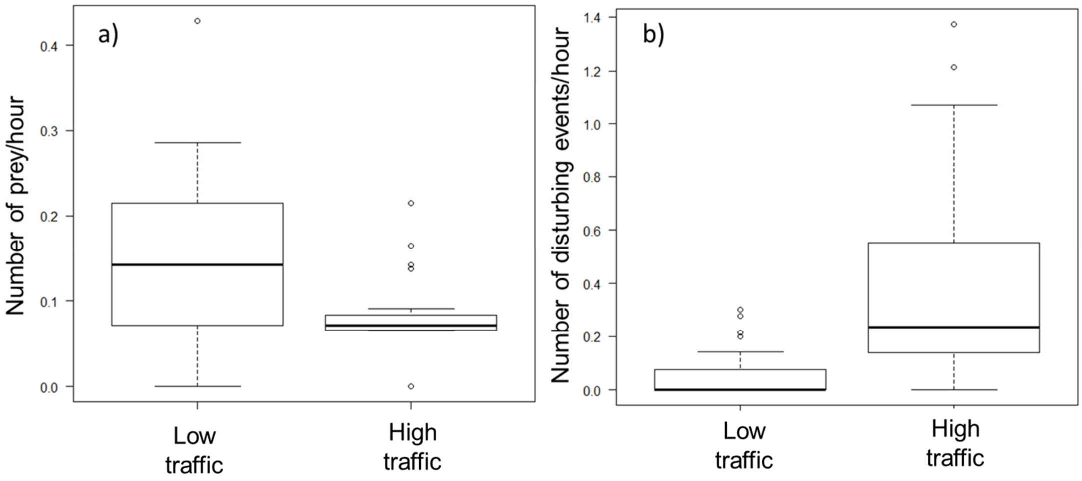

Figure 9: a) number of prey items brought to the nest per hour by male ospreys and b) disturbing events per hour in ‘low traffic’ and ‘high traffic’ areas in Corsica.

To evaluate chick stress levels, we tested both the concentration (ng mg^{-1}) and the temporal expression of corticosterone (ng mm^{-1}). In both cases we found that values for chicks from high traffic areas were significantly higher than those recorded at other nests in

Corsica (Mann-Whitney *U*-Test: U = 1.0; p = 0.027; N = 9) and at non-disturbed nests in general (i.e. including control samples: Mann-Whitney *U*-Test: U = 1.0; p = 0.004; N = 18) (Fig. 10). Values also differed when considering each location separately (Fig. 10; Kruskal-Wallis: $\chi^2 = 11.42$, df = 3, p = 0.010, N = 18).

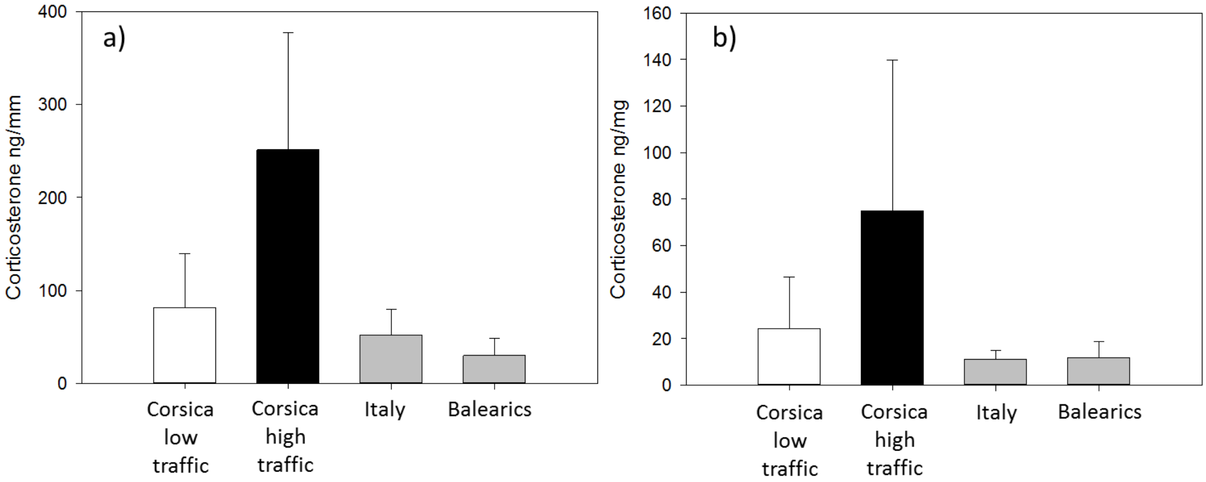

Figure 10: Mean values of a) corticosterone temporal expression (ng/mm) and b) its concentration (ng/mg) for each locality. Corsica has been split in low and high traffic areas (respectively white and black bars); other sites (Italy and Balearics) in grey bars.

10.5. DISCUSSION

Our extensive, long-term and multidisciplinary data set allowed a very detailed investigation of the incidence of the current management of the Scandola MPA on the status of a Mediterranean conservation flagship species, the osprey. This unique information allowed us to validate our two working hypotheses: (1) the MPA had a positive effect on the Corsican osprey population during the first 20 years, yet (2) following recent increase in ship traffic, ospreys breeding at sites facing the Scandola MPA are now being critically disturbed, and their breeding performance has dropped despite the fact that they could rely upon fish resources which are more abundant than elsewhere outside of the reserve. Our case study should motivate improved management of ecotouristic activities linked to charismatic species.

Specifically, we demonstrate that most breeding performance parameters declined over time (especially since 2000) for pairs breeding within reserve's boundaries, compared to those breeding outside of the protected area. Although this may also be partly explained by density-dependent population regulation (Bretagnolle *et al.*, 2008), we show that the reserve played an important role in shaping population trends over time. For instance, Bretagnolle *et al.* (2008) reported a temporal increase in nest density in the central, historical breeding area (from Calvi to Porto). However, this area encompassed both nests located inside, and outside of the reserve (e.g. sites in the Revellata area or south of the reserve). Disturbance by ship traffic and density dependence processes therefore acted simultaneously across these sites. Nevertheless, our results showed evident contrasting trends between the two categories (inside/outside reserve): nests for which breeding parameters were negatively affected were mostly situated inside of the reserve.

In order to clarify the proximate causes of such discrepancies, two lines if thought can be developed:

1) Local prey abundance affects osprey breeding success. As expected, we found that sites within the reserve hosted greater fish populations with larger body size (and, consequently, higher biomass) than sites outside the reserve. This can be explained by the positive effect of fishing banning in the protected area, which favours protection and allows more fish to reach adulthood and reproduce. The role of MPAs as source areas for fish dispersal has been largely acknowledged (Francour *et al.*, 2001), especially in terms of marine population connectivity (Cowen & Sponaugle, 2009). In particular, the Scandola reserve has the reputation to be one of the most important marine biodiversity hotspots in the Western Mediterranean basin. Thanks to long-term underwater protection (Francour, 1994; Ward *et al.*, 1999; Francour *et al.*, 2001), all Scandola marine biotas are well-preserved, and their finely-structured trophic webs maintained (Francour, 1994). These positive MPA effects are substantial for the local fish fauna (Francour *et al.* 2001), including fish predated by ospreys (Francour & Thibault, 1996). Therefore, the MPA played a positive role, by providing abundant food resources to foraging ospreys. These results are coherent with GPS-tracking of breeding adults (Fig. 6), which showed that their feeding home ranges were extremely small, and largely confined to coastal areas adjacent to breeding sites.

2) Massive summer boat traffic within the reserve, linked to sea-based tourism, explains the recent decline in osprey breeding performances.

Focused observations upon the number of boat passages per day were conducted in the summer of 2010 by Morvan (2010) at two specific osprey nest sites located in the reserve. He recorded between 350 and 450 boat passages per day, with peaks occurring between 11:00-12:00 and 15:00-16:00 (Morvan, 2010). Accordingly, we found that touristic boats traffic was much more intense in the reserve than outside (especially in July during the high season). Furthermore, most (74.6%) of the boat passages in the reserve occurs at a reduced distance from the coast (< 250 m), possibly generating greater disturbance to ospreys. To understand how such boat traffic affects osprey behaviour and, ultimately, breeding performance, we tested whether the behaviour of breeding ospreys was negatively affected by the continuous boat passage and noisy stops. Our sample size was limited for nests within the reserve, because in the 3 years of the project many nests failed just before the observation protocol started. However, although most of the traffic is concentrated in the reserve because of the existence of the MPA and the presence of emblematic ospreys, even other places offering marine caves and geologic formations are being targeted by touristic companies during their daily cruises (e.g. Capo Rosso; Fig 1b). At sites located along such boat trips, nautical traffic significantly impacted osprey behaviour (Fig. 9). There, the number of disturbing events per hour was higher, with females at the nest spending more time alarming for boats approaching, and fewer prey-items were brought back to the nest by males (Fig. 9). In this context, time spent alarming or repeatedly flying off the nest may reduce time allocated to other important activities (notably foraging). Further, boats approaching too closely scare parents off the nest, which results in eggs or chicks being left unattended. The absence of parents, even for short durations, may indeed favour predator attacks (Edington & Edington, 1986; Bolduc & Guillemette, 2003), for instance by Yellow-legged gulls *Larus michaellis* or Ravens *Corvus corax*. Nautical traffic may also have further perturbing effects. In particular, epipelagic fish that constitute the main food source for ospreys may change behaviour, by switching daily activity patterns or by swimming deeper to avoid noise and fishing pressure (Bracciali *et al.*, 2012). One may also speculate that boat traffic may enhance the vorticity of surface water, perturbing the epipelagic area upon which ospreys are critically dependent for efficient foraging. Confronted with such perturbed foraging areas, ospreys may move away in search for calm waters, spending more time travelling; this may result in lower rates of food provisioning to the nest, and in lower reproductive performance.

This is supported by the fact that chicks from nests exposed to ship traffic had significantly higher corticosterone levels, indicating physiological stress. This is predicted to have a negative on chick growth and survival rates. Human recreational activities have already been identified as the cause of physiological stress impacting individual fitness. For example, a study conducted on Capercaillie *Tetrao urogallus* populations in Europe, showed that stress hormone levels increased markedly for individuals living close to winter recreational areas (Thiel *et al.*, 2011). However, we cannot attribute with certainty the observed pattern in corticosterone accumulation to boat traffic. Multiple factors can act concurrently to determine stress responses. For examples, higher concentrations of corticosterone could result from the presence of potential predators in the surroundings and/or by conspecific intrusions in the territory of their parents: in these cases female may effectively increase chick stress levels (Bretagnolle & Thibault, 1993). Another cause of stress could be food scarcity. Indeed, former studies showing elevate corticosterone levels in response to stress suggest that birds are thereby able to physiologically cope with food shortages associated with unpredictable food resources (Love *et al.*, 2003).

Management implications

Marine ecotourism is a notable source of environmental disturbance. A global analysis of marine reserve regulations at 91 MPA across 36 countries found that a majority of high-risk activities involved motorised boats (Thurstan *et al.*, 2012). When designing MPAs, anticipating forthcoming touristic fluxes is therefore absolutely essential, to avoid facing acute management crises as in the case of the Scandola MPA. Such anticipatory planning necessarily involves pertinent socio-economic factors analyses (Badalamenti *et al.*, 2000) leading to a consensual regulation of public access and ship traffic. Further, the designation of MPAs must be complemented by a sound management plan, and the allocation of the financial means necessary to its enforcement. Tourism in Corsica actually started in the early 20th century and, based on observed numbers, predictions of current trends have been made: such previous studies already indicated that enhanced ecotourism and related boat traffic may affect marine biodiversity at Scandola in the longer term (Francour, 1994; Francour *et al.*, 2001; Richez & Richez Battesti, 2007; Tavernier, 2010). Other MPA where marine ecotourism has become problematic tried to solve the issue by appropriate mitigation measures. For example, at Asinara Island National Marine Reserve (IT) access has been regulated, no-entry/no-take areas have been implemented, as well as seasonal closures and speed restrictions (Villa *et al.*, 2002; Russ & Alcala, 2004). Such mitigation measures keep impacts to a sustainable level, especially in reserves were particularly sensitive species or communities exists (e.g. aggregations of dolphins and sharks (Heyman *et al.*, 2001; Kelly *et al.*, 2004), sea turtles (Hazel *et al.*, 2007), spawning fish assemblages (Bracciali *et al.*, 2010), seabirds colonies (Velando & Munilla, 2011), or benthic communities (Selig & Bruno, 2010; Lloret *et al.*, 2008)). Crucially, the success of such management strategies was tightly linked to the degree of involvement of the local community (Badalamenti *et al.*, 2000; Baine *et al.*, 2007), and we strongly feel that an improvement of the osprey conservation within the Scandola MPA will only be possible through a collaboration with the local tourism industry. This might be facilitated by the fact that the Regional Natural Park of Corsica is increasingly aiming towards sustainable tourism development, to enhance the value of the biodiversity while reinforcing the sanctuary status of UNESCO World Heritage Site. In this framework, Scandola has a great potential for achieving both goals, yet disturbance caused by enhanced boat traffic has to be carefully managed. Our integrative study is a major incentive for a better integration of terrestrial and marine processes, to achieve an improved protection of this unique site and of the associated Mediterranean osprey population.

11. THE VULNERABLE OSPREY BREEDING POPULATION OF THE AL HOCEIMA NATIONAL PARK, MOROCCO: PRESENT STATUS AND THREATS.

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11.1. ABSTRACT

In the Mediterranean, most areas belonging to the initial distribution range of the Osprey *Pandion haliaetus* have been lost and local populations have disappeared in recent decades because of persecution. Even though direct management actions have allowed local partial recovery, the Mediterranean population currently only holds a few tens of breeding pairs and is still exposed to local extinction risks. One of the last Mediterranean Osprey breeding areas lies along the North African coast between Morocco and Algeria. In this paper, we report new information on the Osprey population within the Al Hoceima National Park, Morocco. The status of the population for 2012 and 2013 is reported and compared with data collected during the period 1983–1990. A reduction in number of nests and breeding pairs was observed and a 35.7% decrease in the population size recorded. In addition, we discuss the main identified threats to Osprey habitats (e.g. dynamite and poison fishing) that affect the Osprey breeding population in this area. In this context, we stress the necessity for urgent measures to be adopted at the local scale for the protection of this vulnerable population in the light of a sound conservation strategy also at the scale of the Mediterranean.

Keywords: conservation, Morocco, *Pandion haliaetus*, population, threats.

11.2. INTRODUCTION

The Osprey *Pandion haliaetus*, is a long-lived raptor distributed on all continents except Antarctica between 49° S and 70° N (Poole, 1989). Although most exclusively a tree-nester in the vicinity of rivers and lakes in northern parts of their Palearctic range, in the Mediterranean area Ospreys choose rocky cliffs for nesting, close to marine or brackish water fishing environments (Poole, 1989). Despite direct management actions carried out in the last decades, allowing a partial recovery in Corsica and in the Balearics (Bretagnolle *et al.*, 2008; Triay & Siverio, 2008), the Mediterranean population still shows an unfavourable conservation status (Muriel *et al.*, 2010), with less than 80 breeding pairs, distributed between Corsica (32 pairs), the Balearic islands (16–18 pairs), Algeria (supposedly 15–17 pairs) and Morocco (supposedly14–18 pairs) (Monti, 2012). Thanks to reintroduction projects the species is now also breeding in mainland Spain and central Italy since 2009 and 2011, respectively (Muriel *et al.*, 2010; Monti, 2012). The Osprey population of Morocco was discovered only in 1983 when the first exhaustive survey was carried out along the Mediterranean coast (Berthon & Berthon, 1984; Thibault *et al.*, 1996). During the period 1983–1990, the population was regularly monitored, as reported by Hodgkins & Beaubrun (1990). This population, scattered along the rocky coast from Cala Iris to Al Hoceima, is thought to be the only reproductive nucleus in Morocco. Only in 1989, two nests were discovered near Jebha, a small town 30 km west of Cala Iris. In the Chafarinas Islands, two breeding pairs of Osprey were present in 1950 (Terrasse & Terrasse, 1997). Since 1994, only one pair inhabits the archipelago, still observed in June 2013 (Triay & Siverio 2008; Monti, 2012; G. Dell'Ariccia, CEFE-CNRS, pers. comm., 2013). A breeding occurrence has never been proved for the Atlantic coast of the country, although it has been strongly suspected in a few places (Thévenot *et al.*, 1985).

In spite of the great importance of such a population for Osprey conservation at the scale of the Mediterranean, no additional census took place after 1990. Only in 2008, a new exploration was conducted by the local non-governmental organisation Association de Gestion Intégrée des Ressources (AGIR), which estimated a total of 14–18 pairs within the same area (Nibani, 2010), nowadays recognised as the protected area of the Al Hoceima National Park (PNAH). Supported by the Mediterranean Small Island Initiative, the 'Haut Commissariat aux Eaux et Forêts et à la Lutte contre la Désertification' took the initiative to realise a global census of the Osprey population of the Al Hoceima National Park in 2012 and 2013. For the first time, nests were thoroughly checked by climbing on the rocky cliffs. Such an approach allowed validation of the occurrence of reproduction and to record the exact number of active nests (number of breeding pairs) and of eggs and/or chicks in the population.

We report the current status of the Osprey population of the Al Hoceima National Park in 2012–2013. These results are compared with historical data from previous surveys conducted between 1983 and 1990 (only published as internal reports; Hodgkins & Beaubrun, 1990). In addition, we describe and quantify the main threats to Ospreys that were identified during our field sessions in the area.

11.3. MATERIAL AND METHODS

a. Study site

The Al Hoceima National Park (Fig. 1) is classified as a semi-arid to arid Mediterranean bioclimatic zone located on the northern coast of Morocco (Al Hoceima, 42°39′ N, 11°05′ E). It consists of both a marine and terrestrial area of 19,600 ha and 28,400 ha, respectively. The protected area, expanding over 40 km of coastline along the Mediterranean Sea from Cala Iris to Al Hoceima, is characterised by high calcareous cliffs, marine caves and small rocky islets close to the seashore, which for the most part belong to Spanish territories (e.g. Peñón de Vélez de la Gomera). The fish fauna is particularly rich and includes both Mediterranean and Atlantic species coming in through the nearby Strait of Gibraltar (Nibani, 2010). Some of these species represent good potential prey for Ospreys, which nest on rocky pinnacles long the sea coast (Thibault *et al.*, 1996).

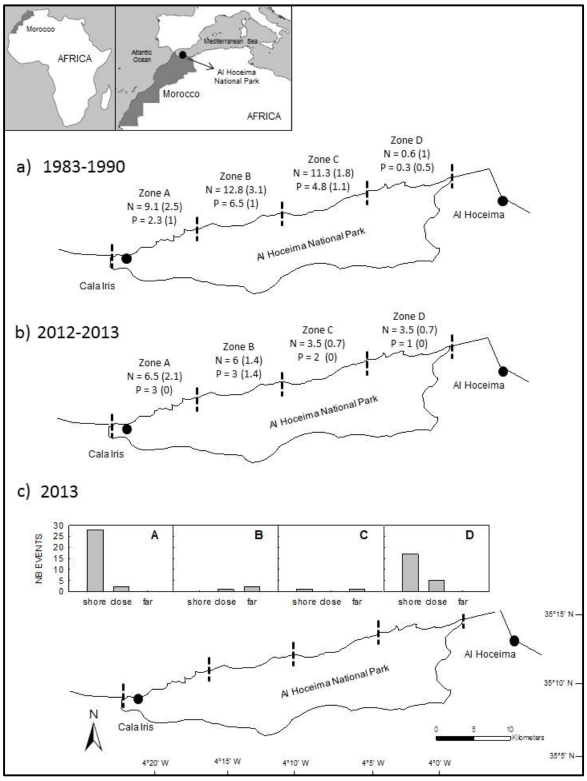

Figure 1: Location of Al Hoceima National Park, Morocco, of which the 40-kilometre coastline was divided into four zones (from A to D; from west to east) of 10 km each. For each sector the following parameters are reported: N = number of nest sites found; P = number of territorial pairs. These parameters are considered both for (a) the historical data collected during the period 1983–1990 and (b) those data collected in 2012–2013. Numbers expressed are the mean with the SD in parentheses. (c) Occurrences of potential threats, counted in 2013, are reported and ranked in three different classes of distance from the coast (shore = 0 m; close = 0 > x < 300 m; far > x < 300 m) for each zone.

b. Census methods

Previous surveys in the period 1983–1990 were performed only via distant observations, using boats at sea or a telescope from land. The position of each nest was recorded on a geographical map and photos taken for better identification (Hodgkins & Beaubrun, 1990). Nests were considered as occupied according to Ospreys' behaviour, i.e. presence of individuals at the nest or in its surroundings when nest content was not visible at all from distance. In other cases, nests were considered as unoccupied if nothing was detected in the nest or no Ospreys were observed in the vicinity of it or as undetermined when no information was available.

The 2012–2013 census took place in May (as in 1983–1990), at the time when most breeding Ospreys are rearing chicks. A team of five observers, lead by JMD who has 30 years of experience in surveying Ospreys in the Mediterranean, participated in the mission. Fieldwork consisted of 4 d of observations each year from land and 4 d of coastal surveys at sea. Osprey nests were searched for along the cliffs during coastal surveys by means of local fishermen's boats, and nest contents were first checked at distance from land using a telescope. Since we covered the whole 40 km of coast included in the protected area, all occupied Osprey territories were surveyed. A nest was considered as active if at least one egg was laid. In order to avoid risks of errors in counting eggs and chicks from above, nest occupancy and nest contents were validated by climbing rocky cliffs to the vicinity of nests. Osprey presence and number of eggs and chicks were hence unequivocally assessed. Hatchlings were measured, weighed and individually marked by a metal ring (CRBPO-MNHN, Paris) and a white-coloured ring with a three-letter code (for long-distance identification).

Both in 2012 and 2013, threats potentially affecting the Osprey population were observed in the protected area. The 40 km of coast along the PNAH were divided into four zones (from A to D; from west to east) of 10 km each. During 4 d of surveys at sea in 2013, we recorded all events representing a potential disturbance, assigned it to the relative geographical sector and noted its distance from the coast according to three different classes of distance (shoreline, within 300 m, or greater than 300 m). Annual quantification of fishing and estimates of each threat were reported also according to the National Office of Fisheries of Morocco (ONP) estimates (http://www.onp.co.ma) and to Nibani (2010); their negative effects on both habitats and species are here reported and discussed. Means ± SD are reported.

11.4. RESULTS AND DISCUSSION

c. Historical data and present population status

A regular census of the Osprey population was carried out during the 1983–1990 period, except for the years 1984 (partial census) and 1988 (Hodgkins & Beaubrun, 1990). In 1983, this population was initially estimated at 10–15 pairs (Berthon & Berthon, 1984; Hodgkins & Beaubrun, 1990). During this early monitoring period, a total of 52 different nest structures were recorded (mean per year = 33.6 ± 6.2) and 14 (± 1.8) territorial pairs were located (Fig. 1). The population maintained a stable trend showing limited variation in numbers during the period 1983–1990 (Tab. 1).

Table 1: Historical (1983–1990) and present (2012–2013) population data recorded in the PNAH. For each year are reported the number of nests (N nests), the number of territorial pairs (N pairs), the number of abandoned nests (Nests abandoned) and number of nests with undetermined status (Nests undet.). For the periods 1983–1990 and 2012–2013, the mean values ± SD are reported. * = Incomplete census in 1984, not included in mean values, ** = number of pairs that actually reproduced in 2012 and 2013.

Year	*N* nests	*N* pairs	Nests abandoned	Nests Undet.
1983	24	14	9	1
1984*	9	6	3	0
1985	32	15	14	3
1986	33	15	17	1
1987	37	16	16	5
1989	33	11	10	12
1990	43	13	31	0
Mean	*33.6 ± 6.2*	*14 ± 1.8*	*16.2 ± 7.9*	*3.7 ± 4.5*
2012	16	8 (5**)	4	4
2013	23	10 (7**)	9	4
Mean	*19.5 ± 4.9*	*9 ± 1.4 (6 ± 1.4**)*	*6.5 ± 3.5*	*4 ± 0*

In 2012 and 2013, a total number of 23 (mean per year = 19.5 ± 4.9) nest structures were recorded in the PNAH, between Cala Iris and Al Hoceima (Fig. 1). In these two years, 6.5 (± 3.5) nests were obviously abandoned (structures were formed by only a few branches and the nests appeared to have been unused for several years). Four nests were occupied by a territorial male alone, whereas another three hosted non-reproductive pairs (no egg/chick observed). A breeding occurrence was only recorded in five and seven sites in 2012 and 2013, respectively (6.0 ± 1.4 for both years). The whole population was estimated at 20–25 adults, to which 8–12 chicks might be added per year. Chicks were about three weeks old at the time of our visits in 2012–2013, meaning that egg laying approximately occurred between March and April and hatching at the end of April (according to an incubation length of 35–42 d reported by Cramp & Simmons, 1980). Only six out of the nine territorial pairs observed were actually breeding, and represented the effective reproductive nucleus of the population in May 2012 and 2013. A reduction in the number of nests and territorial pairs was hence recorded, and a 35.7% decrease in population size has occurred since 1990. In 2012 and 2013, the Osprey population size of Morocco was hence well below the numbers estimated during previous surveys (Berthon & Berthon, 1984; Thibault *et al.*, 1996). Due to the lack of systematic and repeated censuses during the early breeding season, no data on previous nest failure were available, meaning that the actual breeding population may be larger than our estimate based on the number of active nests. For instance, some of the breeding females that might have failed earlier in the season might have already moved away at the time of the census in May. Thus, the population decrease could be perhaps less dramatic than suspected. However, old counts performed during 1983–1990 were carried out also in the month of May during each year and hence at the same breeding stage as those of 2012 and 2013. Therefore, if we assume that rates of breeding failure at incubation stages remained similar between the 1980s and 2010s, the surveys must be comparable. Nevertheless, since we employed a more reliable monitoring method (previous counts being carried out only via distant observations), this might have impacted total numbers. On this basis, our survey strongly suggests that a strong decrease in population size has occurred during the last 20 years from 14–16 pairs in the 1980s to the only six breeding pairs and nine territorial pairs in 2012–2013.

At the same time, the total number of observed nest structures decreased from 52 to 23 nests. Two types of factors could explain why some nest structures have disappeared in recent decades. First, environmental factors such as wind and rain could have destroyed unused nests. Second, inhabitants of the neighbouring villages frequently explore the coastal cliffs to use dynamite fishing and their repeated passages together with continuous explosions can have accelerated the process of nest demolition. Both factors could be responsible for rapid nest destruction. To conclude, a systematic monitoring protocol to record population demographic parameters (e.g. the presence and number of birds and their breeding status, hatching and fledging success) during each breeding season is required and should be adopted as soon as possible by the PNAH. The current situation requires urgent and efficient measures aimed at the preservation of this vulnerable population (Monti, 2012).

d. Potential threats identified within the PNAH

During the field census of 2012–2013 several important threats potentially affecting the Osprey population were observed. In 2013, we counted a total of 62 events during four days of coastal surveys within the park (Fig. 1). The majority of them occurred at the borders of the protected area, in the surroundings of the villages of Cala Iris (zone A = 56.4%, n = 35) and Al Hoceima city (zone D = 35.4%, n = 22). Few cases were detected in the central zones of the park (zone B = 4.8%, n = 3; zone C = 3.2%, n = 2). In total, 95.1% (n = 59) of the events was recorded within 300 m from the coast (0 m = 74.2%, n = 46; 0 > x < 300 m = 20.9%, n = 13), often close to Osprey nests. Only three occurrences (4.9%) were detected at a distance greater than 300 m. These potential threats are listed below: (1) Fishing represented the main cause of both direct and indirect disturbance for Osprey. According to ONP (http://www.onp.co.ma) estimates, a total amountof 5 510 tons of fish has been officially extracted for the harbours of Al Hoceima and Cala Iris, in the first 10 months of 2012. In the same year, intensive trawl fishing was observed near the coast within the PNAH. Some 2,200 tons of fish are estimated to be extracted per year by the 14 vessels that currently work in the area of the PNAH (Nibani, 2010). This practice is likely to strongly disturb the breeding grounds of demersal fish and may deplete breeding fish stocks (Jones, 1992). Even if trawl fishing does not directly deplete Osprey prey (e.g. fishes living close to the sea surface) and/or disturb breeding Ospreys, if vessels work far away from the coast, it is certainly damaging for the whole marine ecosystem and consequently for bird species linked to it.

In several studies, a negative effect of such practices has been described for seabirds (Arcos *et al.*, 2008). Over a long time span, seabirds might run into difficulties satisfying their food requirements, with repercussions on both reproduction and survival (Cury *et al.*, 2011). Trawl fishing is also known to deteriorate marine ecosystems by destroying non-target benthos, causing post-fishing mortality of damaged organisms, and long-term changes to benthic community structure (Jones, 1992). In order to minimise disturbance and negative effects, the number of vessels should be regulated, access to the park forbidden, as well as traffic shifted to a set distance from the coast. In this context, artificial obstacles were placed (in the first months of 2013) on the sea bed to reduce trawlers activities inside the PNAH. Therefore, we only counted three passages of actively fishing trawling vessels (at > 300 m from the coast) in May 2013. (2) Dynamite fishing is commonly used within PNAH (Nibani, 2010). Men sitting on vertical cliffs and watching for a shoal of fish represented the prelude to dynamite fishing (Nibani, 2010). The dynamite is tossed from the cliff in order to kill fish, which come up to the sea surface and are collected by a swimmer. Ten to 15 individuals, mainly in the vicinity of the villages of Al Hoceima and Bades, are estimated to routinely use this illegal technique within the PNAH and to extract 367 tons of fish per year (Nibani, 2010). In 2013 (during 4 d of surveys at sea), we recorded the presence of dynamite fishermen in two different cases. (3) Copper sulphate fishing for octopus *Octopus vulgaris* is commonly used by at least 15 local fishermen (counts according to Nibani, 2010). From an inflatable boat moving slowly on the sea surface, fishermen screened the water until an octopus was detected. They then forced the octopus to come out of its hole by means of copper sulphate spread from the surface. Although this fishing is focused on a single species, sulphate quickly spreads, poisoning marine organisms in the surroundings. (4) Occurrence of scuba spear fishing was also recorded in one case. However, this fishing practice is currently performed by foreign people on powerful motorboats coming into the integral zone of the PNAH from the harbour of Cala Iris (Nibani, 2010). (5) Small-scale commercial or subsistence fishing practices, by means of small boats and traditional techniques such as rod and tackle, throw nets and drag nets, represent the principal economic income for ca. 3,650 fishermen working within the PNAH territory (estimates for the province of Al Hoceima; Nibani, 2010). They are able to extract a total biomass of 1,500 tons of fish per year (Nibani, 2010). In 2013, we recorded a total of 54 cases of presence of local fishermen in close proximity of Osprey nests, within the integral protection zone of the park (Fig. 1). Breeding Ospreys are disturbed both during the phase of territory settlement (adults were frequently observed changing nest structures at the beginning of the breeding season due to the steady presence of local people (Houssine Nibani, unpublished data). This could lead to failures of breeding attempts, i.e. during the incubation or chick-rearing period. Human fishing zones should therefore be limited to areas distant from Osprey nests (e.g. > 500 m to avoid any alarming displays of Ospreys; Bretagnolle & Thibault, 1993). (6) An old garbage dump has been releasing rubbish at sea in the vicinity of an Osprey nest located close to the borders of the National Park and to the harbour of Al Hoceima for decades. Rubbish was partially burnt or dumped directly into the sea. Although the dumping site has been officially moved far away inland, we still recorded occasional activities at this site. The foraging opportunities offered by the site, thanks also to abundant fish discards coming from the activities of the harbour, attract > 1,000 Yellow-legged Gulls *Larus michaellis*. This gull species is known to be a potential threat for breeding seabirds, since gulls can plunder eggs or chicks at the nest (Libois *et al.*, 2012). This Osprey nest was indeed one of the non-active ones in 2012–2013. (7) Disturbance of breeding Ospreys by motor boats is suspected to occur. On the beach of Al Hoceima, 10–14 watercrafts can be rented by tourists from April to September. Tourists are then allowed within the park, where no restrictions are specified. Noises from their boat engines can disturb nesting Ospreys. Such traffic should be forbidden, or at least regulated within the park. (8) During the past, Osprey chick consumption was suspected to occur. As an ancient tradition, reported by elderly residents of villages and confirmed by the AGIR association, fishermen used to retrieve Osprey chicks from the nests to eat them for improving their own skills in fishing. An estimate of the past consumption's occurrences has not been clearly possible. Moreover, it is not known if this practice still occurs nowadays even if it may be directed to other species. Despite this, we recorded that a chick, previously ringed by us in 2012, was collected alive in the nest by a local person and illegally traded (Houssine Nibani, pers. comm.). Environmental education programs should be improved, resulting in a future better knowledge of the local richness in term of habitat and species.

e. Conclusions

The PNAH appears to be strongly exposed to different human pressures that are likely affecting Osprey survival and threatening local biodiversity. Direct effects of such threats, especially those resulting from illegal fishing practices such as dynamite fishing and poisoning, have strong implications for Osprey conservation, but also for the entire marine biodiversity of PNAH. In the past, traditional fishing represented one of the major economic incomes for precarious local people living inside the park's boundaries. From the 1980s, a noteworthy exodus of persons that came from other parts of Morocco to settle in the Rif region occurred. As a result, the province of Al Hoceima witnessed a rapid demographic increase from 54,319 inhabitants in 1960 to 109,990 in 2004 (Nibani, 2010). Better economic possibilities favoured progress in fishing methods (e.g. improvements in technical systems used on boats) that enhanced pressures on the marine environment. Decline of several fish stocks was one of the most evident negative effects exerted by such vessels and by the use of illegal fishing activities such as dynamite fishing and poisoning (Nibani, 2010), even if there is currently no direct evidence of insufficient food supply for the Ospreys. The park is also threatened by possible future coastal development and urbanisation. Local practices in land use (e.g. in 2013, the building of a new harbour started within the PNAH territory) together with the increasing touristic pressure are seriously undermining the natural resources of this area, considered as one of the most representative Mediterranean biotope for its high biodiversity in terms of species and habitats. The park should arrange for administrative authorities to plan strong conservation actions in the area. However, any management action must be based upon a good understanding of the functioning of animal populations. At present, basic information on the spatial ecology of Ospreys in the Mediterranean is still lacking, affecting the possibility of putting into action precise conservation measures for the species at regional scales. Further studies should investigate such topics with particular attention, to test the existence of connectivity between the different Mediterranean Osprey populations. This will help to understand the rate of isolation of these populations and allow evaluation of the extinction risk of each population, such as that of the Al Hoceima National Park.

12. THE OSPREY REINTRODUCTION IN CENTRAL ITALY: DISPERSAL, SURVIVAL AND FIRST BREEDING DATA.

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12.1. ABSTRACT

Capsule Sex-biased dispersal and an age-dependent effect in survival rate accounted for the pattern of first settlement and reproduction in a newly reintroduced Osprey population.

Aims We estimate the survival of translocated individuals, describe juvenile movements and evaluate the success of first breeding events to document the re-establishment of an Osprey breeding population.

Methods Between 2006 and 2010, 32 fledgling Ospreys were reintroduced via hacking techniques in Maremma Regional Park, Italy. We evaluated the effects of age on survival through multistate capture-mark-recapture analyses. Movements were investigated by radiotracking and using records of resightings.

Results Survival was high for juveniles after the release (0.87), markedly decreased during the first winter (0.26), and improved again in subsequent years (annual apparent survival of 0.69 for immatures and 0.93 for adults). Mean distance covered in initial dispersal was greater for females (246.2 km) than for males (38.7 km).

Conclusion Our results provided information on dispersal and survival rate of reintroduced Ospreys in a Mediterranean area. Despite low apparent survival in the first year, the high survival rates found in immatures and adults suggested favourable conditions for this new population. The study of demographic parameters is important for calibrating management actions aimed at the establishment of a self-sustaining Osprey population.

12.2. INTRODUCTION

In any reintroduction project, the pre- and post-release management can affect both post-release survival and dispersal of translocated individuals, ultimately influencing their successful establishment and persistence (Armstrong & Seddon, 2008). The study of the details of reintroduction programmes is therefore important for planning management and conservation strategies aimed at reducing post-fledging mortality (Soutullo *et al.*, 2006). This is especially true for those populations in which movements and dispersal strategies are not well known and can affect an individual's survival rate. For example, in migratory populations the high energetic demands required by long non-stop flights during migratory journeys (Alerstam, 1990) have been proved to be one of the primary causes of death (Sillett & Holmes, 2002; Lok *et al.*, 2013 ; Klaassen *et al.*, 2014).

The Osprey *Pandion haliaetus* is a distinctive bird of prey widely distributed across different biogeographical regions of the world (Poole, 1989). During the 19th and early 20th centuries it faced heavy direct persecution (e.g. shooting, egg-collection and habitat destruction; Poole, 1989; Saurola, 2005). As a result, many local populations disappeared (Cramp & Simmons, 1980; Dennis & Dixon, 2001). It is included in Annex I of the European Directive (2009/147/EC) on the conservation of wild birds and hence, considered a priority species for conservation along its whole distributional range. Around the Mediterranean, the conservation status of the species is nowadays considered unfavourable (Thibault *et al.*, 2001; Muriel *et al.*, 2010), with <80 breeding pairs distributed between Corsica, the Balearics, Morocco and Algeria (Monti, 2012). Although direct management actions allowed a partial recovery in Corsica and in the Balearics, the Mediterranean population is still vulnerable (Thibault & Bretagnolle, 2001; Thibault *et al.*, 2001; Triay & Siverio, 2008; Monti *et al.*, 2013). Furthermore, because of the high philopatry of the species, the natural recolonization of portions of the past range is unlikely to occur within a short time span. Accordingly, recovery of historical breeding sites by means of reintroduction has been considered as a key strategy for Osprey conservation in this area, since reintroductions are one of the best and reliable ways for the re-establishing of populations, once the past causes of extinction have been identified and eliminated (Griffith *et al.*, 1989; Seddon *et al.*, 2007).

Three reintroduction projects for the Osprey were launched in southern Europe: in 2003 in continental Spain, 2006 in Central Italy and 2011 in Portugal (Muriel *et al.*, 2006; CIBIO, 2011; Monti *et al.*, 2012). In Italy, the Osprey became extinct as a breeding species during the last years of the 1960s mainly due to direct persecution (Bulgarini *et al.*, 1998; Brichetti & Fracasso, 2003). The last reproduction events were reported for the islands of Sicily and Sardinia in 1968–1969 and continental Apulia region in 1955 (Frugis & Frugis, 1963;

Thibault & Patrimonio, 1992; Brichetti & Fracasso, 2003). The last known breeding occurrence for Central Italy was in Tuscany (Montecristo Island in 1929; Arrigoni degli Oddi, 1929). In 2006, thanks to collaboration between the Natural Regional Park of Corsica (France) and the Maremma Regional Park (MRP) (Italy), a reintroduction programme was launched in Central Italy, aiming at re-establishing a breeding population that would potentially inter-connect with the nearby Corsican breeding population (Sforzi *et al.*, 2007). Since the Osprey population of the Mediterranean is thought to be mostly sedentary and/or characterized by individuals that perform reduced movements within the basin (Thibault *et al.*, 1996), here we first report the movements and dispersal patterns of translocated birds to assess whether they remained in the area or moved far away. Second, we estimate apparent survival through mark-recapture models. Considering that the hacking technique used for reintroduction may affect the survival rates of young birds because of the lack of direct parental care during the first stages of life, we expected lower values of survival compared to those found in wild populations. In particular, we hypothesized that a high mortality rate in reintroduced birds may reduce the chances of reaching adulthood and in turn affect the success of the reintroduction programme. Finally, we would predict a greater survival rate for this relatively sedentary population compared to Osprey populations of north and central Europe that perform hazardous long-distance migrations (Klaassen *et al.*, 2014).

12.3. MATERIAL AND METHODS

a. Study site and field methods

The choice of the reintroduction site took into account the strategic geographical position of the area as highly suitable for the future Osprey population expansion (Sforzi, 2004; Dominici *et al.*, 2007). The release site (42°39’ N, 11°05’ E) was located in the MRP, at the centre of an extensive coastal wetland system in southern Tuscany. The system (hereafter called ‘study area’) is composed of several protected areas (Fig. 1): Burano Lake (BUR – WWF protected area – 40 km from the release site); Orbetello Lagoon (ORB – WWF natural reserve – 30 km from the release site); Diaccia Botrona Natural Reserve (DBR – 15 km from the release site); Orti-Bottagone Marsh (ORT – WWF natural reserve – 50 km from the release site); Massaciuccoli Lake (MAS – LIPU natural reserve – 140 km from the release site). Lagoons and saltwater marshes provide suitable fishing grounds for Ospreys. The release area is located in one of the Integral Reserves of the MRP at the mouth of the Ombrone River (for further details on this area see Monti *et al.*, 2012).

The Corsican Osprey population was used as the donor population. Although strong direct persecution reduced this population at only three breeding pairs in 1974, local management actions since then allowed a rapid demographic recovery. The population was considered stable with around 30 breeding pairs (Thibault *et al.*, 2001; Bretagnolle *et al.*, 2008). A total of 6–8 chicks was collected per year from the donor population without compromising its survival, for a minimum duration of five years of releases (Dominici *et al.*, 2007). Juvenile Ospreys were taken from nests located along the west coast of Corsica (including the Scandola Natural Marine Reserve; 42°25’ N, 8°36’ E) at an age of 35–42 days from clutches of three eggs. The biggest and oldest chick was taken for translocation, while the younger chicks were left in the nest, where their chances of survival were increased in the absence of one sibling. Chicks were transported by helicopters to the hacking tower in the MRP, where they were kept for approximately three weeks until release. Each individual was marked with both a metal ring and a coloured darvic ring with an alpha-numeric code (for at distance identification) and equipped with a 10 g tail-mounted VHF radiotransmitter (Biotrack Ltd, UK). Behaviour was monitored on a daily basis during the pre-release phase from dawn to dusk. Home ranges and space-use strategies were investigated throughout the post-fledging dependence period (PFDP) by direct observations and intensive VHF radiotracking (for details of the monitoring protocol see Monti *et al.*, 2012). During the PFDP (July–August), juveniles mainly moved within a radius of about 1 km in the surroundings of the release pens. Nonetheless, they performed also greater movements, exploring larger areas (<15 km). Since the radio battery life only lasted a maximum of three months (from June to September), individuals were detected through direct observations by reading ring codes after this period of telemetry. For each site of the study area, the presence of Ospreys was checked once a week, on average. Resightings of ringed birds were used to calculate the minimum distance covered and the main direction of juveniles’ first movements. Breeding attempts were strictly monitored. Early in the season (February–March) each site of the study area was visited two times per week to check for individuals performing courtship displays, constructing nests or trying to mate. Later on, occupied breeding sites were kept under daily surveillance (from April to August). Wild-born chicks were handled at 5–6 weeks after hatching to take body measures and to be marked with a metal ring and a coloured darvic ring with an alpha-numeric code.

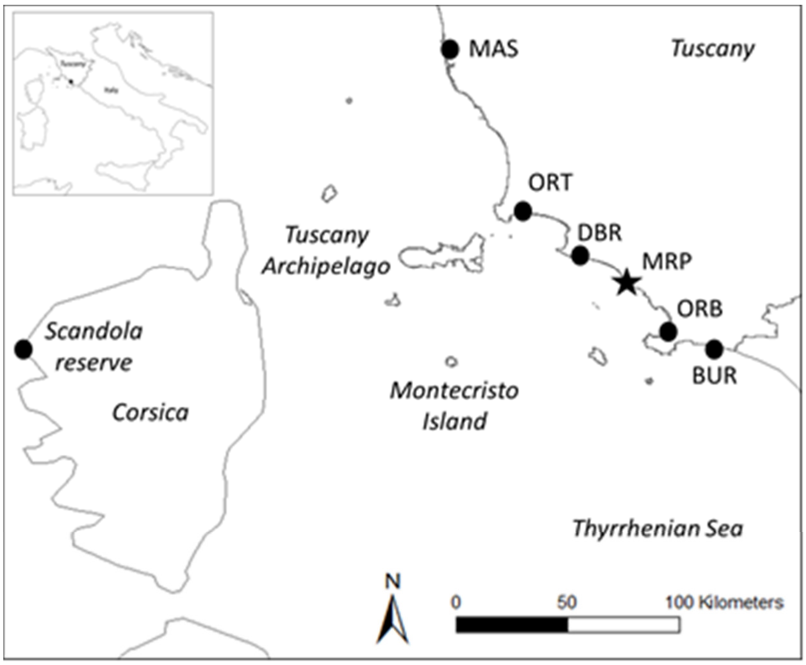


Figure 1: Geographical location and abbreviations of the main sites along the coastal wetlands system in Tuscany: black dots from north to south of Tuscany, Massaciuccoli Lake (MAS), Orti-Bottagone Marsh (ORT), Diaccia Botrona Nature Reserve (DBR), Orbetello Lagoon (ORB) and Burano Lake (BUR). Black star: Maremma Regional Park (MRP = release site). The islands of the Tuscany Archipelago and the Scandola Reserve (black dot) in Corsica are also shown.

b. Estimation of apparent survival

Using records of resightings and recoveries of marked non-territorial and territorial birds, a capture-mark-recapture (CMR) analysis was carried out on reintroduced birds. Overall, 185 resightings were collected during the period 2006–2013. Only four sightings have been reported from areas well beyond the southern Tuscany wetland study area (Fig. 2); these data, obtained without protocol or constant field effort, were not included in the survival analyses. Thus, we considered 181 records. The study area was checked every 1–2 days during the release phases (from June to August) and every week during the rest of the year. We assumed that the loss of coloured rings was rare and unlikely for juveniles and so this was ignored for analyses (Tavecchia *et al.*, 2012; Mihoub *et al.*, 2014).

As we were mainly interested in estimating survival during the first phases of the reintroduction, we set four specific age classes according to the species' biology: (1) juveniles, during summer, before dispersal (from first ringing in July to end of August; 3–4 months of age; 2 months after fledging in the wild); (2) juveniles after dispersal (from September to February; 5–10 months of age); (3) immature birds; 10–20 months old; (4) adults older than 20 months. Two main periods of six months were considered for the analyses: summer (from March to August, corresponding to the breeding season) and winter (from September to February, corresponding to the wintering season). We also considered two sex categories (coded in two groups in our analyses). In addition, we estimated the annual apparent survival for immature and adult birds. Recoveries of dead individuals only occurred in summer periods, in the study area, and only for juveniles at their first summer (no recoveries for immature or adults, or during winter periods), whereas resightings occurred all year round. Each observation (ringing, resighting or recovery) was coded according to three events, describing the status of the individual (dead or alive). We used the multistate framework to code individual encounter histories with one state ‘alive’ and one state ‘dead’, according to Lebreton *et al.* (1999). Model selection was performed using the program E-SURGE (Choquet *et al.*, 2009a) with an Akaike Information Criterion corrected for sample size (QAICc) (Burnham & Anderson, 2002). In our initial model, the survival Φ and resighting p rates are age, time and sex-dependent while recovery r rate was kept constant. Considering that during the PFDP marked birds were closely monitored by intensive radiotracking, we set resighting and recovery rates equal to 1 for this period. Recovery rate was assumed to be zero after the first summer because of the battery failure of VHF transmitters and of no recovery data reported. Goodness-of-fit (GOF) tests of the initial model were performed using U-CARE 2.5 (Choquet *et al.*, 2009b). Estimates are given with 95% confidence intervals following within brackets.

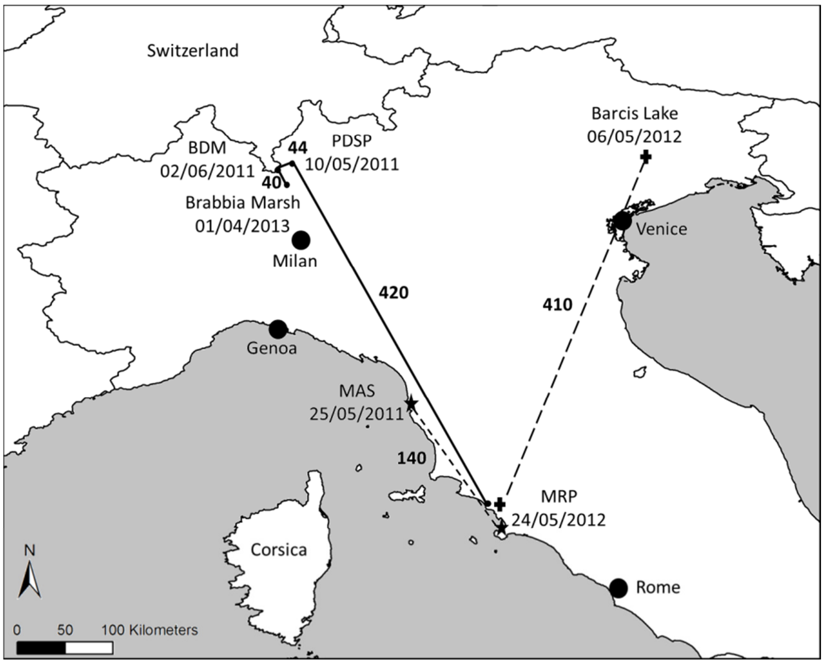


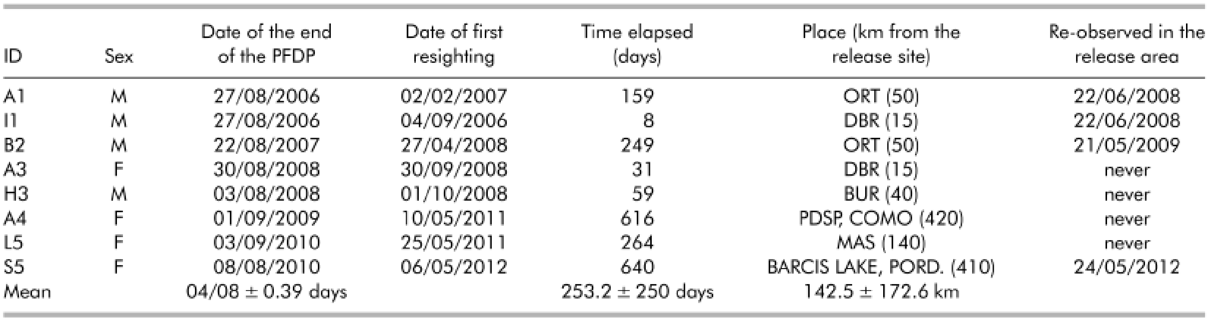
Figure 2: Map showing long-distance dispersal movements of three reintroduced females in Italy: A4, released in 2009 (black dots and solid lines); L5, released in 2010 (black starts and sketched lines); S5, released in 2010 (black cross and dotted lines). For each location the name of the place or its abbreviation, the date of sighting and the minimum line of sight distance from the previous location (in bold and expressed in km) are shown.

c. Movements and dispersal and first breeding events

Between 2006 and 2010, 32 juvenile Ospreys (20 females and 12 males) were translocated from Corsica to MRP (Fig. 3). From September onwards, 71.4% of birds moved and were not detected anymore, whereas 28.6% were observed again. In particular between 2006 and 2013, eight individuals (four males and four females) were recorded outside the release area. Three out of four males were resident during the subsequent years, visiting different wetlands, mainly located along the Tuscany coast (Fig. 1; Tab. 1). The males settled in this area soon after leaving the study site and after it was intensively explored during four to five years, it finally became the place of first breeding attempts and reproduction.

Occasional sightings reported by birdwatchers gave the opportunity to assess the minimum distance covered and the direction of juvenile Ospreys' movements in their first year of life (Tab. 1). Four sightings were reported also from areas well beyond the southern Tuscany wetland study area (Fig. 2). On average the distance covered after leaving the release area was greater for females (246.2 ± 201.5 km) than for males (38.7 ± 16.5 km). All females but one did not come back to the release area (Fig. 2). First breeding attempts were performed in 2010 by three pairs in the study area (Fig. 3). All of them were made up of one reintroduced adult males (mean age = 3.6 ± 0.57 years) and a wild-born female of unknown origin (unmarked). One of these pairs, composed of a male released in 2006 (age 5) and an unmarked female, settled in a salty swamp in the MRP and successfully raised two chicks in 2011, one chick in 2012, two chicks in 2013 and one in 2014 (total of six chicks). Between 2011 and 2013, a second territorial pair settled in the MRP but failed to breed. Finally in 2014, a third pair composed of a reintroduced female (age 4) and a male wearing only a metal ring bred in the Diaccia Botrona Natural Reserve, producing three fledglings (Fig. 3).

Table 1: Furthest movements of the released Ospreys, ID, sex, date of the end of the PFDP, date and time elapsed from the PFDP (days) until the first resighting, and location (with line of sight distance in km from the release site) are reported. For some birds, the date of the first return in the MRP is also reported.



ID	Sex	Date of the end of the PFDP	Date of first resighting	Time elapsed (days)	Place (km from the release site)	Re-observed in the release area
A1	M	27/08/2006	02/02/2007	159	ORT (50)	22/06/2008
I1	M	27/08/2006	04/09/2006	8	DBR (15)	22/06/2008
B2	M	22/08/2007	27/04/2008	249	ORT (50)	21/05/2009
A3	F	30/08/2008	30/09/2008	31	DBR (15)	never
H3	M	03/08/2008	01/10/2008	59	BUR (40)	never
A4	F	01/09/2009	10/05/2011	616	PDSP, COMO (420)	never
L5	F	03/09/2010	25/05/2011	264	MAS (140)	never
S5	F	08/08/2010	06/05/2012	640	BARCIS LAKE, PORD. (410)	24/05/2012
Mean		04/08 ± 0.39 days		253.2 ± 250 days	142.5 ± 172.6 km	

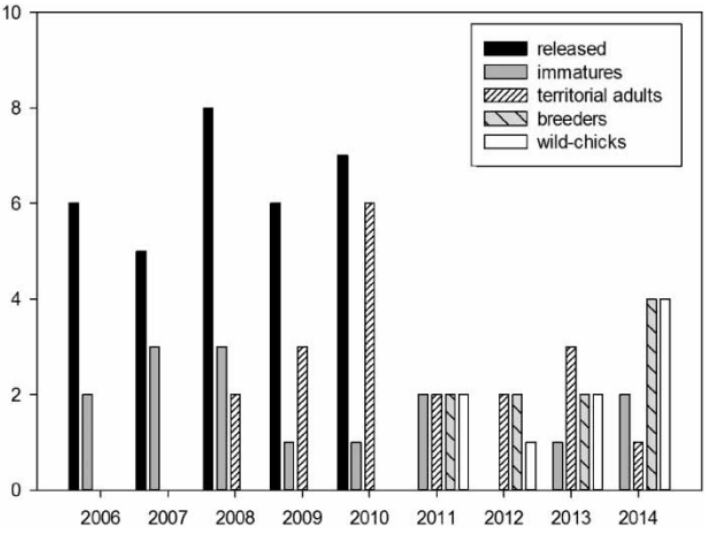
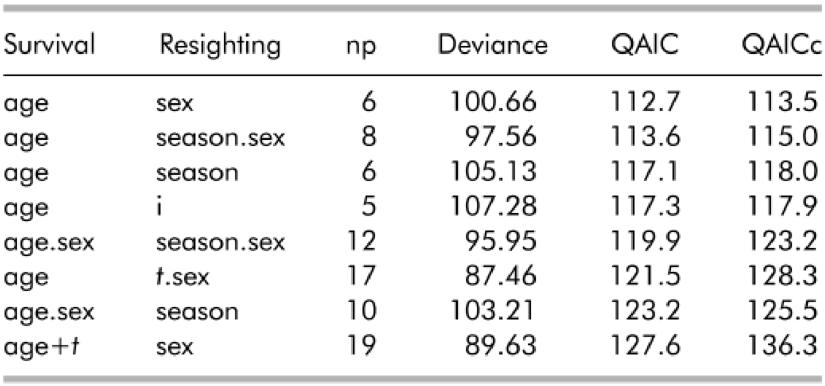


Figure 3: The number of Ospreys observed in the study area per year: juveniles banded and released (black bars), number of immature individuals (grey bars), number of territorial adults that did not reproduce (white bars with skew lines), number of reeders (grey bars with skew lines) and number of wild-born chicks (white bars).

d. Mortality and apparent survival

Four cases of mortality were recorded among the translocated birds (four females and one male). For three fledglings females, the cause of death was predation by carnivores (feathers with chewed up rachis base found). Electrocution occurred in one case: the dead body with burned feather tips was found on the ground under an electric pole located outside the MRP. According to the GOF test, our initial model reasonably fits the data ($\chi 2 = 9.1$, df = 9, P = 0.43). The best model assumes a sex effect for resighting rate (larger for males (0.97 [0.81–0.99]) than for females (0.69 [0.40–0.88])) and an age effect for survival (Tab. 2). Survival rate for juveniles was very high (0.87 [0.71–0.95]) for the first two months (PFDP in the summer) and decreased to 0.26 [0.13–0.46] after dispersal and during their first winter (period September–February). Annual survival was estimated at 0.69 [0.29–0.92] for second-year birds (immature), and at 0.93 [0.65–0.99] for adults. To obtain an estimate comparable with those of other published studies, we pooled our data (originally split in three periods) to build up an annual cluster. We then multiplied survival rate of the first two months (0.87) × survival rate for six months after leaving the area (0.26) × a four-month survival rate extracted from the annual survival rate of immature (the cubic root of 0.69). In this way, we obtained a first-year apparent annual survival of 0.20.

Table 2: Model selection for survival and resighting rates with recovery rate as kept constant. Age consisted of four age classes: juveniles during summer, juveniles during winter, immatures and adults. Season was summer and winter periods, t means a time effect (i.e. variation between years), np is the number of identifiable parameters and QAICc is the Akaike Information Criterion corrected for sample size. Models are ranked by decreasing QAIC values.



Survival	Resighting	np	Deviance	QAIC	QAICc
age	sex	6	100.66	112.7	113.5
age	season.sex	8	97.56	113.6	115.0
age	season	6	105.13	117.1	118.0
age	i	5	107.28	117.3	117.9
age.sex	season.sex	12	95.95	119.9	123.2
age	t.sex	17	87.46	121.5	128.3
age.sex	season	10	103.21	123.2	125.5
age+t	sex	19	89.63	127.6	136.3

12.4. DISCUSSION

In our translocated birds, the PFDP of three months and the onset of longer range dispersal in late August were within the range reported for the species (Cramp & Simmons, 1980). Wild-born male Ospreys are known to be more philopatric than wild-born females and males generally cover shorter distances during the natal dispersal phase (Poole, 1989; Johnson & Melquist, 1991; Martell *et al.*, 2002). Our data on translocated birds confirmed this behaviour, explaining the higher probability (97%) of resighting a male rather than a female (69%) in the study area. Any reintroduction programme can be considered successful when the new population becomes self-sustaining (Schaub *et al.*, 2009).

Ospreys achieve sexual maturity (and start searching for a suitable territory for breeding) at the age of 2–4 years (Poole, 1989; Englund & Greene, 2008). First breeding attempts of inexperienced pairs usually lead to a high percentage of failures, but this failure rate decreases in the following years as their experience grows (Poole, 1989; Muriel *et al.*, 2006). The settlement and breeding of the first pair hence constitutes an important milestone for the formation of a new viable population and for the attraction of other individuals in the area. However in a reintroduction context, modelling population dynamics is an important tool to understand the demographic causes of variation in population sizes which may suggest how to re-orientate conservation actions to enhance the survival probability of young birds, hence enabling the success of a reintroduction project (Sarrazin & Barbault, 1996). That is true especially for long-lived species in which low juvenile and high adult survival rates are expected (Clobert & Lebreton, 1993). A few data are available from individuals released in reintroduction programmes in America (Hammer & Hatcher, 1983; Rymon, 1989; Martell *et al.*, 2002), but no CMR analyses were carried out for a proper estimation of apparent survival. No data have been published so far for the Mediterranean area (whether wild and reintroduced populations). Although our data set of reintroduced individuals was rather small, our results showed a high survival (0.87) in the first stages of the PFDP, between the release and their departure. Such a high survival rate initially seems to exclude the possibility that there were negative effects related to difficulties encountered by fledglings because of the hacking method (e.g. lack of parental care and artificial feeding initially after the release). Analogous causes of mortality (e.g. predation, electrocution) were reported for other Osprey reintroduction projects (Mackrill, 2005; Casado & Ferrer, 2008; CIBIO, 2011).

During the PFDP, young Ospreys returned to the hacking facilities to feed on artificial platforms (where fresh fish was supplied until the definitive departure of birds), while first fishing attempts were generally performed only after the departure (Monti *et al.*, 2012). This is a crucial moment for young birds, which must rapidly learn how to capture live fish to survive to their first winter. In their first winter we found a very low survival rate (0.26), suggesting that juvenile birds likely encountered difficulties in catching prey, finding suitable habitat or dealing with unfavourable environmental conditions. By pooling these values we obtained a first-year annual survival of 0.20, i.e. 2.5 times below the survival probability of 0.50 obtained from wild-born juveniles from a population in continental France (Wahl & Barbraud, 2014). However, the annual survival rate increased to 0.69 for second-year birds (immatures) but it was still lower than the survival rate of 0.87 estimated for the immatures and adults in Continental France (Wahl & Barbraud, 2014). This suggests that reintroduced juveniles may be affected by the lack of parental care in the dispersal phase of their life (for example, fishing lessons from parents during the PFDP may result in higher survival of young once they are dispersed). Thus, it would be more interesting to compare our values with other reintroduced population in Europe (e.g. England) or in the Mediterranean area (e.g. Andalucía and Portugal) as soon as such analyses are available. On the other hand, annual survival rate was relatively high (0.93) for adults compared to other wild populations: in Sweden 0.81 (Ryttmann, 1994), the USA 0.85–0.90 (Spitzer *et al.*, 1983) and continental France 0.87 (Wahl & Barbraud, 2014). This result could be related to the current low population density in the area, and suggests favourable conditions for adults and low levels of competition with conspecifics for food resources and for territories, as already postulated in other reintroductions (Martell *et al.*, 2002). A future increase in population size could possibly account for greater local competition with effects on demographic parameters, as recorded for the wild Corsican population (Bretagnolle *et al.*, 2008). Another factor which could play in favour of a high adult survival in our case might be related to the fact that most of the breeders were resident and did not migrate, but rather spent the winter close to the breeding areas in Italy, thus avoiding the risk and energy costs that accompany a long-distance migration. For northern Ospreys, Klaassen *et al.* (2014) estimated mortality rate six times higher during migration than during stationary periods, suggesting that events during the migration have an important impact on the population dynamics of long-distance migrants.

However, the Mediterranean basin is known to be an area where direct persecution by human (e.g. hunting and poaching) is widespread (millions of birds, including also protected species such as raptors, are killed annually; www.birdlife.org). This aspect might increase mortality for those individuals spending all their lives in the Mediterranean basin, and especially for inexperienced juvenile birds. Finally, these high adult survival rates were calculated on a very small sample size and this young population only contains young adults with high survival expectancy and no senescent individuals as yet. Further studies should include larger Osprey populations in the Mediterranean, monitored for a longer period (e.g. wild populations from Corsica and Balearic islands; reintroduced populations in Andalucía). Reintroduction has proved to be an appropriate method to locally re-establish Osprey populations. Although, results from European experiences (i.e. reintroductions in Rutland Water – England and Andalucía – Spain) show that to achieve the goal of a self-sustaining population which does not require extra human intervention (e.g. supplementary translocations), both a long time span and/or a high number of individuals to be released are needed. In particular, 75 Scottish birds were released between 1996 and 2001 in England before obtaining 7 territorial pairs in 2013 (Tim Mackrill, pers. comm.); 191 northern Ospreys (from Germany, Scotland and Finland) were used between 2003 and 2010 for the reintroduction in Andalucía before obtaining 12 territorial pairs in 2013 (Eva Casado, pers. comm.).

In Italy, despite constraints related to the limited number of chicks available for translocation each year (e.g. the donor population in Corsica being relatively small), two pairs are currently breeding in the area eight years after the start of the project and several territorial individuals are regularly seen in the southern Tuscany coastal wetland study area. This is quite comparable to the projects in England and Andalucía that are classified as successful. Here, we have estimated the apparent survival rate of the re-establishing population as a first step to monitor the demographic parameters of the new population. Once more data and a longer time period of data are available, a proper population viability analyses will allow us to estimate the growth rate and probability of extinction of the population taking into account number of breeding and territorial pairs, fecundity and recruitment of other eventual wild-born individuals. A gradual increase in the number of breeding pairs is now expected in the wetland system of coastal Tuscany. Furthermore, the seven islands included in the Tuscany Archipelago National Park and located midway between Corsica and Tuscany could function as ‘stepping-stones’ and might play a relevant role for the future expansion of the species. The recent building of artificial nests in Corsica, Tuscany (coastal territories and Montecristo Island) and Sardinia (Porto Conte Regional Park, Alghero province) could stimulate the colonization of new sites by mature Ospreys, so favouring the process of natural expansion of the populations. These structures aim to attract floaters and encourage the first phases of settlement and reproduction. This would ensure both local conservation to the species and genetic exchanges between the two breeding populations (Corsica and Italy). Overall this may ensure the re-establishment of at least a part of the ancient range of Osprey distribution in the Central Mediterranean basin.

13. GENERAL CONCLUSIONS

This research demonstrated the great potential of spatio-temporal scale-dependent approaches in ecology and conservation biology, and also clearly shows that such approaches require field and laboratory techniques drawn from a wide range of sub-disciplines, ranging from molecular biology to socio-economical considerations. Overall, this work has led to some important advances with respect to the conservation biogeography of ospreys in the Western Mediterranean, in western Europe, and at a worldwide scale. Those insights are particularly valuable with respect to the effective management of this emblematic species.

Beyond raptor conservation and the management of migratory bird species, all results gathered contribute to the current research effort towards building an integrative framework for biodiversity conservation.

13.1. PRESERVING OSPREYS AT A GLOBAL SCALE:

Notably, our results on sequences of mtDNA highlighted the presence of four distinct evolutive lineages in ospreys, at a worldwide scale (article 1). We evidenced a fully new lineage, originating from East Asia. Importantly, each osprey lineage represents an Evolutionary Significative Unit and should hence be treated and managed separately from other populations from other lineages. Thus, in the framework of reintroduction projects we recommend that no translocations should be conducted using source populations belonging to another distinct lineage. In other words, translocations in Europe should not use birds originating from the Americas, Oceania and East Asia.

The population genetic study using microsatellite markers confirmed the existence of at least three out of the four groups identified by mtDNA. Populations from America, Australia and Palearctic were found isolated, without significant gene flow. On the contrary, in the Palearctic we found that osprey populations, even if geographically distant, were partially connected by gene flow. However, this group presented an evident sub-structuration into two entities, following a north-south gradient (continental Europe vs Mediterranean). The absence of further distinction in the Mediterranean basin accounted for connectivity between local populations. Despite occasional inter-changes at the Palearctic scale due to dispersal events, the strong sub-structuration suggests that osprey populations living at different latitudes have developed specific genetic information. This could be the result of different evolutionary histories (e.g. the existence of refugial areas during last glaciations), and/or due to their respective habitat matrices: nests in continuous forested habitats vs fragmented coastal marine environments. Therefore, populations of the Palearctic, although belonging to the same lineage (no differences at the mtDNA level), present recent dissimilarities displayed at the level of nuclear genes.

In addition, we also found strong divergences in the migratory strategies and ranging behaviour between ospreys from continental Europe and the Mediterranean: the former carried out long-distance journeys until western African grounds south of the Sahara (making stopovers along the way) and the latter were partially migratory with 30% individuals mainly remaining in the Mediterranean basin. On the basis of behavioural differences in migration and in the genetic structuration at nuclear loci I recommend to pay attention to these aspects in the management of these populations. In particular I think that restoration of small populations, or the reconstitution of breeding nuclei by means of reintroduction or restocking programmes should rely primarily on source populations sharing similar characteristics and that evolved under similar environmental constraints. Indeed, reintroduction and reinforcement programmes would aim at restoring wild populations at sustainable levels with identical biological characteristics to the original population (Armstrong & Seddon, 2008). In the case of osprey, long-distance migratory populations with specific genetic information should not be primarily used to reconstruct populations in the Mediterranean region and Atlantic islands, where local populations show both differential genetic structuring and behaviour. Inadequate human interventions may provoke modifications in these populations and produce changes which are not in synchrony with natural evolutionary processes, neither with the habitat matrix and related environmental constrains. For instance, Villers *et al.* (2011) demonstrated, by means of an experiment, that captive-bred Little Bustard *Tetrax tetrax* originating from eggs collected in Spain (in an area where the local population is numerous but sedentary) and released in France (where a local population is migratory), did not subsequently migrate. This suggests that a sound conservation strategy should take into great consideration not only the ensuring of a rapid population recovery, but also the expression of migratory movements to maintain the integrity of native population.

13.2. PRESERVING OSPREYS AT A REGIONAL SCALE:

Furthermore, I found that Mediterranean ospreys mostly spend the winter at temperate latitudes showing a high plasticity in habitat selection. The use of marine bays, coastal lagoons/marshland, and freshwater sites located inland requires a broad approach for the protection of key areas, during the inter-breeding period. At the same time, since the wintering grounds are largely spread over the coasts of the western parts of the Mediterranean basin (Italy, North Africa, France and Spain), rather than concentrated in one single area (like e.g. the Wadden Sea or Camargue as crucial wintering and stopver sites for many shorebirds and waterbirds), an harmonization of the management protocols of these wetlands is required for different countries. On this basis, I recommend the necessity of implementing an international institution for the osprey conservation and/or the creation of a specific network/foundation which oversee the management actions and local protection aspects across countries. First common collaborations between France, Italy, Spain, and Morocco have been launched in the framework of this PhD project, but further actions and related decisions should be activated in the optics of a future connected network for osprey conservation in the whole Mediterranean basin. The implementation of such international foundations/specialist groups represents indeed an effective tool commonly used for the study, monitoring, managing and conserving specific emergencies and/or taxonomic groups (Richardson & Whittaker, 2010; Ladle & Whittaker, 2011). For example: a) the Vulture Conservation Foundation (VCF) is an international NGO committed to the conservation of the European vulture species aiming at limiting the negative effects of threats such as illegal poisoning, lack of food availability and collisions at wind farms and powerlines that are putting the incipient recovery of some populations at risk ("http://www.4vultures.org"). Similarly, Species Specialist Groups aim to actively promote research and conservation worldwide by developing conservation national or international Action Plans for the most threatened species and by encouraging information exchange and cooperation amongst these specialists, and with other relevant organisations, particularly the WWF, Wetlands International, BirdLife International and IUCN Species Survival Commission (SSC) (www.wetlands.org). An official institution like these should also be created for the osprey.

13.3. SPECIFIC MANAGEMENT RECOMMENDATIONS AT LOCAL SCALE:

More specifically the results gathered during this PhD can be used to implement management and conservation action at local scale. Considering the three specific cases in the Mediterranean, some practical considerations can be outlined. In Corsica, applied measures can be advised for the management of Scandola Natural Reserve and the conservation of its iconic species, the osprey. For instance it would be worth to regulate the daily boat traffic in terms of accesses and visits allowed per day. Big numbers and high density of ships at specific nesting sites should be avoided and strictly controlled to keep at a minimum limit disturbance to ospreys. Boats should not be allowed to approach to the coast in front of osprey nests, but should rather respect a buffer zone of a minimum of 300 m radius in order to avoid any disturbance to ospreys and to let the males fishing efficiently. To delimit such areas, waypoint buoys could be placed at sea. Such regulations could be adaptive and change every year, according to the ospreys' active nest distribution (and even change in course of the season after a nest failure). Enlarging the reserve boundaries would allow diluting disturbance: a project proposal in this sense has already been advanced by the Parc Naturel Régional de Corse for many years. Wardening should be maintained high to avoid negative effects of boat disturbance also at other osprey sites. Such kinds of measures have been already implemented in MPAs and allowed important success for the restoring of bird and fish communities (Heyman *et al.*, 2001; Velando & Munilla, 2011), often obtaining the support of local stakeholders (Badalamenti *et al.*, 2000).

In the Al Hoceima National Park (PNAH, Morocco), repeated census revealed the vulnerability of this population restricted in a narrow stretch of coast which is heavily exploited and disturbed by human activities (e.g. exploitation of the coastal habitat and dynamite fishing activities). The urgency of wardening and adequate regulations for a better securing of this nucleus is urgently needed and should be implemented by the National Park, with the help of local agencies (HCEFLCD) and NGOs.

In the framework of the Italian reintroduction project in the Maremma Regional Park and surrounding areas of southern Tuscany, translocations have been temporarily suspended due to scarce breeding success in Corsica recorded in the last years. However, other direct management actions have been foreseen and are currently ongoing. Managers planned the installation of new artificial nests at key sites (e.g. Capraia Island between Corsica and mainland Italy, Massaciuccoli Lake in Tuscany, and Omodeo Lake in Sardinia) to favour the settlement of floaters in the region and promote the natural recolonization of adjacent areas, to ultimately support other populations within the Mediterranean basin.

13.4. RESEARCH PERSPECTIVES

Towards a broad conservation of the osprey and a better knowledge on the species, we suggest some new research avenues, which should be considered as priorities. Researchers and managers should hence address the following topics:

a) update the census of the Asian populations, to be integrated with genetic analyses in order to delimitate distributional boundaries of each ESU (see the case of overlapping areas in article 1), with particular attention to the Japanese and Siberian populations for which a limited number of samples has been analysed. As the majority of these samples are museums specimens the current existence of this clade in East Asia and Indonesia needs to be confirmed from samples of living individuals;

b) on the same line, the migratory behaviour of Asiatic populations needs to be investigated to understand their main migratory routes, wintering grounds in tropical areas of

Indonesia and to search for eventual resident or partial migratory nuclei along the latitudinal gradient in Asia (from Kamtchatka to Australia). This would be of notable interest also for conservation issues documented for migratory birds across the East Asian–Australasian flyway (EAAF: Bamford *et al.*, 2008; Amano *et al.*, 2010);

c) investigate the migratory behaviour of the *ridgwayi* subspecies by tagging individuals from Caribbean to check for the existence of a resident or partial migratory population with distinctive migratory strategies than those reported for north American populations (i.e. Martell *et al.*, 2014);

d) Finally at the Mediterranean scale, demographic analyses should be conducted on poorly-known wild populations (e.g. Morocco and Algeria, Balearics) or reintroduced ones (e.g. Andalucia). This will allow compiling a better scenario of population health in this region. For instance, analysing survival and breeding success in Corsica and Balearics since 1975, could help to understand to what extent the recolonization of Corsica could be due to emigration of Balearic birds, by using simulation demographic models with Leslie matrix (Caswell, 2014).

Beyond the evolutionary history which accounted for the species range as we know it today, what does really shape migratory habits of ospreys across their extensive distributional range, in current times? By plotting the mean temperature of the coldest quarter of the year on a globe map and, at the same time, the geographical distribution of osprey populations, it is striking to note how thermal gradients match with the range of populations exhibiting different migratory behaviours (Poole, 1989). Ospreys are probably not affected directly by cold temperatures *per se*, but temperatures may indirectly influence migratory decisions at the population-level. As a piscivorous bird, the osprey is strictly dependent upon the accessibility of fish which, being cold-blooded organisms, are sensitive to thermal switches in the water column (Brett, 1956). In the Northern Hemisphere, ospreys mainly breed in forested habitats at freshwater sites; cold temperatures during winter may provoke the freezing of lakes and rivers, preventing birds to plunge to access their prey, ultimately leading individuals to migrate towards more temperate areas with unfrozen waters. At temperate or tropical latitudes instead, warmer conditions present year-round probably guarantees the accessibility to water bodies that do not freeze and the availability of cold-blooded prey close to the water surface, also in winter (Poole, 1989). Finally, at geographical places where intermediate conditions occur (e.g. Baja California, Florida, Mediterranean coasts, Red Sea and Persian Gulf), populations are partially migratory with short-distance migratory, or even resident individuals (Martell *et al.*, 2004). In these cases, partial erratism or regional movements may be dictated by local variations in food availability.

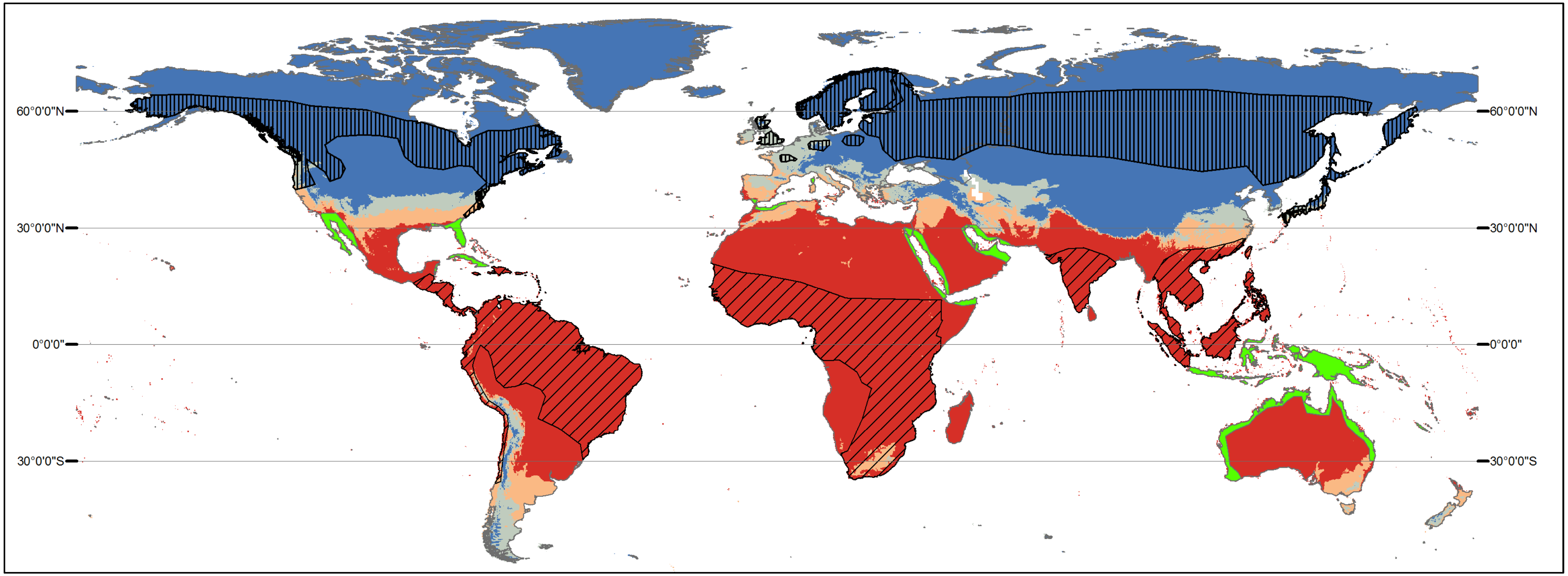


Figure 11: Map of the globe representing: a) the Mean Temperature of Coldest Quarter (BIO$_{11}$), as downloaded from WorldClim – Global Climate Data (www.worldclim.org). Thermal gradients are reported in different ranges of colours: blue from -50° to 0° C, grey from 0° to +5° C, orange from +5° to 10° and red from +10° to +30°. b) Geographical distribution of osprey populations around the world: vertical stripes are for breeding areas, skew lines for wintering areas and green color-filled zones represent areas with resident or partially migratory populations.

At the scale of the Mediterranean, mean temperatures during the colder period of the year vary locally: for example, in the Balearic Islands, southern Spain and north African coasts temperatures are >10° C on average, whereas along the northern coasts of the Mediterranean, in Corsica and central Italy mean temperatures are <10° C (Fig. 12). Interestingly, we found that a higher proportion of tagged adults (80%) where resident in Balearics compared to Corsica and Italy where only 33.3% of tagged adults did not migrate. Therefore, migratory decisions could be dictated and regulated by local weather conditions during the year, and especially during the coldest period.

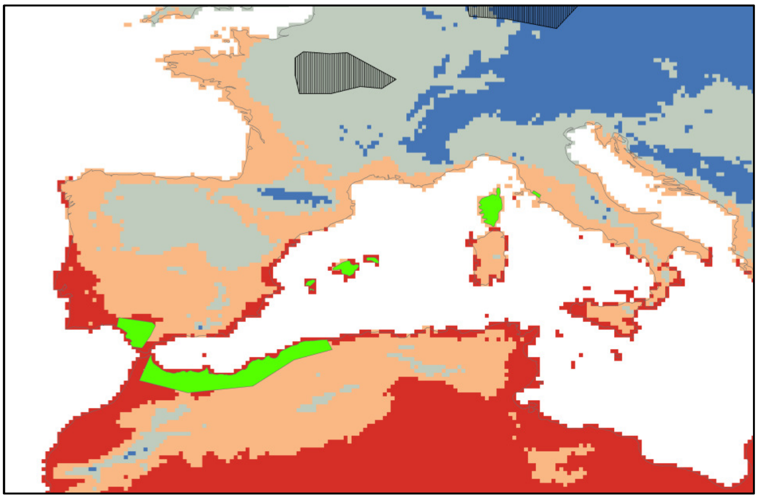


Figure 12: Mean temperatures of the colder quarter of the year (BIO_{11}) in the Western Mediterranean basin and distribution of local osprey populations (green color-filled zones). Colour code refers to temperatures as in Fig. 11.

Historically, climate change has led to shifts in phenology in many species, ultimately shaping their distribution and life histories (e.g. glacial periods) (Visser & Both, 2005). Since the last decades, we are currently experiencing a global warming process that starts to trigger shifts in the phenology of species, with important ecological effects on habitats and living organisms (Walther *et al.*, 2002). What hence could we expect for osprey and migratory organisms in general, in the near future? One could assume that migratory animals are resilient to climate change because of their high mobility which allows them to travel towards more suitable climates (Crick, 2004). Notwithstanding, the life cycle of a migratory species is complicated by the fact that individuals deal with a wide range of factors encountered over long-distance journeys encompassing vast geographic areas and a broad set of ecological conditions (Knudsen *et al.*, 2011; Studds & Marra, 2011; Marra *et al.*, 2014). Such complex life-system prevents from clearly predicting biological responses and vulnerability of migratory populations to large-scale changes in climate, and from designing anticipatory conservation measures. However as a general statement, one can forecast that migratory populations may turn into partial migratory or resident ones according to the general warming process characterized by the gradual increase of mean temperatures towards higher latitudes. Many studies demonstrated the association between phenological shifts (e.g. departure dates, stopover frequency, duration of the migratory journey) and recent climate change (Gordo, 2007; Marra *et al.*, 2005). The variation of temperatures and ecological conditions may re-shape the spatial distribution and temporal availability of resources, and consequently affect migratory habits of individuals and populations (i.e. the acquisition of optimal body condition during the days preceeding departure; Gordo, 2007; Møller *et al.*, 2008). But where will future climate change have the greatest influence for animal populations? How species with different ecological requirements will respond to it? To what extent will their populations be affected and at what stage of the annual cycle? Unfortunately, there is a lot of uncertainty among climate models predicting patterns of specific changes (Allen *et al.*, 2000; Tebaldi *et al.*, 2005). For this reason, assessing climate change vulnerability in migratory species requires a methodological approach which takes the full annual cycle into account, and which embraces the complexity of species-specific life histories, so being applicable to many taxa and geographical regions, and ultimately using a multiple scales-set of investigations.

In the specific case of osprey, as it seems that migration and wintering in the Mediterranean basin are risky stages (especially in the north African shores and interior lakes and rivers where several of our tracked birds died or disappeared), an increase of mean temperatures in the region would lead ospreys to become more sedentary and stay nearby their breeding sites, where mortality seems to be very low. This could increase the survival prospect of local populations, even if it is difficult to foresee to what extent other factors such as density, local threats and resource availability will contribute to depict the future scenario of the osprey in the Mediterranean.

In conclusion, this study is structured to be a good model also for studying other species, which share similar characteristics and ecological aspects during the life-cycle, such as large migratory birds (e.g. raptors, storks and cranes) presenting a wide distributional range. For example, migratory birds deserve a multi-scale and multi-populations study approach to achieve sound conservation goals. They indeed, travelling long-distances across different regions, habitats and political boundaries, are potentially threatened by a multitude of factors which affect the survival and persistence on long-term of their populations (Newton, 2010). Evolutionary tracts and adaptive behaviours need to be deeply investigated, namely in the optic of recent rapid changes. In this sense, is mandatory adopting a multiscale integrated approach on targeted species to have a more complete view that allows understanding their evolutive histories, genetics and population limitations, to ultimately advice on their conservation. This is true especially for those populations living within human exploited environments and/or fragmented habitats for the majority of the year cycle, where a lot of threats or ecological constrains are present. The Mediterranean is one of the most exploited regions and hosts high values of biodiversity, thus being a good area where interesting case studies are worth to be tested. Migratory bird populations living at these latitudes (e.g. Mediterranean) would have evolved specific behaviours and may possess unique genetic pools consequence of ancient glacial periods (i.e. these areas functioned as glacial refugia for many species of mammals and birds during glacial times; Vilaça *et al.*, 2014; Hewitt, 2000). Furthermore, populations confined in marine islands, are notoriously of great interest because influenced by a persistent ecological barrier such as the sea which can refrain individuals to cross it and abandon the island (from which are therefore highly dependent). Large migratory raptor and stork populations are interesting in this sense because of their reluctance to cross large water bodies (for the absence of thermal currents at sea), and would be compared with non migratory species (such as vultures for example) to understand how they have been adapted in leaving (or not) islands and what impact we can now observe on the dynamics of their populations, to ultimately design appropriate management programs.

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15. ANNEXES

15.1. ANNEXE 1: ADDITIONAL FILES FROM ARTICLE 1

Additional file 1: Taxon Sampling

Detailed list of samples indicating: sample lab code, subspecies according to morphology classification, country of collection, locality, sample type (tp = toepad; wb = wet blood; db = dry blood; ft = feather; fs = fasta sequence), codes, gene bank number accession for cyt b and ND2 and name of the institution and/or collector (with affiliation).

Sample Code	Subspecies	Country of Collection	Locality	Sample Type	Collection date	Ring/Museum Code	Genebank A.N.	Institution and Reference
70	ridgwayi	Netherland Antilles	Netherlands Antilles-Curacao	museum/tp	01/11/1953	ZMA.AVES.28711		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
71	ridgwayi	Netherland Antilles	Netherlands Antilles-St.Marteen	museum/tp	11/02/1905	RMNH.AVES.162906		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
72	ridgwayi	Netherland Antilles	Netherlands Antilles-Bonaire	museum/tp	25/11/1978	ZMA.AVES.32804		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
73	ridgwayi	Netherland Antilles	Netherlands Antilles-Bonaire	museum/tp	28/11/1951	ZMA.AVES.11423		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
74	ridgwayi	Netherland Antilles	Netherlands Antilles-Bonaire	museum/tp	01/06/1905	ZMA.AVES.33694		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
76	ridgwayi	Bahamas	Great Inagua island	museum/tp	10/03/1888	BMNH 1906.12.7.624		Natural History Museum - London (Mark Adams)
77	ridgwayi	Bahamas	unknown	museum/tp	03/03/1902	BMNH 1924.4.10.188		Natural History Museum - London (Mark Adams)
78	ridgwayi	Bahamas	unknown	museum/tp	06/12/1902	BMNH 1924.4.10.189		Natural History Museum - London (Mark Adams)
79	carolinensis	USA	Florida-Torch key	museum/tp	01/01/1883	RMNH.AVES.162900		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
80	carolinensis	USA	Florida-Spanish key	museum/tp	01/01/1883	RMNH.AVES.163190		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
81	carolinensis	USA	Florida-Spanish key	museum/tp	01/01/1883	RMNH.AVES.163331		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
144	carolinensis	USA	unknown	museum/tp	16/04/1836	RMNH.AVES.162896		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
150	carolinensis	Suriname	Matapica	museum/tp	19/08/1961	RMNH.33233		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
151	carolinensis	Suriname	Matapica	museum/tp	04/08/1962	RMNH.33831		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
154	carolinensis	Caribbean	Netherlands Antilles-Aruba island (origin Virginia)	museum/tp	01/02/1979	608-39789		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
199	carolinensis	USA	Oregon	fresh/wb	01/07/1999	A 2431		University of Greifswald - collection of M. Martell (Martin Haase)
200	carolinensis	USA	Oregon	fresh/wb	01/07/1999	A 2435		University of Greifswald - collection of M. Martell (Martin Haase)
216	carolinensis	USA	Massachussetts-Westport	fresh/db	17/05/2012	0928-09931		Alan Poole (Cornell Lab of Ornithology - Ithaca, NY)
217	carolinensis	USA	Massachussetts-Westport	fresh/db	29/05/2012	no code		Alan Poole (Cornell Lab of Ornithology - Ithaca, NY)
218	carolinensis	USA	Massachussetts-Westport	fresh/db	29/05/2012	0928-09932		Yula Kapetanankos and Alan Poole (Cornell Lab of Ornithology - Ithaca, NY)
219	carolinensis	USA	Massachussetts-Westport	fresh/db	30/05/2012	no code		Yula Kapetanankos and Alan Poole (Cornell Lab of Ornithology - Ithaca, NY)
220	carolinensis	USA	Massachussetts-Westport	fresh/db	30/05/2012	no code		Yula Kapetanankos and Alan Poole (Cornell Lab of Ornithology - Ithaca, NY)
222	carolinensis	USA	Massachussetts-Westport	fresh/db	07/06/2012	no code		Yula Kapetanankos and Alan Poole (Cornell Lab of Ornithology - Ithaca, NY)
224	carolinensis	USA	Massachussetts-Westport	fresh/db	NO DATE	no code		Alan Poole (Cornell Lab of Ornithology - Ithaca, NY)
b	carolinensis	USA	unknown	sequence/fs	NO DATE	no code	AY987232	Lerner & Mindell (2005)
c	carolinensis	USA	unknown	sequence/fs	NO DATE	no code	EU167008	Lerner & Mindell (2005)
165	haliaetus	Russia	Magadan region	museum/tp	20/08/1936	R-31411		Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
166	haliaetus	Russia	Baikal lake	museum/tp	22/05/1959	R-101447		Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
167	haliaetus	Russia	Kuriles islands	museum/tp	01/01/1948	R-79411		Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
170	haliaetus	Russia	Magadan region	museum/tp	20/08/1936	R-31410		Zoological Museum of Moscow, Russia (Mikhail Kalyakin)

171	haliaetus	Russia	Khabarovsky region	museum/tp	05/05/1965	R-92511		Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
173	haliaetus	Russia	Primorskii region	museum/tp	20/04/1962	R-90372		Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
174	haliaetus	Mongolia	unknown	museum/tp	29/06/1903	R-4500		Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
175	haliaetus	Russia	Tuva region	museum/tp	17/08/1902	R-28728		Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
176	haliaetus	Russia	Khabarovsky region	museum/tp	25/06/1910	R-28723		Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
178	haliaetus	Japan	Ota-ku	fresh/ms	22/10/2008	2008-265-NSMT-8057		National Museum of Nature and Science, Amakubo - Japan (Isao Nishiumi)
179	haliaetus	Japan	Hokkaido	fresh/ms	04/07/2008	KUS14-NSMT-9259		National Museum of Nature and Science, Amakubo - Japan (Isao Nishiumi)
180	haliaetus	Japan	Hokkaido	fresh/ms	04/07/2008	KUS15-NSMT-9260		National Museum of Nature and Science, Amakubo - Japan (Isao Nishiumi)
181	haliaetus	Japan	Tokoname-shi	fresh/ms	28/03/2011	2011.106-NSMT-50628		National Museum of Nature and Science, Amakubo - Japan (Isao Nishiumi)
182	haliaetus	Japan	Ota-ku	fresh/ms	06/12/2012	2013.8-NSMT-52035		National Museum of Nature and Science, Amakubo - Japan (Isao Nishiumi)
83	cristatus	Indonesia	Sanghir island	museum/tp	04/08/1865	RMNH.AVES.163187		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
84	cristatus	Indonesia	Java (poeloe lantjang,batavia)	museum/tp	11/08/1927	RMNH.99462		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
85	cristatus	Indonesia	Java (poeloe lang,java sea)	museum/tp	09/09/1906	RMNH.99465		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
86	cristatus	Indonesia	Ceram sea (poeloe kasoeari)	museum/tp	09/06/1910	RMNH.AVES.163098		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
87	haliaetus	Indonesia	Sumatra (korintji,sandaron agang)	museum/tp	14/07/1915	RMNH.AVES.163143		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
90	cristatus	Indonesia	Morotai	museum/tp	31/12/1861	RMNH.AVES.162885		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
91	cristatus	Indonesia	Motie	museum/tp	02/10/1863	RMNH.AVES.162886		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
93	cristatus	Indonesia	Celebes (Buton)	museum/tp	23/09/1948	ZMA.AVES.47906		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
94	cristatus	Indonesia	Sanghir island	museum/tp	24/01/1886	RMNH.AVES.162903		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
95	cristatus	Indonesia	Siau island	museum/tp	NO DATE	RMNH.AVES.163100		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
96	cristatus	Indonesia	Tanahwangko, Minahasa, Celebes	museum/tp	11/01/1940	ZMA.AVES.47510		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
97	cristatus	Indonesia	Ceram, Kaibobo	museum/tp	NO DATE	RMNH.AVES.163238		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
98	cristatus	Indonesia	Borneo (Pagattan)	museum/tp	02/09/1844	RMNH.AVES.162908		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
99	cristatus	Indonesia	Ternate island (Molucca islands)	museum/tp	24/04/1861	RMNH.AVES.162938		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
100	cristatus	Indonesia	Ternate island (Molucca islands)	museum/tp	30/04/1861	RMNH.AVES.163251		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
101	cristatus	Indonesia	Celebes,Mara	museum/tp	20/04/1914	RMNH.AVES.162869		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
102	haliaetus	Indonesia	Pulau Batjan	museum/tp	11/01/1861	RMNH.AVES.163099		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
103	haliaetus	Indonesia	Java,Djampang Koelan	museum/tp	17/02/1929	RMNH.AVES.99451		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
105	cristatus	Indonesia	Java,Bokor,Batavia	museum/tp	15/04/1912	RMNH.AVES.99467		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
106	cristatus	Indonesia	Java,Bokor,Batavia	museum/tp	15/04/1912	RMNH.AVES.99457		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
107	cristatus	Indonesia	Java,Moeara angke	museum/tp	27/12/1912	RMNH.AVES.99458		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
108	cristatus	Indonesia	Java (poeloe lantjang,batavia)	museum/tp	22/05/1922	RMNH.AVES.99468		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
109	cristatus	Indonesia	Java	museum/tp	NO DATE	RMNH.AVES.99463		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
110	cristatus	Indonesia	Java,Bokor,Batavia	museum/tp	06/10/1920	RMNH.AVES.99453		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
111	cristatus	Indonesia	Aru islands	museum/tp	19/06/1865	RMNH.AVES.163322		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
112	cristatus	Indonesia	Babar island	museum/tp	17/04/1898	RMNH.AVES.163357		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
113	cristatus	Indonesia	Buru island (djikoe-merasa N.Beroe)	museum/tp	25/07/1923	RMNH.AVES.cat.29		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
140	cristatus	New Caledonia	unknown	museum/tp	NO DATE	RMNH.AVES.162873		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
141	cristatus	New Guinea	Sentani	museum/tp	25/04/1903	RMNH.AVES.163102		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
142	haliaetus	New Guinea	Dorek	museum/tp	NO DATE	RMNH.AVES.162884		Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
201	cristatus	Australia	New South Wales	fresh/wb	1998	A 2447		University of Greifswald - collection of M. Martell (Martin Haase)
202	cristatus	Australia	New South Wales	fresh/wb	1998	A 2448		University of Greifswald - collection of M. Martell (Martin Haase)
203	cristatus	Australia	New South Wales	fresh/wb	01/08/1998	A 2450		University of Greifswald - collection of M. Martell (Martin Haase)
204	cristatus	Australia	New South Wales	fresh/wb	01/08/1998	A 2451		University of Greifswald - collection of M. Martell (Martin Haase)
205	cristatus	Australia	New South Wales	fresh/wb	01/09/1998	A 2452		University of Greifswald - collection of M. Martell (Martin Haase)
206	cristatus	Australia	New South Wales	fresh/wb	01/09/1998	A 2453		University of Greifswald - collection of M. Martell (Martin Haase)
207	cristatus	Australia	New South Wales	fresh/wb	01/09/1998	A 2454		University of Greifswald - collection of M. Martell (Martin Haase)
208	cristatus	Australia	New South Wales	fresh/wb	01/09/1998	A 2455		University of Greifswald - collection of M. Martell (Martin Haase)
209	cristatus	Australia	New South Wales	fresh/wb	01/09/1998	A 2456		University of Greifswald - collection of M. Martell (Martin Haase)
a	cristatus	Australia	unknown	sequence/fs	NO DATE	no code	DQ780884	Lerner & Mindell (2005)
1	haliaetus	France	Loiret	fresh/ft	2003	BA10505		Rolf Wahl (MNHN)
2	haliaetus	France	Loiret	fresh/ft	2006	BS14320		Rolf Wahl (MNHN)
3	haliaetus	Latvia	Basi	fresh/ft	08/07/2012	ET3579		Aigars Kalvans (LFN)
4	haliaetus	Latvia	Vaidavas lake	fresh/ft	03/07/2012	ET3505		Aigars Kalvans (LFN)

5	haliaetus	France	Corsica-Cape Corse	fresh/db	13/06/2012	BS15569 (CAB)	Jean-Marie Dominici (PNRC)
6	haliaetus	France	Corsica-Scandola Reserve	fresh/db	24/03/2012	BS15576 (A02)	Flavio Monti & Jean-Marie Dominici (CEFE-PNRC)
7	haliaetus	Spain	Canary Islands-Tenerife Island	fresh/db	16/06/2012	(SN)	Manuel Siverio (GOHNIC)
8	haliaetus	Spain	Balearic Islands-Menorca Island	fresh/db	19/06/2012	(HU)	Rafel Triay (IME)
9	haliaetus	Morocco	Al-Hoceima National Park	fresh/db	20/05/2012	BS15552 (AAA)	Flavio Monti & Jean-Marie Dominici (CEFE-PNRC)
10	haliaetus	Morocco	Al-Hoceima National Park	fresh/db	22/05/2012	BS15554 (AAC)	Flavio Monti & Jean-Marie Dominici (CEFE-PNRC)
11	haliaetus	Morocco	Al-Hoceima National Park	fresh/db	23/05/2012	BS15556 (AAE)	Flavio Monti & Jean-Marie Dominici (CEFE-PNRC)
12	haliaetus	France	Corsica-Scandola Reserve	fresh/db	29/05/2012	BS15565	Jean-Marie Dominici (PNRC)
13	haliaetus	France	Corsica	fresh/db	29/05/2012	BS15560	Jean-Marie Dominici (PNRC)
14	haliaetus	France	Corsica	fresh/db	29/05/2012	BS15561	Jean-Marie Dominici (PNRC)
15	haliaetus	France	Corsica-Porto gulf	fresh/db	22/05/2012	BS15573 (CAI)	Jean-Marie Dominici (PNRC)
16	haliaetus	France	Corsica-Galeria gulf	fresh/db	01/05/2012	BS15571	Jean-Marie Dominici (PNRC)
17	haliaetus	France	Corsica-Scandola Reserve	fresh/db	24/05/2012	BS15563	Jean-Marie Dominici (PNRC)
18	haliaetus	France	Corsica	fresh/db	30/05/2012	BS15566	Jean-Marie Dominici (PNRC)
19	haliaetus	Latvia	Pukbu	fresh/ft	02/07/2012	ET3299	Aigars Kalvans (LFN)
20	haliaetus	Latvia	Usmas lake	fresh/ft	06/07/2012	ET3546	Aigars Kalvans (LFN)
21	haliaetus	Latvia	Usmas lake	fresh/ft	06/07/2012	ET3551	Aigars Kalvans (LFN)
22	haliaetus	Latvia	Vecbebri	fresh/ft	10/07/2012	ET3593	Aigars Kalvans (LFN)
23	haliaetus	Latvia	Baltmuiza	fresh/ft	10/07/2012	ET3600	Aigars Kalvans (LFN)
24	haliaetus	Latvia	Apriki	fresh/ft	08/07/2012	ET3574	Aigars Kalvans (LFN)
25	haliaetus	Latvia	Allarmuiza	fresh/ft	09/07/2012	ET3590	Aigars Kalvans (LFN)
26	haliaetus	Latvia	Lubana lake	fresh/ft	30/06/2012	ET3269	Aigars Kalvans (LFN)
27	haliaetus	France	Loiret	fresh/ft	2006	BS14321	Rolf Wahl (MNHN)
28	haliaetus	France	Essonne	fresh/ft	2006	BS14332	Rolf Wahl (MNHN)
29	haliaetus	France	Loir-et-Cher	fresh/ft	2006	BS14301	Rolf Wahl (MNHN)
30	haliaetus	France	Loir-et-Cher	fresh/ft	2006	BS14309	Rolf Wahl (MNHN)
31	haliaetus	France	Loiret	fresh/ft	2006	BS14326	Rolf Wahl (MNHN)
32	haliaetus	France	Loir-et-Cher	fresh/ft	2006	BA10900	Rolf Wahl (MNHN)
33	haliaetus	France	Loiret	fresh/ft	2006	BA10897	Rolf Wahl (MNHN)
34	haliaetus	France	Loiret	fresh/ft	2006	BS14304	Rolf Wahl (MNHN)
35	haliaetus	France	Loiret	fresh/ft	2006	BS14315	Rolf Wahl (MNHN)
36	haliaetus	France	Loiret	fresh/ft	2006	BA10893	Rolf Wahl (MNHN)
37	haliaetus	Spain	Canary Islands-La Gomera	fresh/ft	01/06/2009	S8	Manuel Siverio (GOHNIC)
38	haliaetus	Spain	Canary Islands-Tenerife	fresh/ft	23/05/2009	S7	Manuel Siverio (GOHNIC)
39	haliaetus	Italy	Maremma Regional Park	fresh/ft	11/06/2012	S7	Flavio Monti & Andrea Sforzi (CEFE-MRP)
41	haliaetus	Cape Vert	Boavista	fresh/db	01/04/2012	01	Pedro López-Suárez (NCV)
42	haliaetus	Cape Vert	Boavista	fresh/db	14/04/2012	02	Pedro López-Suárez (NCV)
43	haliaetus	Spain	Canary Islands-Tenerife	fresh/db	16/06/2012	SP	Manuel Siverio (GOHNIC)
44	haliaetus	Morocco	Al-Hoceima National Park	fresh/db	JUNE 2012	BS15558 (AAI)	Houssine Nibani (AGIR-PNAH)
45	haliaetus	Estonia	unknown	fresh/ft	20/07/2012	U4	Urmas Sellis (EOS)
46	haliaetus	Estonia	unknown	fresh/ft	19/07/2012	S8	Urmas Sellis (EOS)
47	haliaetus	Estonia	unknown	fresh/ft	19/07/2012	S9	Urmas Sellis (EOS)
48	haliaetus	Estonia	unknown	fresh/ft	19/07/2012	S7	Urmas Sellis (EOS)
49	haliaetus	Estonia	unknown	fresh/ft	03/08/2012	U5	Urmas Sellis (EOS)
50	haliaetus	Estonia	unknown	fresh/ft	03/08/2012	U6	Urmas Sellis (EOS)
51	haliaetus	Estonia	unknown	fresh/ft	04/08/2012	S0	Urmas Sellis (EOS)
52	haliaetus	Estonia	unknown	fresh/ft	19/07/2012	U2	Urmas Sellis (EOS)
53	haliaetus	Finland	Hame region	fresh/ft	23/07/2012	M-61572	Pertti Saurola (FMNH)
54	haliaetus	Finland	Hame region	fresh/ft	25/07/2012	M-64274	Pertti Saurola (FMNH)
55	haliaetus	Finland	Hame region	fresh/ft	25/07/2012	M-64268	Pertti Saurola (FMNH)
56	haliaetus	Finland	Hame region	fresh/ft	25/07/2012	M-64270	Pertti Saurola (FMNH)
57	haliaetus	Finland	Hame region	fresh/ft	25/07/2012	M-64272	Pertti Saurola (FMNH)
58	haliaetus	Finland	Lapland	fresh/ft	24/07/2012	M-63753	Harri Koskinen (FMNH)
59	haliaetus	Finland	Lapland	fresh/ft	25/07/2012	M-63756	Harri Koskinen (FMNH)

60	haliaetus	Finland	Lapland	fresh/ft	25/07/2012	M-63760	Harri Koskinen (FMNH)
61	haliaetus	Finland	Lapland	fresh/ft	25/07/2012	M-63763	Harri Koskinen (FMNH)
62	haliaetus	Finland	Lapland	fresh/ft	26/07/2012	M-63765	Harri Koskinen (FMNH)
63	haliaetus	Finland	Lapland	fresh/ft	26/07/2012	M-63827	Harri Koskinen (FMNH)
64	haliaetus	Finland	Lapland	fresh/ft	27/07/2012	M-63830	Harri Koskinen (FMNH)
65	haliaetus	Finland	Lapland	fresh/ft	27/07/2012	M-63832	Harri Koskinen (FMNH)
66	haliaetus	Spain	Balearic Islands-Menorca Island	fresh/wb	14/06/2000	A	Rafel Triay (IME)
67	haliaetus	Spain	Balearic Islands-Menorca Island	fresh/wb	18/06/2000	R	Rafel Triay (IME)
68	haliaetus	Spain	Balearic Islands-Menorca Island	fresh/wb	15/06/2000	N	Rafel Triay (IME)
69	haliaetus	Spain	Balearic Islands-Menorca Island	fresh/wb	07/06/2000	Z	Rafel Triay (IME)
82	cristatus	India	Hindustan	museum/tp	NO DATE	RMNH.AVES.163189	Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
114	haliaetus	Italy	Genova, airport (Italy)	museum/tp	01/08/1990	MSNG-54719	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
115	haliaetus	Italy	Genova, Pegli (Italy)	museum/tp	24/03/1957	MSNG-36570	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
116	haliaetus	Italy	Murialdo, Savona (Italy)	museum/tp	28/09/1939	MSNG-33574	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
117	haliaetus	Italy	Genova, Caselli (Italy)	museum/tp	30/03/1949	MSNG-34783	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
118	haliaetus	Italy	Montecastello Tanaro, Alessandria (Italy)	museum/tp	01/09/1946	MSNG-34459	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
119	haliaetus	Italy	Genova, Sestri Levante (Italy)	museum/tp	20/03/1962	MSNG-38664	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
120	haliaetus	Italy	Genova, Busalla torrente Scrivia (Italia)	museum/tp	26/03/1951	MSNG-35046	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
121	haliaetus	Italy	Genova, Sestri Ponente (Italy)	museum/tp	16/04/1879	MSNG-33132	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
122	haliaetus	Italy	Genova, Cornigliano (Italy)	museum/tp	31/03/1960	MSNG-52971	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
124	haliaetus	Italy	Imola, Basso di Poggi (Italia)	museum/tp	NO DATE	MSNG-53333	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
125	haliaetus	Italy	Genova, Borzoli (Italia)	museum/tp	01/04/1875	MSNG-24720	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
127	haliaetus	Pakistan	Baluchistan	museum/tp	22/01/1872	BMNH 1874.11.23.9	Natural History Museum - London (Mark Adams)
128	haliaetus	Yemen	Abdul-Kori	museum/tp	04/12/2012	BMNH 1899.8.11.113	Natural History Museum - London (Mark Adams)
129	haliaetus	Iran	Tunb islands	museum/tp	18/03/1921	BMNH 1924.3.20.74	Natural History Museum - London (Mark Adams)
130	haliaetus	Iraq	Mesopotamia,Tigris,Hamar lake	museum/tp	20/05/1928	BMNH 1933.2.16.304	Natural History Museum - London (Mark Adams)
131	haliaetus	Saudi Arabia	Arabia,Jizan	museum/tp	15/12/1936	BMNH 1937.4.17.244	Natural History Museum - London (Mark Adams)
132	haliaetus	Saudi Arabia	Arabia,Jedda	museum/tp	15/06/1948	BMNH 1948.56.3	Natural History Museum - London (Mark Adams)
133	haliaetus	Saudi Arabia	Arabia,Jedda	museum/tp	08/06/1947	BMNH 1949.24.6	Natural History Museum - London (Mark Adams)
134	haliaetus	Saudi Arabia	Saudi Arabia	museum/tp	09/02/1927	BMNH 1949.24.9	Natural History Museum - London (Mark Adams)
135	haliaetus	Netherland	Texel island	museum/tp	09/05/1907	RMNH.AVES.163191	Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
136	haliaetus	Netherland	Zandvoort	museum/tp	06/05/1919	RMNH.AVES.162901	Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
137	haliaetus	Netherland	Hillegom	museum/tp	03/06/1880	RMNH.AVES.163279	Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
138	haliaetus	Netherland	Noordwijk	museum/tp	05/05/1862	RMNH.AVES.163264	Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
139	haliaetus	Netherland	Leiduin,Vogelenzong	museum/tp	26/04/1878	RMNH.AVES.163268	Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
143	haliaetus	India	Nipaul	museum/tp	NO DATE	RMNH.AVES.163256	Naturalis Biodiversity Centre - Leiden, NL (Becky Desjardins)
149	haliaetus	Italy	Imola, Pontedassio (Italy) (origin Spain)	museum/tp	22/05/1995	MSNG-54785	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
152	haliaetus	Italy	Genova, Lerca (Italy) (origin Sweden)	museum/tp	17/04/1958	MSNG-54788	Museo "Giacomo Doria" - Genova, Italy (Enrico Borgo)
155	haliaetus	Germany	Germany	museum/es	NO DATE	PH1 (red)	Ursula Hofle Hansen (CIA)
156	haliaetus	Germany	Germany	museum/es	NO DATE	PH2 (white)	Ursula Hofle Hansen (CIA)
162	haliaetus	Portugal	Portugal	museum/es	1995	219-95-PH1 (red)	Ursula Hofle Hansen (CIA)
163	haliaetus	Portugal	Portugal	museum/es	1995	PH2 (red)	Ursula Hofle Hansen (CIA)
164	haliaetus	Portugal	Portugal	museum/es	1995	000166-95-PH3 (white)	Ursula Hofle Hansen (CIA)
168	haliaetus	Russia	Urals	museum/tp	11/05/1939	R-50450	Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
169	haliaetus	Russia	Urals	museum/tp	10/06/1940	R-50539	Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
172	haliaetus	Russia	Urals	museum/tp	20/05/1941	R-56829	Zoological Museum of Moscow, Russia (Mikhail Kalyakin)
177	haliaetus	Portugal	Portugal (origin Sweden)	fresh/ft	01/04/2012	P17	Luis Plama (CIBIO)
183	haliaetus	Russia	Russia, Darwin state natural biosphere reserve	fresh/wb	14/07/2013	1	Miroslav Babushkin (DSNBR)
184	haliaetus	Russia	Russia, Darwin state natural biosphere reserve	fresh/wb	14/07/2013	2	Miroslav Babushkin (DSNBR)
185	haliaetus	Russia	Russia, Darwin state natural biosphere reserve	fresh/wb	14/07/2013	3	Miroslav Babushkin (DSNBR)
186	haliaetus	Russia	Russia, Darwin state natural biosphere reserve	fresh/wb	15/07/2013	4	Miroslav Babushkin (DSNBR)
187	haliaetus	Russia	Russia, Darwin state natural biosphere reserve	fresh/wb	15/07/2013	5	Miroslav Babushkin (DSNBR)
188	haliaetus	Russia	Russia, Darwin state natural biosphere reserve	fresh/wb	15/07/2013	6	Miroslav Babushkin (DSNBR)
189	haliaetus	Russia	Russia, Darwin state natural biosphere reserve	fresh/wb	15/07/2013	7	Miroslav Babushkin (DSNBR)

190	haliaetus	Russia	Russia, Darwin state natural biosphere reserve	fresh/wb	14/07/2013	8		Miroslav Babushkin (DSNBR)
191	haliaetus	Russia	Russia, Darwin state natural biosphere reserve	fresh/wb	15/07/2013	9		Miroslav Babushkin (DSNBR)
192	haliaetus	Russia	Russia, National park "Russkiy Sever"	fresh/wb	16/07/2013	10		Miroslav Babushkin (DSNBR)
193	haliaetus	Russia	Russia, National park "Russkiy Sever"	fresh/wb	16/07/2013	11		Miroslav Babushkin (DSNBR)
194	haliaetus	Russia	Russia, National park "Russkiy Sever"	fresh/wb	16/07/2013	12		Miroslav Babushkin (DSNBR)
195	haliaetus	Russia	Russia, National park "Russkiy Sever"	fresh/wb	16/07/2013	13		Miroslav Babushkin (DSNBR)
196	haliaetus	United Arab Emirates	United Arab Emirates	fresh/wb	01/04/1999	A 2418		University of Greifswald - collection of M. Martell (Martin Haase)
197	haliaetus	United Arab Emirates	United Arab Emirates	fresh/wb	01/04/1999	A 2419		University of Greifswald - collection of M. Martell (Martin Haase)
198	haliaetus	United Arab Emirates	United Arab Emirates	fresh/wb	01/04/1999	A 2420		University of Greifswald - collection of M. Martell (Martin Haase)
210	haliaetus	Portugal	Portugal	fresh/wb	NO DATE	A 2457 - ZMUG RA02H		University of Greifswald - collection of M. Martell (Martin Haase)
211	haliaetus	Portugal	Portugal	fresh/wb	NO DATE	A 2458 - ZMUG RA02H		University of Greifswald - collection of M. Martell (Martin Haase)
212	haliaetus	Portugal	Portugal	fresh/wb	NO DATE	A 2459 - ZMUG RA02H		University of Greifswald - collection of M. Martell (Martin Haase)
213	haliaetus	Portugal	Portugal	fresh/wb	NO DATE	A 2460 - ZGMU RA02H		University of Greifswald - collection of M. Martell (Martin Haase)
214	haliaetus	Portugal	Portugal	fresh/wb	NO DATE	A 2461 - ZGMU RA02H		University of Greifswald - collection of M. Martell (Martin Haase)
215	haliaetus	Portugal	Portugal	fresh/wb	NO DATE	A 2462 - ZGMU RA02H		University of Greifswald - collection of M. Martell (Martin Haase)
d	haliaetus	Germany	Germany	sequence/fs	NO DATE	no code	AJ604503	Lerner & Mindell (2005)
e	haliaetus	Israel	Israel	sequence/fs	NO DATE	no code	EU345523	Lerner & Mindell (2005)
w	Aquila rapax	Africa	South Africa	sequence/fs	NO DATE	no code	AY987283	Lerner & Mindell (2005)
z	Haliaeetus albicilla	nd	nd	sequence/fs	NO DATE	no code	AY987315	Lerner & Mindell (2005)
x	Hamirostra melanosternon	Australia	unknown	sequence/fs	NO DATE	no code	AY987243	Lerner & Mindell (2005)
y	Leptodon cayanensis	America	Paraguay	sequence/fs	NO DATE	no code	AY987240	Lerner & Mindell (2005)

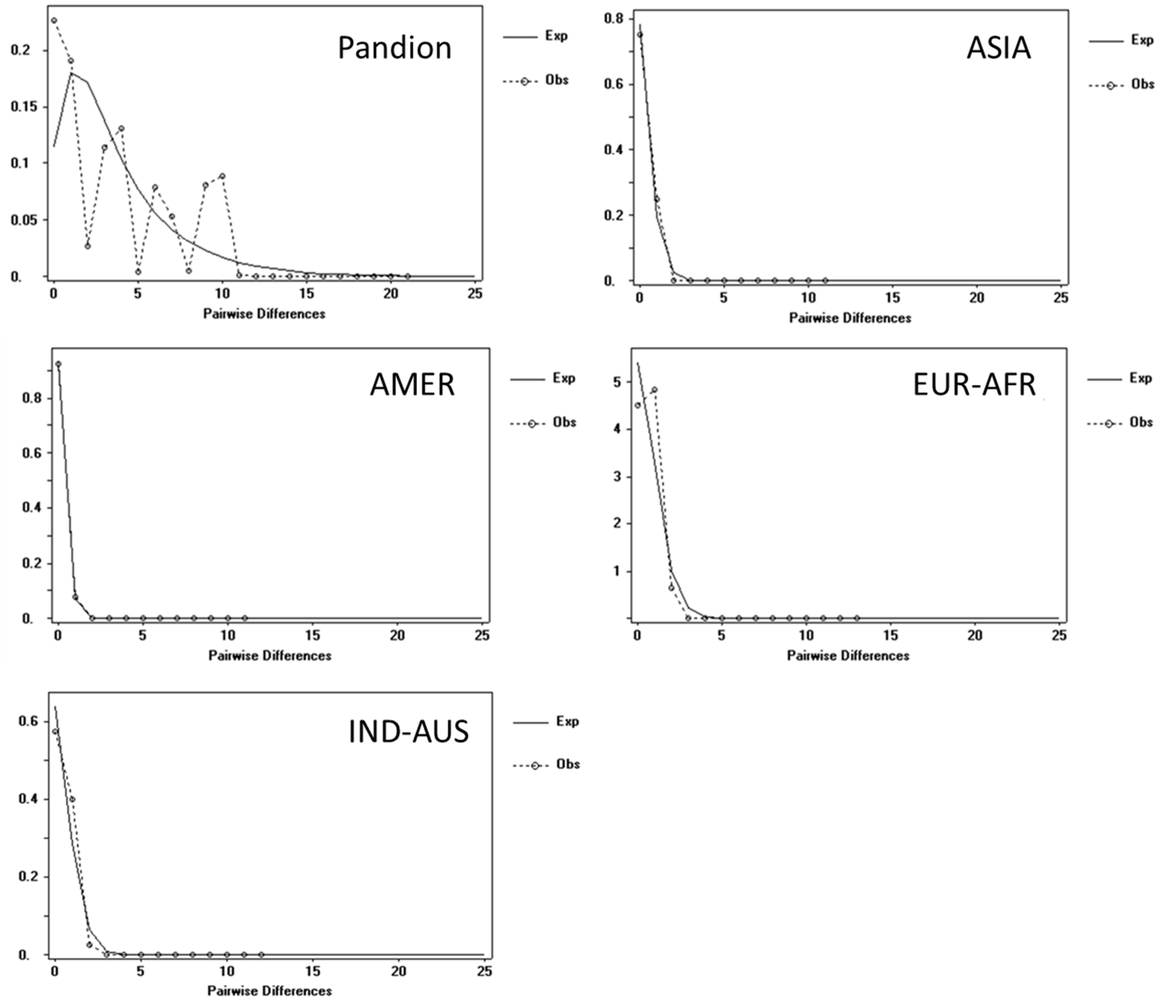
Additional file 2: DNA amplification

Cytochrome *b* and ND2 primer names and sequences for amplification and sequencing.

cyt *b* Primers	Sequence	ND2 Primers	Sequence
PANH-F1	5’- ATGGCCCCCAATCCTCGAAAATCACACC - 3’	F13	5’- CCCATACCCCGAAAATGATGG - 3’
PANH-F2	5’- AGTCAATAACTCCCTAATCGACC - 3’	PHND2-F1	5’- GATCATCAGGACAGTGAGACATCACCC - 3’
PANH-F6	5’- ACACAACCCTAGCCT TCTCATCC - 3’	F17	5’- ACTAACAGGTTTCCTACCTAAGTGGC - 3’
PANH-F10	5’- CCCTCATAGCAACAGCCT TC - 3’	PHND2-R1	5’- GGCCTTCGGTTTTGGTTATCC - 3’
PANH-R1	5’- TCTACTGAGAAACCTCCTCAGGCTC - 3’	PHND2-R2	5’- AGTTGGTAGAGTTGGGGGGTAGTGTG - 3’
PANH-R3	5’- AACAGGTTGGGTGAGAACATGGATAGGG - 3’		

Additional file 3: Mismatch distributions

Mismatch distributions observed in osprey samples and expected in expanding and/or bottleneck populations. Distributions were calculated for the whole Pandion dataset and for each lineage.



15.2. ANNEXE 2: ADDITIONAL FILES FROM ARTICLE 3

Additional file 1: Mean values of migratory parameters for Western Palearctic ospreys, reported for both seasons and age classes.

Season	Bird ID	Sex	Origin	Status	Year	Start	End	day aft start	day aft end	Duration (days)	Migration distance (km)	Distance nest/stop (km)	Maximum distance (km)	DailyDist travel days (km/day)	Straightness Value	Stopover (days)
Autumn	**M52**	**M**	Sweden	adult	2006	30-Aug	29-Oct	90	150	60	6167.75	5530.00	372.00	167.80	0.90	27
				adult	2007	20-Jul	24-Sep	49	115	66	5748.76	5536.00	362.70	177.60	0.96	27
				adult	2008	15-Aug	06-Oct	75	127	52	5794.97	5529.00	565.10	230.10	0.95	32
				adult	2009	30-Jul	17-Sep	59	108	49	5897.99	5531.60	470.10	288.10	0.94	31
				adult	2010	05-Aug	20-Sep	65	111	46	5784.01	5515.60	520.90	223.10	0.95	20
	M77*	**M**	Sweden	adult	2006*	05-Sep	24-Oct	96	145	49	2377.10	1912.10	270.30	113.90	0.80	24
	M57	**M**	Sweden	adult	2009	14-Sep	23-Oct	105	144	39	5816.11	5520.93	498.50	237.40	0.95	18
	F53	**F**	Sweden	adult	2006	02-Aug	28-Sep	62	119	57	6972.45	5919.20	396.70	192.90	0.85	25
				adult	2007	08-Jul	20-Sep	37	111	74	6722.15	5919.60	388.70	169.70	0.88	36
				adult	2008	28-Jul	11-Nov	57	163	106	8784.19	5918.50	460.80	168.30	0.67	66
	F58	**F**	Sweden	adult	2008	15-Aug	29-Sep	75	120	45	6027.57	5585.80	534.80	210.10	0.93	20
	F69	**F**	Sweden	adult	2007	11-Aug	02-Oct	71	123	52	7003.17	6023.30	490.20	204.70	0.86	22
				adult	2008	16-Aug	09-Oct	76	130	54	6476.82	6023.10	499.80	218.30	0.93	26
				adult	2009	27-Jul	29-Sep	56	120	64	6560.26	6023.30	441.80	187.60	0.92	29
	J75-09	**/**	Sweden	adult	2012	11-Sep	10-Nov	102	162	60	4952.70	3998.25	458.68	161.60	0.81	36
	mean ad							71.67	128.79	58.86	6336.35	5612.44	461.48	202.66	0.89	29.64
	J60-07	**/**	Sweden	juv	2007	13-Aug	19-Oct	73	140	67	4391.80	3318.20	477.70	185.70	0.76	47
	J61-07*	**/**	Sweden	juv	2007*	28-Aug	20-Oct	88	141	53	6120.05	4808.43	460.36	168.70	0.79	25
	J63-07*	**/**	Sweden	juv	2007*	30-Aug	30-Sep	90	121	31	489.06	368.40	232.17	185.90	0.75	30
	J60-08*	**/**	Sweden	juv	2008*	18-Aug	19-Oct	78	140	62	2392.10	807.78	474.45	134.08	0.34	40
	J57-08*	**/**	Sweden	juv	2008*	24-Aug	20-Sep	84	111	27	552.10	291.86	224.09	114.60	0.53	20
	J75-09	**/**	Sweden	juv	2009	19-Aug	13-Nov	79	165	86	6527.91	4249.34	576.24	200.70	0.65	49
				juv	2011	11-Jul	03-Sep	40	94	54	4808.14	4005.71	397.61	173.30	0.83	32

J76-09	**/**	Sweden	juv	2009	01-Sep	21-Oct	92	142	50	6217.18	5372.18	426.63	242.30	0.86	27
J60-09	**/**	Sweden	juv	2009	24-Aug	25-Sep	84	116	32	5396.12	4890.72	539.50	249.30	0.91	14
J53-10	**/**	Sweden	juv	2010	30-Aug	12-Nov	90	164	74	6826.91	5170.38	494.37	104.10	0.76	21
J60-10	**/**	Sweden	juv	2010	22-Aug	16-Nov	82	168	86	7991.95	4732.02	685.74	257.90	0.59	58
mean juv							80	141.29	64.14	6022.86	4534.08	513.97	201.90	0.77	35.43
F02	**F**	Corsica	adult	2013	24-Sep	04-Oct	115	125	10	2416.98	2407.43	342.19	249.44	0.99	0
F03	**F**	Corsica	adult	2013	20-Aug	21-Aug	80	81	1	429.52	240.84	219.32	217.63	0.56	0
F04	**F**	Corsica	adult	2013	13-Sep	15-Sep	104	106	2	1608.15	1356.21	741.59	519.47	0.84	0
M05	**M**	Corsica	adult	2013	24-Jun	25-Jun	23	24	1	257.91	239.38	220.32	111.51	0.93	0
	M	Corsica	adult	2014	30-Jun	30-Jun	29	29	1	260.19	237.01	260.19	260.19	0.91	0
F06	**F**	Corsica	adult	2013	10-Aug	15-Aug	70	75	5	1597.77	1326.47	520.90	243.50	0.83	1
	F	Corsica	adult	2014	17-Aug	24-Aug	77	84	7	1748.01	1385.69	362.28	172.26	0.79	0
F08	**F**	Corsica	adult	2013	12-Aug	16-Aug	72	76	4	1524.80	1317.92	398.27	271.50	0.86	0
	F	Corsica	adult	2014	17-Aug	22-Aug	77	82	5	1553.43	1317.75	445.32	231.00	0.85	0
MB5	**M**	Balearics	adult	2009	09-Nov	17-Nov	161	169	8	3525.57	3246.87	603.56	327.44	0.92	0
mean Medit							80.80	85.10	4.40	1492.23	1307.56	411.39	260.39	0.85	0.10
F10	**/**	Italy	juv	2013	30-Jul	06-Aug	59	66	7	886.79	444.60	218.87	110.60	0.50	2
F11	**/**	Balearics	juv	2013	04-Aug	09-Aug	64	69	5	1059.50	976.32	277.99	175.60	0.92	0
F12	**/**	Balearics	juv	2013	28-Aug	02-Sep	88	93	5	1387.26	1129.19	525.76	229.39	0.81	0
F13	**/**	Balearics	juv	2013	10-Aug	16-Aug	70	76	6	1060.46	930.55	368.13	265.05	0.88	2
F14	**/**	Balearics	juv	2013	08-Aug	14-Aug	68	74	6	1116.19	756.69	368.48	194.90	0.68	3
F15	**/**	Balearics	juv	2013	29-Jul	03-Aug	58	63	5	758.79	607.67	246.58	151.70	0.80	2
F16	**/**	Balearics	juv	2013	07-Aug	11-Aug	67	71	4	1072.84	678.98	318.27	202.13	0.63	0
F17	**/**	Corsica	juv	2013	15-Aug	19-Aug	75	79	4	639.09	463.01	204.62	127.84	0.72	0
F18*	**/**	Corsica	juv	2013*	20-Aug	25-Aug	80	85	5	1451.85	952.30	584.27	236.27	0.66	0
F20	**/**	Italy	juv	2013	05-Aug	08-Aug	65	68	3	518.22	425.77	184.92	125.92	0.82	0
JUV1-57	**/**	Balearics	juv	2000	02-Aug	10-Aug	62	70	8	1129.43	690.25	/	/	0.61	/
JUV2-59	**/**	Balearics	juv	2000	01-Aug	05-Aug	61	65	4	/	617.10	/	/	/	/
JUV3-60	**/**	Balearics	juv	2000	17-Aug	16-Oct	77	137	60	2003.13	930.01	/	/	0.46	/
D7_fosp20	**/**	Italy	juv	2014	21-Aug	28-Aug	81	88	7	1087.20	714.08	364.57	135.90	0.66	0

	CIV_fosp21	**/**	Corsica	juv	2014	16-Aug	21-Aug	76	81	5	1301.38	652.46	330.16	217.04	0.50	0
	H7_fosp25	**/**	Italy	juv	2014	17-Aug	28-Aug	77	88	11	2514.36	954.38	386.17	205.17	0.38	0
	E7_fosp27	**/**	Italy	juv	2014	21-Aug	27-Aug	81	87	6	1499.08	722.60	408.34	214.21	0.48	0
	CAP_fosp24	**/**	Corsica	juv	2014	14-Aug	17-Aug	74	77	3	754.84	575.94	165.98	114.29	0.76	0
	mean juv							69.40	73.22	8.56	1174.28	721.74	329.79	181.94	0.66	0.90
Spring	**M52**	**M**	Sweden	adult	2007	28-Mar	01-May	55	89	34	6963.79	5528.60	578.60	208.40	0.79	1
				adult	2008	13-Mar	07-Apr	41	66	25	6049.21	5524.70	470.70	235.60	0.91	4
				adult	2009	13-Mar	03-Apr	40	61	21	5371.65	5519.90	464.10	286.60	1.00	4
				adult	2010	13-Mar	03-Apr	40	61	21	5764.88	5475.80	470.90	268.70	0.95	5
				adult	2011	13-Mar	08-Apr	40	66	26	6111.05	5526.70	488.30	228.80	0.90	5
	F53	**F**	Sweden	adult	2007	04-Mar	15-Apr	31	73	42	7360.03	5918.40	499.20	208.70	0.80	1
				adult	2009*	15-Mar	12-Apr	42	70	28	5631.86	5917.60	468.60	180.10	1.00	7
	F69	**F**	Sweden	adult	2008	13-Mar	14-Apr	41	73	32	6859.00	6023.30	665.60	298.20	0.88	12
				adult	2009	13-Mar	12-Apr	40	70	30	6477.82	6023.30	357.40	218.07	0.93	6
	J75-09	**/**	Sweden	adult	2012	08-Apr	30-Apr	67	89	22	4245.94	4025.71	608.59	242.90	0.95	10
	mean ad							43.70	72.00	28.11	6133.71	5507.38	511.49	244.00	0.91	5.33
	J75-09	**/**	Sweden	juv	2011	10-May	31-May	98	119	21	4927.85	4043.37	494.83	277.01	0.82	6
	J76-09*	**/**	Sweden	juv	2010*	09-Jun	17-Jul	128	166	38	1202.72	5293.51	357.84	204.40	4.40	33
	J60-10*	**/**	Sweden	juv	2012*	08-Apr	23-Apr	67	82	15	4636.57	5482.33	390.10	198.80	1.18	0
	mean juv							97.67	119							
	F02	**F**	Corsica	adult	2014	13-Feb	25-Feb	12	24	12	2646.12	2408.32	271.89	140.00	0.91	0
	F04	**F**	Corsica	adult	2014	16-Feb	20-Feb	15	19	4	1701.22	1370.60	549.70	340.29	0.81	0
	M05	**M**	Corsica	adult	2014	06-Feb	06-Feb	5	5	1	250.00	250.00	250.00	250.00	1.00	0
	F06	**F**	Corsica	adult	2014	19-Feb	24-Feb	18	23	5	1896.76	1380.40	450.23	289.21	0.73	0
	F08	**F**	Corsica	adult	2014	21-Feb	24-Feb	20	23	3	1535.89	1316.10	492.63	369.72	0.86	0
	MB5	**M**	Balearics	adult	2010	25-Mar	04-Apr	52	62	10	3432.39	3244.54	437.06	267.80	0.95	0
	mean Medit							20.33	26	5.83	1910.40	1661.66	408.58	276.17	0.87	0.00

Additional file 2: Origins, category (see materials and methods) and location of both secondary feeding sites and wintering grounds of Western Palearctic ospreys. * represents individuals for which migration was not complete (or when bird died).

Bird	Sex	Origin	Category	Secondary feeding site	Wintering ground
M52	M	Sweden	LDM	NA	Senegal
M77*	M	Sweden	LDM	NA	NA
M57	M	Sweden	LDM	NA	Senegal
F53	F	Sweden	LDM	NA	Ghana
F58	F	Sweden	LDM	NA	Guinea-Bissau
F69	F	Sweden	LDM	NA	Guinea-Bissau
J60-07	JUV	Sweden	LDM	NA	Morocco-Atlantic coast
J61-07*	JUV	Sweden	LDM	NA	Niger
J75-09	JUV	Sweden	LDM	NA	Morocco-Atlantic coast
J76-09	JUV	Sweden	LDM	NA	Senegal
J60-09	JUV	Sweden	LDM	NA	Mauritania
J53-10	JUV	Sweden	LDM	NA	Senegal/Ghana
J60-10	JUV	Sweden	LDM	NA	Ivory Coast
J63-07*	JUV	Sweden	--	--	--
J66-07*	JUV	Sweden	--	--	--
J60-08*	JUV	Sweden	--	--	--
J57-08*	JUV	Sweden	--	--	--
J58-10*	JUV	Sweden	--	--	--
F01	F	Corsica	RES	NA	Corsica
F02	F	Corsica	LDM	Sardinia-Italy	Morocco-Atlantic coast
F03	F	Corsica	SDM	Massaciuccoli Lake-Tuscany	Sardinia-Italy
F04	F	Corsica	LDM	Ponte Leccia-Central Corsica	Andalucia-Spain
M05	M	Corsica	SDM	NA	Sardinia-Italy
F06	F	Corsica	LDM	Aleria-east Corsica	Andalucia-Spain
F08	F	Corsica	LDM	Bonifacio-south Corsica	Morocco-Mediterranean coast
B5M	M	Balearics	LDM/RES	NA	Mauritania/Balearics
L7	M	Italy	RES	NA	Tuscany
BAL1M	M	Balearics	RES	NA	Balearics
BAL4M	M	Balearics	RES	NA	Balearics
BAL3IND	NA	Balearics	RES	NA	Balearics
BAL2F	F	Balearics	RES	NA	Balearics
F10	JUV	Italy	SDM	NA	Abruzzo-Italy
F11	JUV	Balearics	LDM	NA	Andalucia-Spain
F12	JUV	Balearics	LDM	NA	Morocco-Atlantic coast
F13	JUV	Balearics	LDM	NA	Andalucia-Spain
F14	JUV	Balearics	LDM	NA	Morocco-Mediterranean coast
F15	JUV	Balearics	LDM	NA	Andalucia-Spain
F16	JUV	Balearics	LDM	NA	Algeria
F17	JUV	Corsica	SDM	NA	Sardinia-Italy
F18*	JUV	Corsica	LDM	NA	Malta
F20	JUV	Italy	SDM	NA	Campania-Italy
JUV1-57*	JUV	Balearics	LDM	NA	Chad

JUV2-59	JUV	Balearics	LDM	NA	Algeria
JUV3-60	JUV	Balearics	LDM	NA	Algeria
D7_fosp20	JUV	Italy	LDM	NA	Sicily-Italy
CIV_fosp21	JUV	Corsica	LDM	NA	Algeria
H7_fosp25	JUV	Italy	LDM	NA	Algeria
E7_fosp27	JUV	Italy	LDM	NA	Sicily-Italy
CAP_fosp24	JUV	Corsica	LDM	NA	Sicily-Italy
CBB_fosp03*	JUV	Corsica	--	--	--
CBD_fosp13*	JUV	Corsica	--	--	--
CBC_fosp19*	JUV	Corsica	--	--	--
CIZ_fosp22*	JUV	Corsica	--	--	--
C7_fosp23*	JUV	Italy	--	--	--

15.3. ANNEXE 3: ADDITIONAL FILES FROM ARTICLE 5

Additional file 1: Details of the GLMMs performed on the breeding parameters of the Corsican osprey population.

Dependent Variable (n)	Selected Model	Time since reserve	Reserve Out/In	1990 before/after	Variable	Parameter estimates	AICC
a. N eggs laid (541)	no effect	*	*	NA	Intercept	1.04 ± 0.02	1638.2
b. N eggs hatched (730)	yearB*Out/In	*	*	NA	Intercept	0.48 ± 0.10	2383.4
					yearB	0.00 ± 0.00	
					Reserve_IN	0.25 ± 0.17	
					YearB*Reserve_IN	-0.01 ± 0.00	
c. N chicks fledged (744)	yearB*Out/In	*	*	NA	Intercept	0.41± 0.15	2126.8
					yearB	-0.00 ± 0.00	
					Reserve_IN	0.47 ± 0.20	
					YearB*Reserve_IN	-0.03 ± 0.00	
d. Hatching success (538)	yearB*Out/In	*	*	NA	Intercept	-0.04 ± 0.53	596.2
					yearB	0.03 ± 0.02	
					Reserve_IN	4.06 ± 1.25	
					YearB*Reserve_IN	-0.15 ± 0.04	
e. Fledging success (576)	yearB	*	*	NA	Intercept	2.74 ± 0.43	675.7
					yearB	-0.07 ± 0.01	
f. Breeding success (540)	yearB*Out/In	*	*	NA	Intercept	-0.57 ± 0.39	739.9
					yearB	0.02 ± 0.01	
					Reserve_IN	2.19 ± 0.88	
					YearB*Reserve_IN	-0.09 ± 0.03	
a1. N eggs laid threshold (541)	no effect	NA	*	*	Intercept	1.04 ± 0.02	1636.2
b1. N eggs hatched threshold (730)	1990*Out/In	NA	*	*	Intercept	0.67 ± 0.05	2383.6
					1990_before	-0.13 ± 0.09	
					Reserve_IN	-0.17 ± 0.09	
					1990_before*Reserve_IN	0.34 ± 0.15	
c1. N chicks fledged threshold (744)	1990*Out/In	NA	*	*	Intercept	0.15 ± 0.08	2127.0
					1990_before	0.23 ± 0.14	
					Reserve_IN	-0.39 ± 0.14	
					1990_before*Reserve_IN	0.61 ± 0.17	
d1. Hatching success threshold (538)	1990*Out/In	NA	*	*	Intercept	1.09 ± 0.23	599.2
					1990_before	-1.54 ± 0.52	
					Reserve_IN	-0.46 ± 0.27	
					1990_before*Reserve_IN	3.37 ± 1.22	
e1. Fledging success threshold (576)	1990*Out/In	NA	*	*	Intercept	0.50 ± 0.18	668.7
					1990_before	1.70 ± 0.43	
					Reserve_IN	-0.52 ± 0.31	
					1990_before*Reserve_IN	2.14 ± 1.12	
e1. Breeding success threshold (540)	1990*Out/In	NA	*	*	Intercept	0.17 ± 0.14	734.5
					1990_before	-0.86 ± 0.40	
					Reserve_IN	-0.65 ± 0.29	
					1990_before*Reserve_IN	3.72 ± 1.17	

Additional file 2: Estimates of foraging home ranges (UD95%) and core foraging areas (UD50%) of adult ospreys on the west coast of Corsica, according to sex, year and monitoring period.

ID	Sex	Year	Monitoring Period	UD50% (km^2)	UD95% (km^2)
A02	M	2012	24/03-21/04	10.79	183.28
A03	F	2012	27/03-30/04	4.94	56.46
FOSP01	F	2013	27/03-30/06	4.01	32.16
FOSP02	F	2013	17/03-24/05	13.88	183.66
FOSP03	F	2013	23/03-28/05	9.13	94.94
FOSP04	F	2013	23/03/03/04	4.55	28.97
		2014	25/03-20/04	3.93	22.07
FOSP05	M	2013	27/03-24/06	4.11	77.17
		2014	06/02-30/06	4.15	50.43
FOSP06	F	2013	29/03-07/05	5.29	71.82
		2014	24/03-25/06	2.30	11.83
FOSP08	F	2013	05/04-24/06	2.23	9.88
		2014	09/03-08/07	2.22	10.01
Mean				*5.50*	*64.05*
SD				*3.57*	*59.54*

16. RIASSUNTO GENERALE

Introduzione

La biodiversità (intesa come diversità di organismi a livello di specie, di individui, di geni e di ecosistemi) è continuamente minacciata sia da processi naturali sia antropici (Primack, 2000). Mentre i primi operano su lunghi periodi di tempo, durante i quali gli ecosistemi riescono a ripristinare gli equilibri iniziali o a stabilizzarsi gradualmente su nuovi equilibri, i secondi avvengono rapidamente, modificando spesso in modo irreversibile gli ecosistemi e i processi ecologici. Le minacce maggiori alla biodiversità derivano dunque prioritariamente dall'impatto delle attività umane sull'ambiente. Nel corso degli ultimi decenni molti organismi si sono estinti, molti habitat hanno subito drastiche modifiche e/o stanno soffrendo rapidi processi di deterioramento. Per questo i programmi di conservazione e le azioni di gestione improntati su più scale spaziali rivestono un'importanza fondamentale per assicurare la protezione della biodiversità. A causa dell'impossibilità di preservare tutte le aree naturali e le specie esistenti, i piani di conservazione si sono spesso concentrati su habitat ad alta priorità o singole specie (Neel, 2008). Tuttavia, mentre negli ultimi decenni il concetto di biodiversità si riferiva solamente alla ricchezza di specie (e, dunque, ad un sistema di riferimento statico e prevedibile; Poiani *et al.*, 2000), più di recente il concetto di biodiversità è stato esteso ai processi evolutivi operanti a livello di geni, popolazioni, specie ed ecosistemi (Mestolo & Whittaker, 2011; Mace *et al.*, 2012). Di conseguenza, le attuali raccomandazioni per la conservazione della biodiversità pongono l'attenzione sulla necessità di preservare dei modelli ecologici dinamici, operanti a differenti scale, considerando tutti i processi che avvengono all'interno dei sistemi naturali (Richardson & Whittaker, 2010; Mace *et al.*, 2012).

La necessità di scegliere una scala spazio-temporale adeguata è stata in gran parte dettata da questioni pratiche legate all'habitat e alla conservazione della biodiversità, ma quale sia l'approccio teorico generale da seguire é ancora fortemente dibattuto a livello accademico (Dungan *et al.*, 2002). Dato che è impossibile prendere in considerazione l'insieme delle variabili naturali che contemporaneamente agiscono sui sistemi ecologici nello spazio e nel tempo, un programma di conservazione adeguato dovrebbe mirare a scegliere le variabili che restituiscono la massima prevedibilità, dando priorità a quelle scale che corrispondono a questo prerequisito. Ma quale sia la scala giusta da scegliere dipende in parte dalle questioni che si vogliono affrontare. Difficoltà di questo tipo si incontrano quando ci si cimenta con lo studio di specie migratorie, che possiedono un ciclo vitale che include diverse scale spaziali e temporali. La migrazione é uno spostamento periodico (stagionale) attraverso il quale un organismo si muove periodicamente da una regione ad un'altra per meglio soddisfare le proprie esigenze fisiologiche ed ecologiche durante una fase del proprio ciclo vitale (Dingle, 1996; Berthold, 2001). I grandi migratori possono percorrere grandi distanze lungo rotte inter-continentali e attraversare molti habitat diversi durante un intero ciclo di migrazione (Newton, 2010). Nel corso dell'anno i periodi migratori sono alternati dalle fasi di riproduzione in primavera e di svernamento in inverno. Proteggere solo una parte di questo complesso sistema vitale rischierebbe di compromettere il successo dei piani di conservazione, a causa della possibilità che le minacce si verifichino ad altre scale spaziali e temporali non opportunamente considerate. Comprendere l'ecologia delle popolazioni animali e pianificare misure di conservazione adeguate richiede quindi la conoscenza dei meccanismi che agiscono a diverse scale spaziali e temporali, così come l'attuazione di metodi di ricerca integrativi e approcci analitici differenti. In questo contesto, un valido approccio dovrebbe cercare di: (1) selezionare una o piú specie che possa/no servire da modello per numerose altre specie con ecologia, storia evolutiva e/o caratteristiche di distribuzione simili; (2) sviluppare un approccio che consideri piú scale spaziali e temporali contemporaneamente (sensu Wiens, 1995), che abbia un dominio di applicazione specifico e limitato ed (3) integrare questi due approcci (Wiens *et al.*, 1993;. Collins *et al.*, 1993).

In questa tesi ho deciso di seguire questo tipo di approccio, guidato dalla necessità di rispondere a domande specifiche sulla storia evolutiva ed ecologia della specie modello presa in esame.

La specie modello

Con una distribuzione mondiale, il falco pescatore *Pandion haliaetus* è considerato una delle sei specie di uccelli terrestri, insieme all'airone bianco maggiore *Ardea alba*, l'airone guardabuoi *Bubulcus ibis*, il mignattaio *Plegadis falcinellus*, il barbagianni *Tyto alba* e il falco pellegrino *Falco peregrinus* cosmopolite (Newton, 2003a). Il falco pescatore è un rapace di medie dimensioni che ha evoluto caratteristiche fisiche particolari e un comportamento finalizzato alla cattura dei pesci, sue uniche prede (Cramp & Simmons, 1980; Poole, 1989). Essendo una specie specializzata ma allo stesso tempo opportunistica, la dieta comprende sia pesci d'acqua dolce che marini. Mentre nel Nord America e Nord Europa la specie è relativamente comune e nidifica sulla cima di alberi in ambiente di foresta in corrispondenza di ecosistemi d'acqua dolce, nel Mediterraneo il falco pescatore è strettamente legato ad ecosistemi marini, nidificando sulle scogliere a picco sul mare. In questa regione biogeografica le strategie di migrazione del falco pescatore e le aree utilizzate durante la stagione non-riproduttiva (siti di svernamento) non sono mai state indagate con precisione. Con meno di un centinaio di coppie riproduttive distribuite tra la Corsica, le Isole Baleari, il Marocco e l'Algeria, il falco pescatore del Mediterraneo risulta un'entità fragile dal punto di vista conservazionistico. Negli ultimi decenni minacce antropiche hanno portato la specie sull'orlo dell'estinzione in molte parti d'Europa e specialmente nel bacino del Mediterraneo (i.e. Spagna continentale, Italia e Portogallo; Dennis, 2008). A scala regionale la specie è infatti considerata "in pericolo".

STATUS LEGALE

Il falco pescatore è una specie particolarmente protetta in Italia e in tutti i paesi europei. Iscritta nell'appendice 1 della Direttiva Europea per la conservazione degli uccelli selvatici (specie oggetto di misure speciali per la conservazione, in particolare per ciò che concerne il loro habitat: Zone di Protezione Speciale), iscritta nell'allegato 2 della Convenzione di Berna (specie faunistica particolarmente protetta); inclusa nell'allegato 2 della Convenzione di Bonn relativa alla conservazione delle specie migratrici (specie in stato di conservazione sfavorevole, che necessita l'adozione di misure appropriate) e nell'allegato 2 della Convenzione di Washington sul commercio internazionale di specie di fauna e flora selvatiche minacciate di estinzione (CITES) (specie minacciata di estinzione, il commercio è vietato all'interno e all'esterno dell'Unione Europea).

Essendo una specie cosmopolita e migratrice, il falco pescatore *Pandion haliaetus* rappresenta un buon modello di studio per investigare come adattamenti comportamentali evolutisi in popolazioni geograficamente distanti e in diverse condizioni ecologiche, possano aver condizionato l'attuale distribuzione geografica, la divergenza genetica, la connettività e le strategie migratorie e di alimentazione delle singole popolazioni. Per meglio indagare questi aspetti, questo studio ha adottato un approccio multidisciplinare, articolato su diverse scale spaziali e temporali. Le discipline di cui ci si è avvalsi spaziano dalla ecologia molecolare, l'ecologia trofica valutata attraverso l'analisi degli isotopi stabili, l'ecologia spaziale attraverso l'uso di innovativi strumenti di biotelemetria, così come l'analisi di dinamica di popolazione, i censimenti di specie ittiche e le valutazioni dei livelli di disturbo antropico.

Il progetto é strutturato seguendo un approccio eco-regionale, attraverso 3 diverse scale spaziali: livello globale, regionale e locale. Allo stesso tempo, questo progetto è stratificato su 3 scale temporali, con diversi livelli di risoluzione: tempi evolutivi, storici e recenti. Seguendo un approccio a differenti scale spaziali e temporali, si è cercato di rispondere alle seguenti domande:

<u>*Scala globale e tempi evolutivi*</u>

- Qual è il grado di divergenza genetica tra le popolazioni falco pescatore e qual è la storia evolutiva della specie a scala mondiale?

- Qual è il grado di connettività tra le popolazioni a livello globale?

<u>*Scala regionale e tempi storici*</u>

- Esistono diverse strategie di migrazione per le diverse popolazioni di falco pescatore nel Paleartico occidentale? Quali?

- Dove trascorrono l'inverno i falchi pescatori mediterranei e dove si disperdono i giovani?

<u>*Scala locale e tempi recenti*</u>

- Qual è l'intensità degli scambi tra le popolazioni del bacino del Mediterraneo?

- I trend demografici delle popolazioni del Mediterraneo sono influenzati da variabili ambientali e/o antropiche?

<u>Articolo 1:</u> **Essere cosmopoliti: storia evolutiva e filogeografia di un rapace specializzato, il falco pescatore.**

A livello globale, ho studiato la diversità genetica (DNA mitocondriale) del falco pescatore con lo scopo di chiarire la struttura filogeografica e lo status tassonomico della specie. É stato proposto un ipotetico scenario evolutivo che spiega come la distribuzione e la differenziazione della specie abbia avuto luogo e come un rapace così specializzato sia stato in grado di colonizzare la maggior parte del globo. Il falco pescatore é risultato strutturato in quattro gruppi genetici principali, distribuiti su quattro aree geografiche differenti (America, Indo-Australasia, Europa-Africa e Asia). Ogni lignaggio evolutivo, pur comprendendo individui di popolazioni provenienti da aree geografiche molto distanti, ha mostrato una scarsa variabilità genetica interna. Al contrario, una grande differenza nucleotidica é stata registrata tra i quattro lignaggi. Le ricostruzioni demografiche suggeriscono che tre delle quattro linee evolutive presentano trend stabili o caratterizzati da lievi incrementi. Le datazioni molecolari hanno stimato che la scissione iniziale tra i vari lignaggi possa essere fatta risalire a circa 3,1 milioni d'anni fa, durante il Pliocene. Questo studio suggerisce quindi uno scenario evolutivo di colonizzazione a partire dal continente americano (dove la specie sembra essersi verosimilmente originata) verso il Vecchio Mondo, passando per lo stretto di Bering. Periodi glaciali sfavorevoli avrebbero poi portato la specie a spostarsi verso zone piú miti: dall'Asia quindi il falco pescatore avrebbe colonizzato prima l'Indonesia e l'Australia (rimanendovi isolato per molto tempo) per poi, durante i successivi periodi interglaciali, colonizzare l'est asiatico (Siberia e Giappone) e tutto il Paleartico occidentale, attraverso due ondate di coloniozzazione differenti. Le popolazioni del Paleartico occidentale rappresentano dunque l'ultimo stadio di tale storia evolutiva. È importante sottolineare che, ogni lignaggio evolutivo rappresenta una ESU (Unitá Evolutiva Significativa) e dovrebbe quindi essere trattato e gestito separatamente dalle altre popolazioni provenienti da altri lignaggi. La sistematica della specie (e anche della famiglia Pandionidae) dovrebbe quindi essere rivista alla luce di questi risultati recenti. Questo studio ha apportato nuove conoscenze genetiche essenziali per la gestione e le strategie di conservazione della specie. Nell'ambito di progetti di ripopolamento/reintroduzione suggeriamo di effettuare traslocazioni utilizzando popolazioni appartenenti allo stesso clade di origine. In altre parole, traslocazioni in Europa non dovrebbero utilizzare individui provenienti dalle Americhe, Oceania e Asia orientale.

Articolo 2: **Connettivitá tra popolazioni di falco pescatore rivelate mediante tecniche di genotipizzazione: un approccio multi-scalare usando i microsatelliti.**

La variabilità e la struttura genetica delle popolazioni di falco pescatore sono state studiate usando marcatori genetici del DNA nucleare (microsatelliti). Venti loci microsatellitari sono stati analizzati su un totale di 200 campioni provenienti da tutto il mondo. Lo studio di genetica di popolazione mediante l'utilizzo di microsatelliti ha confermato l'esistenza di almeno tre dei quattro gruppi individuati con l'analisi del DNA mitocondriale. Popolazioni provenienti dall'America, Australia e dal Paleartico sono risultati essere isolate tra loro, formando quindi dei gruppi non connessi da flusso genico. Vaste barriere geografiche, come ad esempio le distese oceaniche, possono aver favorito l'isolamento di questi gruppi, impedendone la connettivitá. Al contrario, nel Paleartico abbiamo trovato che popolazioni di falco pescatore, anche geograficamente distanti, sono parzialmente connesse da flusso genico. Tuttavia, quest'ultimo gruppo ha presentato un'ulteriore sub-strutturazione in due entità distinte (Europa continentale vs regione Mediterranea). Nonostante scambi occasionali a livello del Paleartico dovuti ad eventi di dispersione, la forte sub-strutturazione porta a pensare che le popolazioni di falco pescatore che vivono a latitudini diverse abbiano sviluppato delle informazioni genetiche specifiche. Pertanto, popolazioni del Paleartico, pur appartenendo allo stesso clade evolutivo (a livello mtDNA), presentano diversità recenti a livello dei geni nucleari. Nel Mediterraneo, inoltre, l'assenza di ulteriore strutturazione sembra indicare che le popolazioni di questa regione sono connesse tra di loro. In questo senso, comportamenti come la dispersione, la migrazione e la filopatria potrebbero quindi aver agito contemporaneamente, plasmando la struttura genetica e la diversità delle diverse popolazioni. Una migliore comprensione di questi comportamenti si è resa quindi necessaria in quanto potrebbe contribuire a ricostruire la dinamica delle popolazioni, fornendo informazioni essenziali per la gestione e la conservazione delle specie, in particolare nell'area del Mediterraneo.

Articolo 3: **Migrazione scala-dipendente: effetti spazio temporali contrastanti tra popolazioni e classi d'etá nel falco pescatore.**

Oltre ad una differenza di struttura genetica nel Paleartico, abbiamo anche trovato forti divergenze nelle strategie migratorie adottate. Cinquantaquattro falchi pescatori provenienti da popolazioni scandinave (Svezia) e mediterranee (Corsica, Baleari, Italia) sono stati monitorati grazie all'ausilio della telemetria satellitare (GPS): un totale di 70 tracciati ottenuti hanno permesso di indagare la variazione del comportamento migratorio lungo un gradiente latitudinale, in individui di diverse classi di età e in relazione ad una vasta gamma di fattori ecologici (come ad esempio la presenza di barriere geografiche i.e. il Mar Mediterraneo, o i venti, ecc). In particolare, abbiamo scoperto che gli individui del Nord Europa (i.e. Svezia) mostrano un comportamento migratorio relativamente omogeneo, compiendo lunghi viaggi migratori (ca 6000 km) attraverso l'Europa, passando per lo Stretto di Gibilterra ed attraversando la grande barriera del deserto del Sahara prima di raggiungere i siti di svernamento sulle coste occidentali dell'Africa sub-Sahariana. I falchi pescatori del Mediterraneo, invece, hanno mostrato un comportamento piú diversificato ed eterogeneo, tipico di una popolazione parzialmente migratrice. Il 46% degli individui monitorati é rimasto presso i siti di nidificazione durante tutto l'anno senza migrare (residenti), 15.5% ha compiuto brevi spostamenti (<500 km) ed il restante 38.5% ha migrato percorrendo distanze maggiori (ca. 1000 km). I siti di svernamento sono comunque collocati principalmente nel bacino del Mediterraneo. I falchi mediterranei che hanno intrapreso una migrazione hanno eseguito lunghi voli non-stop sul mare aperto, cosa che non é stata osservata negli individui scandinavi. La maggior eterogeneitá nella scelta delle rotte migratorie, nelle date di partenza e nei siti per lo svernamento osservata nella popolazione mediterranea potrebbe essere dovuta alla presenza di condizioni ecologiche più favorevoli nella regione Mediterranea durante l'anno. All'interno di ciascuna popolazione abbiamo notato che individui adulti sono in grado di percorrere maggiori distanze giornaliere, seguendo rotte meno sinuose rispetto a quelle osservate per individui di giovane etá, suggerendo quindi che le prestazioni migratorie sono fortemente condizionate dall'etá e migliorano con l'esperienza acquisita nel tempo (Sergio *et al.*, 2014). I giovani hanno inoltre mostrato capacitá ridotte nell'utilizzare correnti e venti favorevoli per attraversare i tratti di mare aperto. Nel complesso, il nostro studio dimostra che la conoscenza dettagliata dei flussi migratori a diverse scale spazio-temporali è di fondamentale importanza per la progettazione dei piani di conservazione e per la gestione delle popolazioni vulnerabili di specie migratorie.

Articolo 4: **Plasticitá comportamentale nell'ecologia invernale del falco pescatore del Mediterraneo come rilevato dalle analisi di isotopi stabili e monitoraggio con telemetria GPS.**

Per comprendere l'ecologia invernale dei falchi pescatori del Mediterraneo abbiamo usato un duplice approccio complementare, utilizzando sia il monitoraggio mediante telemetria GPS, sia l'analisi degli isotopi stabili a partire da campioni di piume. Inizialmente sono state campionate le piume di circa 80 individui provenienti da popolazioni diverse, lungo un vasto gradiente latitudinale (dalla Lapponia all'Africa), al fine di valutare la variabilità negli isotopi stabili di carbonio, azoto e zolfo (δ13C, δ15N e δ34S): ció ha permesso di distinguere i vari siti di riproduzione e i differenti tipi di habitat delle popolazioni del Paleartico occidentale. Le analisi isotopiche sono state poi ripetute su un campione sperimentale di 18 falchi adulti provenienti dalla Corsica, per stimare la latitudine dei siti di svernamento ed il tipo di habitat utilizzato durante il periodo invernale. Inoltre 12 falchi pescatori adulti sono stati dotati di dispositivi GPS e monitorati durante la migrazione e lo svernamento. Combinando le due tecniche abbiamo confermato che la popolazione Mediterranea é una popolazione parzialmente migratoria, con individui sia residenti che migratori. I falchi pescatori del Mediterraneo hanno trascorso l'inverno a latitudini temperate (i siti di svernamento sono risultati essere dislocati lungo le coste di diversi paesi del bacino del Mediterraneo, e non concentrati in un'unica zona) e hanno mostrato una marcata plasticità nella selezione dell'habitat, facendo uso di baie marine, lagune costiere e paludi o siti d'acqua dolce dell'entroterra. I movimenti giornalieri e gli home range sono risultati ridotti nel corso della stagione invernale. Tale plasticità comportamentale inter-individuale nella scelta del luogo e del tipo di habitat suggerisce la necessità di adottare un approccio a larga scala per la protezione del falco pescatore nel periodo invernale. Per contribuire a garantire un giusto livello di conservazione delle popolazioni di falco pescatore del bacino del Mediterraneo è dunque necessaria una armonizzazione dei protocolli di gestione delle zone umide tra i diversi paesi interessati.

Articolo 5: **Il prezzo del successo: impatto negativo di un area marina protetta su una popolazione di rapace emblematico del Mediterraneo.**

Negli ultimi decenni, il disturbo arrecato alla fauna selvatica da parte delle attivitá umane è diventato una delle principali preoccupazioni per i biologi conservazionisti. Nel Mediterraneo, in particolare, il turismo nautico e le relative attività ricreative sono andate aumentando rapidamente negli ultimi anni, soprattutto all'interno delle aree marine protette (AMP) dove si registrano i maggior livelli di biodiversitá in termini di habitat e specie. In questo studio abbiamo studiato l'impatto dell'area marina protetta di Scandola (Corsica, Mediterraneo centrale), sulla popolazione di un rapace emblematico, il falco pescatore. Analizzando i dati lungo un periodo di 37 anni si é riscontrato il paradosso conservazionistico secondo il quale i parametri riproduttivi delle coppie nidificanti all'interno dell'area marina protetta hanno subito un decremento significativo rispetto a quelli registrati per le coppie nidificanti nel resto della Corsica, all'esterno dell'area protetta. Allo stesso tempo, i flussi turistici (numero di barche e turisti) sono andati aumentando rapidamente soprattutto all'interno della riserva. Grazie ad un'analisi della distribuzione della risorsa trofica mediante 98 transetti in kayak e videoregistrazioni subacquee dentro e fuori la riserva, abbiamo potuto constatare che tale paradosso non é causato da un deficit alimentare: all'interno della riserva infatti abbiamo registrato una maggior biomassa e pesci di taglia piú grande rispetto ai siti di riproduzione esterni alla riserva. Al contrario, uno studio dettagliato del traffico nautico effettuato tra il 2013 e il 2014 ha rivelato che il numero complessivo di passaggi d'imbarcazioni turistiche e i passaggi a distanze inferiori ai 250 m dai nidi falco pescatore sono significativamente maggiori all'interno della riserva di Scandola rispetto alle zone di controllo esterne. Infine, ripetute osservazioni comportamentali dei falchi pescatori al nido, secondo un protocollo di monitoraggio effettuato tra il 2012 e il 2014, hanno dimostrato che il traffico nautico locale modifica in modo significativo il *time budget* giornaliero, riducendo la percentuale di prede riportate al nido dai maschi, e aumentando il tempo trascorso dalle femmine in comportamenti di allarme. Nel complesso, quindi, la nostra valutazione integrativa di analisi demografiche, ecologia del movimento (analisi di tracciati GPS), ecologia comportamentale ed indagini sull'abbondanza delle prede dimostra chiaramente l'impatto del turismo locale sulla popolazione di falco pescatore. Questo studio sottolinea l'importanza, a livello mondiale, di attuare rigorose misure di ecoturismo sostenibile.

Articolo 6: **Status e minacce della popolazione vulnerabile di falco pesactore del Parco Nazionale di Al Hoceima (Marocco).**

La maggior parte delle aree storiche di distribuzione del falco pescatore nel Mediterraneo sono andate perse e alcune popolazioni locali si sono estinte a causa del disturbo e della persecuzione diretta. Anche se delle azioni di gestione diretta hanno consentito a livello locale un parziale recupero di alcune popolazioni, la popolazione mediterranea é attualmente composta da poche decine di coppie nidificanti ed è, per questo, considerata come un'entitá a rischio sotto il profilo conservazionistico. Uno degli ultimi nuclei riproduttivi si trova lungo le coste Nord-africane del Marocco e dell'Algeria. In questo lavoro riportiamo nuove informazioni sulla popolazione di falco pescatore del Parco Nazionale del Al Hoceima (Marocco). I dati raccolti durante le missioni effettuate nel 2012 e 2013 sono stati confrontati con i dati storici raccolti durante il periodo 1983-1990, al fine di stimare il trend demografico della popolazione nel corso delle ultime decadi. In particolare, abbiamo registrato una riduzione del numero di nidi e delle coppie riproduttive ed una diminuzione generale della popolazione del 35,7%. In questo lavoro, riportiamo inoltre le principali minacce individuate durante le missioni di campo: la popolazione nidificante di falco pescatore del Parco Nazionale d'Al Hoceima é localmente minacciata da attivitá umane impattanti quali la pesca per mezzo della dinamite e/o con sostanze velenose (e.g. solfato di rame) e la pesca a strascico lungo costa. In questo contesto, sottolineiamo l'urgenza di pianificare misure urgenti di gestione per la protezione di questa popolazione vulnerabile, alla luce di una strategia di conservazione della specie anche a livello del bacino del Mediterraneo.

Articolo 7: **Reintroduzione del falco pescatore in Italia centrale: dispersione, sopravvivenza e primi dati di nidificazione.**

In questo articolo presentiamo i risultati delle prime fasi di dispersione giovanile e le stime dei tassi di sopravvivenza di individui di falco pescatore traslocati nell'ambito di un progetto di reintroduzione della specie in Italia. Tra il 2006 e il 2010, 32 giovani falchi pescatori sono stati reintrodotti mediante la tecnica dell'hacking all'interno del Parco Regionale della Maremma. Attraverso analisi di cattura-marcaggio-ricattura abbiamo valutato gli effetti dell'età sulla sopravvivenza. I movimenti esplorativi e di dispersione invece sono stati studiati mediante telemetria tradizionale ed utilizzando dati di riletture di anelli. La disperisone é risultata essere sbilanciata in funzione del sesso: la distanza media percorsa è stata maggiore per le femmine (246,2 km) che per i maschi (38,7 km). La sopravvivenza invece é mutata secondo un effetto età-dipendente: i valori sono risultati essere maggiori per individui giovani nei primi mesi dopo il rilascio (0,87), minimi per gli individui giovani al primo inverno (0,26), e relativamente migliori negli anni successivi (sopravvivenza apparente annuale di 0,69 per immaturi e di 0,93 per gli adulti). Tali risultati hanno fornito informazioni importanti sulla dispersione e sul tasso di sopravvivenza di falchi pescatori reintrodotti. Nonostante il basso tasso di sopravvivenza apparente registrato durante il primo anno, gli alti tassi di sopravvivenza riscontrati in individui immaturi ed adulti suggeriscono la presenza di condizioni locali favorevoli per questa popolazione di nuovo insediamento. Lo studio dei parametri demografici e della dispersione sono risultati importanti per calibrare le azioni di gestione finalizzate alla creazione di una nuova popolazione. In tal senso, i primi eventi di riproduzione (2011-2014), rappresentano, dopo circa 40 anni d'assenza, il ritorno di questa specie sul territorio italiano. L'obiettivo primario del lavoro dei prossimi anni sarà quindi favorire un incremento graduale del numero di coppie nidificanti nella Toscana meridionale, al fine di creare una popolazione in grado di auto-sostenersi nel tempo.

Conclusioni

Questa ricerca ha dimostrato come un approccio basato su un analisi spazio-temporale dipendente possa essere efficace in ecologia e biologia della conservazione, mostrando anche che tale metodologia di ricerca richiede l'applicazione congiunta di tecniche di campo e di laboratorio, attraverso la consocenza di una vasta gamma di discipline che vanno dalla biologia molecolare a considerazioni di tipo socio-economico. Nel complesso, questo lavoro ha portato ad alcuni importanti progressi per la biogeografia della conservazione del falco pescatore nel Mediterraneo occidentale, in Europa occidentale e a scala mondiale. Queste nuove informazioni sono particolarmente utili per quanto riguarda la gestione efficace di questa specie emblematica.

Sulla base di differenze comportamentali e di una differente strutturazione genetica del DNA nucleare, si suggerisce di valutare soluzioni specifiche nella gestione delle differenti popolazioni: in particolare pensiamo che il ripristino delle popolazioni limitate, o la ricostituzione di nuclei per mezzo di programmi di reintroduzione o ripopolamento dovrebbero fare uso principalmente di inidividui aventi la stessa origine e presentanti caratteristiche analoghe evolutesi sotto circostanze ambientali simili. Infatti, i programmi di reintroduzione sono finalizzati a ricostituire le popolazioni selvatiche a livelli sostenibili con caratteristiche biologiche identiche alla popolazione originaria. Nel caso del falco pescatore, popolazioni migratrici a lunga distanza caratterizzate da specifiche informazioni genetiche non dovrebbero essere prioritariamente utilizzate per ricostruire popolazioni della regione del Mediterraneo e delle isole atlantiche, dove le popolazioni locali mostrano struttura genetica e comportamento differenti. Interventi umani inadeguati possono provocare modificazioni in queste popolazioni e produrre cambiamenti che non sono in sintonia con i processi evolutivi naturali, né con la matrice dell'habitat locale. Una corretta strategia di conservazione dovrebbe mirare non solo a garantire un rapido recupero della popolazione, ma anche a preservarne le caratteristiche migratorie per mantenere l'integrità della popolazione nativa.

Nel Mediterraneo, dal momento che le aree di svernamento del falco pescatore non risultano essere concentrate in un'unica area (come ad esempio il mare di Wadden o la Camargue che coistituiscono i principali siti di svernamento per molti limicoli ed acquatici del Nord Europa), ma sono ampiamente distribuite lungo le coste della parte occidentale del bacino del Mediterraneo (Italia, Nord Africa, Francia e Spagna), si ritiene necessaria un'armonizzazione dei protocolli di gestione di queste aree umide. La gestione di questa specie richiede quindi uno sforzo congiunto tra i vari paesi del Mediterraneo. A livello regionale quindi, si consiglia l'istituzione di una fondazione internazionale per la conservazione della specie e / o la creazione di una specifica rete di specialisti che sorvegli le azioni di gestione e gli aspetti di protezione locali nei vari paesi, collaborando unitamente. Prime concrete collaborazioni tra la Francia, l'Italia, la Spagna e il Marocco sono state avviate nell'ambito di questo progetto di dottorato, ma ulteriori azioni e relative decisioni dovrebbero essere attivate nell'ottica di un futuro network operativo per la conservazione del falco pescatore in tutto il bacino del Mediterraneo.

Più in particolare i risultati ottenuti nel corso di questo dottorato possono essere utilizzati per implementare la gestione e le strategie di conservazione della specie a scala locale.

Facendo riferimento ai tre casi specifici nel Mediterraneo, alcune considerazioni pratiche possono essere delineate. In Corsica, ed in particolare nella Riserva Naturale di Scandola, risulta necessario limitare il traffico giornaliero di barche, regolando gli accessi e il numero di visite consentite, al fine di ridurre al minimo il disturbo ai falchi pescatori, soprattutto nel periodo di riproduzione. Non dovrebbe essere consentito alle barche di avvicinarsi alla costa di fronte ai nidi. Sarebbe inoltre opportuno rispettare una zona cuscinetto (minimo di 300 m di raggio) al fine di evitare qualsiasi disturbo di falchi pescatori. La vigilanza dovrebbe essere mantenuta alta per evitare gli effetti negativi del traffico nautico. Tali tipi di misure sono già state attuate in altre aree marine protette e hanno permesso importanti miglioramenti delle comunità di uccelli e pesci.

In Marocco, la vulnerabilità della popolazione locale ristretta in una stretta striscia di costa che è pesantemente sfruttata e disturbata da attività umane (ad esempio la pesca con la dinamite) richiede urgentemente azioni di vigilanza e norme adeguate, per una migliore preservazione di questo nucleo riproduttivo.

Nell'ambito del progetto di reintroduzione italiano sono previste altre azioni di gestione diretta. I gestori hanno pianificato l'installazione di nuovi nidi artificiali in siti chiave (ad esempio nelle isole dell'Arcipelago Toscano ed in Sardegna) per favorire l'insediamento di nuovi individui e favorire la ricolonizzazione naturale di aree adiacenti, al fine di sostenere la popolazione del bacino del Mediterraneo.

In conclusione, questo studio è strutturato per essere un buon modello anche per altre specie che condividano con il falco pescatore caratteristiche e aspetti ecologici simili, come i grandi uccelli migratori ad ampia distribuzione. Gli uccelli migratori necessitano infatti di un approccio di studio improntato su differenti scale e su piú popolazioni per raggiungere efficaci obiettivi di conservazione. Viaggiando su lunghe distanze attraverso diverse regioni, habitat e confini politici, essi sono infatti potenzialmente minacciati da una moltitudine di fattori che influenzano la sopravvivenza e la persistenza a lungo termine delle loro popolazioni. Tratti evolutivi e comportamenti adattativi devono essere quindi indagati in dettaglio, soprattutto alla luce dei recenti cambiamenti climatici globali. In questo senso, è obbligatoria l'adozione di un approccio multiscala integrato su specie modello per avere una visione più completa che permetta di comprendere le storie evolutive, la genetica e i fattori limitanti delle popolazioni, al fine di una loro corretta strategia di conservazione. Questo vale soprattutto per le popolazioni che vivono in ambienti umani sfruttati e / o in habitat frammentati per la maggior parte dell'anno, dove possono essere presenti svariate minacce. Il Mediterraneo è una delle regioni più sfruttate in questo senso. Ospitando al contempo alti valori di biodiversità, esso costituisce quindi un interessante contesto dove investigare casi di studio di questo tipo.

17. Résumé Générale

Introduction

La biodiversité (considérée comme la diversité des organismes au niveau de l'espèce, des individus, des gènes et des écosystèmes) est constamment menacée à la fois par des processus naturels ou d'origine humaine (Primack, 2000). Alors que les processus naturels agissent sur de longues périodes de temps, au cours de laquelle les écosystèmes sont en mesure de rétablir l'équilibre initial ou stabiliser progressivement sur un nouvel équilibre, les perturbations anthropiques se produisent rapidement, souvent modifiant de façon irréversible les écosystèmes et les processus écologiques. Les principales menaces sur la biodiversité résultent donc principalement de l'impact des activités humaines sur l'environnement. Au cours des dernières décennies, de nombreuses espèces se sont éteintes, de nombreux habitats ont subi des changements radicaux et/ou souffrent de processus de détérioration rapide. Pour assurer la protection de la biodiversité, il est essentiel de développer des programmes de conservation et des mesures de gestion à plusieurs échelles spatiales. En raison de ressources financières limitées, il est impossible de conserver tous les domaines naturels et des espèces existantes, et de ce fait, les plans de conservation se concentrent souvent sur des habitat hautement menacés ou sur quelques espèces, souvent charismatiques, emblématiques ou avec un rôle particulièrement structurant dans les écosystèmes (espèce clé de voute et/ou espèce parapluie) (Neel, 2008). Cependant, tandis que dans les dernières décennies, le concept de biodiversité se référait uniquement à la richesse en espèces (et, par conséquent, à un système de référence « statique » et relativement prévisible; Poiani *et al.*, 2000), plus récemment le concept de biodiversité a été étendu à processus évolutifs opérant également au niveau des gènes, des populations, et des écosystèmes (Louche & Whittaker, 2011; Mace *et al.*, 2012). Par conséquent, les recommandations actuelles pour la conservation de la biodiversité insistent sur la nécessité de préserver des écosystèmes dynamiques, incluant la totalité des processus opérant à différentes échelles (Richardson & Whittaker, 2010; Mace *et al.*, 2012).

La nécessité de choisir une échelle spatio-temporelle adéquate a été largement dictée par les problèmes pratiques liés à chaque habitat et chaque espèce considérés séparément, mais l'approche théorique générale à suivre est encore fortement débattue dans les milieux académiques (Dungan *et al.*, 2002). Comme il est impossible de considérer l'ensemble des variables qui agissent simultanément sur les systèmes écologiques naturels dans l'espace et le temps, un programme de conservation devrait concentrer ses efforts sur les variables qui résument et appréhendent le mieux la complexité et la fonctionnalité des écosystèmes. Mais le choix de la bonne échelle dépend aussi en partie des questions à traiter. Ces difficultés sont particulièrement importantes dans le cas d’espèces migratrices, qui ont un cycle de vie qui comprend plusieurs échelles spatiales et temporelles. La migration constitue un mouvement périodique (saisonnier) par lequel un organisme se déplace régulièrement d'une région à l'autre pour adapter ses contraintes physiologique et écologiques (Dingle, 1996; Berthold, 2001). Les grands migrateurs peut parcourir de grandes distances le long des routes inter-continentales et traverser de nombreux habitats différents cours d'un cycle de migration (Newton, 2010). Au cours de l'année, les périodes de mouvement migratoire alternent avec des phases de sédentarité au moment de la reproduction au printemps-été et en hiver. Protéger une partie seulement de ce système vital complexe risque de compromettre le succès des plans de conservation, car les menaces survenant à d'autres échelles spatiales et temporelles pourraient ne pas être correctement prises en compte. Comprendre l'écologie des populations animales et planifier des mesures de conservation nécessite donc une connaissance suffisante des mécanismes qui agissent à différentes échelles spatiales et temporelles, ainsi que la mise en œuvre de méthodes de recherche intégrative et des approches analytiques différentes. Dans ce contexte, une approche valable devrait chercher à: (1) sélectionner une ou plusieurs espèces qui peuvent servir de modèles pour beaucoup d'autres espèces dont l'écologie, l'histoire évolutive et /ou les caractéristiques de distribution sont similaires; (2) développer une approche qui considère plusieurs échelles spatiales et temporelles simultanément (*sensu* Wiens, 1995) et (3) d'intégrer ces deux approches (Wiens *et al.*, 1993; Collins *et al.*, 1993). Dans cette thèse, j’ai décidé de suivre cette approche, motivée par la nécessité de répondre à des questions précises sur l'histoire évolutive et l'écologie d’une espèce d’oiseau migrateur menacée, le balbuzard pêcheur *Pandion haliaetus*.

Le modèle balbuzard

Le Balbuzard pêcheur est le seul représentant du genre Pandion, lui-même unique genre de la famille des Pandionidae, séparée des autres familles de rapaces (Accipitridae, Cathartidae, Falconidae). Avec une distribution mondiale, le balbuzard pêcheur est considéré comme l'une des six espèces d'oiseaux terrestres cosmopolites, ainsi que le héron *Ardea alba*, l'héron guard beu *Bubulcus ibis*, ibis falcinelle *Ibis falcinelle*, la chouette effraie *Tyto alba* et le faucon pèlerin *Falco peregrinus* (Newton, 2003). Le Balbuzard pêcheur est un rapace de grande taille au régime exclusivement piscivore, qui a développé des caractéristiques physiques et comportementales particulières pour la capture du poisson (Cramp & Simmons, 1980; Poole, 1989). Il demeure néanmoins opportuniste car son régime alimentaire comprend plusieurs espèces de poissons d'eau douce et marins vivant près de la surface de l’eau. Alors qu'en Amérique du Nord et en Europe du Nord, l'espèce est relativement commune et niche au sommet des arbres dans des écosystèmes d'eau douce, le balbuzard en Méditerranée est étroitement lié aux écosystèmes marins, nichant sur les falaises surplombant la mer. Les stratégies de migration de balbuzards pêcheurs et les zones utilisées au cours de la saison de non-reproduction (sites d'hivernage) n’ont jamais été étudiés précisément. Avec moins d'une centaine de couples reproducteurs répartis entre la Corse, les Baléares, le Maroc et l'Algérie, la population de balbuzard pêcheur en Méditerranée reste fragile. Au cours des dernières décennies, les menaces anthropiques ont conduit l'espèce au bord de l'extinction dans de nombreuses régions d'Europe et en particulier dans le bassin méditerranéen (Espagne continentale, Italie et Portugal; Dennis, 2008). A l'échelle régionale, l'espèce est en fait considérée comme "en danger", bien qu’elle soit protégée en particulier dans tous les pays européens.

Étant une espèce cosmopolite et migratrice, le Balbuzard pêcheur constitue un modèle biologique intéressant pour étudier comment les adaptations comportementales ont évolué dans des populations géographiquement éloignées et dans différentes conditions écologiques (entre l’Arctique et les tropiques). J’ai adopté une approche multidisciplinaire, articulée autour de différentes échelles spatiales et temporelles, en utilisant des outils empruntés à l'écologie moléculaire, l’écologie trophique (évaluée par analyse des isotopes stables et recensements des proies), l’écologie spatiale (biotélémétrie), ainsi que la dynamique des populations.

Le projet a été structuré selon trois échelles spatiales différentes (niveaux mondial, régional et local) et sur trois échelles de temps (temps évolutif, temps historiques et années récentes). J’ai essayé de répondre aux grandes questions suivantes:

Niveau mondial et temps évolutifs

- Quel est le degré de divergence génétique entre les populations de balbuzard et quelle est l'histoire évolutive de l’espèce à l'échelle mondiale? La systématique actuellement utilisée reflète t’elle cette histoire évolutive ?

- Quel est le degré de connectivité entre les populations à l'échelle mondiale?

Niveau régional et temps historiques

- existe-t’il différentes stratégies de migration pour les différentes populations de balbuzards dans le Paléarctique occidental?

- Quels sont les sites et habitats d’hivernages utilisés par les balbuzards nichant en Méditerranée?

Niveau local et années récentes

- Quelle est l'intensité des échanges entre les populations de balbuzards du bassin méditerranéen et avec les autres populations Européennes?

- Les tendances démographiques observées dans les populations Méditerranéennes sont-elles influencées par des contraintes environnementales naturelles et/ou d'origine anthropique?

Article 1: **Être cosmopolite: phylogéographie et évolution d'un rapace spécialiste, le balbuzard pêcheur.**

A partir de 200 échantillons de plumes et de sang, prélevés sur des balbuzards du monde entier (sur des spécimens vivant ou de musée), j'ai effectué le séquençage de deux gènes de l'ADN mitochondrial pour étudier la diversité génétique et la structure phylogéographique de l'espèce. L'espèce est structurée en quatre groupes génétiques distincts, répartis dans quatre régions du monde (Amérique, Indonésie-Australasie, Europe-Asie centrale-Afrique, et Asie de l'est). A l'intérieur de chaque lignée évolutive, bien que couvrant de larges zones géographiques, on remarque un relatif manque de variabilité génétique. En revanche, une grande différence nucléotidique a été enregistrée parmi les quatre lignées. Des reconstructions démographiques suggèrent que trois des quatre lignées sont stables (tous à part le lignée d'Asie), voire en légères augmentation. Les datations moléculaires ont estimé que la répartition initiale entre différentes lignées remonterait à environ 3,1 millions d'années, pendant le Pliocène. Nous proposons un scénario évolutif hypothétique pour expliquer comment un rapace hautement spécialisé a été capable de coloniser le monde entier. Nous pensons que l'espèce est originaire du continent américain (de part la position basale des spécimens américains sur les arbres phylogénétiques) et qu'elle a colonisé l'Ancien Monde via l'Asie en traversant le détroit de Béring. Des périodes glaciaires défavorables auraient alors forcé l'espèce à se déplacer vers des zones au climat plus clément: l'Asie, puis le balbuzard serait descendu vers le sud, trouvant refuge en Indonésie et en Australie. Elle serait restée isolée pendant une longue période glaciaire avant de reprendre une expansion vers le nord pendant les périodes interglaciaires suivantes. Il semble qu'elle ait colonisé l'Asie de l'Est (Sibérie et Japon) et tout le Paléarctique occidental, à travers deux routes de colonisation distinctes, séparées par l'Himalaya. Les populations du Paléarctique occidental représenteraient donc la dernière étape de cette histoire évolutive. Surtout, chaque lignée évolutive représente une ESU (Unité Evolutive Significative) et devrait donc être traitées et gérée séparément des autres populations provenant d'autres lignées. La systématique de l'espèce (et même la famille Pandionidae) devraient être réexaminées à la lumière de ces nouvelles découvertes. Cette étude apporte des nouvelles connaissances génétiques essentielles pour les stratégies de gestion et de conservation de l'espèce. Dans le cadre de projets de repeuplement / réintroduction, nous suggérons de limiter les translocations en utilisant uniquement des individus appartenant aux populations issues de la même lignée évolutive. En d'autres termes, des translocations en Europe ne devraient pas utiliser d'oiseaux originaires d'Amérique, d'Océanie et d'Asie de l'Est.

Article 2: **La connectivité entre les populations de balbuzard pêcheur révélée par le génotypage: une approche multi-échelle en utilisant des microsatellites.**

La structure et la variabilité génétique des populations de balbuzards ont été étudiées en utilisant des marqueurs génétiques de l'ADN nucléaire (microsatellites). Vingt loci microsatellites ont été analysés sur un total de 200 échantillons provenant du monde entier. L'analyse par microsatellites a confirmé l'existence d'au moins trois des quatre lignées identifiés par l'analyse de l'ADN mitochondrial (voir article 1). Les populations d'Amérique, d'Australie et du Paléarctique forment trois groupes bien isolées les uns des autres, non reliés par des flux de gènes. De vastes barrières géographiques, comme les océans, ont certainement favorisé l'isolement de ces groupes. Au contraire, les populations de balbuzards pêcheurs du Paléarctique occidental, bien que géographiquement éloignées de plusieurs centaines ou milliers de km, sont partiellement reliées par des flux de gènes. Cependant, ce groupe Paléarctique présente une sous-structuration en deux entités distinctes (Europe continentale vs région méditerranéenne). Malgré des échanges occasionnels, cette forte sous-structuration conduit à penser que les populations de balbuzards pêcheurs vivant à des latitudes différentes ont développé une information génétique spécifique. Par conséquent, les populations de Paléarctique, bien qu'appartenant à la même lignée évolutive (au niveau de l'ADN mitochondrial, article 1), sont différentes au niveau de gènes nucléaires, et cette différenciation a du se produire plus récemment. Dans le bassin Méditerranéen, l'absence de structuration semble indiquer que les populations de cette région sont encore reliées entre elles. En ce sens, les comportements tels que la dispersion, la migration et la philopatrie pourraient donc avoir agi simultanément, pour façonner la structure génétique et la diversité des différentes populations. Une meilleure compréhension de ces comportements est donc devenue nécessaire, car elle pourrait aider à reconstruire la dynamique des populations, fournissant des informations essentielles pour la gestion et la conservation de l'espèce, notamment en Méditerranée.

Article 3: **Variations spatio-temporelles du comportement migratoire entre populations et classes d'âge chez le balbuzard pêcheur.**

Suite aux différences constatées dans la structure génétique des balbuzards au sein du Paléarctique, nous avons étudié si les stratégies de migration différaient entre le nord et le sud de l'Europe. Cinquante-quatre balbuzards de populations scandinave (Suède) et de

Méditerranée (Corse, Îles Baléares, Italie) ont été suivis à l'aide de la télémétrie par satellite (GPS). Nous avons analysé un total de 70 trajets migratoires qui ont mis en évidence un comportement migratoire différent entre les individus scandinaves et méditerranéens. Les individus de l'Europe du Nord ont un comportement migratoire relativement homogène, ce qui rend les longs trajets migratoires (ca 6000 km) à travers l'Europe, à travers le détroit de Gibraltar et en traversant la grande barrière du désert Sahara avant d'atteindre les sites d'hivernage sur la côte ouest de l'Afrique subsaharienne. En revanche les balbuzards Méditerranéens montrent un comportement plus diversifié et hétérogène, typique d'une population partiellement migratrice. En effet, 46% des oiseaux sont restés à proximité des sites de nidification toute l'année sans migrer (résidents); 15,5% ont réalisé des courts voyages migratoires (<500 km) et les 38,5% restant ont migré sur de plus grandes distances (1296.5 ± 740.9 km). Leurs sites d'hivernage sont toujours situés dans le bassin méditerranéen. Les oiseaux Méditerranéens qui ont entrepris une migration ont effectué de longs vols non-stop (d’une durée maximale de 23 h) au-dessus de la mer, alors que de telles traversées maritimes n’ont pas été observées chez les individus scandinaves. Pour la population Méditerranéenne, la grande hétérogénéité dans le choix des routes migratoires, des dates de départ et des sites d'hivernage pourrait être due à la présence de conditions écologiques plus favorables dans la région méditerranéenne tout au long de l'année. Au sein de chaque population, nous avons remarqué que les individus adultes peuvent parcourir de plus grandes distances quotidiennes, en suivant des itinéraires plus directs et moins sinueux que les jeunes oiseaux, ce qui suggère que la performance de la migration est fortement affecté par l’expérience acquise au fil des années (Sergio *et al.*, 2014). Les jeunes ont également montré une capacité réduite à utiliser des vents porteurs et favorables pour traverser la mer. Dans l'ensemble, notre étude montre que la connaissance détaillée des flux migratoires à différentes échelles d'espace et de temps est cruciale pour la conception de plans de conservation et de gestion des espèces migratrices.

Article 4: **Plasticité comportementale et écologie hivernale du balbuzard pêcheur en Méditerranée détectée par l'analyse des isotopes stables et la télémétrie GPS.**

Pour comprendre l'écologie en hiver des balbuzards nichant en Méditerranée, nous avons utilisé une approche complémentaire, utilisant à la fois le suivi individuel via la télémétrie par GPS, et par l'analyse des isotopes stables à partir d'échantillons de plumes. Des plumes de 98 poussins de balbuzard issus de différentes populations ont été échantillonnées le long d'un large gradient latitudinal (de la Laponie à l'Afrique), afin d'évaluer la variabilité des isotopes stables du carbone, de l'azote et le soufre ($d^{13}C$, $\delta^{15}N$ et $\delta^{34}S$). Cette analyse a permis de distinguer les différents sites de reproduction et les différents types d'habitats utilisés par les populations du Paléarctique occidental. Les analyses isotopiques ont ensuite été répétées sur un échantillon expérimental de 18 balbuzards adultes nichant en Corse, pour tenter d'estimer la latitude de leurs sites d'hivernage et le type d'habitat utilisé pendant l'hiver. En outre 12 balbuzards adultes ont été équipés de balises GPS pour suivre leur migration et hivernage. En combinant les deux techniques, nous avons confirmé que la population de Méditerranée est une population partiellement migratrice, mêlant des individus résidents et migrateurs. Les balbuzards méditerranéens ont tous passé l'hiver sous des latitudes tempérées (répartis le long des côtes de plusieurs pays du bassin méditerranéen, et non concentrés dans quelques zones). Ils ont montré une grande plasticité dans la sélection l'habitat, utilisant des baies marines, des lagunes côtières, et des marais intérieurs. Les mouvements quotidiens et les domaines vitaux ont été réduits au cours de la saison d'hiver. Cette plasticité comportementale inter-individuelle dans le choix du site d'hivernage et du type d'habitat suggère la nécessité d'adopter une approche à grande échelle pour la protection du balbuzard en hiver. Pour aider à assurer un niveau approprié de conservation des populations de balbuzards dans le bassin méditerranéen, il est nécessaire d'harmoniser les mesures de gestion des zones humides entre les différents pays concernés.

Article 5: **La rançon du succès: impact négatif du tourisme dans une réserve naturelle sur une population de rapaces emblématique de Méditerranée.**

Au cours des dernières décennies, la perturbation causée à la faune par les activités humaines est devenue une préoccupation majeure pour les biologistes de la conservation. En Méditerranée, le tourisme nautique et les activités récréatives associées ont augmenté dramatiquement depuis les années 1980, en particulier dans les aires marines protégées (AMP). Nous avons étudié l'impact de l'aire marine protégée de Scandola (Corse) sur la population du balbuzard pêcheur, emblème de la réserve. L'analyse des données sur une période de 37 ans a montré un paradoxe de conservation selon lequel les paramètres de reproduction des couples reproducteurs dans la zone de protection marine ont progressivement diminué par rapport à ceux enregistrés pour les couples reproducteurs dans le reste de la Corse, en dehors de la zone protégée. Dans le même temps, l'afflux de touristes (en termes de nombre de bateaux) a connu une croissance rapide, en particulier dans la réserve. Grâce à une analyse sur la répartition de la ressource trophique (abondance de poissons de surface) via 98 transects en kayak avec enregistrement vidéo sous-marin, nous avons trouvé que ce paradoxe n'était certainement pas causé par un déficit alimentaire: la biomasse et la taille des poissons sont supérieures dans la réserve par rapport au reste du littoral. Par ailleurs, une étude détaillée sur la circulation des bateaux réalisée entre 2013 et 2014 a révélé que le nombre de bateaux touristiques passant à moins de 250 m des nids de balbuzards sont 2 fois plus importants dans la réserve de Scandola par rapport aux zones témoin à l'extérieur. Enfin, des observations comportementales des nids de balbuzard, entre 2012 et 2014, ont montré que le trafic local des bateaux change de manière significative le budget quotidien de temps, en réduisant le nombre de proies rapporté au nid par le mâle, et en augmentant le temps passé par les femmes en alarme. Enfin, à partir de plumes prélevées sur des poussins au moment de leur baguage, nous trouvons des niveaux de corticostérone (qui est une hormone de stress) 2.5 fois plus élevés chez les poussins issus des zones à fort trafic de bateau par rapport à des zones plus calmes en Corse ou ailleurs (Italie, Baléares). Globalement, donc, notre analyse combinant un bilan démographique, l'écologie des proies, les observations comportementales et les hormones de stress suggère un fort impact du tourisme sur la population locale de balbuzard pêcheur. Cette étude souligne l'importance, dans le monde, la mise en œuvre de mesures strictes afin de mettre en place un écotourisme durable.

Article 6: **Statut et menaces de la population vulnérable du balbuzard pêcheur du parc national d'Al Hoceima (Maroc).**

La plupart des zones de distribution historique du Balbuzard en Méditerranée ont été perdues et certaines populations locales se sont éteintes en raison de persécutions directes. L'un des derniers noyaux de reproduction est situé le long de la côte de l'Afrique du Nord au Maroc et en Algérie. Dans ce travail, nous rapportons de nouvelles informations sur la population de balbuzard pêcheur du parc national d'Al Hoceima (Maroc). Les données recueillies au cours des missions effectuées en 2012 et 2013 ont été comparés avec les données historiques recueillies au cours de la période 1983-1990, afin d'estimer l'évolution démographique de la population au cours des dernières décennies. En particulier, nous avons constaté une réduction du nombre de nids et couples reproducteurs et une diminution globale de la population de 35,7%. Les principales menaces pesant sur cette population de balbuzard sont les activités humaines comme la pêche à la dynamite et/ou avec des substances toxiques (par exemple le sulfate de cuivre), plongée pêche sous-marine, perturbation par les bateaux à moteur (jet-ski) et le chalutage le long de la côte. Dans ce contexte, nous soulignons la nécessité d'entreprendre un plan d'action pour la protection de cette population vulnérable, à la lumière d'une stratégie de conservation de l'espèce au niveau du bassin méditerranéen.

Article 7: **Réintroduction du balbuzard pêcheur dans le centre de l'Italie: dispersion, survie et premières données de nidification.**

Dans cet article, nous présentons les résultats sur la survie et la dispersion des jeunes balbuzards pêcheurs relâchés dans le Parc Naturel Régional de la Maremma, dans le cadre d'un projet de réintroduction de l'espèce en Italie. Entre 2006 et 2010, 32 jeunes balbuzards prélevés dans des nids en Corse ont été relâchés par la technique du « taquet ». Grâce à un important travail de suivi sur le terrain, en utilisant les données de télémétrie VHF et de relectures de bagues, nous avons évalué les effets de l'âge sur la survie par une analyse de capture-marquage-recapture. La dispersion varie selon le sexe: la distance moyenne parcourue au cours des premiers mois a été supérieure chez les femelles (246,2 km) par rapport aux males (38,7 km). La probabilité de survie varie en fonction de l'âge: les valeurs étaient plus élevées pour les jeunes individus dans les 3 premiers mois après la libération (0,87), puis chutaient lors du premier hiver (0,26), avant de réaugmenter les années suivantes (la survie apparente annuelle était estimée à 0,69 pour les immatures et 0,93 pour les adultes). Malgré le faible taux de survie apparente enregistrée au cours de la première année, les taux élevés de survie observés suggèrent la présence de conditions locales favorables pour cette nouvelle population. L'étude des paramètres démographiques et les résultats de dispersion sont importants pour calibrer les mesures de gestion visant à créer une nouvelle population. En ce sens, les premiers événements de reproduction (2011-2014) signalent le retour de cette espèce sur le territoire italien après 40 ans d'absence. L'objectif principal du travail des années à venir sera donc de favoriser une augmentation progressive du nombre de couples reproducteurs dans le sud de la Toscane, dans le but de créer une population capable de se perpétuer au fil du temps.

Conclusions

Cette étude a montré qu'une approche fondée sur une analyse á plusieures échelles peut être efficace en écologie et biologie de la conservation. Cette méthode de recherche nécessite de combiner des techniques de terrain et de laboratoire, à travers un large éventail de disciplines allant de la biologie moléculaire jusqu'à des considérations socio-économiques. Dans l'ensemble, ce travail a conduit à des avancées importantes pour la connaissance de la biogéographie du balbuzard pêcheur dans la Méditerranée occidentale, Europe de l'Ouest et dans le monde. Ces nouvelles informations seront particulièrement utiles pour décider de mesures de gestion efficaces pour cette espèce emblématique.

Sur la base des différences de comportement et de structure génétique de l'ADN nucléaire, je suggère de considérer des mesures de gestion spécifiques pour les différentes populations. En particulier, je pense que le renforcement des petites populations de balbuzards, par la reconstitution des noyaux à travers des programmes de translocation, devrait faire usage principalement des individus ayant la même origine évolutive car elles présentent des caractéristiques similaires, ayant évolué sous certaines conditions environnementales similaires. Dans le cas du balbuzard pêcheur, les populations migratrices à longue distance, caractérisées par une information génétique spécifique, ne devraient pas être utilisées pour reconstituer les populations de la région Méditerranéenne et des îles de l'Atlantique, où les populations locales montrent une structure génétique et un comportement différents. Des interventions humaines inadéquates pourraient provoquer des changements dans ces populations qui ne sont pas en phase avec les processus évolutifs naturels, ni avec de la matrice d'habitat local. Une stratégie de conservation appropriée doit viser non seulement à assurer une augmentation rapide de la population, mais aussi de préserver les caractéristiques de la migration pour maintenir l'intégrité de la population indigène.

En Méditerranée, les aires d'hivernage de balbuzard pêcheur ne semblent pas être concentrées dans des sites circonscrits (tels que la mer des Wadden ou la Camargue pour de nombreux échassiers et canards d’Europe du Nord), mais sont largement réparties le long des côtes de la partie occidentale du bassin méditerranéen (Italie, Afrique du Nord, France et Espagne). Je pense qu’il est alors nécessaire d’harmoniser les mesures de gestion et protocoles de suivi de ces zones humides. La gestion de cette espèce nécessite donc un effort conjoint entre les différents pays autour de la Méditerranée. À l'échelle régionale par conséquent, nous recommandons la mise en place d'une fondation internationale pour la conservation du balbuzard et/ou la création d'un réseau spécifique de spécialistes de l’espèce qui supervisent les actions locales dans les différents pays, en travaillant ensemble. Des collaborations concrètes entre la France, l'Italie, l'Espagne et le Maroc ont été entreprises dans ce projet de doctorat, mais de nouvelles mesures et décisions pertinentes devraient être activées en vue d'un futur réseau pour la conservation du balbuzard pêcheur autour du bassin méditerranéen.

Plus précisément, les résultats obtenus dans le cadre de cette thèse peuvent être utilisés pour mettre en œuvre des stratégies de gestion et de conservation de l'espèce à l'échelle locale. En référence aux trois cas spécifiques en Méditerranée, certaines considérations pratiques peuvent être décrites. En Corse, et en particulier dans la réserve naturelle de Scandola, il est nécessaire de limiter le trafic quotidien de bateaux, en réglementant l'accès et le nombre de visites autorisées, afin de perturber le moins possible les balbuzards, en particulier dans la saison de reproduction. Il ne devrait pas être permis aux bateaux de se rapprocher de la côte devant les nids. Il serait même souhaitable de se conformer à une zone tampon (minimum de

300 m de rayon) afin d'éviter toute perturbation des balbuzards nicheurs. La surveillance doit être maintenue à un niveau élevé pour éviter ces effets négatifs de la circulation des bateaux. En revanche ces mesures pourraient être adaptées au cours de la saison, en permettant l'accès à des secteurs sans nids, ou après l'échec d'un couple. Ces types de mesures ont déjà été mises en œuvre dans d'autres zones marines protégées et ont permis aux communautés d'oiseaux et de poissons de se reconstituer.

Au Maroc, la vulnérabilité de la population locale de balbuzards est restreinte à une bande étroite de la côte qui est fortement exploitée et perturbée par les activités humaines (comme la pêche à la dynamite), nécessitant de toute urgence des mesures de contrôle et des normes appropriées, pour une meilleure conservation de ce noyau de reproduction.

Dans le projet de réintroduction italienne, d'autres actions de translocation ne sont pas prévues dans un futur proche. Les gestionnaires ont prévu l'installation de nouveaux nids artificiels dans les sites clés (par exemple dans les îles de l'archipel toscan et la Sardaigne) afin d'encourager la recolonisation naturelle des zones adjacentes, afin de soutenir la population du bassin méditerranéen.

En conclusion, cette étude a été conçue pour éventuellement servir de modèle pour les autres espèces qui partagent des caractéristiques écologiques similaires comme les grands oiseaux migrateurs à large aire de répartition. L'étude des oiseaux migrateurs nécessite une approche qui se fonde sur différentes échelles spatiales et temporelles pour atteindre des objectifs de conservation efficaces. Voyageant sur de longues distances à travers différentes régions, habitats et au-delà des frontières politiques, ils sont en fait potentiellement menacés par une multitude de facteurs qui influent sur la survie et la persistance à long terme de leurs populations. Ces traits comportementaux adaptatifs doivent donc être étudiés en détail, en particulier vis à vis du récent changement climatique mondial. En ce sens, il est important d'adopter une approche multi-échelle pour avoir une vue plus complète qui permet de comprendre les histoires évolutives, et les facteurs limitant les populations, afin d'assurer leur stratégie de conservation appropriée. Cela est particulièrement vrai pour les espèces animales qui vivent dans des environnements anthropisés exploités et/ou des habitats fragmentés, qui peuvent présenter diverses menaces. La Méditerranée est l'une des régions du monde les plus exploitées, mais représente également un point-chaud de biodiversité : elle constitue donc un contexte intéressant pour ce type de cas d'études.

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