

## Brief report

# Immunocompetence and the prevalence of haematozoan parasites in two long-lived seabirds

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## 1. Introduction

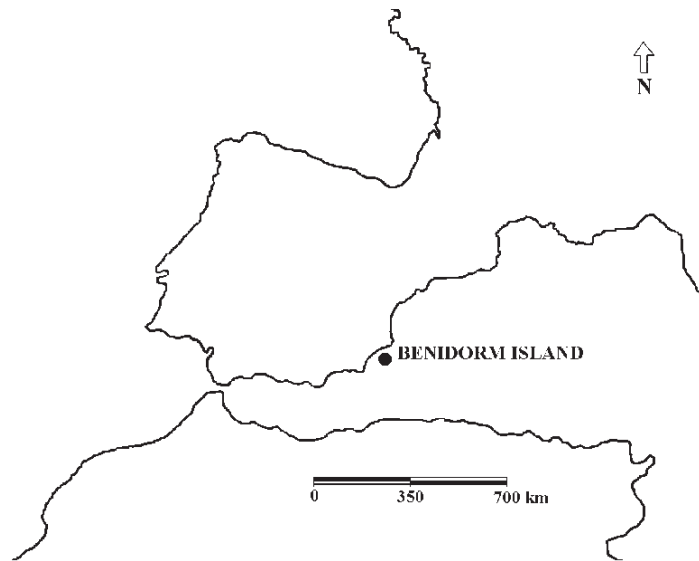
Factors affecting infection of birds by haemoparasites have received much attention during the last decade, since blood parasites can affect fitness components (Merino *et al.* 2000), community structure (Minchella & Scott 1991, Hudson & Greenman 1998) and evolution (Hamilton & Zuk 1982, Howard 1991). Infection can be influenced by three factors (a) presence of the appropriate vector (Bennett *et al.* 1992, Little & Earlé 1995, Piersma 1997, Figuerola 1999, Sol *et al.* 2000, Jovani *et al.* 2001), (b) presence of the right host-parasite assemblage (Earlé & Underhill 1993, Little & Earlé 1994, Stewart *et al.* 1997, Blanco *et al.* 1998, Jones & Shellam 1999) since avian haematozoa are specific to host families or subfamilies (Ricklefs 1992), and (c) immunological capabilities of the host (Ricklefs 1992, Figuerola *et al.* 1996, Tella *et al.* 1999). Here we compare the competence of the immune system of European Storm-petrels *Hydrobates pelagicus* with that of

syntopically breeding Mediterranean Yellow-legged Gulls *Larus argentatus michahellis*. Blood smears of the European Storm-petrel, as well as ca. 30 other species of the order Procellariiformes (Peirce 1981, González-Solís & Abella 1997) have been examined to detect haematozoan presence, with negative results (Merino & Mínguez 1998). In contrast, Yellow-legged Gulls, as well as other species of gulls, are known to have a high prevalence of blood parasites (Ruiz *et al.* 1995, Bosch *et al.* 1997, Merino 1998, Ruiz *et al.* 1998, Martínez-Abraín *et al.* 2002).

We provide evidence in favour of the hypothesis developed by Ricklefs (1992) and supported by Tella *et al.* (1999) that bird species with long embryonic growth periods (which typically are long-lived species) have strong immune responses and that this feature is likely associated with the prevention of infection by blood parasites.

Additionally, we provide evidence supporting the evolutionary theory of aging according to which long-lived birds, such as tubenoses, with a

Fig. 1. Map showing the location of Benidorm Island in the western Mediterranean, where syntopically breeding European Storm-petrels and Yellow-legged Gulls were sampled with the PHA assay.



low reproductive investment, should have powerful repair mechanisms compared to birds that invest more in reproduction and have shorter life spans (Stearns 1997).

## 2. Material and methods

### 2.1. Study area

Storm-petrels and Yellow-legged Gulls were sampled on the small island of Benidorm, Alicante, western Mediterranean (see Fig. 1). The island of Benidorm (6,5 ha.) is a small limestone outcrop located approx. 3 km off the coast of Benidorm (38°30'N, 0°08'W) and has a maximum elevation of 73 m. Annual rainfall averages approximately 300 mm and mean monthly temperature fluctuates from 12 °C in January–February to 27 °C in July–August. Vegetation is dominated by small Mediterranean shrubs adapted to arid conditions and high salinity, although exotic *Opuntia* is very widespread. There are no sources of freshwater on the island which holds one of the largest colonies of European Storm-petrels in the western Mediterranean, with ca. 500 pairs (Mínguez 2003).

### 2.2. Parasite sampling procedure

Absence of blood parasites in Storm-petrels from

Benidorm Island was determined by Merino and Mínguez (1998) working with 63 nestlings and 55 adults. Additionally, in 2002 we sampled 25 nestlings (35–45 days old) from two adjacent small colonies (Mitjana I. and Tabarca I.) for haematozoan prevalence. Mitjana is located 6 Km further north of Benidorm and 100 m from the mainland coast. Tabarca is located ca. 50 km south of Benidorm and ca. 10 km off the mainland coast.

Adult Yellow-legged Gulls from the island are known to have high prevalence and intensity of *Haemoproteus lari* (Martínez-Abraín *et al.* 2002). Thirty-four chicks of the Yellow-legged Gull were sampled for blood parasites at the Island of Benidorm in late May and early June 2003. Chicks were 2–4 weeks old, their age exceeding the prepatent period reported on chicks of other avian species (Merino & Potti 1995). Samples were immediately smeared, air dried and, within a few hours, fixed in absolute ethanol for one minute. Smears were stained with Giemsa solution in the laboratory fifteen days after collection. Blood parasites were screened microscopically using 500× and 1000× magnification.

### 2.3. Measurement of immunocompetence: the PHA test

The phytohaemagglutinin (PHA) test measures a T-lymphocyte-dependent component of immuno-

competence, and has been used in many bird studies (Lochmiller *et al.* 1994, Sorci *et al.* 1997, Moreno *et al.* 1998, Soler *et al.* 1999). Several studies on domestic fowl show that this assay offers a reliable measurement of cellular response *in vivo* (Goto *et al.* 1978, McCorkle *et al.* 1980) and it is an indicator of health and condition in birds (Saino *et al.* 1997, Alonso & Tella 2001). This test does not produce an adverse effect on birds sampled, as the reaction is transient and local (Merino *et al.* 1999a), and the response to PHA antigen requires little energy expenditure (Hörak *et al.* 2000). More specifically the PHA test assays the response to an intradermal inoculation of phytohaemagglutinin (PHA) in an isotonic saline solution. The injection of this antigen results in a dense perivascular accumulation of lymphocytes, as it is a powerful mitogen that activates lymphocytes cells in peripheral blood, causing a temporary inflammation of the zone injected.

The response to the PHA assay is known to be age dependent (Møller *et al.* 2001, Tella *et al.* 2002), being higher in chicks than in adults as a rule. Hence, we only compared the response of nestlings of the two species, as their capture is straightforward and there is no risk of nest abandonment.

We injected 23 two-month-old nestlings of the European Storm-petrel *Hydrobates pelagicus* at the island of Benidorm during the second fortnight of July 2001 with 0.04 mL of a concentration of 500 µg/mL of PHA (20 µg) (after Fair *et al.* 1999, González *et al.* 1999, Johnsen & Zuk 1999) in the wing patagium. Some authors (Smits *et al.* 1999, Tella *et al.* 2002) have shown that the injection of the same amount of isotonic saline in the right wing web as a control is not necessary (i.e. results do not vary between experiments using or not the control isotonic saline injection), and hence we did not perform this control, reducing the stress caused to nestlings (see Merino *et al.* 1999a). Point of injection was premarked in order to measure the thickness of the wing immediately before and 24 hours after inoculation, using a pressure-sensitive spessimeter with an accuracy of 0.01 mm. The wing web index (WWI) or cellular immune response index, was calculated as the difference between measures before and after injection in the patagium injected with the PHA solution.

We sampled 25 chicks of the Yellow-legged

Gull on 22–23 May and 2–3 June 2003. Chicks were 2–4 weeks old. To detect gull chicks after 24 h we ringed them after first trapping and look for them afterwards around the same area. Chicks were not kept enclosed to prevent possible depression of their immune function. Considering that the response to PHA depends on the body mass of the species sampled (Tella *et al.* 2002), and that chicks of the Yellow-legged Gull were much heavier than storm-petrel chicks, we injected the amount of PHA known to elicit the maximum response in this species (Tella *pers. com.*), which is 0.05 mL of a concentration of 2000 µg/mL (100 µg of PHA).

#### 2.4 Statistical methods

The distributions of WWI values both for gulls and storm-petrels did not significantly depart from a normal distribution, according to Kolmogorov-Smirnov tests ( $P = 0.953$  and  $0.963$  respectively), but variances were unequal after performing Levene's test. Hence, immunocompetence of the two species was compared using the non-parametric Mann-Whitney U test.

### 3. Results

All 25 chicks of the storm-petrel from Mitjana and Tabarca Islands were free of blood parasites. One of the 34 chicks of the Yellow-legged Gull scanned was severely infected with *Babesia bennetti*. Although no specific sampling procedure was applied, storm-petrel chicks were found to be highly ectoparasitized by fleas when handled and ticks were found on Yellow-legged Gull chicks.

The difference in the immunocompetence index between storm-petrels and Yellow-legged Gulls was highly significant ( $U = 15.5$ ,  $P < 0.001$ ). The average response of storm-petrels was ca. three times higher ( $1.62 \pm 0.44$ ) than the response of Yellow-legged Gulls ( $0.62 \pm 0.29$ ) (see Fig. 2).

### 4. Discussion

In a previous study at Benidorm Island we found a high prevalence of *Haemoproteus lari* on adult

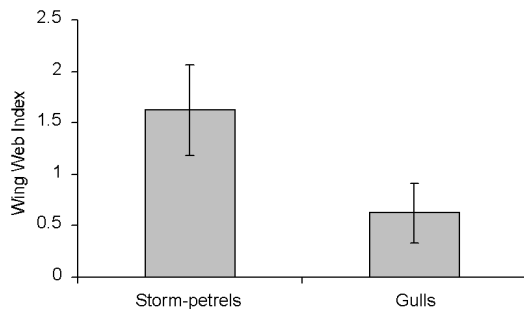


Fig. 2. Mean (and SE) response to the PHA assay (Wing Web Index) by Yellow-legged Gulls and European Storm-petrels breeding syntopically at the island of Benidorm.

Yellow-legged Gulls but did not capture any dipteran insect known to be vector of the haemoparasite found infecting gulls (Martínez-Abraín *et al.* 2002). Hence, we concluded that adult gulls became infected when feeding at mainland refuse dumps. The absence of infections with *Haemoproteus* in pre-fledging gull chicks is consistent with the apparent absence of dipteran vectors on the island. Infection with *Babesia* is consistent with the presence of their vectors (i.e. ticks) on gulls. Presence of this blood parasite on Yellow-legged Gull chicks was already established in this colony by Merino (1998) who found one pre-fledging chick infected. Merino and Mínguez (1998) also found some haematozoan vectors (ticks) on the island of Benidorm and suggested that the absence of blood parasites in storm-petrels from Benidorm could be due either to the absence of suitable vectors or to a natural resistance of these birds to blood parasites. However, Merino *et al.* (1999b) found one storm-petrel chick infected with a soft tick, in addition to infection by fleas, dermanyssid mites and lice. Our results support the idea that storm-petrels have a natural resistance to infection by blood parasites, since they showed high immunocompetence (i.e. strong PHA responses), although scarcity of proper vectors do not allow fully excluding the role of the absence of proper vectors. Cory's Shearwaters *Calonectris diomedea* from the Chafarinas Islands, located 4.5 km off the North African coast, were also free of blood parasites despite the presence of vectors for *Haemoproteus* parasites and infected gull chicks on the island (Ruiz *et al.* 1995, González-Solís & Abella 1997). Differences in PHA response cannot

be attributed to differences in age because we compared chicks of both species at the same stage of development, within the time frame of the ontogeny of each species.

Ricklefs (1992) analyzed haemoparasite prevalence in a large number of bird species, finding that prevalence was inversely related to incubation period. This correlation may arise from a direct relation between immunocompetence and the embryonic development period. The enhanced immune system should help to prevent or control haemoparasitic infections during postnatal development or even during adult life. Møller *et al.* (2001) and Tella *et al.* (2002) found that cell-mediated immunity (measured by means of the PHA test) in chicks is directly related to the length of the nestling period. They also found a strong correlation between adult cell-mediated immunity and a number of life-history traits (i.e. longevity, body size, development rate, colony size), and suggested that cell-mediated immunity in adults is plausibly responsive to selection for prevention and repair mechanisms related to long life span. However, Tella *et al.* (2002) did not find any correlation between prevalence of blood parasites and immune response, working with ca. 50 species of birds, ranging from Passeriformes to seabirds. This negative result might have reflected the fact that prevalence was obtained from the literature, consisting of data from several different populations. Hence, because prevalence may vary depending on many ecological factors, as well as the methods for determining parasite prevalence, a relationship with immune function could be obscured. Our work, although restricted to two seabird species, clearly shows a link between immune function and blood parasite prevalence when working with data on both prevalence and immunocompetence collected in the same colony, hence working at a finer scale.

We suggest, in accordance with Ricklefs (1992) and Tella *et al.* (1999), that Procellariiformes are commonly free of blood parasites because they possess a well-developed immune system linked to their relatively long embryonic development, and in comparison with seabirds that have much shorter periods of embryonic development (e.g. Yellow-legged Gulls). Absence of blood parasites in tubenoses may be mainly a by-product of selection for a strong immune system

(i.e. for good repair mechanisms) in long-lived species. This strong immune system would allow oceanic seabirds to breed on islands located close to the mainland where vectors may be present and remain free of blood parasites as in the case of the European Storm-petrel in the colonies of Benidorm, Mitjana and Tabarca, which are located close to the mainland coast. Also, a strong immune system could help seabirds to cope with heavy infestations of ectoparasites, typical of burrow nests that are reused during subsequent years (see e.g. Møller & Erritzøe 1996, Merino *et al.* 1999b, Martin *et al.* 2001). Additionally, a strong immune system could be adaptive in the marine environment to protect seabirds against bacterial and viral infections (Moreno *et al.* 1998). Alternatively, storm petrels from Benidorm could be free of *Babesia* parasites owing to some sort of incompatibility (e.g. physiological or behavioural) between host and parasite (see Martínez-Abraín *et al.* 2004). Our results are also consistent with the evolutionary theory of aging, since species with a lower reproductive investment can invest more in immunocompetence (see Stearns 1997, Owens & Wilson 1998). From an evolutionary perspective the marine environment would have originally selected for mainland bird species with long incubation periods, because this life-history trait drives the development of strong cell-mediated immune responses, good repair mechanisms, and leads to long life spans (see Tella *et al.* 2002). A long life span is the best strategy to maximize life-time reproductive output (i.e. fitness) when dealing with the unpredictability of the seas (Moreno 2003). Further research on bird immunocompetence on islands with abundant presence of haemoparasite vectors is needed to account for its actual magnitude in preventing blood parasite infection.

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## Selostus: Immuneetti ja veriloisten esiintyminen etelänharmaalokilla ja merikeijulla.

Merikeijulla, kuten muillakaan ulappalintujen lahkoon kuuluvilla lajeilla, ei toistaiseksi ole todettu esiintyvän veriloisia. Ilmiö voi johtua sopivien väli-isäntien tai isäntä-loisyhteisöjen puuttumisesta tai isäntälajin luonnollisesta vastustuskyvystä. Artikkelin kirjoittajat vertasivat merikeijujen pesäpoikasten immuunikykyä samalla tutkimusalueella Välimerellä pesivien etelänharmaalokkien pesäpoikasten immuunikyvyn. Merikeijun pesäpoikasilla havaittiin huomattavasti voimakkaampi reaktio immuneettikykyä mittaavaan PHA- testiin kuin etelänharmaalokin poikasilla. Kirjoittajat esittävät, että hyvä immuneettikyky voisi selittää veriloisten puuttumisen merikeijulla ja muilla putkinokkaisilla. Vaihtoehtoisia selityksiä veriloisten puuttumiselle voivat olla sopivien väli-isäntien puute tai loisten ja isäntälajin yhteensopimattomuus toisilleen. Tutkimustulokset tukevat hypoteesia, jonka mukaan lintulajit, joiden alkiokehitys on hidas, omaavat monimutkaisen immuneettisysteemin. Tulokset tukevat myös oletusta, jonka mukaan pitkäikäisillä lajeilla, joiden lisääntymispanostus on alhainen, täytyy olla tehokkaammat puolustusmekanismit kuin lyhytikäisillä lajeilla, jotka panostavat enemmän lisääntymiseen.

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