Original article

Infestation of small seabirds by *Ornithodoros maritimus* ticks: Effects on chick body condition, reproduction and associated infectious agents

Ana Sanz-Aguilar[a,b,*], Ana Payo-Payo[c], Andreu Rotger[a], Lena Yousfi[d], Sara Moutailler[d], Cecile Beck[e], Marine Dumarest[e], José Manuel Igual[a], Miguel Ángel Miranda[b], Mariana Viñas Torres[f], Virginia Picorelli[g], Amandine Gamble[h], Thierry Boulinier[h]

[a] *Animal Demography and Ecology Unit, IMEDEA (CSIC-UIB), Miguel Marques 21, Esporles, Spain*
[b] *Applied Zoology and Animal Conservation Group, University of Balearic Islands, Ctra Valldemosa km 7.5, Palma, Spain*
[c] *School of Biological Sciences, Zoology Building, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK*
[d] *UMR BIPAR, ANSES, INRA, Ecole Nationale Vétérinaire d'Alfort, Université Paris-Est, 14 Rue Pierre et Marie Curie, 94700 Maisons-Alfort, France*
[e] *UMR Virologie, ANSES, INRA, Ecole Nationale Vétérinaire d'Alfort, Université Paris-Est, 14 Rue Pierre et Marie Curie, 94700 Maisons-Alfort, France*
[f] *Servei de Planificació del Medi Natural, Conselleria de Medi Ambient, Gremi Corredors 10, Palma, Spain*
[g] *RRNN es Vedrà, es Vedranell i els illots de Ponent, Carrer de Múrcia 6, Eivissa, Spain*
[h] *CEFE CNRS Université Montpellier, Campus CNRS, Montpellier, France*

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ABSTRACT

Ticks can negatively affect their host by direct effects as blood feeding causing anaemia or discomfort, or by pathogen transmission. Consequently, ticks can have an important role in the population dynamics of their hosts. However, specific studies on the demographic effects of tick infestation on seabirds are still scarce. Seabird ticks have also the potential to be responsible for the circulation of little known tick-borne agents, which could have implications for non-seabird species. Here, we report the results of investigations on potential associations between soft tick *Ornithodoros maritimus* load and reproductive parameters of storm petrels *Hydrobates pelagicus* breeding in a large colony in a cave of Espartar Island, in the Balearic archipelago. We also investigated by molecular analyses the potential viral and bacterial pathogens associated with *O. maritimus* ticks present at the colony. Lower nestling survival was recorded in the most infested area, deep in the cave, compared to the area near the entrance. The parasite load was negatively associated with the body condition of the nestlings. One pool of ticks tested positive for West Nile virus and 4 pools tested positive for a *Borrelia* species which was determined by targeted nested PCR to have a 99% sequence identity with *B. turicatae*, a relapsing fever *Borrelia*. Overall, these results show that further investigations are needed to better understand the ecology and epidemiology of the interactions between ticks, pathogens and Procellariiform species.

1. Introduction

Animals coexist with numerous parasite species with important ecological and evolutionary consequences (Hamilton and Zuk, 1982; Møller et al., 1993; Moore, 2002; Poulin, 2011). Hematophagous ectoparasites such as ticks, in particular, can damage their hosts directly by the fact of biting (i.e. dermatoid process) and blood-feeding (i.e. blood loss and associated anaemia) and/or indirectly through pathogen transmission (Jongejan and Uilenberg, 2004; Brites-Neto et al., 2015).

Ticks are divided into two groups: hard ticks (Ixodidae) and soft ticks (Argasidae). Both families can potentially transmit numerous pathogens of medical and veterinary interest (Dietrich et al., 2011 and references therein). However, those transmitted by soft ticks have been less studied due to the specialization of Argasidae to hidden habitats (i.e. crevices) and the short time they spend for blood feeding on the host compared to hard ticks (Vial, 2009). Some of the most common worldwide diseases caused by pathogens transmitted by soft ticks include: human tick-borne relapsing fever (TBRF); viral encephalitis transmitted by ticks infesting seabirds, shore birds and roosting birds; the African swine fever virus (ASFV); fowl spirochetosis infections or anaplasmosis-like diseases and epizootic bovine abortion (Vial, 2009). Such pathogens can induce both lethal and sub-lethal effects on their hosts (Niebylski et al., 1999; Gray and Bradley, 2006). Organisms present a wide range of responses to tick-induced pathogens (Ramamoorthi et al., 2005). For instance, transmitted pathogens infecting the host might induce behavioral and/or physiological
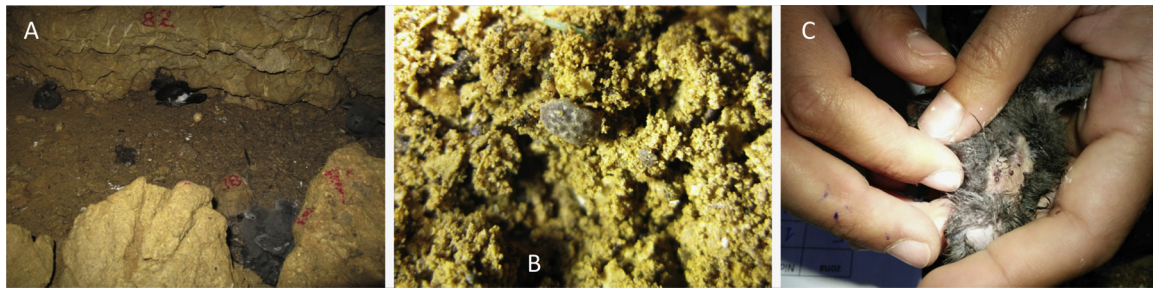
Fig. 1. A) Inner chambers of the storm petrel colony at Espartar Island. In the photograph can be observed one incubating adult, three alive nestlings and two dead nestlings; B) adults soft tick (*O. maritimus*) and C) larvae of soft tick (*O. maritimus*) on a storm petrel nestling.

modifications which interfere with immunity response, gene expression or energy allocation, thus potentially affecting fitness. However, the effects of tick infestation and tick-borne diseases on host populations are often poorly understood and quantifications of sub-lethal effects are hard to assess (Nemeth et al., 2006; Dietrich et al., 2011).

Seabirds can be predictable hosts for ticks because they show colonial breeding habits resulting in large and dense aggregations, high nest site fidelity and extended breeding periods (Ramos et al., 2001; Schreiber and Burger, 2001). These breeding characteristics of seabirds facilitate transmission and contribute to the support of large and dense populations of ticks (Dietrich et al., 2011 and references therein). Severe infestations of seabird colonies by ticks can have heavy negative effects (Duffy, 1983; Ramos et al., 2001; Dietrich et al., 2011). For example, Duffy (1983) documented a large-scale nest desertion in Peruvian guano seabirds (cormorants, boobies and pelicans) due to *Ornithodoros amblus* soft tick infestation at breeding sites. Similarly, high densities of *O. capensis* s.s. seemed to be responsible for Sooty tern (*Sterna fuscata*) egg and newly hatched chick desertion at Seychelles in 1972 (Feare, 1976). Colony abandonment or dispersal could be used as a strategy to avoid the negative effects of parasites (Boulinier and Danchin, 1996). In fact, some cases of lethal effects of ticks (i.e. *Ixodes uriae*) on seabirds have been documented, especially for young nestlings (Boulinier and Danchin, 1996; Ramos et al., 2001) but very rarely in adults (Gauthier-Clerc et al., 1998). On the other hand, non-lethal but also negative effects have been documented on seabird body condition and/or nestlings' growth rates (e.g. Bosch and Figuerola, 1999; McCoy et al., 2002; Hipfner et al., 2019). However, sometimes the effects of ticks are not evident until infestation levels are very high (Gauthier-Clerc et al., 2003; Hipfner et al., 2019) or may depend on the environmental conditions experienced by the hosts (McCoy et al., 2002). Although ticks can have an important role in seabird population dynamics, specific studies on the demographic effects of tick infestation on seabirds are still scarce (Dietrich et al., 2011; Rodríguez et al., 2019). Moreover, the ultimate mechanisms responsible for lethal and sub-lethal effects of ticks (i.e. direct effect or indirect effect due to pathogen transmission) on seabirds remain largely unknow (Yabsley et al., 2012).

Espartar Island, located on the Balearic archipelago, hosts the largest Spanish colony of Mediterranean storm petrel (*Hydrobates pelagicus melitensis*), estimated at 750-1250 breeding pairs (Picorelli, unpublished data). Other seabirds (gulls and shearwaters) also breed in the island, but storm petrels mainly concentrate in cliff caves, sharing their breeding habitat with Balearic shearwaters (*Puffinus mauretanicus*). Balearic shearwaters breed in lower densities and start reproduction much earlier (i.e. February; Guilford et al., 2012) than storm petrels (i.e. May-June; Ramírez et al., 2016). A systematic monitoring of the breeding parameters of storm petrels breeding at the largest colony of the island, the cap de Migdia cave (˜250 breeding pairs) started in 2014 (Mínguez et al., 2015). During the first years of monitoring (2014–2016), we detected a high mortality of nestlings (see results) compared to other storm petrel Mediterranean colonies in which nestling survival typically varies between 90–95% (Sanz-Aguilar

et al., 2009). Moreover, we experienced recurrent tick bites during the daylight monitoring of the cave, a very unusual fact when compared with our experience monitoring other storm petrel colonies. Ticks were identified as the soft tick *O. maritimus* (Vermeil and Marguet, 1967), included in the complex *O. (Carios) capensis* that includes eight described species that parasitize tropical and temperate colonial seabird species (Hoogstraal et al., 1979; Khoury et al., 2011; Dupraz et al., 2016). Identification of *O. maritimus* was performed by morphology (i.e. median dorsal line of mamillae disc short) and confirmed by an expert on the group (Dupraz, M., personal communication). In addition, based on Dupraz et al. (2016) *O. maritimus* is mostly restricted to the West Palearctic Region where its presence parasitizing seabirds have been extensively reported (Dietrich et al., 2011). The species *O. capensis* s.s. is also present in the same region, but almost exclusively associated with pigeons and it can be morphologically separated from *O. maritimus* (Khoury et al., 2011).

Both bites to researchers and mortality of storm petrel chicks were especially evident in a particular area of the cave, the inner chambers. Consequently, in 2018 we evaluated the potential differences of *O. maritimus* load between different parts of the colony as a proxy of tick abundance and evaluated their effects on nestlings' body condition. We used this information to infer the potential effect of ticks on the reproductive parameters of storm petrels. Moreover, we also used a high throughput real-time PCR system to test for the presence in *O. maritimus* ticks of the most common tick-borne infectious agents including bacteria, parasites and viruses potentially harboured by ticks.

2. Methods

2.1. Species, study site, field data and tick collection

The Mediterranean storm petrel is one of the smallest seabirds of the Order Procellariiformes (average body mass, 28 g, Warham, 1990). Storm petrels breed in caves, burrows, under boulders or in crevices where they lay a single egg directly on the floor (Fig. 1A). The egg is incubated by both partners for 40 days and chick rearing lasts for about 63–70 days (Scott, 1970; Mínguez, 1994). The earliest clutches are laid in the second half of April and the last eggs are laid in the first week of July (Ramírez et al., 2016). Most fledglings leave colonies in August (Mínguez, 1994).

The study was conducted on Espartar Island, a 20.5 ha island located within the Es Vedrà, Es Vedranell i els illots de Ponent Natural Reserve (38° 57′ 31″ N, 1° 11′ 44″ E, Fig. 2). Espartar Island has a characteristic steep relief with gentle slopes facing south and cliffs in the north. Storm petrels breed all around the island at low densities but concentrate at high densities in natural caves. Our study site was located in one of those caves, Cap des Migdia cave, that annually hosts around 250–300 storm petrel breeding pairs. The cave has two areas with different characteristics (Fig. 2): the entrance and the inner chambers. The entrance of the cave is a wide and illuminated area where nests are located in small cavities and rock debris. The inner chambers are narrow, dark and humid; in this area, nests are located in
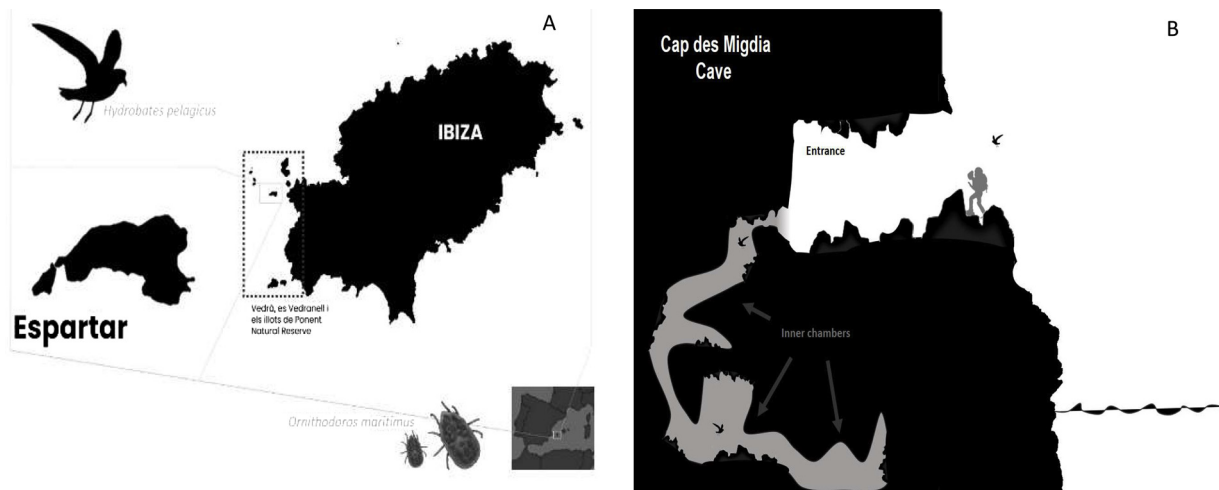
Fig. 2. A) Map of the study area and the studied species; B) Infographic of Cap des Migdia Cave study areas: Entrance and inner chambers. Representation is informative but not topologically accurate.

small cavities, rock debris and dusty soil (Fig. 1A). The inner chamber of the cave also hosts 12 pairs of breeding Balearic shearwaters.

From 2014 to 2018, all accessible nests found in the study site (N = 312) were marked (Fig. 2A) and inspected every 2 weeks along the breeding season (May-September) to record reproductive parameters: hatching success (No.$_{eggs\ hatched}$/No.$_{eggs\ laid}$), fledgling success (No.$_{chicks\ fledged}$/No.$_{eggs\ hatched}$) and breeding success (No.$_{chicks\ fledged}$/No.$_{eggs\ laid}$) (Mínguez et al., 2015). In addition, during the 2018 breeding season, we inspected for parasites some adults and all the nestlings located alive and alone in accessible nests at each monitoring visit. Given the extended breeding period of storm petrels and the mortality of some of the young nestlings inspected (N = 6), some nestlings were inspected once and others multiple times. In total we performed 62 inspections of 23 nestlings reared at the entrance of the cave and 92 inspections of 37 nestlings reared at the inner chambers. 28 breeding adults nesting at the entrance and 24 nesting at the inner chambers were inspected once during the breeding season. We did not capture the nestlings during the brooding period (i.e. when adults are covering the nestling) to avoid potential negative manipulation effects during this critical stage (Mínguez and Oro, 2003). We visually inspected nestlings and adults pushing aside the down and feathers and counted the number of tick larvae attached to their skin (Fig. 1C). However, in order to avoid disturbance of breeding birds, inspections of adults were faster and less exhaustive than inspections to nestlings. We measured their wing length, tarsus and body mass using a ruler, a digital calliper and a digital balance. Handling time was reduced to the minimum possible.

Assessing tick density in the cave is extremely challenging. *O. maritimus* ticks (Fig. 1B) are mainly nocturnal, feeding on the host rapidly at night in the nymphal and adult life stages (Vial, 2009). During the day, they are buried in the ground and located in small crevices and under stones (Vial, 2009). On the contrary, tick larvae can be attached for a much longer time on their hosts, their blood meal lasting from hours to several days (Vial, 2009). Therefore, in order to evaluate the potential differences in tick density on the different areas of the cave (i.e. entrance vs. inner chambers) we used the data on the larvae tick load (i.e. the number of tick larvae attached to the skin) of storm petrel nestlings (Fig. 2C) as a proxy of the amount of ticks on the two areas of the cave. At the end of the 2017 breeding season, ticks were searched on the ground of the cave, under stones and in crevices, and collected for molecular analyses. Ticks found together (i.e. under the same stone) were pooled. Until analysed, they were stored at −70 °C. Note that adult ticks were only found in the inner parts of the cave (i.e. the chambers) although the entrance was also inspected.

2.2. Statistical analyses

First, we evaluated the effect of the area of the cave on the parasite load (i.e. no. of attached tick larvae) experienced by the nestlings at each monitoring visit conducted in 2018. Based on field observations, we also accounted for nestling age as a predictor of parasite load. Nestling age was determined at first capture using the relationship between age and wing length (Age = (0.424*wing length) + 6.6102; R^2 = 0.95; Sanz-Aguilar, unpublished data) estimated at Benidorm Island (Spain), where an intense monitoring in 2015 allowed to establish the exact hatching date for 30 nestlings. This analysis was carried out using a generalized linear mixed model GLMM with a Poisson distribution (log link function) and considering nestling identity as a random effect.

Nestling and adult body condition was estimated individually using the residuals from a least squares (OLS) linear regression of body mass against the measure of wing (Owen and Cook, 1977). The residual indices were used as a proxy of body condition: the individuals with positive residual values were considered in better body condition than individuals with negative values (Jakob et al., 1996). We assessed the association between the nestling body condition and the nesting area (entrance vs. inner chambers) and the individual tick load (on a log scale). For tick load, we considered either the actual tick load (No. of tick larvae) presented by each individual at each monitoring or the maximum tick load, the maximum number of tick larvae recorded for a given individual during any of the monitoring visits carried out during the study period. As nestlings remain in the same nest during their development, this last fixed individual covariate may be related with the tick load in the nest and potentially experienced by the individual. This analysis was carried out using a GLMM with a normal distribution (identity link function) and considering nestling identity as a random effect. Adults were only measured once during the study period and no tick larvae was found attached to them. Consequently, a GLM with a normal distribution (identity link function) was used to analyse the potential differences in their body condition between the entrance and inner chambers breeding areas.

Finally, we evaluated the potential effect of the year and area of the cave on reproductive parameters: hatching success, fledgling success and breeding success. This analysis was carried out using a GLMM with a binomial distribution (logit link function) and considering nest identity as a random effect.

Model selection was performed using the Akaike's information criterion (AIC; Burnham and Anderson, 2002). We considered models to be equivalent when the difference in AIC with the best model (ΔAIC) was < 2 (Burnham and Anderson, 2002).

Table 1

Prevalence of *O. maritimus* larvae on storm petrel nestlings during the monitoring period at the entrance and the inner part of the colony (sample size in parentheses).

	03/07/19	12/07/19	19/07/19	02/08/19	23/08/19
Entrance	100% (3)	57% (7)	28% (18)	5% (21)	0% (13)
Inner chambers	100% (6)	78% (9)	89% (19)	52% (33)	8% (25)

2.3. RNA and genomic DNA extraction from the ticks

After collection and storage, ticks were identified and used for DNA/RNA extractions. The extraction protocols used on the ticks followed those outlined in Michelet et al. (2014) and Gondard et al. (2018). All ticks were washed for 5 min in an ethanol bath, 10 min in two successive water baths and placed individually in sterile tube and crushed in 300 μl of Dulbecco's modified eagle medium (DMEM) with 10% fetal calf serum using Precellys®24 Dual homogenizer (Bertin, France). The supernatant was divided in 3 fractions: 100 μl for the DNA extraction, 100 μl for the RNA extraction and the rest was used as back-up and conserved at −80 °C. Genomic tick DNA was then extracted using the Wizard genomic DNA purification kit (Promega, France) according to the manufacturer's instruction. Purified DNA and RNA were eluted into 50 μl of rehydration solution and 50 μl of RNase-free water, respectively, and conserved at −80 °C. Tick DNA and RNA quality was assessed via the amplification of the ITS2 region and COI gene respectively (Michelet et al., 2014; Gondard et al., 2018).

2.4. High throughput real-time PCR system

Ticks were analysed for the most common tick-borne infectious agents using the BioMark real-time PCR amplification system. The DNA primer chip developed by Michelet et al. (2014) includes primers for detecting 28 bacterial species, 12 parasite species and the RNA primer chip developed by Gondard et al. (2018) includes 21 viruses. Twenty-two ticks pooled in eleven pools of a maximum of five adult ticks and were analysed by both systems in duplicate.

All RNAs were reverse transcribed into cDNAs using random primers and oligos (dT). The remaining methods followed those of Michelet et al. (2014) and Gondard et al. (2018). DNA and cDNA pre-amplifications were performed using the TaqMan PreAmp Master Mix (Applied Biosystems, France) according to the manufacturer's instructions. Primers for bacteria or viruses were pooled by combining equal volumes of each primer to have 200 nM of each. The pre-amplification was performed in a final volume of 5 μL containing 2.5 μL TaqMan PreAmp Master Mix (2x), 1.2 μL pooled primer mix (0.2) and 1.3 mL DNA. Thermal cycling conditions were as follows: one cycle at 95 °C for 10 min, 14 cycles at 95 °C for 15 s and 4 min at 60 °C (Michelet et al., 2014; Gondard et al., 2018).

The quantitative PCR reactions were then performed using 6-carboxyfluorescein (FAM) and black hole quencher (BHQ1)-labeled TaqMan probes (Michelet et al., 2014; Gondard et al., 2018) with TaqMan Gene expression Master Mix, in accordance to the manufacturer's instructions (Applied Biosystem, France). PCR cycling comprised 5 min at 95 °C, 45 cycles at 95 °C for 10 s, 15 s at 60 °C and 10 s at 40 °C. Data were acquired on the BioMark real-time PCR system and analyzed using the Fluigdim real-time PCR Analysis software. The assays were performed in duplicate using two negative water controls per chip and *Escherichia coli* strain EDL933 was added in each run to control for internal inhibition (Michelet et al., 2014).

A nested PCR using primers for the detected bacteria (targeting *Borrelia* gene *fla*; Loh et al., 2016) and a real-time PCR using primers for the detected virus (targeting the NS5 region from all flavivirus; (Weissenböck et al., 2002) were used to confirm the presence of the detected infectious agent in the samples. Amplicons were sequenced by

Eurofins MWG Operon (Germany) and assembled using the BioEdit software (Ibis Biosciences, Carlsbad). An online BLAST (National Center for Biotechnology Information) was used to identify the sequenced organism.

3. Results

3.1. Tick infestation

We did not find tick larvae attached to adult birds. In the case of nestlings, we observed that tick larvae were mainly attached to the skin under the wings, the head and the neck, which are the areas with lower density of down and feathers (own observation). 80% of the nestling inspected at the inner chambers (N = 37) and 43% of the nestling inspected at the entrance (N = 23) presented at least one tick larvae during the study period. The percentage of nestlings infested by at least one tick larvae showed temporal variations and decreased at the end of the study period, especially at the entrance (Table 1).

Tick larvae load (i.e. No. of tick larvae on storm petrel nestlings) was higher in the inner chambers (mean 4.18 ± 0.69 SE) of the cave than at the entrance (mean 1.13 ± 0.85 SE), and decreased as nestlings became older (Table 2, Fig. 3). The age-related decrease in tick load was more rapid at the entrance of the cave (Fig. 3). Six of the nestlings inspected died (one at the entrance and 5 at the inner chambers). Nestlings found dead presented high numbers of tick larvae on them at the last alive inspection (mean = 20.8; range 2–40). We documented the mortality of 10% of the nestlings detected as infested by ticks at the entrance (No.$_{dead}$/No.$_{infested}$ = 1/10) and the mortality of 17% of the nestlings infested by ticks in the inner chambers (No.$_{dead}$/No.$_{infested}$ = 5/30).

3.2. Body condition

Adults showed no differences in body condition at the entrance and the inner chambers (Table 3). For nestlings, differences in body condition were mainly related with individual tick load (either the actual or the maximum tick load) and not with the effect the zone *per se* (Table 2). The best model indicated that nestlings with the highest tick larvae recorded during any of the monitoring visits (i.e. the maximum tick load) experienced lower body condition (Table 2, Fig. 4).

3.3. Reproductive parameters of storm petrels

Individuals breeding in the entrance of the cave showed higher mean performances in breeding parameters, especially regarding the fledging success (Table 4; HS$_{entrance}$ = 0.771 ± 0.025 SE; FS$_{entrance}$ = 0.903 ± 0.021 SE; BS$_{entrance}$ = 0.682 ± 0.028 SE) than individuals breeding in the inner chambers (HS$_{chambers}$ = 0.646 ± 0.018 SE; FS$_{chambers}$ = 0.516 ± 0.025 SE; BS$_{chambers}$ = 0.320 ± 0.018 SE). Hatching success and fledgling success varied over time and between zones, the additive models being preferred over the models with interactions (Table 4). The differences between zones were higher for fledgling success (i.e. nestling survival)

Table 2

Model selection of the effects of zone and age on tick *O. maritimus* load of storm petrel nestlings in Cap des Migdia Cave. df, degrees of freedom; Dev, Deviance; ΔAIC, Akaike's information criterion difference with the best model; "+", additive effect.

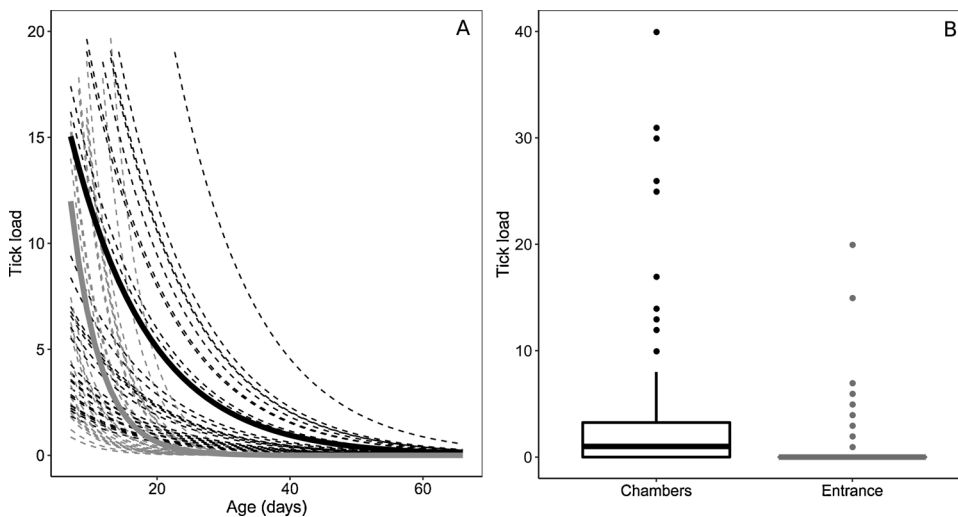| Effect	df	Dev	ΔAIC
Chick age*Zone	5	613.204	0
Chick age+Zone	4	634.002	18.797
Chick age	3	648.081	30.877
Zone	3	927.631	310.426
Null model	2	939.427	320.222

Fig. 3. A) Relationship between tick *O. maritimus* larvae load and storm petrel nestlings age. Dotted lines are the individuals' predictions and solid lines the mean prediction, Table 2, with grey and black lines representing the entrance and the inner chambers of the colony respectively. B) Boxplot showing the distribution of the number of tick *O. maritimus* larvae per storm petrel nestlings at the entrance and the inner chambers of the colony.

Table 3

Model selection of the effects of the zone, the individual tick *O. maritimus* larvae load and the maximum tick larvae load recorded for each individual during the monitoring period on body condition index of storm petrel nestlings and adults. Note that for adults, only the zone is tested as no tick larvae were found attached to them. Notation as in Table 1.

Group	Effect	df	Dev	ΔAIC
Nestlings	Log(Maximum No. ticks +1)	3	−34.232	0
Nestlings	Log(Maximum No. ticks +1)+Zone	4	−34.246	1.986
Nestlings	Log(No.ticks +1)*Zone	5	−35.808	2.424
Nestlings	Log(No.ticks +1)	3	−31.538	2.694
Nestlings	Log(Maximum No. ticks +1)*Zone	5	−34.268	3.964
Nestlings	Null model	2	−28.061	4.172
Nestlings	Log(No.ticks +1)+Zone	4	−31.779	4.454
Nestlings	Zone	3	−29.136	5.096
Adults	Null model	1	0.446	0
Adults	Zone	2	0.440	1.627

Table 4

Model selection of the effects of year and zone on the reproductive parameters: Hatching success (HS), fledgling success (FS) and breeding success (BS) of storm petrels breeding in Cap des Migdia Cave. Notation as in Table 1.

Parameter	Effect	df	Dev	ΔAIC
HS	Year + Zone	7	1180.305	0
HS	Year*Zone	11	1178.445	6.141
HS	Zone	3	1197.818	9.513
HS	Year	6	1194.097	11.793
HS	Null model	2	1210.125	19.820
FS	Year + Zone	7	661.359	0
FS	Year*Zone	11	654.552	1.193
FS	Zone	3	679.188	9.829
FS	Year	6	734.838	71.480
FS	Null model	2	756.804	85.446
BS	Zone	3	1130.221	0
BS	Year + Zone	7	1127.815	5.595
BS	Year*Zone	11	1120.786	6.565
BS	No effect (constant)	2	1203.073	70.852
BS	Year	6	1200.250	76.029

than for hatching success (Fig. 5). Breeding success showed the lowest temporal variation, the constant model being preferred over the temporal models (Table 4, Fig. 5).

3.4. Molecular analyses

The West Nile virus (WNV) was detected in duplicate on one tick pool over the 11 that were analysed with the design targeting the genotype 3 of this virus (known to be transmitted by ticks). Although the Pan-Flavivirus real-time PCR did not succeed to confirm this result, nor the attempt of viral isolation into mammalian cell culture, this may be expected if the concentration of the virus is low. This was the only virus detected among the 21 that were searched. Four pools of ticks tested positive for a *Borrelia* sp. which was determined by targeted nested PCR to have a 99% sequence identity with *B. turicatae*, a relapsing fever *Borrelia* (GenBank accession number: MK732470).

4. Discussion

In this study, we show spatially explicit differences of infestation by the soft tick *O. maritimus* of nestling storm petrels in a large cave on Espartar Island. Tick infestation was higher in the cave chambers and less so in the entrance. The within-colony differences in parasite density may be related to different and not exclusive factors as the presence of Balearic shearwaters breeding in the inner parts of the cave and the *per*
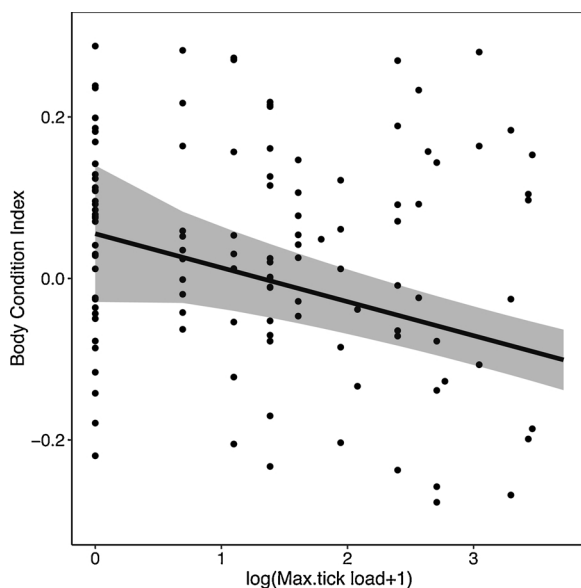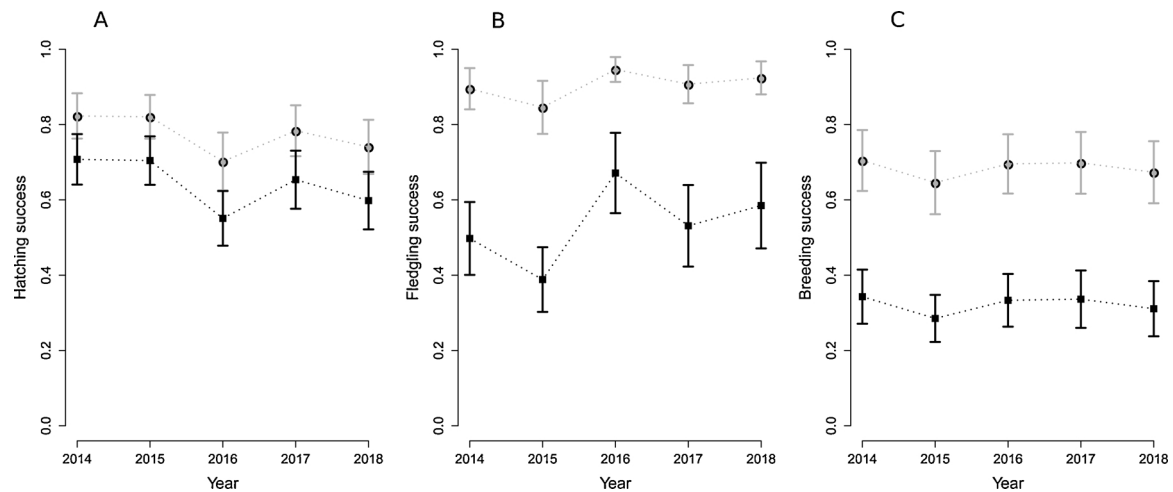

Fig. 4. A) Relationship between body condition index of storm petrel nestlings and the maximum tick *O. maritimus* load (log scale) detected for each individual (Table 3), the black line represent the estimate of the model and the grey area the SE. Points indicate the individual predictions.

Fig. 5. A) Annual mean (and 95% CI) of hatching success (HS), B) fledgling success (FS) and C) breeding success (BS) of storm petrels breeding in the entrance (grey) and the inner chambers (black) of Cap des Migdia Cave between 2014 and 2018. Estimates from the model including the additive effects of zone and year (Table 4).

se characteristics of the cave (Dietrich et al., 2011 and references therein). First, tick population dynamic parameters (notably survival) are known to depend on local climatic conditions such as temperature and humidity (Sonenshine and Roe, 2013). The studied colony is located inside a cave where environmental conditions (especially deep in the cave) are expected to be quite stable. Moreover, humidity is higher at the inner chambers than at the entrance (own observation). These factors can facilitate oviposition and tick survival, especially in the inner chambers (Vial, 2009. Second, although parasites and infectious agents responsible for diseases are often strongly host-specific, *Ornithodoros* spp. show indiscriminate host feeding and short time for feeding completion compared to hard ticks (Vial, 2009). Balearic shearwaters start to visit the colonies in November and the last chicks fledge in June (Guilford et al., 2012). Storm petrels start to visit the colonies in March and the last chicks fledge in late September (Ramírez et al., 2016). Moreover, storm petrels show asynchronous breeding: the first eggs are laid at the end of April and the last at the beginning of July (Mínguez, 1994). Reproductive asynchrony of storm petrels may promote the reproduction of ticks (Møller et al., 1993). Consequently, hosts can be available in the cave during 11 months per year and seabird nestlings during 4–5 months, favouring the production of several tick generations per year and high local population densities (Vial, 2009).

Nestling body condition was negatively correlated with tick load, which may explain the higher nestling mortality in the inner parts of the colony. Our results agree with other studies on seabirds that have detected high nest desertion and/or nestling mortalities of individuals infested by ticks or in areas with high tick densities (e.g. Feare, 1976; Duffy, 1983; Ramos et al., 2001; Reeves et al., 2006; Deschamps, 2013). In addition, nestling mortality in the inner parts of the cave, where tick prevalence was higher and ticks were more abundant, was very high (48%). We cannot directly analyse the effect of tick infestation on nestling survival due to our reduced sample size of nestlings inspected before dying (i.e. for which we had counted the number of tick larvae attached to them when alive). This is because the majority of the mortalities occurred during the first two weeks after hatching and many nestlings were born and died between our monitoring visits (own observation). However, the low survival of storm petrel nestlings located in the inner parts of the Espartar's cave matches with the high mortality estimates provided for other seabird species nestlings infested by ticks (Feare, 1996; Ramos et al., 2001). For example, Ramos et al. (2001) documented a mortality of 63% of roseate terns (*Sterna dougallii*) infested by hard ticks *Amblyomma loculosum* versus a nestling mortality of 17% of non-infested nestlings during a harsh breeding season with food shortage. On the contrary, during a breeding season with benign environmental conditions, only 24% of the tick-infested nestlings died

(Ramos et al., 2001).

In fact, environmental conditions may influence the effect of parasites on hosts (Ramos et al., 2001; McCoy et al., 2002). At Espartar's colony, during our 5 years of monitoring the fledgling success showed annual variations that may be related with differential tick densities or environmental conditions affecting storm petrels and or interacting with their parasites (McCoy et al., 2002), factors that should be studied in the future. However, mortality in the inner chambers of the cave was always very high. On the contrary, at the entrance of the cave, nestling mortality showed the usual low levels (~10%) estimated at other storm petrel colonies (Sanz-Aguilar et al., 2009) where tick prevalence is lower (Merino et al., 1999). For example, in Benidorm Island only one of the 34 storm petrel nestlings inspected by Merino et al. (1999) in 1996 presented a single *Ornithodoros* spp. tick larvae. Additionally, a tick larvae was detected only on 4 out of 27 storm petrel nestlings inspected at Benidorm Island on 25th June 2019 (tick larvae prevalence of 15%; own unpublished data). Monitoring of tick infestation could be conducted to explore potential increase over time, such as that reported as a function of warmer winter temperature in an arctic population of Brünnich's guillemots (*Uria lomvia*) (Deschamps, 2013). Prevalence of ticks infesting seabirds has been shown to be very variable among seabird species, breeding colonies and/or years ranging 0% and > 70% (Feare, 1974; Merino et al., 1999; Ramos et al., 2001; Deschamps, 2013; Hipfner et al., 2019). However, the effects of ticks on hosts depend also on the tick load, the host species, the age of the host (e.g. the effects can be different for nestlings and adults), and/or the environmental conditions, as explained above (Feare, 1974; Merino et al., 1999; Ramos et al., 2001; McCoy et al., 2002; Deschamps, 2013; Hipfner et al., 2019). For example, Feare (1976) documented a 67% of tick prevalence on Sooty terns and high mortality effects, while Hipfner et al. (2019) documented tick prevalence > 60% for two species of auklet nestlings without relevant survival effects.

Although we cannot determine the ultimate cause of nestlings' death, there are several non-exclusive possibilities (Dietrich et al., 2011; Yabsley et al., 2012). First, anaemia caused by blood loss could be lethal for young nestlings infested by high numbers of ticks (Wanless et al., 1997). In fact, several studies have evidenced negative effects of ticks on nestlings' body condition, at least during harsh environmental conditions or high parasite loads (Morbey, 1996; Bosch and Figuerola, 1999; McCoy et al., 2002; Hipfner et al., 2019). Given their very small body size (< 10 g when hatching, Davis, 1957), storm petrel nestlings could be more susceptible to ectoparasites as ticks than larger seabird species (Merino et al., 1999). In fact, no mortality was recorded for nestlings of Balearic shearwaters (adult weight ~500 g) at the study site during the study period. In this vein, Hipfner et al. (2019) did not find

negative effects of tick infestation on survival or nestlings growth of two medium size seabird species (adult weight 150–500 g), *Cerorhinca monocerata* and *Ptychoramphus aleuticus*, at normal tick *I. uriae* loads; but they found negative effects at very high tick loads, in accordance with other evidences in other seabird species (Deschamps, 2013). In agreement with our results, they also documented a reduction in tick load with nestling age, being essentially zero at fledging (Hipfner et al., 2019). However, contrary to hard *Ixodes* ticks, soft *Ornithodoros* nymph and adult ticks are only attached to their hosts for very short blood meals, at night (Vial, 2009), making it difficult to establish the actual tick load experienced by the hosts. We could only count the larvae ticks feeding on nestlings during the monitoring visits, while adults and nymphs were likely also feeding on both adult and chick storm petrels. However, adults breeding in both areas showed similar body condition, which suggest that ticks may have higher negative effects on nestlings than on adults.

Second, the low survival of nestlings in the inner part of the cave may be a consequence of a massive adult abandonment of young nestlings as a result of high parasite load, as shown in other species (Feare, 1976; King et al., 1977; Duffy, 1983; Reeves et al., 2006). However, we think that this possibility is highly improbable. Some field observations using camera trapping indicates that the adults came back to feed the chicks when they were already dead. Moreover, neighbours of already dead chicks continued incubating or rearing their surviving chicks (Fig. 1A).

Finally, ticks may be hosting infectious agents pathogenic for their seabird hosts, at least for young nestlings. Our study shows that two tick-borne agents known for their pathogenic potential for vertebrates were detected among the 11 pools of ticks that were analysed. We detected RNA from WNV genotype 3, which suggests that the virus is circulating in the storm petrel population, possibly relatively independently from other cycles of that virus in the area. West Nile virus can cause high mortality in birds, particularly in naïve populations (George et al., 2015), and neurological disorders in horses and humans. Moreover, the virus could also cause sub-lethal unknown negative effects (Nemeth et al., 2006). The major vector species of WNV are mosquitoes, in particular ornithophilic species such as *Culex pipiens* (Calistri et al., 2010; Brugman et al., 2018). To date, little information is available on the mosquito species associated to nesting areas of storm petrels, as well as the possible role of soft ticks in the virus circulation. WNV genotype 3 have been previously isolated from *O. maritimus* (Hoogstraal et al., 1976) and proved to be transmitted in laboratory by other soft tick species, such as *O. moubata* and *O. capensis* (Lawrie et al., 2004; Hutcheson et al., 2005). Consequently, the *O. capensis* complex may act as a reservoir of this genotype of WNV (Lawrie et al., 2004) and deserve to be further investigated. Moreover, DNA from a *Borrelia* sp. very similar to relapsing fever *B. turicatae* was detected. It may appear surprising to detect DNA of such as bacteria in ticks from storm petrel because *B. turicatae* has been mostly reported in the southern USA and Latin America (Schwan et al., 2005). Nevertheless, relapsing fever *Borrelia* has also been detected in ticks from a storm petrel and shearwater colony in Japan (Takano et al., 2009), in African penguins (*Spheniscus demersus*) (Yabsley et al., 2012), in brown pelicans (*Pelecanus occidentalis*) (Reeves et al., 2006) and recently in ticks from yellow-legged gull (*Larus michahellis*) nests in Algeria (Lafri et al., 2017). The detection of those *Borrelia* with a very similar sequence to *B. turicatae* thus suggests that further investigations are required to understand how widespread are relapsing fever *Borrelia* in seabird ticks (Kim et al., 2016). In addition, considering the lack of host specificity of *Ornithodoros* spp. and the pathogenic potential of relapsing fever *Borrelia* for other vertebrates, including humans, the question of the extent of the circulation of such bacteria arises. Given the growing interest in relapsing fever *Borrelia* in America and the biomedical tools that may become available to detect the past exposure of hosts by tracking antibody levels (Lopez et al., 2013), investigations could rely on the detection of the *Borrelia* in ticks, but also sometime more efficiently by

detecting antibodies in the vertebrate host (Armstrong et al., 2018).

5. Conclusions

In conclusion, our study provides evidence that *O. maritimus* may directly or indirectly be the responsible of the extremely high mortality of storm petrel nestlings observed at the study site. Our results open exciting questions that need to be addressed for a better understanding of the ecology and epidemiology of the interactions between soft ticks and breeding Procellariiform species. Is the case of Espartar unique among storm petrel colonies? What is the ultimate cause of nestling mortality? Will the colony persist under such high levels of parasite infestation and nestling mortality? Is it possible to detect any sub-lethal effects of ticks or pathogens on adults? How do tick population dynamics work? Are the tick-borne virus and bacteria circulating in the storm petrel population relatively independently from other vertebrate host populations? Understanding how these host–parasite systems function in space and time will require further challenging but potentially rewarding multidisciplinary collaborations (taxonomy, ecology, and epidemiological studies).

Data accessibility

Data will be available via the Spanish National Research Council data repository. Sanz-Aguilar, A., Payo-Payo, A., Igual, J. M., Rotger, A., Viñas Torres, M., Picorelli, V., 2019. Storm petrel data sets. https://doi.org/10.20350/digitalCSIC/.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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