

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

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25 **Abstract:**

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

45

46 **Keywords:** *Borrelia*, *Ornithodoros*, mortality, storm petrels, soft ticks, tick-borne  
47 pathogens, WNV.

48 **Introduction**

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

55           Ticks are divided into two groups: hard (Ixodidae) and soft (Argasidae). Both  
56 families can potentially transmit numerous pathogens of medical and veterinary  
57 interest (Dietrich et al., 2011 and references herein). However, those transmitted by  
58 soft ticks have been generally less studied due to the specialization of Argasidae to  
59 hidden habitats (i.e. crevices) and the short time they spend for blood feeding on the  
60 host compared to hard ticks (Vial, 2009). Some of the most common worldwide  
61 pathogens transmitted by soft ticks include: human tick-borne relapsing fever (TBRF);  
62 viral encephalitis transmitted by ticks infesting seabirds, shore birds and roosting birds;  
63 the African swine fever virus (ASFV); fowl spirochetosis or anaplasmosis-like infections  
64 and epizootic bovine abortion (Vial, 2009). Such pathogens can induce both lethal and  
65 sub-lethal effects on their hosts (Niebylski et al., 1999; Gray and Bradley, 2006).

66           Organisms present a wide range of responses to tick induced pathogens (Ramamoorthi  
67 et al., 2005). For instance, transmitted pathogens infecting the host might induce  
68 behavioral and/or physiological modifications which interfere with immunity response,  
69 gene expression or energy allocation, thus potentially affecting fitness. However, the  
70 effects of tick-borne infections on host populations are often poorly understood and

71 quantifications of sub-lethal effects are hard to assess (Nemeth et al., 2006; Dietrich et  
72 al., 2011).

73           Seabirds can be predictable hosts for ticks because they show colonial breeding  
74 habits resulting in large and dense aggregations, high nest site fidelity and extended  
75 breeding periods (Ramos et al., 2001; Schreiber and Burger, 2001). These breeding  
76 characteristics of seabirds facilitate transmission and contribute to the support of large  
77 and dense populations of ticks (Dietrich et al., 2011 and references herein). Severe  
78 infestations of seabird colonies by ticks can have heavy negative effects (Duffy, 1983;  
79 Ramos et al., 2001; Dietrich et al., 2011). For example, Duffy (1983) documented a  
80 large-scale nest desertion in Peruvian guano seabirds (cormorants, boobies and  
81 pelicans) due to *Ornithodoros amblus* soft tick infestation at breeding sites. Similarly,  
82 high densities of *O. capensis* s.s. seemed to be responsible for Sooty tern (*Sterna*  
83 *fuscata*) egg and newly hatched chick desertion at Seychelles in 1972 (Feare, 1976).  
84 Colony abandonment or dispersal could be used as potential strategies to avoid the  
85 negative effects of parasites (Boulinier and Danchin, 1996). In fact, some cases of lethal  
86 effects of ticks (i.e. *Ixodes uriae*) on seabirds have been documented, especially for  
87 young nestlings (Boulinier and Danchin, 1996; Ramos et al., 2001) but very rarely in  
88 adults (Gauthier-Clerc et al., 1998). On the other hand, non-lethal but also negative  
89 effects have been documented on seabird body condition and/or nestlings' growth  
90 rates (e.g. Bosch and Figuerola, 1999; McCoy et al., 2002; Hipfner et al., 2019).  
91 However, sometimes the effects of ticks are not evident until infestation levels are  
92 very high (Gauthier-Clerc et al., 2003; Hipfner et al., 2019) or may depend on the  
93 environmental conditions experienced by the hosts (McCoy et al., 2002). Although  
94 ticks can have an important role in seabird population dynamics, specific studies on

95 the demographic effects of tick infestation on seabirds are still scarce (Dietrich et al.,  
96 2011; Rodríguez et al., 2019). Moreover, the ultimate mechanisms responsible for  
97 lethal and sub-lethal effects of ticks (i.e. direct effect or indirect effect due to pathogen  
98 transmission) on seabirds remain largely unknown (Yabsley et al., 2012).

99         Espartar Island, located on the Balearic archipelago, hosts the largest Spanish  
100 colony of Mediterranean storm petrel (*Hydrobates pelagicus melitensis*), estimated at  
101 750-1250 breeding pairs (Picorelli, unpublished data). Other seabirds (gulls and  
102 shearwaters) also breed in the island, but storm petrels mainly concentrate in cliff  
103 caves, sharing their breeding habitat with Balearic shearwaters (*Puffinus*  
104 *mauretanicus*), this last species breeding in lower densities and starting reproduction  
105 much earlier (i.e. February; Guilford et al., 2012) than storm petrels (i.e. May-June;  
106 Ramírez et al., 2016). A systematic monitoring of the breeding parameters of storm  
107 petrels breeding at the largest colony of the island, the cap de Migdia cave (~250  
108 breeding pairs) started in 2014 (Mínguez et al., 2015). During the first years of  
109 monitoring (2014-2016), we detected a high mortality of nestlings (see results)  
110 compared to other storm petrel Mediterranean colonies in which nestling survival  
111 typically varies between 90-95% (Sanz-Aguilar et al., 2009). Moreover, we experienced  
112 recurrent tick bites during the daylight monitoring of the cave, a very unusual fact  
113 when compared with our experience monitoring other storm petrel colonies. Ticks  
114 were identified as the soft tick *O. maritimus* (Vermeil and Marguet, 1967), included in  
115 the complex *O. (Carios) capensis* that includes eight described species that parasitize  
116 tropical and temperate colonial seabird species (Hoogstraal et al., 1979; Khouri, 2011;  
117 Dupraz et al., 2016). Identification of *O. maritimus* was performed by morphology (i.e.  
118 median dorsal line of mamillae disc short) and confirmed by an expert on the group

119 (Dupraz, M., personal communication). In addition, based on Dupraz et al., (2016) *O.*  
120 *maritimus* is mostly restricted to the West Palearctic Region where its presence  
121 parasitizing seabirds have been extensively reported (Dietrich et al., 2011). The species  
122 *O. capensis* s.s is also present in the same region, but almost exclusively associated to  
123 pigeons and it can be morphologically separated from *O. maritimus* (Khoury et al.,  
124 2011).

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

## 134 **Methods**

### 135 *Species, study site, field data and tick collection*

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

143           The study was conducted on Espartar Island, a 20.5 ha island located within the  
144 Es Vedrà, Es Vedranell i els illots de Ponent Natural Reserve (38° 57' 31" N, 1° 11' 44" E,  
145 Fig. 2). Espartar Island has a characteristic steep relief with gentle slopes facing south  
146 and cliffs in the north. Storm petrels breed all around the island at low densities but  
147 concentrate at high densities in natural caves. Our study site was located in one of  
148 those caves, Cap des Migdia cave, that annually hosts around 250-300 storm petrel  
149 breeding pairs. The cave has two areas with different characteristics (Fig. 2): the  
150 entrance and the inner chambers. The entrance of the cave is a wide and illuminated  
151 area where nests are located in small cavities and rock debris. The inner chambers are  
152 narrow, dark and humid; in this area, nests are located in small cavities, rock debris  
153 and dusty soil (Fig. 1A). The inner chamber of the cave also hosts 12 pairs of breeding  
154 Balearic shearwaters.

155           From 2014 to 2018, all accessible nests found in the study site (N = 312) were  
156 marked (Fig. 2A) and inspected every 2 weeks along the breeding season (May-  
157 September) to record reproductive parameters: hatching success ( $\text{No.eggs hatched}/\text{No.eggs}$   
158  $\text{laid}$ ), fledgling success ( $\text{No.chicks fledged}/\text{No.eggs hatched}$ ) and breeding success ( $\text{No.chicks}$   
159  $\text{fledged}/\text{No.eggs laid}$ ) (Mínguez et al., 2015). In addition, during the 2018 breeding season,  
160 we inspected for parasites some adults and all the nestlings located alive and alone in  
161 accessible nests at each monitoring visit. Given the extended breeding period of storm  
162 petrels and the mortality of some of the young nestlings inspected (n = 6), some  
163 nestlings were inspected once and others multiple times. In total we performed 62  
164 inspections of 23 nestlings reared at the entrance of the cave and 92 inspections of 37  
165 nestlings reared at the inner chambers. 28 breeding adults nesting at the entrance and  
166 24 nesting at the inner chambers were inspected once during the breeding season. We

167 did not capture the nestlings during the brooding period (i.e. when adults are covering  
168 the nestling) to avoid potential negative manipulation effects during this critical stage  
169 (Mínguez and Oro, 2003). We visually inspected nestlings and adults pushing aside the  
170 down and feathers and counted the number of tick larvae attached to their skin (Fig.  
171 1C). However, in order to avoid disturbance of breeding birds, inspections of adults  
172 were faster and less exhaustive than inspections to nestlings. We measured their wing  
173 length, tarsus and body mass using a ruler, a digital calliper and a digital balance.  
174 Handling time was reduced to the minimum possible.

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

189 *Statistical analyses*



190 First, we evaluated the effect of the area of the cave on the parasite load (i.e.  
191 no. of attached tick larvae) experienced by the nestlings at each monitoring visit  
192 conducted in 2018. Based on field observations, we also accounted for nestling age as  
193 a predictor of parasite load. Nestling age was determined at first capture using the  
194 relationship between age and wing length ( $\text{Age} = (0.424 * \text{wing length}) + 6.6102$ ;  $R^2 =$   
195  $0.95$ ; Sanz-Aguilar, unpublished data) estimated at Benidorm Island (Spain), where an  
196 intense monitoring in 2015 allowed to establish the exact hatching date for 30  
197 nestlings. This analysis was carried out using a generalized linear mixed model GLMM  
198 with a Poisson distribution (log link function) and considering nestling identity as a  
199 random effect.

200 Nestling and adult body condition was estimated individually using the  
201 residuals from a least squares (OLS) linear regression of body mass against the  
202 measure of wing (Owen and Cook, 1977). The residual indices were used as a proxy of  
203 body condition: the individuals with positive residual values were considered in better  
204 body condition than individuals with negative values (Jakob et al., 1996). We assessed  
205 the association between the nestling body condition and the nesting area (entrance vs.  
206 inner chambers) and the individual tick load (on a log scale). For tick load, we  
207 considered either the actual tick load (No. of tick larvae) presented by each individual  
208 at each monitoring or the maximum tick load, the maximum number of tick larvae  
209 recorded for a given individual during any of the monitoring visits carried out during  
210 the study period. As nestlings remain in the same nest during their development, this  
211 last fixed individual covariate may be related with the tick load in the nest and  
212 potentially experienced by the individual. This analysis was carried out using a GLMM  
213 with a normal distribution (identity link function) and considering nestling identity as a

214 random effect. Adults were only measured once during the study period and no tick  
215 larvae was found attached to them. Consequently, a GLM with a normal distribution  
216 (identity link function) was used to analyse the potential differences in their body  
217 condition between the entrance and inner chambers breeding areas.

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

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

#### 225 *RNA and genomic DNA extraction from the ticks*

226 After collection and storage, ticks were identified and used for DNA/RNA  
227 extractions. The extraction protocols used on the ticks followed those outlined in  
228 Michelet et al. (2014) and Gondard et al. (2018). All ticks were washed for 5 min in an  
229 ethanol bath, 10 min in two successive water baths and placed individually in sterile  
230 tube and crushed in 300  $\mu$ l of Dulbecco's modified eagle medium (DMEM) with 10%  
231 fetal calf serum using Precellys®24 Dual homogenizer (Bertin, France). The supernatant  
232 was divided in 3 fractions: 100  $\mu$ l for the DNA extraction, 100  $\mu$ l for the RNA extraction  
233 and the rest was used as back-up and conserved at  $-80^{\circ}\text{C}$ . Genomic tick DNA was then  
234 extracted using the Wizard genomic DNA purification kit (Promega, France) according  
235 to the manufacturer's instruction. Purified DNA and RNA were eluted into 50  $\mu$ l of  
236 rehydration solution and 50  $\mu$ l of RNase-free water, respectively, and conserved at -

237 80°C. Tick DNA and RNA quality was assessed via the amplification of the ITS2 region  
238 and COI gene respectively (Michelet et al., 2014; Gondard et al., 2018).

239 *High throughput real-time PCR system*

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

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

256 The quantitative PCR reactions were then performed using 6-  
257 carboxyfluorescein (FAM) and black hole quencher (BHQ1)-labeled TaqMan probes  
258 (Michelet et al., 2014; Gondard et al., 2018) with TaqMan Gene expression Master  
259 Mix, in accordance to the manufacturer's instructions (Applied Biosystem, France). PCR  
260 cycling comprised 5 min at 95°C, 45 cycles at 95°C for 10 s, 15 sec at 60°C and 10 s at

261 40°C. Data were acquired on the BioMark Real-Time PCR system and analyzed using  
262 the Fluidigm Real-Time PCR Analysis software. The assays were performed in duplicate  
263 using two negative water controls per chip and *Escherichia coli* strain EDL933 was  
264 added in each run to control for internal inhibition (Michelet et al., 2014).

265 A nested PCR using primers for the detected bacteria (targeting *Borrelia* gene  
266 *fla*; Loh et al., 2016) and a Real-Time PCR using primers for the detected virus  
267 (targeting the NS5 region from all flavivirus; (Weissenböck et al., 2002) were used to  
268 confirm the presence of the detected infectious agent in the samples. Amplicons were  
269 sequenced by Eurofins MWG Operon (Germany) and assembled using the BioEdit  
270 software (Ibis Biosciences, Carlsbad). An online BLAST (National Center for  
271 Biotechnology Information) was used to identify the sequenced organism.

## 272 **Results**

### 273 *Tick infestation*

274 We did not find tick larvae attached to adult birds. In the case of nestlings, we  
275 observed that tick larvae were mainly attached to the skin under the wings, the head  
276 and the neck, which are the areas with lower density of down and feathers (own  
277 observation). 80.41% of the nestling inspected at the inner chambers (N=37) and  
278 43.48% of the nestling inspected at the entrance (N=23) presented at least one tick  
279 larvae during the study period. The percentage of nestlings infested by at least one tick  
280 larvae showed temporal variations and decreased at the end of the study period,  
281 especially at the entrance (Table 1).

282 Tick larvae load (i.e. No. of tick larvae on storm petrel nestlings) was higher in  
283 the inner chambers (mean  $4.18 \pm 0.69$  SE) of the cave than at the entrance (mean  $1.13$   
284  $\pm 0.85$  SE), and decreased as nestlings became older (Table 2, Fig. 3). The age-related

285 decrease in tick load was more rapid at the entrance of the cave (Fig. 3). Six of the  
286 nestlings inspected died (one at the entrance and 5 at the inner chambers). Nestlings  
287 found dead presented high numbers of tick larvae on them at the last alive inspection  
288 (mean = 20.8; range 2-40). We documented the mortality of 10% of the nestlings  
289 detected as infested by ticks at the entrance ( $\text{No.}_{\text{dead}}/\text{No.}_{\text{infested}} = 1/10$ ) and the  
290 mortality of 16.67% of the nestlings infested by ticks in the inner chambers  
291 ( $\text{No.}_{\text{dead}}/\text{No.}_{\text{infested}} = 5/30$ ).

### 292 *Body condition*

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

### 299 *Reproductive parameters of storm petrels*

300 Individuals breeding in the entrance of the cave showed higher mean  
301 performances in breeding parameters, especially regarding the fledging success (Table  
302 4;  $\text{HS}_{\text{entrance}} = 0.771 \pm 0.025 \text{ SE}$ ;  $\text{FS}_{\text{entrance}} = 0.903 \pm 0.021 \text{ SE}$ ;  $\text{BS}_{\text{entrance}} = 0.682 \pm 0.028 \text{ SE}$ )  
303 than individuals breeding in the inner chambers ( $\text{HS}_{\text{chambers}} = 0.646 \pm 0.018 \text{ SE}$ ;  $\text{FS}_{\text{chambers}}$   
304  $= 0.516 \pm 0.025 \text{ SE}$ ;  $\text{BS}_{\text{chambers}} = 0.320 \pm 0.018 \text{ SE}$ ). Hatching success and fledgling  
305 success varied over time and between zones, the additive models being preferred over  
306 the models with interactions (Table 4). The differences between zones were higher for  
307 fledgling success (i.e. nestling survival) than for hatching success (Fig. 5). Breeding

308 success showed the lowest temporal variation, the constant model being preferred  
309 over the temporal models (Table 4, Fig. 5).

### 310 *Molecular analyses*

311 The West Nile virus (WNV) was detected in duplicate on one tick pool over the  
312 11 that were analysed with the design targeting the genotype 3 of this virus (known to  
313 be transmitted by ticks). Although the Pan-Flavivirus Real-Time PCR did not succeed to  
314 confirm this result, nor the attempt of viral isolation into mammalian cell culture, this  
315 may be expected if the concentration of the virus is low. This was the only virus  
316 detected among the 21 that were searched. Four pools of ticks tested positive for a  
317 *Borrelia* sp. which was determined by targeted nested PCR to have a 99% sequence  
318 identity with *B. turicatae*, a relapsing fever *Borrelia* (GenBank accession number:  
319 MK732470).

### 320 **Discussion**

321 In this study, we show spatially explicit differences of infestation by the soft tick  
322 *O. maritimus* of nestling storm petrels in a large cave on Espartar island. Tick  
323 infestation was higher in the cave chambers and less so in the entrance. The within-  
324 colony differences in parasite density may be related to different and not exclusive  
325 factors as the presence of Balearic shearwaters breeding in the inner parts of the cave  
326 and the *per se* characteristics of the cave (Dietrich et al., 2011 and references herein).  
327 First, tick population dynamic parameters (notably survival) are known to depend on  
328 local climatic conditions such as temperature and humidity (Sonenshine and Roe,  
329 2013). The studied colony is located inside a cave where environmental conditions  
330 (especially deep in the cave) are expected to be quite stable. Moreover, humidity is  
331 higher at the inner chambers than at the entrance (own observation). These factors

332 can facilitate oviposition and tick survival, especially in the inner chambers (Vial, 2009).  
333 Second, although parasites and infectious agents responsible for diseases are often  
334 strongly host-specific, *Ornithodoros* spp. soft ticks show indiscriminate host feeding  
335 and short time for feeding completion compared to hard ticks (Vial, 2009). Balearic  
336 shearwaters start to visit the colonies in November and the last chicks fledge in June  
337 (Guilford et al., 2012). Storm petrels start to visit the colonies in March and the last  
338 chicks fledge in late September (Ramírez et al., 2016). Moreover, storm petrels show  
339 asynchronous breeding: the first eggs are laid at the end of April and the last at the  
340 beginning of July (Mínguez, 1994). Reproductive asynchrony of storm petrels may  
341 promote the reproduction of ticks (Møller et al., 1993). Consequently, hosts can be  
342 available in the cave during 11 months per year and seabird nestlings during 4-5  
343 months, favouring the production of several tick generations per year and high local  
344 population densities (Vial, 2009).

345 Nestling body condition was negatively correlated with tick load, which may  
346 explain the higher nestling mortality in the inner parts of the colony. Our results agree  
347 with other studies on seabirds that have detected high nest desertion and/or nestling  
348 mortalities of individuals infested by ticks or in areas with high tick densities (e.g.  
349 Feare, 1976; Duffy, 1983; Ramos et al., 2001; Reeves et al., 2006; Descamps 2013). In  
350 addition, nestling mortality in the inner parts of the cave, where tick prevalence was  
351 higher and ticks were more abundant, was very high (48.4 %). We cannot directly  
352 analyse the effect of tick infestation on nestling survival due to our reduced sample  
353 size of nestlings inspected before dying (i.e. for which we had counted the number of  
354 tick larvae attached to them when alive). This is because the majority of the mortalities  
355 occurred during the first two weeks after hatching and many nestlings were born and

356 died between our monitoring visits (own observation). However, the low survival of  
357 storm petrel nestlings located in the inner parts of the Espartar's cave matches with  
358 the high mortality estimates provided for other seabird species nestlings infested by  
359 ticks (Feare, 1996; Ramos et al., 2001). For example, Ramos et al. (2001) documented a  
360 mortality of 62.5 % of roseate terns (*Sterna dougallii*) infested by hard ticks  
361 *Amblyomma loculosum* versus a nestling mortality of 16.7 % of non-infested nestlings  
362 during a harsh breeding season with food shortage. On the contrary, during a breeding  
363 season with benign environmental conditions, only 24.3 % of the tick-infested nestlings  
364 died (Ramos et al., 2001).

365 In fact, environmental conditions may influence the effect of parasites on hosts  
366 (Ramos et al., 2001; McCoy et al., 2002). At Espartar's colony, during our 5 years of  
367 monitoring the fledgling success showed annual variations that may be related with  
368 differential tick densities or environmental conditions affecting storm petrels and or  
369 interacting with their parasites (McCoy et al., 2002), factors that should be studied in  
370 the future. However, mortality in the inner chambers of the cave was always very high.  
371 On the contrary, at the entrance of the cave, nestling mortality showed the usual low  
372 levels (~0.1) estimated at other storm petrel colonies (Sanz-Aguilar et al., 2009) where  
373 tick prevalence is lower (Merino et al., 1999). For example, in Benidorm Island only one  
374 of the 34 storm petrel nestlings inspected by Merino et al. (1999) in 1996 presented a  
375 single *Ornithodoros* spp. tick larvae. Additionally, a tick larvae was detected only on 4  
376 out of 27 storm petrel nestlings inspected at Benidorm Island on 25<sup>th</sup> June 2019 (tick  
377 larvae prevalence of 14.81%; own unpublished data). Monitoring of tick infestation  
378 could be conducted to explore potential increase over time, such as that reported as a  
379 function of warmer winter temperature in an arctic population of Brünnich's



380 guillemots (*Uria lomvia*) (Descamps, 2013). Prevalence of ticks infesting seabirds has  
381 been shown to be very variable among seabird species, breeding colonies and/or years  
382 ranging 0 % and > 70 % (Feare, 1974; Merino et al., 1999; Ramos et al., 2001;  
383 Descamps, 2013; Hipfner et al., 2019). However, the effects of ticks on hosts depend  
384 also on the tick load, the host species, the age of the host (e.g. the effects can be  
385 different for nestlings and adults), and/or the environmental conditions, as explained  
386 above (Feare, 1974; Merino et al., 1999; Ramos et al., 2001; McCoy et al., 2002;  
387 Descamps, 2013; Hipfner et al., 2019). For example, Feare (1976) documented a 66.7 %  
388 of tick prevalence on Sooty terns and high mortality effects, while Hipfner et al. (2019)  
389 documented tick prevalence > 60 % for two species of auklet nestlings without  
390 relevant survival effects.

391         Although we cannot determine the ultimate cause of nestlings' death, there are  
392 several non-exclusive possibilities (Dietrich et al., 2011; Yabsley et al., 2012). First,  
393 anaemia caused by blood loss could be lethal for young nestlings infested by high  
394 numbers of ticks (Wanless et al., 1997). In fact, several studies have evidenced  
395 negative effects of ticks on nestlings' body condition, at least during harsh  
396 environmental conditions or high parasite loads (Morbey, 1996; Bosch and Figuerola,  
397 1999; McCoy et al., 2002; Hipfner et al., 2019). Given their very small body size (< 10 g  
398 when hatching, Davis, 1957), storm petrel nestlings could be more susceptible to  
399 ectoparasites as ticks than larger seabird species (Merino et al., 1999). In fact, no  
400 mortality was recorded for nestlings of Balearic shearwaters (adult weight ~500 g) at  
401 the study site during the study period. In this vein, Hipfner et al. (2019) did not find  
402 negative effects of tick infestation on survival or nestlings growth of two medium size  
403 seabird species (adult weight 150-500 g), *Cerorhinca monocerata* and *Ptychoramphus*

404 *aleuticus*, at normal tick *I. uriae* loads; but they found negative effects at very high tick  
405 loads, in accordance with other evidences in other seabird species (Descamps, 2013).  
406 In agreement with our results, they also documented a reduction in tick load with  
407 nestling age, being essentially zero at fledging (Hipfner et al., 2019). However, contrary  
408 to hard *Ixodes* ticks, soft *Ornithodoros* nymph and adult ticks are only attached to their  
409 hosts for very short blood meals, at night (Vial, 2009), making it difficult to establish  
410 the actual tick load experienced by the hosts. We could only count the larvae ticks  
411 feeding on nestlings during the monitoring visits, while adults and nymphs were likely  
412 also feeding on both adult and chick storm petrels. However, adults breeding in both  
413 areas showed similar body condition, which suggest that ticks may have higher  
414 negative effects on nestlings than on adults.

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

423         Finally, ticks may be hosting infectious agents pathogenic for their seabird  
424 hosts, at least for young nestlings. Our study shows that two tick-borne agents known  
425 for their pathogenic potential for vertebrates were detected among the 11 pools of  
426 ticks that were analysed. We detected RNA from WNV genotype 3, which suggests that  
427 the virus is circulating in the storm petrel population, possibly relatively independently

428 from other cycles of that virus in the area. West Nile virus can cause high mortality in  
429 birds, particularly in naïve populations (George et al., 2015), and neurological disorders  
430 in horses and humans. Moreover, the virus could also cause sub-lethal unknown  
431 negative effects (Nemeth et al., 2006). The major vector species of WNV are  
432 mosquitoes, in particular ornithophilic species such as *Culex pipiens* (Calistri et al.,  
433 2010; Brugman et al., 2018). To date, little information is available on the mosquito  
434 species associated to nesting areas of storm petrels, as well as the possible role of soft  
435 ticks in the virus circulation. WNV genotype 3 have been previously isolated from *O.*  
436 *maritimus* (Hoogstraal et al., 1976) and proved to be transmitted in laboratory by  
437 other soft tick species, such as *O. moubata* and *O. capensis* (Lawrie et al., 2004;  
438 Hutcheson et al., 2005). Consequently, the *O. capensis* complex may act as a reservoir  
439 of this genotype of WNV (Lawrie et al., 2004) and deserve to be further investigated.  
440 Moreover, DNA from a *Borrelia* sp. very similar to relapsing fever *B. turicatae* was  
441 detected. It may appear surprising to detect DNA of such as bacteria in ticks from  
442 storm petrel because *B. turicatae* has been mostly reported in the southern USA and  
443 Latin America (Schwan et al., 2005). Nevertheless, relapsing fever *Borrelia* has also  
444 been detected in ticks from a storm petrel and shearwater colony in Japan (Takano et  
445 al., 2009), in African penguins (*Spheniscus demersus*) (Yabsley et al., 2012), in brown  
446 pelicans (*Pelecanus occidentalis*) (Reeves et al., 2006) and recently in ticks from yellow-  
447 legged gull (*Larus michahellis*) nests in Algeria (Lafri et al., 2017). The detection of  
448 those *Borrelia* with a very similar sequence to *B. turicatae* thus suggests that further  
449 investigations are required to understand how widespread are relapsing fever *Borrelia*  
450 in seabird ticks (Kim et al., 2016). In addition, considering the lack of host specificity of  
451 *Ornithodoros* spp. soft ticks and the pathogenic potential of relapsing fever *Borrelia* for

452 other vertebrates, including humans, the question of the extent of the circulation of  
453 such bacteria arises. Given the growing interest in relapsing fever *Borrelia* in America  
454 and the biomedical tools that may become available to detect the past exposure of  
455 hosts by tracking antibody levels (Lopez et al., 2013), investigations could rely on the  
456 detection of the *Borrelia* in ticks, but also sometime more efficiently by detecting  
457 antibodies in the vertebrate host (Armstrong et al., 2018).

#### 458 **Conclusions**

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

472

#### 473 **Conflict of interest**

474 The authors declare no conflicts of interest.

475

476 **Ethical statement**

477 This study was authorized by the Reserves des Vedrà es Vedranell i els illots de Ponent,  
478 Balearic Government. All aspects of the study were performed according to guidelines  
479 established for the ethical treatment of animals and complied with current Spanish  
480 regulations. The collection of ticks was authorized by the Balearic Government  
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482

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495

496 **Data accessibility**

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

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681

682 **Table 1.** Prevalence of *O. maritimus* larvae on storm petrel nestlings during the  
 683 monitoring period at the entrance and the inner part of the colony (sample size in  
 684 parentheses).

	03/07/19	12/07/19	19/07/19	02/08/19	23/08/19
Entrance	100% (3)	57.14% (7)	27.78% (18)	4.76% (21)	0% (13)
Inner chambers	100% (6)	77.78% (9)	89.47% (19)	51.52% (33)	8% (25)

685

686

687 **Table 2.** Model selection of the effects of zone and age on tick *O. maritimus* load of  
 688 Storm petrel nestlings in Cap des Migdia Cave. df, degrees of freedom; Dev, Deviance;  
 689  $\Delta$ AIC, Akaike’s information criterion difference with the best model; “+”, additive  
 690 effect.

691

Effect	df	Dev	$\Delta$ AIC
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

692

693

694 **Table 3.** Model selection of the effects of the zone, the individual tick *O. maritimus*  
695 larvae load and the maximum tick larvae load recorded for each individual during the  
696 monitoring period on body condition index of storm petrel nestlings and adults. Note  
697 that for adults, only the zone is tested as no tick larvae were found attached to them.  
698 Notation as in Table 1.  
699

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

700

701



702 **Table 4.** Model selection of the effects of year and zone on the reproductive  
 703 parameters: Hatching success (HS), Fledgling success (FS) and Breeding Success (BS) of  
 704 storm petrels breeding in Cap des Migdia Cave. Notation as in Table 1.

705

Parameter	Effect	df	Dev	$\Delta$ AIC
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

706

707 **Figure 1.** A) Inner chambers of the storm petrel colony at Espartar Island. In the  
708 photograph can be observed one incubating adult, three alive nestlings and two dead  
709 nestlings; B) adults soft tick (*O. maritimus*) and C) larvae of soft tick (*O. maritimus*) on a  
710 storm petrel nestling.

711

712 **Figure 2.** A) Map of the study area and the studied species; B) Infographic of Cap des  
713 Migdia Cave study areas: Entrance and inner chambers. Representation is informative  
714 but not topologically accurate.

715

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

722

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

727

728 **Figure 5.** A) Annual mean (and 95%CI) of hatching success (HS), B) fledgling success (FS)  
729 and C) breeding success (BS) of storm petrels breeding in the entrance (grey) and the  
730 inner chambers (black) of Cova des Migdia colony between 2014 and 2018. Estimates  
731 from the model including the additive effects of zone and Year (Table 4).  
732