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

3

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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* load and reproductive parameters of storm petrels *Hydrobates pelagicus*  
34 breeding in a large colony in a cave of Espartar Island, in the Balearic archipelago. We  
35 also investigated by molecular analyses the potential viral and bacterial pathogens  
36 associated with *O. maritimus* ticks present at the colony. Lower nestling survival was  
37 recorded in the most infested area, deep in the cave, compared to the area near the  
38 entrance. The parasite load was negatively associated with the body condition of the  
39 nestlings. One pool of ticks tested positive for West Nile virus and 4 pools tested  
40 positive for a *Borrelia* species which was determined by targeted nested PCR to have a  
41 99% sequence identity with *B. turicatae*, a relapsing fever *Borrelia*. Overall, these  
42 results show that further investigations are needed to better understand the ecology  
43 and epidemiology of the interactions between ticks, pathogens and Procellariiform  
44 species.

45

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

47

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 pathogen transmission  
54 (Jongejan and Uilenberg, 2004; Brites-Neto et al., 2015).

55           Ticks are divided into two groups: hard ticks (Ixodidae) and soft ticks  
56 (Argasidae). Both families can potentially transmit numerous pathogens of medical and  
57 veterinary interest (Dietrich et al., 2011 and references therein). However, those  
58 transmitted by soft ticks have been less studied due to the specialization of Argasidae  
59 to hidden habitats (i.e. crevices) and the short time they spend for blood feeding on  
60 the host compared to hard ticks (Vial, 2009). Some of the most common worldwide  
61 diseases caused by pathogens transmitted by soft ticks include: human tick-borne  
62 relapsing fever (TBRF); viral encephalitis transmitted by ticks infesting seabirds, shore  
63 birds and roosting birds; the African swine fever virus (ASFV); fowl spirochetosis  
64 infections or anaplasmosis-like diseases and epizootic bovine abortion (Vial, 2009).  
65 Such pathogens can induce both lethal and sub-lethal effects on their hosts (Niebylski  
66 et al., 1999; Gray and Bradley, 2006). Organisms present a wide range of responses to  
67 tick-induced pathogens (Ramamoorthi et al., 2005). For instance, transmitted  
68 pathogens infecting the host might induce behavioral and/or physiological  
69 modifications which interfere with immunity response, gene expression or energy  
70 allocation, thus potentially affecting fitness. However, the effects of tick infestation  
71 and tick-borne diseases on host populations are often poorly understood and

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

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

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

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

120 (Dupraz, M., personal communication). In addition, based on Dupraz et al., (2016) *O.*  
121 *maritimus* is mostly restricted to the West Palearctic Region where its presence  
122 parasitizing seabirds have been extensively reported (Dietrich et al., 2011). The species  
123 *O. capensis* s.s. is also present in the same region, but almost exclusively associated  
124 with pigeons and it can be morphologically separated from *O. maritimus* (Khoury et al.,  
125 2011).

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

## 135 **Methods**

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

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

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

156 From 2014 to 2018, all accessible nests found in the study site (N = 312) were  
157 marked (Fig. 2A) and inspected every 2 weeks along the breeding season (May-  
158 September) to record reproductive parameters: hatching success ( $\text{No.}_{\text{eggs hatched}}/\text{No.}_{\text{eggs}}$   
159  $\text{laid}$ ), fledgling success ( $\text{No.}_{\text{chicks fledged}}/\text{No.}_{\text{eggs hatched}}$ ) and breeding success ( $\text{No.}_{\text{chicks}}$   
160  $\text{fledged}/\text{No.}_{\text{eggs laid}}$ ) (Mínguez et al., 2015). In addition, during the 2018 breeding season,  
161 we inspected for parasites some adults and all the nestlings located alive and alone in  
162 accessible nests at each monitoring visit. Given the extended breeding period of storm  
163 petrels and the mortality of some of the young nestlings inspected (N = 6), some  
164 nestlings were inspected once and others multiple times. In total we performed 62  
165 inspections of 23 nestlings reared at the entrance of the cave and 92 inspections of 37  
166 nestlings reared at the inner chambers. 28 breeding adults nesting at the entrance and  
167 24 nesting at the inner chambers were inspected once during the breeding season. We

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

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

190 *Statistical analyses*

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

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

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

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

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

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

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

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

#### 240 *High throughput real-time PCR system*

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

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

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

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

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

## 273 **Results**

### 274 *Tick infestation*

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

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

286 decrease in tick load was more rapid at the entrance of the cave (Fig. 3). Six of the  
287 nestlings inspected died (one at the entrance and 5 at the inner chambers). Nestlings  
288 found dead presented high numbers of tick larvae on them at the last alive inspection  
289 (mean = 20.8; range 2-40). We documented the mortality of 10% of the nestlings  
290 detected as infested by ticks at the entrance ( $\text{No.}_{\text{dead}}/\text{No.}_{\text{infested}} = 1/10$ ) and the  
291 mortality of 17% of the nestlings infested by ticks in the inner chambers  
292 ( $\text{No.}_{\text{dead}}/\text{No.}_{\text{infested}} = 5/30$ ).

### 293 *Body condition*

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

### 300 *Reproductive parameters of storm petrels*

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

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

### 311 *Molecular analyses*

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

### 321 **Discussion**

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

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

346 Nestling body condition was negatively correlated with tick load, which may  
347 explain the higher nestling mortality in the inner parts of the colony. Our results agree  
348 with other studies on seabirds that have detected high nest desertion and/or nestling  
349 mortalities of individuals infested by ticks or in areas with high tick densities (e.g.  
350 Feare, 1976; Duffy, 1983; Ramos et al., 2001; Reeves et al., 2006; Descamps 2013). In  
351 addition, nestling mortality in the inner parts of the cave, where tick prevalence was  
352 higher and ticks were more abundant, was very high (48%). We cannot directly analyse  
353 the effect of tick infestation on nestling survival due to our reduced sample size of  
354 nestlings inspected before dying (i.e. for which we had counted the number of tick  
355 larvae attached to them when alive). This is because the majority of the mortalities  
356 occurred during the first two weeks after hatching and many nestlings were born and

357 died between our monitoring visits (own observation). However, the low survival of  
358 storm petrel nestlings located in the inner parts of the Espartar's cave matches with  
359 the high mortality estimates provided for other seabird species nestlings infested by  
360 ticks (Feare, 1996; Ramos et al., 2001). For example, Ramos et al. (2001) documented a  
361 mortality of 63% of roseate terns (*Sterna dougallii*) infested by hard ticks *Amblyomma*  
362 *loculosum* versus a nestling mortality of 17% of non-infested nestlings during a harsh  
363 breeding season with food shortage. On the contrary, during a breeding season with  
364 benign environmental conditions, only 24% of the tick-infested nestlings died (Ramos  
365 et al., 2001).

366 In fact, environmental conditions may influence the effect of parasites on hosts  
367 (Ramos et al., 2001; McCoy et al., 2002). At Espartar's colony, during our 5 years of  
368 monitoring the fledgling success showed annual variations that may be related with  
369 differential tick densities or environmental conditions affecting storm petrels and or  
370 interacting with their parasites (McCoy et al., 2002), factors that should be studied in  
371 the future. However, mortality in the inner chambers of the cave was always very high.  
372 On the contrary, at the entrance of the cave, nestling mortality showed the usual low  
373 levels (~10%) estimated at other storm petrel colonies (Sanz-Aguilar et al., 2009)  
374 where tick prevalence is lower (Merino et al., 1999). For example, in Benidorm Island  
375 only one of the 34 storm petrel nestlings inspected by Merino et al. (1999) in 1996  
376 presented a single *Ornithodoros* spp. tick larvae. Additionally, a tick larvae was  
377 detected only on 4 out of 27 storm petrel nestlings inspected at Benidorm Island on  
378 25<sup>th</sup> June 2019 (tick larvae prevalence of 15%; own unpublished data). Monitoring of  
379 tick infestation could be conducted to explore potential increase over time, such as  
380 that reported as a function of warmer winter temperature in an arctic population of

381 Brünnich's guillemots (*Uria lomvia*) (Descamps, 2013). Prevalence of ticks infesting  
382 seabirds has been shown to be very variable among seabird species, breeding colonies  
383 and/or years ranging 0% and > 70% (Feare, 1974; Merino et al., 1999; Ramos et al.,  
384 2001; Descamps, 2013; Hipfner et al., 2019). However, the effects of ticks on hosts  
385 depend also on the tick load, the host species, the age of the host (e.g. the effects can  
386 be different for nestlings and adults), and/or the environmental conditions, as  
387 explained above (Feare, 1974; Merino et al., 1999; Ramos et al., 2001; McCoy et al.,  
388 2002; Descamps, 2013; Hipfner et al., 2019). For example, Feare (1976) documented a  
389 67% of tick prevalence on Sooty terns and high mortality effects, while Hipfner et al.  
390 (2019) documented tick prevalence > 60% for two species of auklet nestlings without  
391 relevant survival effects.

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

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

416         Second, the low survival of nestlings in the inner part of the cave may be a  
417 consequence of a massive adult abandonment of young nestlings as a result of high  
418 parasite load, as shown in other species (Feare, 1976; King et al., 1977; Duffy, 1983;  
419 Reeves et al., 2006). However, we think that this possibility is highly improbable. Some  
420 field observations using camera trapping indicates that the adults came back to feed  
421 the chicks when they were already dead. Moreover, neighbours of already dead chicks  
422 continued incubating or rearing their surviving chicks (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. and the pathogenic potential of relapsing fever *Borrelia* for other  
452 vertebrates, including humans, the question of the extent of the circulation of such

453 bacteria arises. Given the growing interest in relapsing fever *Borrelia* in America and  
454 the biomedical tools that may become available to detect the past exposure of hosts  
455 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 do 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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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% (7)	28% (18)	5% (21)	0% (13)
Inner chambers	100% (6)	78% (9)	89% (19)	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

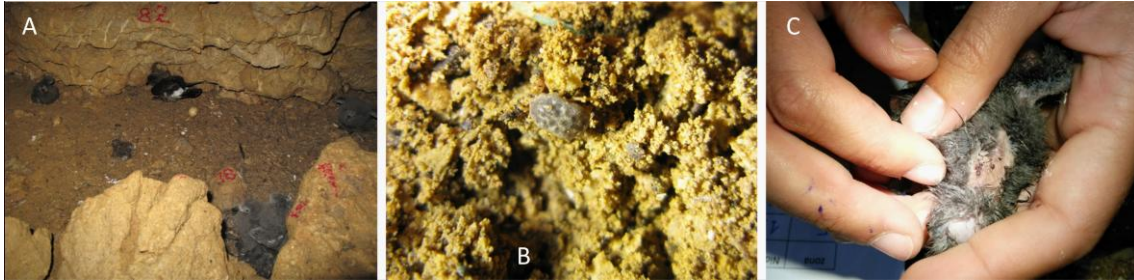
702 **Table 4.** Model selection of the effects of year and zone on the reproductive  
 703 parameters: Hatching success (HS), Fledgling success (FS) and Breeding Success (BS) of  
 704 storm petrels breeding in Cap des Migdia Cave. Notation as in Table 1.

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

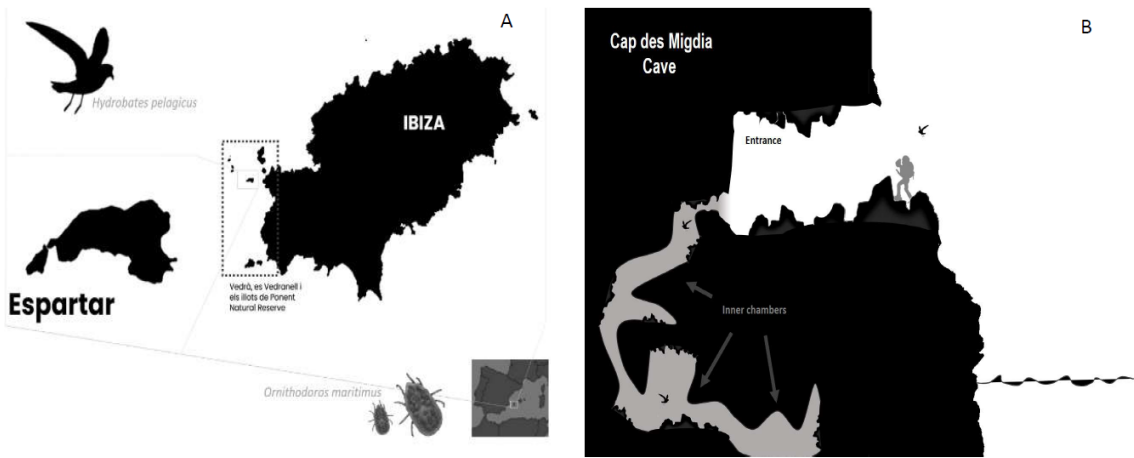
706

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.



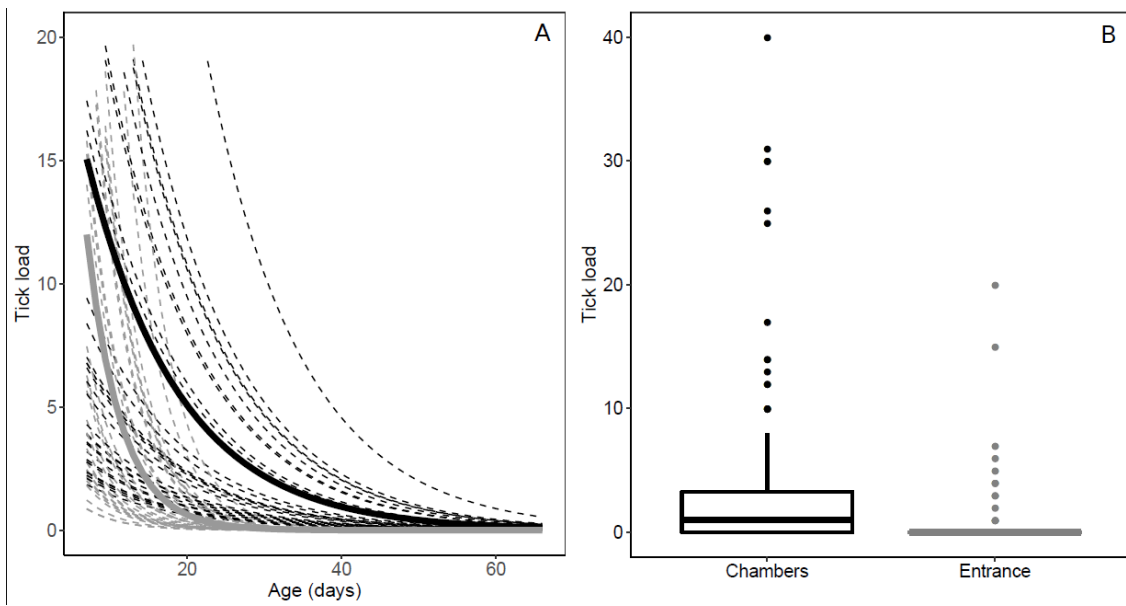
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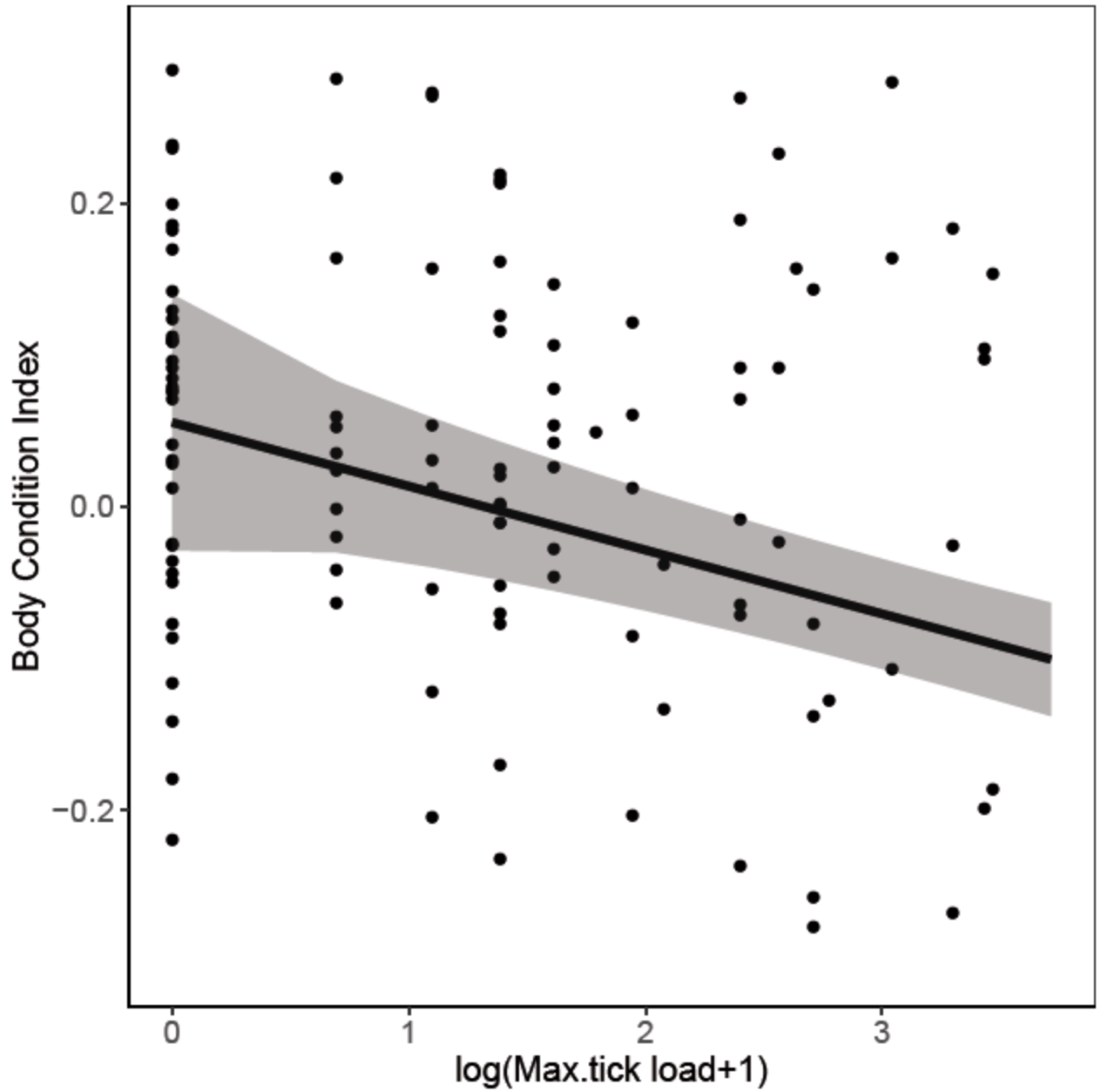
719 **Figure 3.** A) Relationship between tick *O. maritimus* larvae load and storm petrel  
720 nestlings age. Dotted lines are the individuals' predictions and solid lines the mean  
721 prediction, Table 2, with grey and black lines representing the entrance and the inner  
722 chambers of the colony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



725

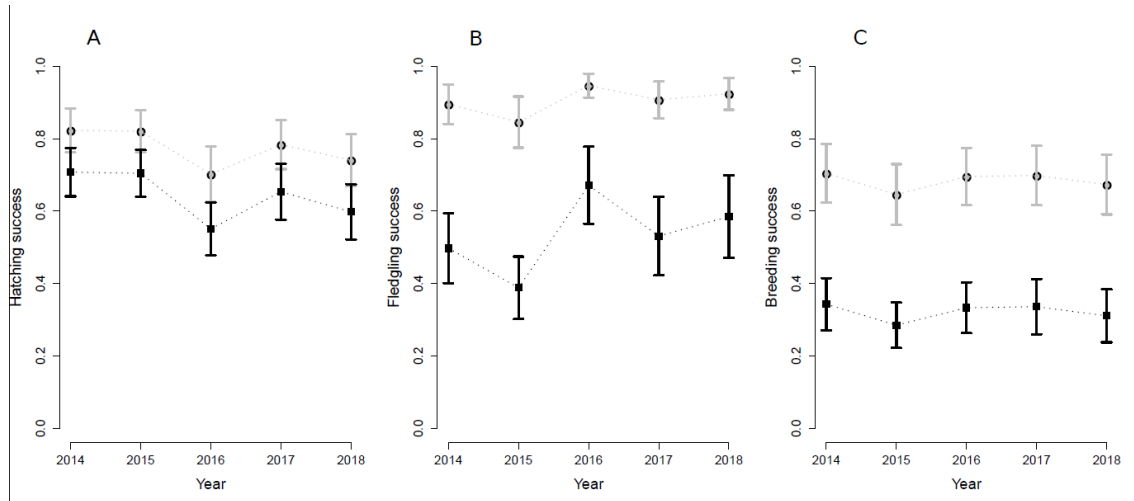
726

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



731  
732

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



737