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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 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 (ΔAIC) 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 μ 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 μ l for the DNA extraction, 100 μ l for the RNA extraction
234 and the rest was used as back-up and conserved at -80°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 μ l of
237 rehydration solution and 50 μ 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 ± 0.69 SE) of the cave than at the entrance (mean 1.13
285 ± 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 25th 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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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% (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 Δ AIC, Akaike's information criterion difference with the best model; "+", additive
690 effect.

691

Effect	df	Dev	Δ 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

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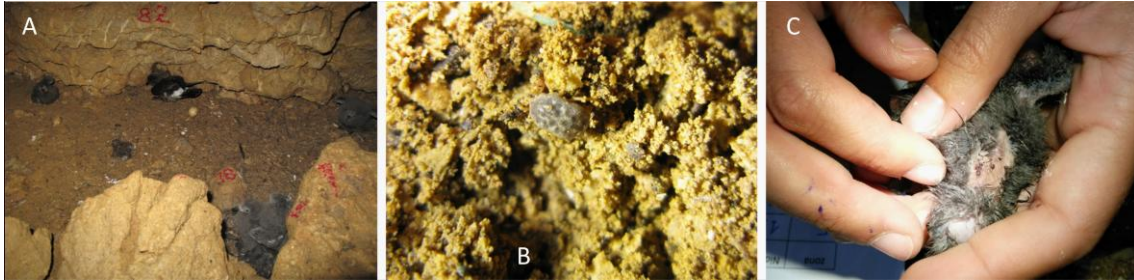
702 **Table 4.** Model selection of the effects of year and zone on the reproductive
 703 parameters: Hatching success (HS), Fledgling success (FS) and Breeding Success (BS) of
 704 storm petrels breeding in Cap des Migdia Cave. Notation as in Table 1.

705

Parameter	Effect	df	Dev	Δ 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

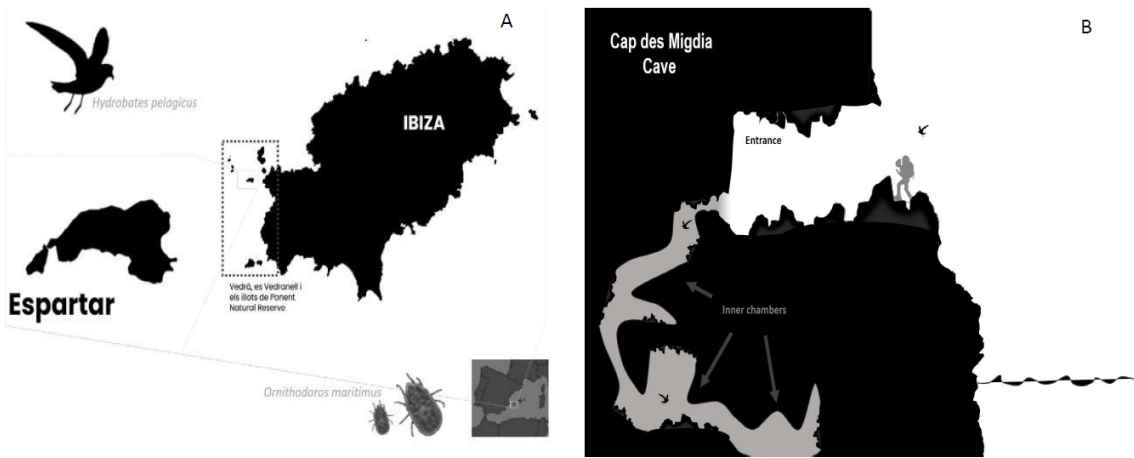
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707 **Figure 1.** A) Inner chambers of the storm petrel colony at Espartar Island. In the
708 photograph can be observed one incubating adult, three alive nestlings and two dead
709 nestlings; B) adults soft tick (*O. maritimus*) and C) larvae of soft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



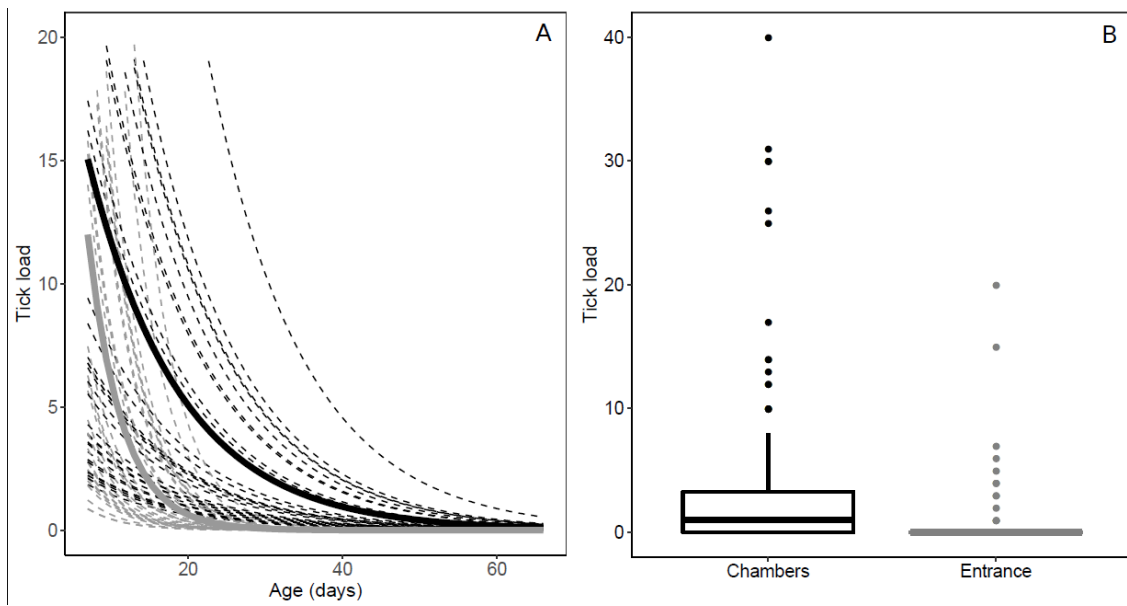
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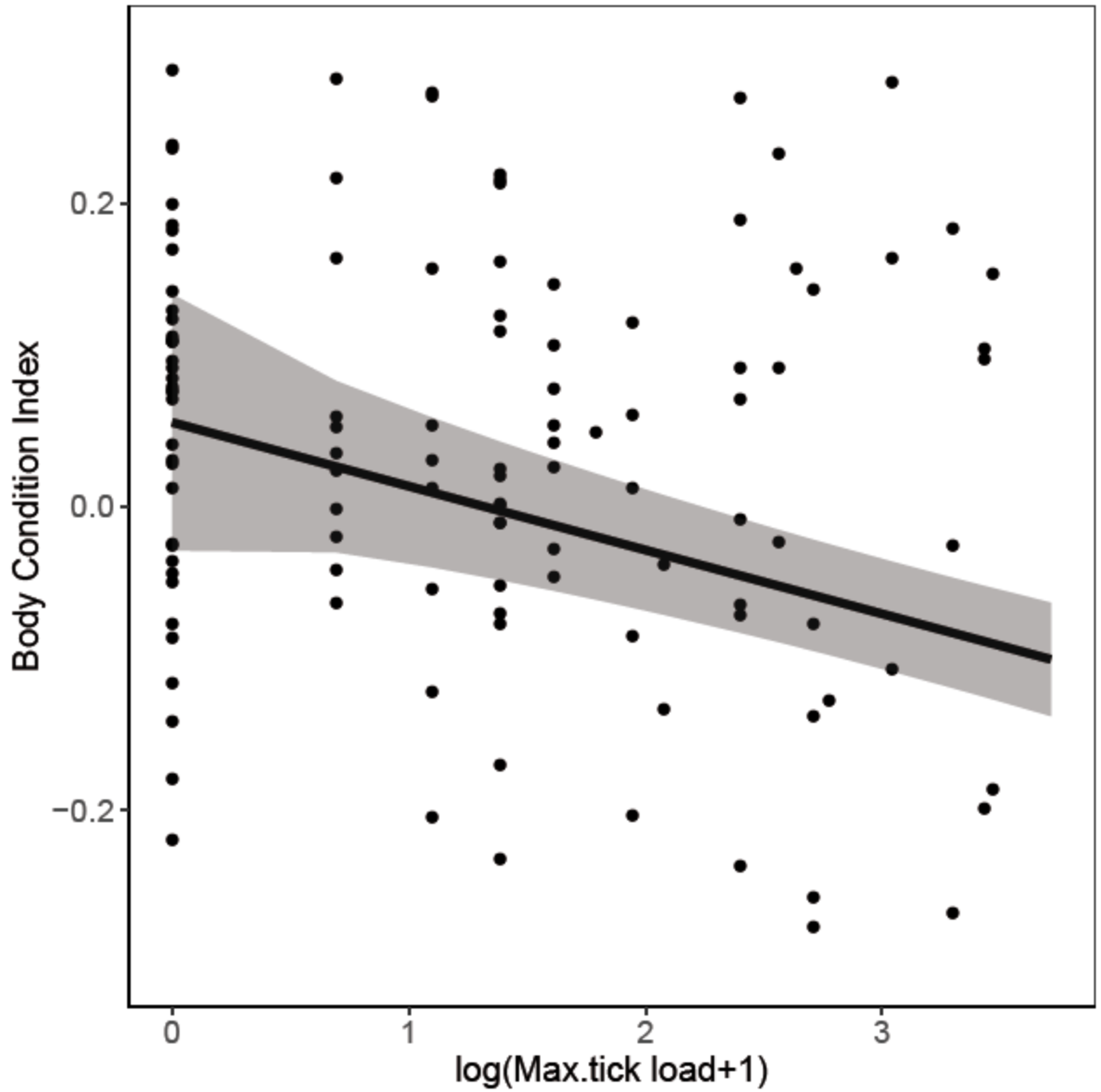
719 **Figure 3.** A) Relationship between tick *O. maritimus* larvae load and storm petrel
720 nestlings age. Dotted lines are the individuals' predictions and solid lines the mean
721 prediction, Table 2, with grey and black lines representing the entrance and the inner
722 chambers of the colony respectively.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

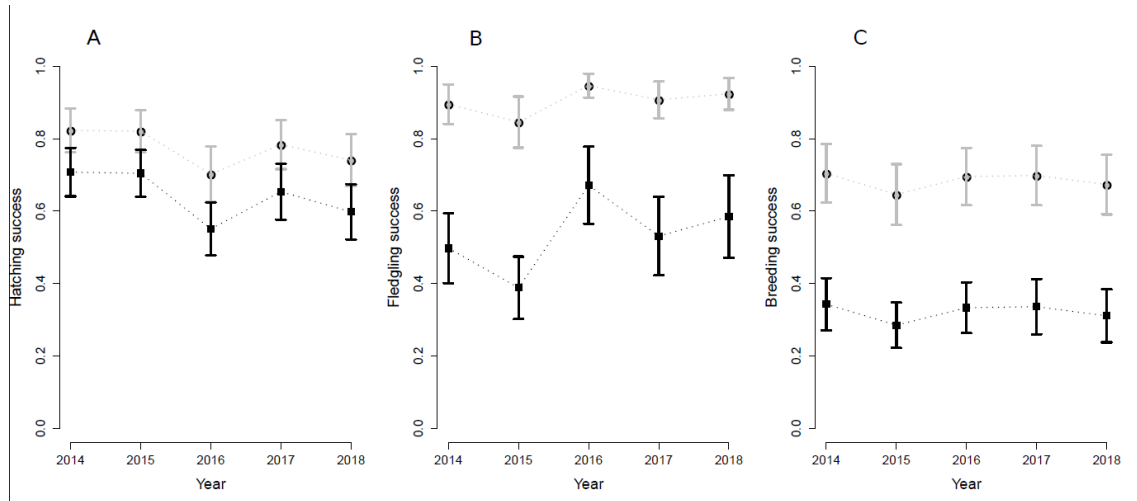
726

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