



## Exposure of yellow-legged gulls to *Toxoplasma gondii* along the Western Mediterranean coasts: Tales from a sentinel

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### ABSTRACT

Efficiently tracking and anticipating the dynamics of infectious agents in wild populations requires the gathering of large numbers of samples, if possible at several locations and points in time, which can be a challenge for some species. Testing for the presence of specific maternal antibodies in egg yolks sampled on the colonies could represent an efficient way to quantify the exposure of breeding females to infectious agents, particularly when using an abundant and widespread species, such as the yellow-legged gull (*Larus michahellis*). We used such an approach to explore spatio-temporal patterns of exposure to *Toxoplasma gondii*, a pathogenic protozoan responsible of toxoplasmosis in humans and other warm-blooded vertebrates. First, we tested the validity of this approach by exploring the repeatability of the detection of specific antibodies at the egg level using two different immunoassays and at the clutch level using an occupancy model. Then, samples gathered in 15 colonies from France, Spain and Tunisia were analysed using an immunoassay detecting antibodies specifically directed against *T. gondii*. Prevalence of specific antibodies in eggs was overall high while varying significantly among colonies. These results revealed that *T. gondii* circulated at a large spatial scale in the western Mediterranean yellow-legged gull population, highlighting its potential role in the maintenance community of this parasite. Additionally, this study illustrates how species commensal to human populations like large gulls can be used as wildlife sentinels for the tracking of infectious agents at the human-wildlife interface, notably by sampling eggs.

### 1. Introduction

*Toxoplasma* infection (toxoplasmosis) is being recognized as one of the most common parasitic zoonotic diseases in the world, notably in relation to the worldwide distribution and wide range of hosts of its causative agent, *Toxoplasma gondii* (Tenter et al., 2000). However, the complex pathogenicity and life cycle of this parasite remain elusive. While felids are considered as the only definitive hosts of *T. gondii*, it is supposed that any warm-blooded vertebrate could be an intermediate

host (Dubey, 1998a), potentially playing an important role in the parasite circulation. For instance, migratory geese are suspected to be responsible for the introduction of the parasite in Arctic cat-free islands (Prestrud et al., 2007). Hence, more studies are needed to better understand the cycle of the parasite, notably at the interface between wild fauna and human populations where public health but also conservation issues can arise (e.g., the suspicion of *T. gondii* introduction via feral cats *Felis catus* populations on remote islands; Work et al., 2002; Deem et al., 2010).

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In this context, studies focusing on wild species commensal to human populations should help to identify potential relevant hotspots of circulation of *T. gondii* to then investigate potential risk factors. In particular, predator and/or scavenger species like gulls may be especially interesting to consider. Indeed, gulls could be exposed to *T. gondii* via oocysts found in the environment, notably water soiled with felids' faeces (Dubey, 2009), but also via other sources considering their opportunistic feeding behaviour (Appendix A, Figure S1.1). Gulls can be exposed to bradyzoites by preying or scavenging on infected intermediate hosts. Notably, large gulls can feed on smaller birds (Oro and Martínez-Abraín, 2007). Also, because gulls live in close proximity to human populations, they have access to refuse tips (Ceia et al., 2014; Navarro et al., 2016; Ramos et al., 2009; Real et al., 2017; Steigerwald et al., 2015). Recently, it has also been suggested that marine prey, and filter feeders in particular, may accumulate oocysts (Massie et al., 2010), explaining therefore the ubiquity of this parasite in the marine environment (Dubey et al., 2005; Honnold et al., 2005; van de Velde et al., 2016; Verma et al., 2018).

Despite the potential wide exposure of gulls to *T. gondii*, only a few studies have reported information on this topic (Burridge et al., 1979; Karakavuk et al., 2018; Prestrud et al., 2007; Uterák et al., 1992). Notably, a recent study revealed the detection of anti-*T. gondii* antibodies in yellow-legged gull (*Larus michahellis*) nestlings using the modified agglutination test (MAT; Cabezón et al., 2016). However, in that study nestling age was not accounted for, making it difficult to interpret the results because measured antibody levels could arise from a mixture between maternally inherited and autogenous antibodies (Boulinier and Staszewski, 2008). Capturing adults of such species for the collection of biological samples requires particular efforts and skills. Testing for the presence of specific maternal antibodies in egg yolk samples could represent an efficient way to quantify the exposure of breeding females to various infectious agents, while avoiding the difficult interpretation of nestling serological profiles. Indeed, in birds, antibodies (IgY) can be transmitted from breeding females to hatchlings through the egg yolk (Boulinier and Staszewski, 2008) from which they can be quantified using classical immunoassays. This approach has already proven efficient to detect exposure of yellow-legged gulls to avian influenza viruses (Hammouda et al., 2014) and flaviviruses (Arnal et al., 2014), and the exposure of great cormorants (*Phalacrocorax carbo*) to Newcastle disease virus (Alekseev et al., 2014). Moreover, eggs also contain information on the diet of breeding females. Because food is likely to be the main source of exposure of adults to *T. gondii*, one could expect that different feeding behaviours of individual gulls can lead to different risks of exposure to the parasite. This factor is particularly important to consider in species exhibiting a wide variety of feeding behaviours such as gulls (Ramos et al., 2009a). For instance, individuals feeding on refuse tips and sewage water could be expected to be more at risk of exposure than individuals feeding at sea (Cabezón et al., 2011, 2016). Since the 1990s, stable isotopes analysis has been used as a powerful approach to study animal diet (Ramos and González-Solís, 2012). This approach relies on the assumption that the isotopic signature of the tissues of a consumer reflects that of its diet (Bearhop et al., 2004), and can be used on egg components. For instance, specific isotopic ratios are known to differ among habitats: marine ecosystems have higher isotopic ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$  or  $\delta^{13}\text{C}$ ), nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ;  $\delta^{15}\text{N}$ ), and sulphur ( $^{34}\text{S}/^{32}\text{S}$ ;  $\delta^{34}\text{S}$ ) than terrestrial and freshwater environments (Hobson, 1999; Hobson et al., 1994; Peterson and Fry, 1987). One could thus expect individuals with egg isotopic signatures indicating the consumption of items from refuse tips or other terrestrial resources to be more likely to be anti-*T. gondii* antibody positive compared to individual with isotopic signatures associated to a marine diet (Ramos et al., 2009; Cabezón et al., 2016), on the condition that dietary and immunological information are integrated on the same time scale.

In the present study, we sampled eggs of an abundant and widely distributed species of the Mediterranean coasts, the yellow-legged gull,

to track its exposure to *T. gondii* at a hierarchy of spatial scales and to explore potential relations with diet. Yellow-legged gulls are well-known consumers of anthropogenic food sources, such as fishery discards and leftovers from refuse tips (Duhem et al., 2008; Ramos et al., 2009, 2011). This feeding behaviour makes the species a potential relevant sentinel to monitor disease circulation and infection risks for human and/or wild populations. The fact that in some colonies, populations are controlled by egg sterilisation to limit interactions with other species and/or human populations (Oro and Martínez-Abraín, 2007) facilitates access to eggs. In addition, because there is some confusion regarding the identity of *T. gondii*-like parasites in wild birds (Dubey, 2002), we also collected tissues from dead gulls in order to confirm the circulation of the parasite and characterize the circulating strain. The Mediterranean area, at the crossroad between continents, is particularly interesting to consider given the structure of *T. gondii* populations at the world scale and the potential relationship between strain and pathogenicity (Galal et al., 2018; Xiao and Yolken, 2015). The expected results of this study may have important implications regarding the potential role of large gulls in the maintenance community of *T. gondii*.

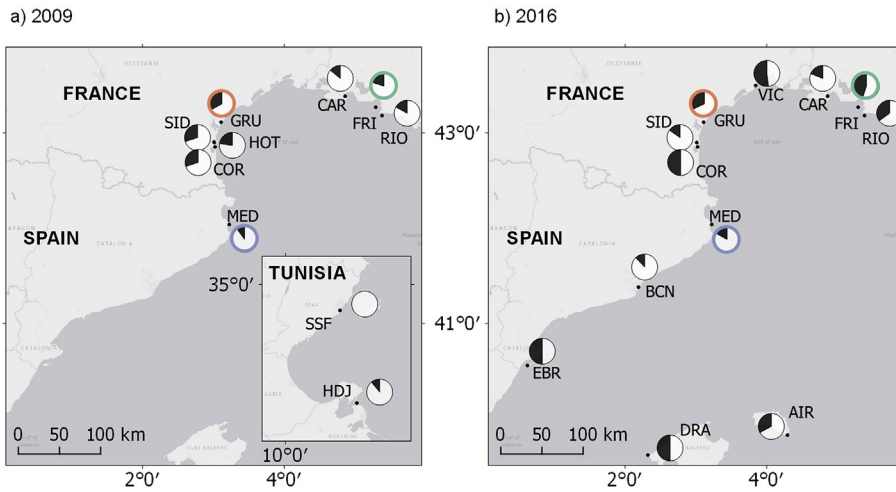
## 2. Material and methods

### 2.1. Study population and sampling protocols

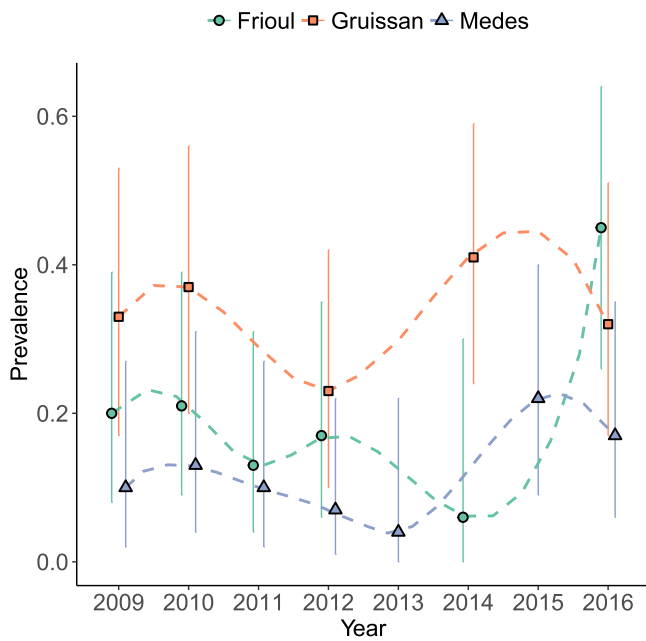
Yellow-legged gull nest in colonies that are mostly established in marshlands and on natural and man-made islets. Some are also found on city roofs. The field sampling was conducted between 2009 and 2016. Each year, a set of colonies located on the Mediterranean coasts of France, Spain and Tunisia was visited during the peak of the laying period (late March-early April) for fresh egg collection (Appendix A, Table S1.1). Incubation stage was assessed by a brief immersion of eggs in water and only one fresh egg per clutch (generally composed of three eggs) was collected (Hays and LeCroy, 1971); freshly laid eggs do not float and non-collected eggs were put back in the nest. This method insured an efficient sampling of eggs with minimum impact on gull reproduction. The sampling protocol was approved by the relevant local institutions (Direction régionale de l'Environnement, de l'Aménagement et du Logement Provence-Alpes-Côte d'Azur and Languedoc-Roussillon; Syndicat Mixte des Etangs Littoraux; Parc National des Calanques; Conservatoire Etudes des Ecosystèmes de Provence-Alpes du sud; Mairie de Marseille; Marie de Grissan; Parc Natural de El Montgrí, les Illes Medes i el Baix Ter; Parc Natural del Delta de l'Ebre; Forest service of the Tunisian Ministry of Agriculture).

Spatial patterns in the prevalence of anti-*T. gondii* antibodies were first explored in the samples collected in 2009 and 2016, including 10 and 12 colonies, respectively (Fig. 1). Second, temporal patterns were explored in three specific breeding sites (Frioul, Gruissan and Medes Islands), using samples collected along the period 2009–2016 (Fig. 2). Third, in the two Tunisian colonies sampled in 2009, the three eggs of every nest had been sampled ( $n = 201$  eggs from 67 nests) in order to assess if the detection of antibodies from any egg provides equivalent information to infer the occurrence of antibodies in the mother using a patch occupancy model (Appendix A). Fourth, in order to explore the pattern of exposure at a smaller scale (within a colony), we used sampled nest locations recorded using the global position system (GPS) in colonies sampled in 2016 (Appendix A, Figure S4.1). Once at the laboratory, within the few days following collection, yolk and albumen were separated and stored at  $-20\text{ }^{\circ}\text{C}$  until analysed.

Complementarily, and in an attempt to isolate *T. gondii* from yellow-legged gulls, necropsies were also conducted on three adults found freshly dead in three colonies (Frioul, Sidrière and Ebro Delta) in 2017. Brain and cardiac muscle tissues were collected and kept at  $4\text{ }^{\circ}\text{C}$  for less than 72 h. Intra-cardiac blood was also collected using a heparinized syringe and centrifuged a few hours after collection; the plasma was stored at  $-20\text{ }^{\circ}\text{C}$  until analysed.



**Fig. 1.** Map of prevalences of anti-*T. gondii* antibodies in yellow-legged gull egg yolk samples in 2009 (a) and 2016 (b) illustrating the spatial variability. RIO: Riou; FRI: Frioul; CAR: Carreau; VIC: Vic-la-Gardiole; GRU: Gruissan; HOT: Hortel; SID: Sidrière; COR: Corrège; MED: Medes; BCN: Barcelona; EBR: Ebro Delta; DRA: Dragonera; AIR: Illa de l’Aire; SSF: Sfax; HDJ: Djerba. Coloured circles highlight the colonies in which temporal variations were explored (Fig. 2). Sample sizes and confidence intervals are given in Appendix A, Table S1.1. Base map: esri®. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 2.** Limited temporal variations of the prevalence of anti-*T. gondii* antibody in yellow-legged gull egg yolk samples between 2009 and 2016 in three colonies: Frioul, Gruissan and Medes Islands. Curves correspond to cubic splined fitted to the yearly prevalences for visualisation purposes only. Bars indicate 95% Clopper-Pearson confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**2.2. Anti-*T. gondii* antibody detection**

Specific antibody levels were measured in yolk samples to infer the past exposure of breeding females to *T. gondii* using a commercially available indirect enzyme-linked immunosorbent assay (ELISA) designed to detect chicken IgY directed against the *T. gondii* p30 antigen (ID Screen® Avian Toxoplasmosis Indirect, IDvet, France). This study is the first to investigate the detection of anti-*T. gondii* antibodies in gull egg yolk samples, and one of the few to investigate their detection in seabirds in general (Dubey, 2002 for a review). We thus included several validation procedures on a subset of samples in our laboratory analyses. Notably, as no gold standard is available for the detection of anti-*T. gondii* antibodies in wild birds, a second assay was run on a subset of samples (Enøe et al., 2000). The MAT was chosen as it is

frequently used for *T. gondii* serosurveys, notably in wild birds (Cabezón et al., 2016; Sandström et al., 2013), although, to our knowledge, it has never been used on egg yolk samples before. Details are presented in the supplementary material (Appendix A). Overall, the ELISA was proven repeatable at a much higher level than the MAT at the sample scale (i.e., repeated analyses conducted on a given sample). At the nest scale (i.e., repeated sampling of eggs from a given nest), the detection probability was suitable. In addition, the ELISA and the MAT lead to the same prevalence of specific antibodies at the colony scale. These results are in line with previous studies suggesting that ELISA are generally suitable to detect anti-*T. gondii* antibodies in domestic and wild animals (e.g., Dubey et al., 2005; Gamble et al., 2005). Plasma samples from necropsied individuals were analysed by MAT with positivity threshold at dilution 1/20.

**2.3. Stable isotope analysis and trophic inferences**

$\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  were measured in the albumen sample as indicators of breeding females’ diet.  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  are used primarily to determine sources of primary production and are useful to trace the input of these elements into the food webs (Richards et al., 2003). In particular, the use of  $\delta^{34}\text{S}$  is recommended when the origin of the diet is heterogeneous.  $\delta^{15}\text{N}$  suffers considerable fractionation with each trophic level, making it suitable for assessing in which trophic level the consumer feeds. That is, consumers at the top of a long trophic chain show a higher  $\delta^{15}\text{N}$  than those that feed on impoverished food chains, as might be the case for individuals feeding on refuse tips (Ramos and González-Solís, 2012).

Albumen samples were first dried in an oven at 60 °C to constant mass, and subsequently ground to fine powder with a mortar. Subsamples of powdered materials were weighed to the nearest  $\mu\text{g}$ , placed in tin capsules and crimped for combustion. Samples were oxidized at the Serveis Científico-Tècnics of the University of Barcelona (SCT-UB; Spain) by means of elemental analysis-isotope ratio mass spectrometry, using a Thermo Finnigan Flash 1112 (CE Elantech, Lakewood, NJ, USA) elemental analyser coupled to a Delta-C isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Finnigan MAT, Bremen, Germany). Stable isotope ratios were expressed in the standard  $\delta$ -notation (in ‰) relative to Vienna Pee Dee Belemnite (VPDB;  $\delta^{13}\text{C}$ ), atmospheric  $\text{N}_2$  (VAIR;  $\delta^{15}\text{N}$ ) and Vienna Cañon Diablo Troilite (VCDT;  $\delta^{34}\text{S}$ ). Isotopic ratio mass spectrometry facility at the SCT-UB applies international inorganic standards (IAEA CH7, IAEA CH6 and USGS 24 for C, IAEA N1, IAEA N2 and IAEA NO3 for N and IAEA-S1, IAEA-S2, IAEA-S3, NBS-127 and YCEM for S) as well as internal organic standards (Human Hair CRM 397, Community Bureau of Reference, Commission of the European Community;  $\delta^{13}\text{C}$ ;

$-19.5 \pm 0.2$ ,  $\delta^{15}\text{N}$ :  $10.0 \pm 0.2$ ,  $\delta^{34}\text{S}$ :  $4.5 \pm 0.2$ ) inserted every 12 samples to calibrate the system and compensate for any drift over time. Replicate assays of standard materials indicated measurement errors of  $\pm 0.1$ ,  $\pm 0.2$  and  $\pm 0.2\%$ , for C, N and S respectively but these are likely underestimates of true measurement errors for complex organics like albumen samples.

#### 2.4. Statistical analyses

We calculated the prevalence of anti-*T. gondii* antibodies in a sampled colony as the proportion of nests containing at least one positive egg over the total number of nests sampled. We then assessed variation in antibody prevalence among years and colonies by means of logistic regression models. Two separate regressions were carried out: (1) one focusing on 2009 and 2016 to explore spatial patterns and (2) one focusing on the temporal series collected on Frioul, Gruissan and Medes to explore temporal patterns. Best models were selected based on Akaike information criteria (AIC) with a threshold of  $\Delta\text{AIC} = 2$  (Burnham and Anderson, 2002).

Spatial auto-correlation of antibody prevalence (as a continuous variable) among colonies was assessed using 2009 and 2016 data, by calculating the Moran's I (Moran, 1950). Within colonies, spatial auto-correlation of nest antibody status (anti-*T. gondii* antibody-negative or -positive as a binary variable) was assessed by calculating the BB joint-count statistic (Cliff and Ord, 1981). These statistics were calculated using the 'spdep' R package and p-values were corrected for multiple tests using the Bonferroni correction. Maps were drawn with QGIS 2.18.21 and the 'QuickMapServices' plugin.

The relationship between nest status (antibody-negative or -positive) and mother's diet was explored by comparing the distribution of the isotopic ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$ ) between antibody-negative and -positive nests. To confirm observations, a logistic regression with the three isotopic signatures as fixed effects, the year and site as random effects and nest status as the response variable was run on the subset of samples for which all measures were available.

More details regarding the run models are given in Appendix A. All statistical analyses were conducted in R 3.3.3 and codes are available in Appendix B.

#### 2.5. *T. gondii* isolation and genotyping

Tissues for parasite isolation were collected from the necropsied gulls that tested positive for anti-*T. gondii* antibodies. Brains and cardiac muscles were prepared and inoculated intraperitoneally to three female Swiss mice (*Mus musculus*) following the procedure described in Mercier et al. (2010). Given the low parasitic burden found in the tissues of most *T. gondii* hosts, mouse bioassay was necessary for an upstream amplification of parasitic burden to reach the sensitivity thresholds of most genotyping techniques. Considering the risk of bacterial proliferation due to the sampling conditions and the decomposition state of the gull samples, those were treated with an antibiotic solution (1000 U/ml penicillin and 100  $\mu\text{g}$  streptomycin/ml in saline solution) before inoculation. A 200  $\mu\text{l}$  aliquot of the gull brain and heart preparations was also used for DNA extraction using the QIAamp<sup>®</sup> DNA MiniKit (Qiagen, France) and quantification of *T. gondii* by real-time PCR targeting the *T. gondii* 529 bp repetitive element as previously described (Homan et al., 2000). The mice were monitored daily and all surviving mice were euthanized at four weeks post-inoculation. Brain from the MAT-positive mice were screened for cysts by microscopic examination. Live parasites were cryopreserved until analysed. DNA from 200  $\mu\text{l}$  of mouse brain tissue was extracted and *T. gondii* isolates were genotyped using the length polymorphism of 15 multilocus microsatellite markers located on 11 different chromosomes in a multiplex PCR assays as detailed in Ajzenberg et al. (2010). All experimental procedures in mice were conducted according to European guidelines for animal care ("Journal Officiel des Communautés Européennes", L358, December

18, 1986) and approved by the Regional Ethics Committee Limousin (Registration Number: CREEAL 3-07-2012).

### 3. Results

#### 3.1. Spatio-temporal variation in antibody prevalence

In total, 1122 egg yolk samples from 988 nests were screened for anti-*T. gondii* antibodies by ELISA (Appendix A, Table S1.1). Anti-*T. gondii* antibodies were detected in 233 screened nests, corresponding to an overall antibody prevalence [95% confidence interval] of 0.19 [0.14; 0.24] in 2009 and 0.33 [0.29; 0.39] in 2016 (note that the set of sampled colonies is different between the two years). Prevalence ranged from 0.03 to 0.52 among years and colonies (Fig. 1). In 2009 and 2016, year and colony had significant effects on the occurrence probability of anti-*T. gondii* antibodies (year:  $\chi^2 = 5.8$ , d.f. = 1,  $p = 0.02$ ; site:  $\chi^2 = 46.5$ , d.f. = 6,  $p < 0.01$ ;  $n = 414$  samples; Appendix A, Table S3.1). When focusing on Frioul, Gruissan and Medes (for which temporal series were analysed), year did not provide a significant predictor of antibody occurrence probability, as it was not included or not significant in the selected models (Appendix A, Table S3.2). However, there was a significant difference between colonies ( $\chi^2 = 23.1$ , d.f. = 2,  $p < 0.01$ ;  $n = 525$  samples). Overall, these results suggest some large scale spatial variation but limited local temporal variation in gull exposure to *T. gondii*.

There was no significant spatial auto-correlation of the prevalence among colonies (2009:  $I = 0.46$ ,  $p = 0.12$ ,  $n = 10$ ; 2016:  $I = -0.30$ ,  $p = 1.00$ ,  $n = 12$ ). Similarly, there was no significant spatial auto-correlation of nest status within colonies (all  $p > 0.10$  for the five concerned colonies; Appendix A, Table S4.1; Fig. 3).

#### 3.2. Relationship between exposure to *T. gondii* and diet

Complete antibody and isotopic data were available for 129 egg yolk samples (Appendix A, Table S1.2). Distributions of the isotopic ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ ) largely overlapped between negative and positive nests (Fig. 4), suggesting no relationship between nest antibody status and inferred breeding females' diet. This observation was confirmed by the fact that none of the isotopic signature had a significant effect on the nest status in the selected models (Appendix A, Table S3.3;  $n = 129$  samples).

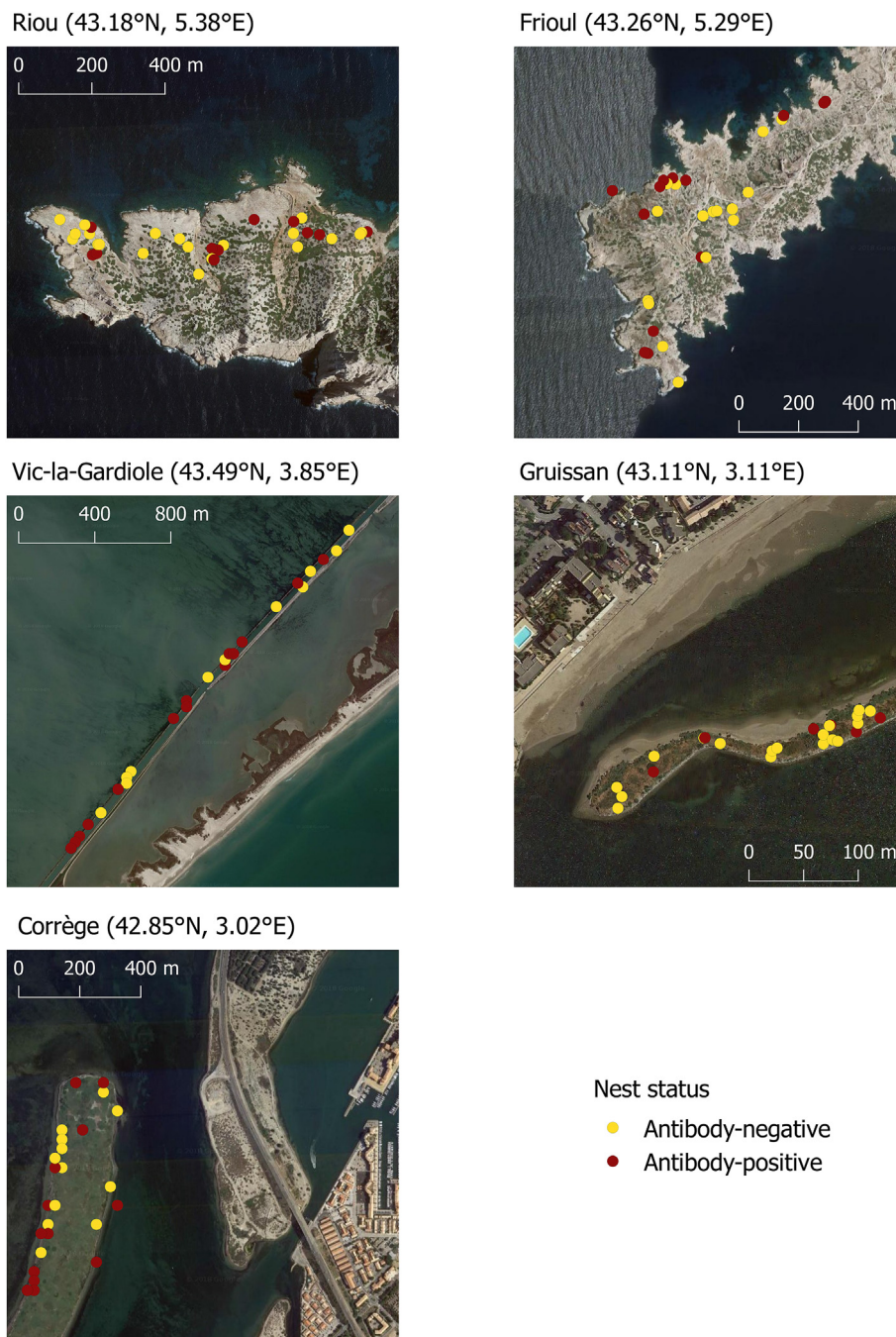
#### 3.3. *T. gondii* isolation and genotyping

The three necropsied gulls were seropositive for anti-*T. gondii* antibodies. Two individuals showed positive real-time PCR (heart and brain) with threshold values (CT) of 30.50 and 34.74 respectively for individuals 170329Lmic01 ES (from Ebro Delta, Spain) and 170402Lmic0111 (from Sidrière, France). One live strain was isolated from the individual originating from Ebro Delta, Spain, as indicated by the observation of *T. gondii* tissue-cysts under the microscope in brain samples from inoculated mice. This isolate was genotyped as type II (Appendix A, Table S5.1).

### 4. Discussion

Using egg sampling and an easily available immunoassay, we explored patterns of yellow-legged gull exposure to *T. gondii* at different spatial and temporal scales. The results revealed a wide exposure of this species to the parasite, which highlights its broad presence at the wildlife-human interface of the western Mediterranean Sea, in line with results obtained from other wild avian species (e.g., Cabezón et al., 2011). Additionally, this study suggests that large gulls could potentially play a role in the reservoir community of this zoonotic parasite, notably through predation or scavenging by other intermediate hosts (e.g., other gulls or rodents) or by felids, although this would be





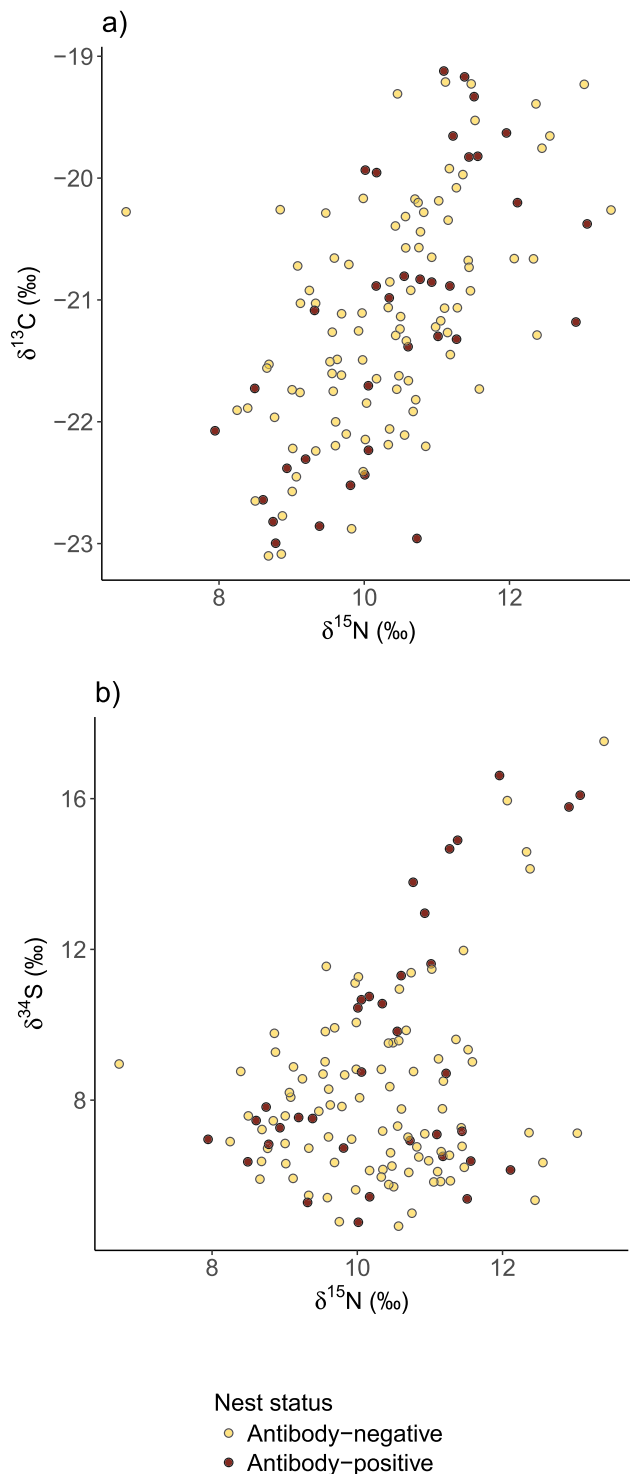
**Fig. 3.** Location and status of yellow-legged gull nests sampled in 2016 and screened for anti-*T.gondii* antibodies. Base map: Google<sup>®</sup>. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

difficult to quantify and may vary depending on the characteristics of the colonies (e.g., predators are likely to be absent from most of the colonies that are on small islets). It also stresses that gulls can be used as general wildlife sentinels for tracking certain infectious agents at the wildlife-human interface, especially through egg sampling.

In gulls, reports of *T. gondii* isolation are scarce (Karakavuk et al., 2018; Literák et al., 1992). Isolating *T. gondii* from its intermediate hosts is difficult, notably because it relies on the availability of fresh internal tissues. For detection and exposure quantification purposes, serology offers a useful tool (Dubey et al., 2005; Elmore et al., 2016, 2014; Opsteegh et al., 2011) but does not allow genetic characterization. In the present study, identification of a type II *T. gondii* isolated from a yellow-legged gull using mouse bioassay confirmed that gulls can be infected by *T. gondii*. Type II is the main lineage infecting

humans in Europe and is also the predominant lineage among both domestic and wild fauna on this continent (Shwab et al., 2014; Ajzenberg et al., 2015). The genotype described in this study using 15 MS genotyping exhibited an unreported combination of alleles within the diversity of type II lineage which supports the substantial genetic diversity within the Type II lineage (Shwab et al., 2018). Future work using molecular epidemiology approaches should thus aim at exploring the phylogenetic relationship between the strains circulating among gulls and the strains infecting other *T. gondii* hosts, including humans. This approach may be useful to quantify parasitic exchanges between these species and thus the potential involvement of yellow-legged gulls in the reservoir community of this parasite.

As mentioned above, yellow-legged gulls could be exposed to *T. gondii* through various sources of exposures notably in relation to their



**Fig. 4.** Biplots of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (a) and  $\delta^{34}\text{S}$  and  $\delta^{15}\text{N}$  (b) representing the isotopic variability of yellow-legged gull albumen samples as a response of their immunological status against *T. gondii*. Isotopic signatures (as a proxy of females' diet) and egg/nest immunological status (as a proxy of females' exposure to the parasite) does not appear to be related. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

opportunistic feeding behaviour. The spatial variations of the prevalence of antibodies against *T. gondii* could be linked to differences in environmental conditions among the considered colonies. Notably, the dryer and hotter climate in Tunisia compared to the European coasts

could limit the environmental transmission of *T. gondii*, leading to lower prevalences (Brouat et al., 2018; Dubey, 1998b). If the impact of climate on *T. gondii* transmission was confirmed, it would suggest that environmental transmission of oocysts may represent a relatively important source of exposure for gulls, in line with a previous study conducted on yellow-legged gulls (Cabezón et al., 2016). The diversity of sources of exposure could explain the absence of an evident relationship between the past exposure to the parasite and diet preferences of the individuals and colonies (inferred through  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ ), highlighting the complexity of the epidemiology of *T. gondii*. In addition, immunological information contained in egg yolk and diet information contained in albumen may represent processes happening at different time scales, blurring potential relationships. Yellow-legged gulls exhibit individual specialization, leading to some consistency of individual diets, and thus of individual isotopic signatures, at the inter-annual scale (Ceia et al., 2014). However, individual diets and isotopic signatures are expected to vary among and within years in relation to food availability. Gulls have been described as income breeders, i.e., females rely on local nutrient sources for egg formation during the clutch production period (Ramírez et al., 2011; Ruiz et al., 2000; Saino et al., 2010). Therefore, the isotopic composition of egg albumens should reflect the females' diet consumed during the egg laying period. In contrast, high anti-*T. gondii* antibody levels are considered to be maintained over the years following exposure and could thus reflect an exposure event that happened several years before laying. This methodological constraint may mask potential links between inferred diet and immunological status. Knowledge on the dynamics of the specific immune response against *T. gondii* however remains elusive. Although protective antibody levels are considered to be maintained life-long in humans, some evidence suggest high seroreversion rates in wild mammals and birds (Opsteegh et al., 2011; Sandström et al., 2013). The lack of knowledge on the specific antibody kinetics after exposure to *T. gondii* makes it difficult to interpret temporal patterns of seroprevalences, which would require the longitudinal monitoring of marked individuals (e.g., Sandström et al., 2013). In addition, clinical cases of toxoplasmosis have been reported from wild and domestic avian species (Dubey, 2002), but the impact of the parasite on wild populations has never been quantified. In particular, because vertical transmission is frequent in humans, leading to abortions and congenital malformations (Tenter et al., 2000), the presence of *T. gondii* in eggs and its impact on breeding should be further investigated. A first step in the exploration of the possibility of vertical transmission in wild birds could be to screen egg yolk samples for parasitic DNA (e.g., Rahaus et al., 2008).

Beyond *T. gondii* epidemiology, understanding the ecology and evolution of infectious diseases in wildlife has been acknowledged as critical for public health and biodiversity conservation (Daszak et al., 2000). However, wildlife investigations have been often hampered by the difficulties in collecting enough data at broad spatial and temporal scales, which has limited our current knowledge on eco-epidemiological dynamics. In this epidemiological context, tracking infectious agents in some wild species may be more rewarding than in others, depending on their epidemiological and ecological characteristics (Halliday et al., 2007). Here we illustrate how gulls could be used as sentinel to report spatial patterns of exposure to a zoonotic infectious agent, notably by sampling eggs. This approach could be applied to quantify the circulation of a wide range of other infectious agents of interest for human or animal populations (e.g., avian influenza viruses: Hammouda et al., 2011; tick-borne viruses: Arnal et al., 2014). In addition, eggs can be used to quantify exposure to various mineral and organic pollutants (Ackerman et al., 2016; Bertolero et al., 2015; Morales et al., 2016, 2012; Ramos et al., 2013; Vicente et al., 2012) and to study food availability around colonies in interaction with density-dependence strength (Real et al., 2017). Because large gulls are abundant and widely distributed (e.g., Hebert, 2015; Navarro et al., 2016), this approach could be applied at larger spatial scales to track exposure

to various infectious agents susceptible to circulate at the human-wildlife and terrestrial-marine interfaces. Finally, implementing longitudinal monitoring programs in sentinel populations (e.g., by sampling eggs of marked female gulls) could allow biological processes to be better characterized. For instance, longitudinal sampling and measurement of the immune response inform on the persistence of antibody levels, which is a key factor to infer infection dynamics both at the individual and population scales (Grenfell and Dobson, 1995).

### Data accessibility

Supplementary data associated with this article can be found on the OSU OREME only repository at <https://doi.org/10.15148/42c9bb4e-ed9b-4373-82ee-e096fdc809ca>.

### Declarations of interest

None.

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### Appendices. Supplementary data

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