LONG-TERM PREVALENCE DATA REVEALS SPILLOVER DYNAMICS IN A MULTI-HOST (*ARTEMIA*), MULTI-PARASITE (MICROSPORIDIA) COMMUNITY

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ABSTRACT

In the study of multi-host parasites, it is often found that host species contribute asymmetrically to parasite transmission, with cascading effects on parasite dynamics and overall community structure. Yet, identifying which of the host species contribute to parasite transmission and maintenance is a recurring challenge. Here, we approach this issue by taking advantage of natural variation in the community composition of host species. We studied the horizontally transmitted microsporidians *Anostracospora rigaudi* and *Enterocytospora artemiae* in a Southern French metacommunity of their brine shrimp hosts, *Artemia franciscana* and *Artemia parthenogenetica*. Within the metacommunity, patches can contain either or both of the *Artemia* host species, so that long-term prevalence data can provide a direct link between the presence of the two host species and the persistence of the two parasites. First, we show that the microsporidian *A. rigaudi* is a spillover parasite: it was unable to persist in the absence of its maintenance host *A. parthenogenetica*. This result was particularly striking in light of *A. rigaudi*'s high prevalence (in the field) and high infectivity (when tested in the lab) in both hosts. Moreover, *A. parthenogenetica*'s seasonal presence imposed seasonality on the rate of spillover, causing cyclical pseudo-endemics in the spillover host *A. franciscana*. Second, while our prevalence data was sufficient to identify *E. artemiae* as either a spillover or a facultative multi-host parasite, we could not distinguish between the two possibilities. This study supports the importance of studying the community context of multi-host parasites, and demonstrates that in appropriate multi-host systems, sampling across a range of conditions and host communities can lead to clear conclusions about the drivers of parasite persistence.

Keywords: *Artemia*, host specificity, microsporidians, multi-host, reservoir host, seasonality, spillovers

INTRODUCTION

Although many parasites infect multiple host species within their community (Cleaveland et al. 2001, Taylor et al. 2001), not all hosts are created equal. Host species vary widely in their degree of exposure to the parasite (e.g. Kilpatrick et al. 2006), competence (e.g. LoGiudice et al. 2003, Auld et al. 2017), and population density (e.g. Dobson 1995, Rhodes et al. 1998, Searle et al. 2016). These factors affect their contribution to the parasite's overall transmission, and thus to the maintenance of a persistent parasite population (Dobson 2004, Streicker et al. 2013).

Quantifying the relative contribution of each host species to the persistence of a multi-host parasite is important for several reasons. Host species that contribute substantially to the parasite's maintenance have a strong influence on its epidemiology (Viana et al. 2014) and evolutionary trajectory (Holt and Hochberg 2002, Benmayor et al. 2009, Ching et al. 2013), and can be pinpointed in the development of disease control strategies (Fenton and Pedersen 2005, Streicker et al. 2013). Furthermore, asymmetrical transmission of parasites between host species can feed back into community structure (Hatcher et al. 2006).

Unfortunately, identifying which host species contribute to the persistence of a parasite population is notoriously difficult (Viana et al. 2014). Pathogens can persist in a host population if their basic reproduction number R_0, which represents the number of secondary cases caused by a single infected host, is greater than one (Dobson 2004, Streicker et al. 2013). The host is then said to maintain the parasite population. In multi-host communities, each host species contributes to the parasite's overall R_0, and an R_0 greater than one can mask a lot of variation in those contributions. For example, Fenton et al. (2015) considered a parasite infecting a simple two-host community, finding that it may fall into three underlying categories: *i)* a facultative multi-host parasite, which can be maintained by either host in the absence of the other (R_0 would still be

greater than one if either host was removed from the community); *ii)* an obligate multi-host parasite, which can only persist when both hosts are present (R_0 would be lower than one if either host was removed); *iii)* a spillover parasite, which can be maintained indefinitely by one of the host species, but not by the other (R_0 in isolation would be respectively greater and lower than one). In the final case, the parasite's presence in the second, 'spillover' host is dependent on regular reintroductions from the 'reservoir' or 'maintenance' host (Ashford 1997, Haydon et al. 2002). Distinguishing between these fundamentally distinct, but superficially similar categories is challenging. Lab-based tests of host competence are insufficient, as the epidemiology of the system is key (Searle et al. 2016); solutions therefore include the establishment of epidemiological, statistical, or genetic models, which may be labor-intensive and sensitive to assumptions (Viana et al. 2014).

Another way to identify into which category a multi-host parasite falls is to exploit variation in the composition of natural host communities (Fig. 1). For example, a parasite which can be found in communities containing both hosts *A* and *B*, but also in isolated populations of host *A* or host *B*, is clearly a facultative multi-host parasite. In contrast, a parasite that spills over from host *A* to host *B* can be identified by its presence in communities containing host *A* or hosts *A* and *B*, but repeated absence in communities containing only host *B*. This type of observation explicitly links the presence or absence of hosts to the presence or absence of disease, and can therefore lead to direct conclusions. Previously, such conclusions have mostly been drawn from human interventions, such as the vaccination or cull of a suspected reservoir (e.g. Dobson 1995, Caley et al. 1999, MacInnes et al. 2001, Nugent 2005, Serrano et al. 2011), or through the experimental construction of host communities (e.g. Power and Mitchell 2004, Searle et al. 2016). However, the approach need not be limited to created variation: we can also look for 'natural experiments',

79 host communities whose composition varies in the field. Of course, as natural experiments are
80 not planned, due caution must be taken with regards to potential confounding factors. For
81 instance, the epidemiology of a focal parasite may be shaped by an environmental variable (e.g.
82 temperature, Altizer et al. 2006, Dunn et al. 2006), which happens to covary with the presence of
83 a certain host. To ensure that the conclusions are robust to any such effects, the observations
84 must be repeated across a range of relevant field conditions (e.g. different temperatures).

85 We illustrate the natural experiment approach using a two-host, two-parasite system: the brine
86 shrimp *Artemia franciscana* and *Artemia parthenogenetica*, and their microsporidian parasites
87 *Anostracospora rigaudi* and *Enterocytospora artemiae*. These species occur in sympatry in the
88 French saltern of Aigues-Mortes (Rode et al. 2013a), whose interconnected basins form a
89 metacommunity with patchy species composition. Taking advantage of this variation, we use
90 long-term prevalence data to place the two parasites into the epidemiological categories
91 described above (spillover, facultative multi-host, or obligate multi-host parasites), adding
92 experimental tests of infectivity to investigate some of our conclusions in more depth. We find
93 that the first microsporidian is a spillover parasite, whose spillover dynamics shape its seasonal
94 prevalence. In contrast, we are unable to identify conclusively whether the second
95 microsporidian is a spillover or a facultative multi-host parasite, and we discuss the relative
96 merits of our approach compared to projections based on epidemiological models.

97 METHODS

98 Host-parasite system

99 *Artemia* (Branchiopoda: Anostraca), also called brine shrimp, is a genus of small crustaceans
100 whose members populate salt lakes and salterns around the world. In Southern France, two

Artemia species coexist: *A. parthenogenetica* and *A. franciscana*. *A. parthenogenetica* is a parthenogenetic clade native to the area, while *A. franciscana* is a bisexual species native to the New World (Thiéry and Robert 1992, Amat et al. 2005). *A. franciscana* was first introduced to this region in the 1970's (Rode et al. 2013c).

A. rigaudi and *E. artemiae* are microsporidian parasites of *Artemia* (Rode et al. 2013a). Although highly prevalent in Southern France (Rode et al. 2013c), they have only recently been described and little is known about their ecology. Both species parasitize the gut epithelium and are continuously transmitted to new hosts via free-living spores (Rode et al. 2013b, 2013a). Previously, in a 'snapshot' sampling effort of the Mediterranean coast, *A. rigaudi* was found to be more prevalent in *A. parthenogenetica*, while *E. artemiae* was more prevalent in *A. franciscana* (Rode et al. 2013c).

We studied *A. rigaudi* and *E. artemiae* infecting *A. franciscana* and *A. parthenogenetica* in the saltern of Aigues-Mortes, in Southern France. This is a seasonal system, where both temperature (Fig. 2A) and salinity (Fig. 2B) vary throughout the year. *Artemia* hosts are present year-round in large quantities, but their average density varies by more than an order of magnitude between late winter and early summer (estimated at respectively ≤ 1 and 10-15 individuals/L; J. P. Rullmann & P. Grillas, personal communication). The species composition of the *Artemia* community also varies seasonally: *A. parthenogenetica* are entirely absent in winter, but form the majority of the population in summer (Fig. 2C).

The Aigues-Mortes saltern forms a metacommunity of *Artemia* hosts with patchy species composition. The saltern is made up of a network of large interconnected basins, between which water is allowed to flow or not as a function of the salt production process. This causes environmental factors such as salinity and food quality to vary, leading to variation in the

124 outcomes of inter-host competition: *A. franciscana* or *A. parthenogenetica* can outcompete one
125 another or coexist (Browne 1980, Browne and Halanych 1989, Barata et al. 1996b). At any given
126 time, therefore, adjoining basins can contain different host communities, though gene flow
127 between the basins is regular enough that there is no genetic spatial structure in the *Artemia*
128 populations (Nougué et al. 2015). Within basins, *Artemia* populations are well-mixed (Lenz and
129 Browne 1991), so that we can assume that the microsporidians' spore pools are shared among the
130 host species (cf. Fels 2006).

131 **Long-term field data**

132 *Data collection*

133 We obtained prevalence data for *A. rigaudi* and *E. artemiae* from 94 samples of *Artemia* spp.,
134 collected at 14 different sites in the Aigues-Mortes saltern between 2008 and 2015 (Table 1).
135 There was no visible swarming behavior at any of the sampled locations at the time of collection
136 (swarming skews microsporidian prevalence, Rode et al. 2013b). Samples were either processed
137 immediately after collection, or stored in 96% ethanol and processed later; this prevented any
138 infection-specific mortality from skewing the results. We tested a random subset of adult *A.*
139 *parthenogenetica* and/or *A. franciscana* from each sample for the presence of *A. rigaudi* and *E.*
140 *artemiae* (mean = 26.8 *Artemia* individuals/sample, *sd* = 24.3). In samples which contained both
141 *A. parthenogenetica* and *A. franciscana*, we usually tested for infection in both host species.
142 Testing was done by PCR using species-specific microsporidian primers, following Rode et al.
143 (2013a).

144 In addition to prevalence data, we had environmental and demographic data for most of the
145 samples; we call these variables "sample-specific variables". First, we knew whether each

146 sample came from a low-, middle- or high-salinity site. The absolute salinity at any given site
147 can change dramatically from one day to the next if the water flow in the saltern is redirected.
148 However, the structure of the saltern means that the salinity at some sites is always lower, higher,
149 or roughly equal to the average salinity of the saltern at that time. We classified these as low-,
150 high-, and middle-salinity sites, respectively (n = 7, 3, and 3 sites; Table 1). This relative
151 classification acts as a residual of salinity after seasonal effects are taken into account, and is not
152 sensitive to the large variability of the absolute salinity measures. We were unable to assign a
153 classification to Site 5, for which we lacked salinity information, or to the 8 samples with an
154 unknown sampling site. Second, we had information on the species composition of each sample:
155 whether it contained both *A. franciscana* and *A. parthenogenetica* (n = 65 samples), only *A.*
156 *franciscana* (n = 25 samples), or only *A. parthenogenetica* (n = 3 samples). One sample had an
157 unknown species composition.

158 *Statistical analyses*

159 The goal of our statistical analyses was to identify whether host community composition affected
160 the prevalence of *A. rigaudi* and *E. artemiae*, while controlling for the two major environmental
161 factors in the saltern (temperature and salinity). To do this, we analyzed the prevalence data of
162 each microsporidian species in two steps: first, a general model of the prevalence over time;
163 second, a more complex model that included sample-specific variables. All analyses were
164 performed using generalized additive mixed models (in R version 3.1.3, R Core Team 2015,
165 package "gamm4" Wood and Scheipl 2017), with the number of infected vs. non-infected hosts
166 as the response variable (binomial response with logit link). Model comparison was done using
167 the corrected AIC (Hurvich and Tsai 1989).

First, we constructed models to describe each microsporidian's yearly prevalence curve, using all of the collected data (n = 138 observations, of which 72 in *A. franciscana* and 66 in *A. parthenogenetica*). These models simply describe the seasonal dynamics of each parasite, i.e. they control for seasonal variation in temperature. We modelled $Prevalence_{ij} = Host\ species_i$ x s($Month_j$) + $Sample_j$, where $Prevalence_{ij}$ represents the proportion of infected host species *i* in sample *j*, s($Month_j$) represents a smoothing function of the continuous *Month* variable (the degree of smoothness is adjusted automatically, Wood and Scheipl 2017), and $Sample_j$ is a random effect controlling for the non-independence of prevalences in *A. franciscana* and *A. parthenogenetica* from the same sample. (We did not include the sampling site in our analyses because working salterns regularly re-distribute the water between basins, so *Artemia* sampled from the same site may or may not have the same genetic background.) The random effect $Sample_j$ was retained in all of the compared models. The resulting optimal models are hereafter referred to as the 'general models'.

Second, we investigated whether prevalence was affected by host species composition (our variable of interest) and the sample-specific salinity (which we wish to control for), independently of the variation already explained by our general models. We restricted our dataset to observations of *A. franciscana*, because we had no power to test the effect of species composition on prevalence in *A. parthenogenetica* (the latter was only present by itself in three samples). We further restricted our dataset to sites where the salinity classification and the species composition were known (n = 62 observations in total). For each microsporidian species, we used the general model from the previous section as a null model, and compared it to alternative models with added sample-specific terms. We modelled $Prevalence_{ij}$ = (fixed terms of the general model) x ($Presence\ of$ A. parthenogenetica$_j$ + $Relative\ salinity_j$) + $Sample_j$, where

191 *Presence of* A. parthenogenetica$_j$ and *Relative salinity*$_j$ are the sample-specific variables of
192 sample *j*, and *Sample*$_j$ is an observation-level random effect accounting for heterogeneity across
193 samples (overdispersion, Harrison 2015). Because *Host species*$_i$ was always *A. franciscana*, it
194 was no longer necessary to include this fixed effect, as we did in the general models. The other
195 fixed terms of the general model and the random effect *Sample*$_j$ were retained in all of the
196 compared models.

197 In addition, we tested whether coinfection rates (with both *A. rigaudi* and *E. artemiae*) were
198 significantly higher or lower than expected, as may be the case when parasites inhibit or facilitate
199 each other, or when certain classes of hosts are more or less vulnerable. We used Cochran-
200 Mantel-Haenszel tests (package "stats" in R version 3.1.3, R Core Team 2015) to test the
201 independence of *A. rigaudi* and *E. artemiae* prevalence across samples; these tests were executed
202 separately for *A. franciscana* and *A. parthenogenetica*.

203 **Experimental tests of microsporidian infectivity**

204 The results of our long-term field data revealed variation in prevalence among host species and
205 on a seasonal basis (see Results). Since field patterns may not reflect functional relationships, we
206 performed two experiments testing the infectivity of *A. rigaudi* and *E. artemiae* in each host and
207 at two temperatures. In both cases, we detected infection using the PCR protocol described by
208 Rode et al. (2013a), and we relied on their finding that an infection is detectable 5-6 days after
209 the host has been exposed to parasite spores.

210 *Experiment 1: Effect of temperature and host species/genotype on transmission*

211 We tested the effects of temperature and recipient host species on parasite transmission by
212 exposing non-infected *A. franciscana* or *A. parthenogenetica* to *A. rigaudi* and *E. artemiae* at 15

213 or 25°C. The purpose of this experiment was firstly to examine the effects of temperature on
214 transmission, and secondarily to provide a rough idea of variation in susceptibility across host
215 species. We did not aim to investigate asymmetric transmission (so we did not add a crossed
216 'donor host' factor), nor to thoroughly document variation in susceptibility across host genotypes
217 (so we used a logistically feasible subset).

218 The non-infected, 'recipient' hosts were adult *Artemia* spp. pulled from laboratory stock
219 collections of Aigues-Mortes lineages. For *A. parthenogenetica*, prior evidence suggests that
220 microsporidian infectivity depends on host genotype (Rode et al. 2013b), so the recipient hosts
221 were collected from four clones, P6 to P9 (P6 and P7 correspond to PAM6 and PAM7 in Nougué
222 et al. 2015). For each clone, we used females from two lines, which had been maintained
223 separately for several generations to standardize maternal effects. We named these recipient
224 groups P6.1, P6.2, P7.1, P7.2, P8.1, P8.2, P9.1, and P9.2. For *A. franciscana*, we formed four
225 replicate recipient groups, F1 to F4.

226 From each recipient group, 10 individuals were exposed to infection at 15°C, 10 individuals
227 were exposed to infection at 25°C, 5 individuals served as negative controls at 15°C, and 5
228 individuals served as negative controls at 25°C. The recipient individuals were infected via
229 exposure to infected 'donor' hosts. Donor hosts were a mixed group of *A. parthenogenetica* and
230 *A. franciscana*, collected from sites in the Aigues-Mortes saltern with high prevalences of *A.*
231 *rigaudi* and *E. artemiae* (as ascertained by preliminary PCRs). Using a field-sampled group of
232 mixed donors mimicked natural transmission conditions. Groups of 15 donors were placed in
233 strainers above the jars of 10 recipient hosts. This allowed spores to pass through, but kept
234 donors and recipients from mixing (Rode et al. 2013b). The strainers were rotated every 45
235 minutes to ensure randomized exposure. Exposure lasted for 9 hours, followed by a 6-day

incubation period. On day 6 of the incubation period, the surviving individuals were sacrificed and tested for the presence of *A. rigaudi* and *E. artemiae* as described previously.

Experiment 2: Effect of temperature and incubation time on A. rigaudi *detection*

In experiment 1, both microsporidians had low transmission success at 15°C (see Results). However, the factors underlying this temperature effect were unclear: the reduced transmission could have been caused by a direct effect of temperature on the microsporidians (e.g. on spore germination, Undeen et al. 1993), or by indirect effects of temperature on the ectothermic hosts. Cool temperatures lower the metabolic rate of *Artemia* (Engel and Angelovic 1968), thereby also lowering their defecation and ingestion rates (which would have reduced the effective inoculum size, Burns 1969, Larsen et al. 2008) and dampening their cellular metabolism (which could have slowed the accumulation of microsporidian DNA in the host, Dunn et al. 2006). Since *E. artemiae* was present in the field in winter (see Results), we could infer that its low transmission success at 15°C must be due, at least in part, to indirect effects on the hosts. However, for *A. rigaudi*, which was not found in the field in winter (see Results), it was important to disentangle these confounding effects. To do this, we designed an experiment to compare the effects of temperature and incubation time on (detected) infectivity, while maintaining a standardized spore dose. We limited this experiment to testing *A. rigaudi* infecting *A. franciscana*.

In this experiment, we allowed *A. rigaudi* infections to incubate at 15°C or 25°C for different lengths of time (6 days vs. 12 days). We exposed adult *A. franciscana* from an uninfected laboratory stock population to feces containing *A. rigaudi* spores. The feces were collected from a laboratory stock of *Artemia* spp. infected with *A. rigaudi*; the spore concentration was unknown. Exposure occurred in groups: six groups of 20 hosts were placed in 50 mL autoclaved brine and 2.8 mL fecal solution was added. Four groups were exposed at 15°C, while two groups

259 were exposed at 25°C. Exposure lasted two days, during which time all spores could be ingested
260 (Reeve 1963). After two days, hosts were separated and each individual was placed in a
261 hemolymph tube containing 2.5 mL brine; this prevented between-recipient infections later in the
262 experiment. The infection was allowed to incubate at the exposure temperature for four days,
263 after which half of the surviving hosts from each group were sacrificed, and two of the groups
264 exposed at 15°C were moved to 25°C. After a further six days of incubation, all remaining hosts
265 were sacrificed and tested for the presence of *A. rigaudi*.

266 *Statistical analyses*

267 Statistical analyses of the experiments were performed using generalized linear mixed models
268 (package "lme4", Bates et al. 2015, in R version 3.1.3, R Core Team 2015), with the number of
269 infected vs. non-infected hosts as the response variable (binomial response with logit link). The
270 significance of the predictors was tested using likelihood ratio tests.

271 For Experiment 1, we analyzed the probability of detecting an infection separately for *A. rigaudi*
272 and *E. artemiae*. Fixed effects included *Temperature*, a *Species/Genotype* factor, and their
273 interaction. As we expected to find differences among the *A. parthenogenetica* clones (Rode et
274 al. 2013b), but could not distinguish between the mixed-together *A. franciscana* families, the
275 *Species/Genotype* factor comprised 5 levels: *A. franciscana*, *A. parthenogenetica* P6, *A.*
276 *parthenogenetica* P7, *A. parthenogenetica* P8, and *A. parthenogenetica* P9. We included
277 *Recipient group* as a random effect to control for shared genetic and environmental effects.

278 For experiment 2, our statistical analyses used *Exposure temperature*, *Periods incubating at*
279 *15°C*, and *Periods incubating at 25°C* as fixed effects (with one period equal to six days), with
280 *Host group* as a random variable controlling for pseudo-replication. Since the sensitivity of our

PCR was fixed, an increase in detectability over time reflects an increase in the quantity of parasite DNA present in the host (i.e. intra-host parasite reproduction).

RESULTS

Long-term field data

First, we described the prevalence dynamics for *A. rigaudi* and *E. artemiae* throughout the year (Fig. 3, Supp. Table 1). *Anostracospora rigaudi* was strongly seasonal: it was highly prevalent from August to October, but absent in winter (*Month* effect, $\Delta AICc \geq 52.7$; Fig. 3A & B). These seasonal dynamics were not different in the two hosts, but its prevalence was higher in *A. parthenogenetica* (effect of *Host species*, $\Delta AICc \geq 156.2$; Fig. 3A vs. B). The prevalence of *E. artemiae* was highly variable and this microsporidian was not strongly seasonal; nevertheless there was statistical support for temporal effects. The precise dynamics depended on the host species: *E. artemiae*'s prevalence increased more steeply towards the end of the year in *A. parthenogenetica* than in *A. franciscana* (interaction between *Host species* and *Month* effect, $\Delta AICc \geq 4.8$; Fig. 3C & D). Overall, *E. artemiae* was more prevalent in *A. franciscana* (Fig. 3D vs. C).

Next, we introduced sample-specific effects to the general models, investigating the effects of salinity and the presence of *A. parthenogenetica* on the prevalence of the microsporidians in *A. franciscana* (Fig. 4, Supp. Table 2). For *A. rigaudi*, the species composition had a strong effect ($\Delta AICc \geq 5.0$): in the absence of *A. parthenogenetica*, the prevalence of *A. rigaudi* was almost always 0%, and never higher than 10% (Fig. 4B). In contrast, in the presence of *A. parthenogenetica* the prevalence could be very high, and the seasonal dynamics found in the general model reappeared (Fig. 4A). There was good support for an additional effect of salinity

303 classification (ΔAICc = 2.1), with the high-salinity sites typically having lower prevalences. For
304 *E. artemiae*, the salinity classification had no effect, and there was little support for an effect of
305 species composition (ΔAICc $\leq$ -2.1).

306 Finally, coinfection rates varied from 0% to 83% in our samples. Infection with *A. rigaudi* and *E.*
307 *artemiae* was independent for *A. parthenogenetica* (Mantel-Haenszel $\chi^2(1) < 0.1$, $p = 0.83$,
308 common odds ratio = 0.8), but was positively associated for *A. franciscana* (slightly more
309 coinfection observed than expected; Mantel-Haenszel $\chi^2(1) = 5.1$, $p = 0.02$, common odds ratio =
310 1.9).

311 **Experimental tests of microsporidian infectivity**

312 *Experiment 1: Effect of temperature and host/genotype on transmission*

313 The P8 genotype of *A. parthenogenetica* and the F4 replicate of *A. franciscana* were previously
314 infected (all controls tested positive for *A. rigaudi* and *E. artemiae*, respectively), so these
315 recipient groups were removed from the analysis. Surprisingly, the previous infections appeared
316 to have an inhibitory effect: none of the exposed P8s were infected with *E. artemiae* at the end of
317 the experiment ($n = 38$), and none of the exposed F4s were infected with *A. rigaudi* ($n = 21$).

318 Temperature had a clear effect on the probability of infection of both *A. rigaudi* and *E. artemiae*;
319 neither infected well at low temperatures ($\chi^2(1) = 136.3$ and 46.5, $p < 0.001$ in both cases; Fig.
320 5). *Anostracospora rigaudi* infected all three genotypes of *A. parthenogenetica* and *A.*
321 *franciscana* equally well ($\chi^2(3) = 0.8$, $p = 0.85$), but infectivity was dependent on
322 *Species/Genotype* in *E. artemiae* ($\chi^2(3) = 18.1$, $p < 0.001$; Fig. 5B). Post-hoc Tukey tests
323 indicated that *A. franciscana* and P9 were equally susceptible to *E. artemiae*, while P6 and P7

were much less susceptible ($z \leq -2.9$, $p < 0.01$). There were no significant interaction effects between temperature and *Species/Genotype*.

Experiment 2: Effect of temperature and incubation time on A. rigaudi *detection*

The probability of detecting *A. rigaudi* in *A. franciscana* – i.e. the within-host accumulation of parasite DNA – increased significantly with incubation at 25°C, but not at 15°C ($\chi^2(1) = 31.9$ and 1.9, $p < 0.001$ and $p = 0.18$, respectively; Fig. 6). There was no significant effect of the exposure temperature on infectivity ($\chi^2(1) = 0.0$, $p = 0.91$). Therefore, the apparent reduction in infectivity of *A. rigaudi* at 15°C during experiment 1 was at least partly caused by slower parasite reproduction inside the hosts, delaying its detectability. The lower rates of detection after 6 days of incubation at 25°C in this experiment compared to experiment 1 could be explained by a lower initial spore dose.

DISCUSSION

When a parasite infects multiple host species, we cannot gain an adequate understanding of its epidemiology and evolution without knowing which of the host species actually contributes to its transmission and maintenance. In this study, we exploit natural variation in the composition of *Artemia* host communities to identify their microsporidian parasites as either spillover, facultative multi-host, or obligate multi-host parasites (Fenton et al. 2015). Our primary result is that *A. rigaudi* is a spillover parasite, whose high infectivity in both hosts causes pseudo-endemic dynamics. Secondarily, we show that *E. artemiae* may be a spillover or a facultative multi-host parasite, and speculate that it is the first. Finally, we reflect on the link between the microsporidians' host specificity and seasonal dynamics, and discuss the merits of our method.

A. rigaudi *is (secretly) a spillover parasite*

Our long-term prevalence data revealed that *A. rigaudi* is a spillover parasite: it cannot persist on *A. franciscana* in the absence of *A. parthenogenetica* (Fig. 4). The presence of the parasite in *A. franciscana* must therefore be dependent on regular re-introductions from *A. parthenogenetica*, its maintenance host. This result is robust to variation in temperature (the effect can be found in every season, Fig. 3) and salinity (the effect is found across all salinity categories, results not shown), which are the main environmental factors that affect this system. Under the relevant natural conditions, populations of *A. franciscana* are unable to maintain *A. rigaudi*.

Parasites are unable to persist in a host species if that host provides little or no transmission, which may occur if its susceptibility, abundance, and/or lifetime spore production are low (Dobson 2004, Streicker et al. 2013). Our experiments ruled out the first possibility for *A. franciscana* and *A. rigaudi*, as both hosts were equally susceptible to the microsporidian (Fig. 5A). The second possibility, that sites containing only *A. franciscana* have consistently lower host densities, we also consider to be unlikely. Although the dataset used here does not contain demographic information, both observation and available evidence show that *A. franciscana*-only sites can reach similar densities as sites containing both hosts (J. P. Rullmann & P. Grillas, unpublished data). Instead, *Artemia* biomass is mainly constrained by food availability (Browne 1980), temperature (Barata et al. 1996a), and salinity (Wear and Haslett 1986), which are seasonal environmental factors (see Fig. 1). We therefore predict that the third possibility is most probable, namely that *A. franciscana* is a poor host for *A. rigaudi* because infected hosts produce few spores. Further experimental studies will be needed to confirm this hypothesis.

The contrast between *A. rigaudi*'s generalist infectivity and status as a spillover parasite is particularly interesting. At first glance, its high infectivity in the two hosts, as tested in the lab

and reflected in the field prevalences (Figs. 4A, 2A & B), may tempt us to conclude that *A. rigaudi* is a generalist parasite. Only a more detailed analysis of the field data belies this generalist infectivity, and reveals *A. rigaudi*'s 'secret' host specificity. This result is a good demonstration of the dangers of using only infectivity as an indicator of parasite specialization and long-term success (Agosta et al. 2010, Lange et al. 2015). In addition, the high infectivity of *A. rigaudi* in *A. franciscana* means that spillovers from *A. parthenogenetica* to *A. franciscana* occur frequently enough that the parasite appears to be independently present in both hosts (Fig. 2A & B). Some authors have termed such frequent spillovers 'apparent multi-host' or 'pseudo-endemic' dynamics (Fenton and Pedersen 2005, Viana et al. 2014)(cf. Dobson 1995, Rhodes et al. 1998), terms which explicitly indicate the difficulty of distinguishing such dynamics from true endemism. This makes the danger of misinterpreting the epidemiology and evolution of pseudo-endemic parasites very high, if their community context is not investigated. *Anostracospora rigaudi* itself is a good example of this problem. Based on previously available information, an earlier paper proposed that *A. rigaudi* could overwinter by infecting the invasive *A. franciscana*, thereby increasing its negative impact on the population of native *A. parthenogenetica* (a process known as "spillback", Rode et al. 2013c). In contrast, our current conclusion suggests that *A. franciscana* individuals act as inhibitory hosts, absorbing more *A. rigaudi* spores than they produce (Holt et al. 2003), and thereby diluting the effect of *A. rigaudi* on *A. parthenogenetica* (Ostfeld and Keesing 2000, Hall et al. 2009).

E. artemiae *may be a spillover or a facultative multi-host parasite*

Our prevalence data identified *E. artemiae* as either a spillover or a facultative multi-host parasite, but was insufficiently powerful to distinguish between the two possibilities. We were unable to analyze the ability of *E. artemiae* to persist on *A. parthenogenetica* because only three

391 of our samples did not contain *A. franciscana*. Although these three samples were *E. artemiae*-
392 free, this may have been due to chance. Based on our experimental test of infectivity, however,
393 we suspect that *E. artemiae* is also a spillover parasite, in this case from *A. franciscana* to *A.*
394 *parthenogenetica*. At high temperatures, *E. artemiae* is very infective in *A. franciscana* and the
395 *A. parthenogenetica* genotype P9, but not at all in the genotypes P6 and P7 (Fig. 5B). These
396 findings are consistent with the genotype-dependent prevalence data collected earlier by Rode et
397 al. (2013b) in natural populations. Since *A. parthenogenetica* populations are a mix of different
398 genotypes (Nougué et al. 2015), we can expect *E. artemiae* to infect *A. parthenogenetica* less
399 well on average. (This interpretation is consistent with our field observations, but some caution is
400 still merited. Since we only tested a small number of genotypes, it is possible that our results
401 would change given a more thorough evaluation of the within-host genetic variation for
402 resistance (cf. Luijckx et al. 2014).) The spillover effect of *E. artemiae* might therefore be even
403 stronger than that of *A. rigaudi*, as the former appears to be more specifically infective than the
404 latter. In the absence of conclusive evidence, however, this microsporidian serves as an excellent
405 example of the difficulty of using observational data to identifying host contributions to parasite
406 success (see discussion below).

407 *Presence of maintenance hosts shapes parasite seasonality*

408 When comparing the seasonal prevalence patterns of *A. rigaudi* and *E. artemiae* (Fig. 2) with the
409 seasonal changes in host community composition (Fig. 1C), it quickly becomes clear that the two
410 are linked. *Anostracospora rigaudi*'s maintenance host, *A. parthenogenetica*, is entirely seasonal,
411 being completely absent in winter and highly prevalent in late summer and autumn; logically,
412 therefore, *A. rigaudi* is also strongly seasonal. In contrast, *E. artemiae* is able to persist
413 throughout the year, with no overarching seasonal prevalence pattern. By testing infectivity at

warm and cool temperature, and comparing the two parasites, we can gain further insight into *A. rigaudi*'s absence in winter. Although low temperatures did slow parasite reproduction within the host when tested in *A. rigaudi* (Fig. 5), they did not make infection impossible for either parasite (*A. rigaudi*: filled triangle in Fig. 5; *E. artemiae*: open circles in Fig. 4B), and clearly do not preclude persistence of *E. artemiae* (Fig. 2D). Therefore, it is possible that *A. rigaudi* could persist in winter, if given the opportunity to do so by the presence of *A. parthenogenetica*. We therefore conclude that the general prevalence patterns of *A. rigaudi* and *E. artemiae* are predominantly shaped by host specialization, and not by environmental factors.

The seasonal cycles of *A. rigaudi* in its spillover host *A. franciscana* are of particular interest (Fig. 2B). These are clearly caused by the seasonal presence of the maintenance host *A. parthenogenetica*, which enforces seasonality on *A. rigaudi* and therefore on the frequency of spillovers. Seasonal cycles in prevalence occur in many host-parasite systems, and are often explained by factors such as climate, host behavior, and host immunity (Hosseini et al. 2004, Duffy et al. 2005, 2009, Grassly et al. 2005, Altizer et al. 2006, Lass and Ebert 2006). In contrast, our study presents a rare example of seasonal infections within a focal host (*A. rigaudi* in *A. franciscana*) being caused by the seasonality of an entirely different host (*A. parthenogenetica*). We know of one other description of such spillover-driven seasonality. Amman et al. (2012) investigated the prevalence of Marburg virus in bats, which causes severe hemorrhagic disease when it spills over to humans. They found that viral infections in bats peaked during birthing seasons, and that 83% of spillovers to humans occurred during these peaks. Such cases highlight the interconnected nature of communities hosting multi-host parasites, and are obviously of particular interest for the establishment of control strategies.

Extrapolating host contributions from observational data

437 In this study, we used variation in the composition of natural host communities to investigate
438 how each host contributes to the maintenance of a shared parasite. The strength of this approach
439 is illustrated by the results for *A. rigaudi*: we obtained direct evidence that under natural
440 conditions, it is a spillover parasite dependent on *A. parthenogenetica* and unable to persist in *A.*
441 *franciscana*. This conclusion, which we could not have drawn from tests of infectivity alone, has
442 crucial consequences for our interpretation of the dynamics and evolution of both parasite and
443 host. On the other hand, the results for *E. artemiae* highlight an important weakness of the
444 method, namely that all possible combinations of communities must be sampled in order to
445 obtain conclusive evidence. If one combination does not occur in the field, this constraint is
446 inescapable. In this case, methods based on the construction of full epidemiological models, such
447 as those described by Fenton et al. (2015), may be more useful. These methods first quantify
448 each host's contribution to parasite transmission using observed data on host abundance, parasite
449 prevalence, and parasite shedding; they then infer the consequences for persistence from these
450 results. While these techniques require the accurate measurement of all relevant parameters and
451 an adequate mathematical description of the epidemiology, they are not dependent on wide-scale
452 sampling across communities. Such methods are therefore useful where large-scale sampling is
453 difficult or community composition is invariable, while the natural experiment approach could
454 be particularly suited to highly variable and regularly sampled multi-host systems (e.g. *Daphnia*,
455 Ebert 2008). Independently of the method used, these (and our) studies consistently demonstrate
456 the importance of studying a parasite within its entire host community before making inferences
457 about its host specificity, epidemiological drivers, and selection environment.

ACKNOWLEDGEMENTS

The authors thank F. Gout, O. Nougué, E. Flaven, and L.-M. Chevin for help with sampling, E. Flaven for help with testing of sampled *Artemia*, and E. Decaestecker, M. A. Duffy and C. L. Murall for their constructive comments on a previous version of the manuscript. We also thank the Salins d'Aigues-Mortes for access to the saltern. We acknowledge support from the QuantEvol (206734) grant from the European Research Council.

LITERATURE CITED

Agosta, S. J., N. Janz, and D. R. Brooks. 2010. How specialists can be generalists: resolving the "parasite paradox" and implications for emerging infectious disease. Zoologia 27:151–162.

Altizer, S., A. Dobson, P. Hosseini, P. Hudson, M. Pascual, and P. Rohani. 2006. Seasonality and the dynamics of infectious diseases. Ecology Letters 9:467–484.

Amat, F., F. Hontoria, O. Ruiz, A. J. Green, M. I. Sánchez, J. Figuerola, and F. Hortas. 2005. The American brine shrimp as an exotic invasive species in the western Mediterranean. Biological Invasions 7:37–47.

Amman, B. R., S. A. Carroll, Z. D. Reed, T. K. Sealy, S. Balinandi, R. Swanepoel, A. Kemp, B. R. Erickson, J. A. Comer, S. Campbell, D. L. Cannon, M. L. Khristova, P. Atimnedi, C. D. Paddock, R. J. Kent Crockett, T. D. Flietstra, K. L. Warfield, R. Unfer, E. Katongole-Mbidde, R. Downing, J. W. Tappero, S. R. Zaki, P. E. Rollin, T. G. Ksiazek, S. T. Nichol, and J. S. Towner. 2012. Seasonal pulses of Marburg virus circulation in juvenile *Rousettus aegyptiacus* bats coincide with periods of increased risk of human infection. PLoS Pathogens 8.

479 Ashford, R. W. 1997. What it takes to be a reservoir host. Belgian Journal of Zoology 127:85–
480 90.
481 Association Infoclimat. 2001. Infoclimat. www.infoclimat.fr.
482 Auld, S. K. J. R., C. L. Searle, and M. A. Duffy. 2017. Parasite transmission in a natural
483 multihost – multiparasite community. Philosophical Transactions of the Royal Society B
484 372:20160097.
485 Barata, C., F. Hontoria, and F. Amat. 1996a. Estimation of the biomass production of *Artemia*
486 with regard to its use in aquaculture: temperature and strain effects. Aquaculture 142:171–
487 189.
488 Barata, C., F. Hontoria, F. Amat, and R. Browne. 1996b. Competition between sexual and
489 parthenogenetic *Artemia*: temperature and strain effects. Journal of Experimental Marine
490 Biology and Ecology 196:313–328.
491 Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. lme4: Linear mixed-effects models
492 using Eigen and S4. R package version 1.1-9.
493 Benmayor, R., D. J. Hodgson, G. G. Perron, and A. Buckling. 2009. Host Mixing and Disease
494 Emergence. Current Biology 19:764–767.
495 Browne, 1980. 1980. Competition experiments between parthenogenetic and sexual strains of the
496 brine shrimp, *Artemia salina*. Ecology 61:471–474.
497 Browne, R. A., and K. M. Halanych. 1989. Competition between sexual and parthenogenetic
498 *Artemia*: a re-evaluation (Branchiopoda, Anostraca). Crustaceana 57:57–71.
499 Burns, C. W. 1969. Relation between filtering rate, temperature, and body size in four species of
500 *Daphnia*. Limnology and Oceanography 14:693–700.

501 Caley, P., G. J. Hickling, P. E. Cowan, and D. U. Pfeiffer. 1999. Effects of sustained control of
502 brushtail possums on levels of *Mycobacterium bovis* infection in cattle and brushtail possum
503 populations from Hohotaka, New Zealand. New Zealand Veterinary Journal 47:133–142.
504 Ching, J., S. A. Musheyev, D. Chowdhury, J. A. Kim, Y. Choi, and J. J. Dennehy. 2013.
505 Migration enhances adaptation in bacteriophage populations evolving in ecological sinks.
506 Evolution 67:10–17.
507 Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic
508 mammals: pathogen characteristics, host range and the risk of emergence. Philosophical
509 Transactions of the Royal Society B: Biological Sciences 356:991–9.
510 Dobson, A. 2004. Population dynamics of pathogens with multiple host species. The American
511 Naturalist 164 Suppl:S64–S78.
512 Dobson, A. P. 1995. The Ecology and Epidemiology of Rinderpest Virus in Serengeti and
513 Ngorongoro Conservation Area. Page 665 *in* A. R. E. Sinclair and P. Arcese, editors.
514 Serengeti II: Dynamics, Management, and Conservation of an Ecosystem. University of
515 Chicago Press, Chicago, USA.
516 Duffy, M. A., S. R. Hall, C. E. Cáceres, and A. R. Ives. 2009. Rapid evolution, seasonality, and
517 the termination of parasite epidemics. Ecology 90:1441–1448.
518 Duffy, M. A., W. K. Kellogg, S. R. Hall, A. J. Tessier, and M. Huebner. 2005. Selective
519 predators and their parasitized prey: Are epidemics in zooplankton under top-down control?
520 Limnology and Oceanography 50:412–420.
521 Dunn, A. M., J. C. Hogg, and M. J. Hatcher. 2006. Transmission and burden and the impact of
522 temperature on two species of vertically transmitted microsporidia. International Journal for
523 Parasitology 36:409–14.

524 Ebert, D. 2008. Host-parasite coevolution: Insights from the *Daphnia*-parasite model system.
525 Current Opinion in Microbiology 11:290–301.
526 Engel, D. W., and J. W. Angelovic. 1968. The influence of salinity and temperature upon the
527 respiration of brine shrimp nauplii. Comparative Biochemistry and Physiology 26:749–752.
528 Fels, D. 2006. Transmission of the microsporidian *Glugoides intestinalis* in relation to spatial
529 structure of the host *Daphnia magna*. Archiv für Hydrobiologie 165:455–467.
530 Fenton, A., and A. B. Pedersen. 2005. Community epidemiology framework for classifying
531 disease threats. Emerging Infectious Diseases 11:1815–1821.
532 Fenton, A., D. G. Streicker, O. L. Petchey, and A. B. Pedersen. 2015. Are All Hosts Created
533 Equal? Partitioning Host Species Contributions to Parasite Persistence in Multihost
534 Communities. The American Naturalist 186:610–622.
535 Grassly, N. C., C. Fraser, and G. P. Garnett. 2005. Host immunity and synchronized epidemics
536 of syphilis across the United States. Nature 433:417–421.
537 Hall, S. R., C. R. Becker, J. L. Simonis, M. A. Duffy, A. J. Tessier, and C. E. Cáceres. 2009.
538 Friendly competition: evidence for a dilution effect among competitors in a planktonic host-
539 parasite system. Ecology 90:791–801.
540 Harrison, X. A. 2015. A comparison of observation-level random effect and Beta-Binomial
541 models for modelling overdispersion in Binomial data in ecology & evolution. PeerJ
542 3:e1114.
543 Hatcher, M. J., J. T. A. Dick, and A. M. Dunn. 2006. How parasites affect interactions between
544 competitors and predators. Ecology Letters 9:1253–1271.
545 Haydon, D. T., S. Cleaveland, L. H. Taylor, and M. K. Laurenson. 2002. Identifying reservoirs
546 of infection: a conceptual and practical challenge. Emerging Infectious Diseases 8:1468–73.

547 Holt, R. D., A. P. Dobson, M. Begon, R. G. Bowers, and E. M. Schauber. 2003. Parasite
548 establishment in host communities. Ecology Letters 6:837–842.
549 Holt, R. D., and M. E. Hochberg. 2002. Virulence on the edge: a source-sink perspective. Pages
550 104–119 *in* U. Dieckmann, J. A. J. Metz, M. W. Sabelis, and K. Sigmund, editors. Adaptive
551 dynamics of infectious diseases: in pursuit of virulence management. Cambridge University
552 Press, Cambridge, UK.
553 Hosseini, P. R., A. A. Dhondt, and A. Dobson. 2004. Seasonality and wildlife disease: how
554 seasonal birth, aggregation and variation in immunity affect the dynamics of *Mycoplasma*
555 *gallisepticum* in house finches. Proceedings of the Royal Society of London, Series B
556 271:2569–2577.
557 Hurvich, C. M., and C. L. Tsai. 1989. Regression and Time-Series Model Selection in Small
558 Samples. Biometrika 76:297–307.
559 Kilpatrick, A. M., P. Daszak, M. J. Jones, P. P. Marra, and L. D. Kramer. 2006. Host
560 heterogeneity dominates West Nile virus transmission. Proceedings of the Royal Society of
561 London, Series B 273:2327–2333.
562 Lange, B., A. P. Kaufmann, and D. Ebert. 2015. Genetic, ecological and geographic covariables
563 explaining host range and specificity of a microsporidian parasite. Journal of Animal
564 Ecology 84:1711–1719.
565 Larsen, P. S., C. V Madsen, and H. U. Riisgård. 2008. Effect of temperature and viscosity on
566 swimming velocity of the copepod *Acartia tonsa*, brine shrimp *Artemia salina* and rotifer
567 *Brachionus plicatilis*. Aquatic Biology 4:47–54.
568 Lass, S., and D. Ebert. 2006. Apparent seasonality of parasite dynamics: analysis of cyclic
569 prevalence patterns. Proceedings of the Royal Society of London, Series B 273:199–206.

570 Lenz, P. H., and R. A. Browne. 1991. Ecology of *Artemia*. Pages 237–253 *in* R. A. Browne, P.
571 Sorgeloos, and C. N. A. Trotman, editors. *Artemia* Biology. CRC Press, Boca Raton, FL.
572 Lievens, E. J. P., G. J. B. Henriques, Y. Michalakis, and T. Lenormand. 2016. Maladaptive sex
573 ratio adjustment in the invasive brine shrimp *Artemia franciscana*. Current Biology
574 26:1463–1467.
575 LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious
576 disease: effects of host diversity and community composition on Lyme disease risk.
577 Proceedings of the National Academy of Sciences of the United States of America
578 100:567–71.
579 Luijckx, P., D. Duneau, J. P. Andras, and D. Ebert. 2014. Cross-species infection trials reveal
580 cryptic parasite varieties and a putative polymorphism shared among host species.
581 Evolution 68:577–586.
582 MacInnes, C. D., S. M. Smith, R. R. Tinline, N. R. Ayers, P. Bachmann, D. G. Ball, L. A.
583 Calder, S. J. Crosgrey, C. Fielding, P. Hauschildt, J. M. Honig, D. H. Johnston, K. F.
584 Lawson, C. P. Nunan, M. A. Pedde, B. Pond, R. B. Stewart, and D. R. Voigt. 2001.
585 Elimination of rabies from red foxes in eastern Ontario. Journal of Wildlife Diseases
586 37:119–132.
587 Nougué, O., N. O. Rode, R. Jabbour-zahab, A. Ségard, L.-M. Chevin, C. R. Haag, and T.
588 Lenormand. 2015. Automixis in *Artemia*: solving a century-old controversy. Journal of
589 Evolutionary Biology 28:2337–2348.
590 Nugent, G. 2005. The Role of Wild Deer in the Epidemiology and Management of Bovine
591 Tuberculosis in New Zealand. Lincoln University.

592 Ostfeld, R. S., and F. Keesing. 2000. Biodiversity and Disease Risk : the Case of Lyme Disease
593 14:722–728.
594 Power, A. G., and C. E. Mitchell. 2004. Pathogen spillover in disease epidemics. The American
595 Naturalist 164:S79–S89.
596 R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for
597 Statistical Computing, Vienna, Austria.
598 Reeve, M. R. 1963. The filter-feeding of *Artemia* I. In pure cultures of plant cells. The Journal of
599 Experimental Biology 40:195–205.
600 Rhodes, C. J., R. P. Atkinson, R. M. Anderson, and D. W. Macdonald. 1998. Rabies in
601 Zimbabwe: reservoir dogs and the implications for disease control. Philosophical
602 Transactions of the Royal Society B: Biological Sciences 353:999–1010.
603 Rode, N. O., J. Landes, E. J. P. Lievens, E. Flaven, A. Segard, R. Jabbour-Zahab, Y. Michalakis,
604 P. Agnew, C. P. Vivarès, and T. Lenormand. 2013a. Cytological, molecular and life cycle
605 characterization of *Anostracospora rigaudi* n. g., n. sp. and *Enterocytospora artemiae* n. g.,
606 n. sp., two new microsporidian parasites infecting gut tissues of the brine shrimp *Artemia*.
607 Parasitology 140:1168–85.
608 Rode, N. O., E. J. P. Lievens, E. Flaven, A. Segard, R. Jabbour-Zahab, M. I. Sanchez, and T.
609 Lenormand. 2013b. Why join groups? Lessons from parasite-manipulated *Artemia*. Ecology
610 letters 16:493–501.
611 Rode, N. O., E. J. P. Lievens, A. Segard, E. Flaven, R. Jabbour-Zahab, and T. Lenormand.
612 2013c. Cryptic microsporidian parasites differentially affect invasive and native *Artemia*
613 spp. International Journal for Parasitology 43:795–803.

614 Searle, C. L., M. H. Cortez, K. K. Hunsberger, D. C. Grippi, I. A. Oleksy, C. L. Shaw, S. B. de la
615 Serna, C. L. Lash, K. L. Dhir, and M. A. Duffy. 2016. Population density, not host
616 competence, drives patterns of disease in an invaded community. The American Naturalist
617 188:554–566.
618 Serrano, E., P. C. Cross, M. Beneria, A. Ficapal, J. Curia, X. Marco, S. Lavín, and I. Marco.
619 2011. Decreasing prevalence of brucellosis in red deer through efforts to control disease in
620 livestock. Epidemiology and Infection 139:1626–1630.
621 Streicker, D. G., A. Fenton, and A. B. Pedersen. 2013. Differential sources of host species
622 heterogeneity influence the transmission and control of multihost parasites. Ecology Letters
623 16:975–984.
624 Taylor, L. H., S. M. Latham, and M. E. J. Woolhouse. 2001. Risk factors for human disease
625 emergence. Philosophical Transactions of the Royal Society B: Biological Sciences
626 256:983–989.
627 Thiéry, A., and F. Robert. 1992. Bisexual populations of the brine shrimp *Artemia* in Sète-
628 Villeroy and Villeneuve Saltworks (Languedoc, France). International Journal of Salt Lake
629 Research 1:47–63.
630 Undeen, A. H., M. A. Johnson, and J. J. Becnel. 1993. The Effects of Temperature on the
631 Survival of *Edhazardia aedis* (Microspora: Amblyosporidae), a Pathogen of *Aedes aegypti*.
632 Journal of Invertebrate Pathology 61:303–307.
633 Viana, M., R. Mancy, R. Biek, S. Cleaveland, P. C. Cross, J. O. Lloyd-Smith, and D. T. Haydon.
634 2014. Assembling evidence for identifying reservoirs of infection. Trends in Ecology and
635 Evolution 29:270–279.

636 Wear, R. G., and S. J. Haslett. 1986. Effects of temperature and salinity on the biology of
637 *Artemia franciscana* Kellogg from Lake Grassmere, New Zealand. 1. Growth and mortality.
638 Journal of Experimental Marine Biology and Ecology 98:153–166.
639 Wood, S., and F. Scheipl. 2017. gamm4: Generalized Additive Mixed Models using "mgcv" and
640 "lme4". R package version 0.2-5.

641 # TABLES

642 **Table 1.** Sampling sites in the Aigues-Mortes saltern.

Site	GPS coordinates	Salinity classification	*n*(samples)
Caitive Nord	43° 31′ 10.84″ N, 4° 14′ 26.13″ E	middle	3
Caitive Sud	43° 31′ 10.47″ N, 4° 14′ 26.12″ E	low	1
Fangouse	43° 30′ 16.04″ N, 4° 13′ 28.75″ E	high	6
Pont de Gazette	43° 31′ 04.63″ N, 4° 10′ 48.56″ E	low	15
Puit Romain	43° 30′ 17.83″ N, 4° 13′ 27.16″ E	middle	6
Site 1	43° 29′ 53.03″ N, 4° 14′ 23.06″ E	low	7
Site 3	43° 31′ 02.65″ N, 4° 14′ 29.53″ E	low	5
Site 4	43° 32′ 24.55″ N, 4° 13′ 25.90″ E	middle	10
Site 5	43° 32′ 14.96″ N, 4° 12′ 41.52″ E	(unknown)	1
Site 8	43° 31′ 37.17″ N, 4° 10′ 37.77″ E	low	3
Site 9	43° 32′ 40.31″ N, 4° 09′ 16.59″ E	high	20
Site 10	43° 32′ 40.10″ N, 4° 09′ 17.16″ E	high	3
Site 12	43° 31′ 55.66″ N, 4° 10′ 23.92″ E	low	1
St. Louis	43° 32′ 56.78″ N, 4° 10′ 07.95″ E	low	5
(unknown)	(unknown)	(unknown)	8

643

FIGURE LEGENDS

Figure 1. Using variation in the composition of host communities to categorize multi-host parasites. Given similar environmental conditions, the occurrence (gray squares) or absence (white squares) of persistent parasite populations can lead to direct conclusions regarding the parasite's dependence on its different hosts. Categories following Fenton et al. (2015).

Figure 2. Seasonality in the Aigues-Mortes saltern. Each point represents one data point; overlapping points shade to black. A) Average monthly temperature between 2008 and 2015. Temperature data was collected at the nearby meteorological station Le Grau-du-Roi – Repausset-Levant (Association Infoclimat 2001). The line traces the mean temperature per month. B) Salinity in the Aigues-Mortes saltern, as recorded at various sites between 2008 and 2015 (n = 193 observations). The line traces the mean salinity per month. C) Species composition of the *Artemia* community, expressed as the proportion of the population that is *A. parthenogenetica* (figure reprinted from Lievens et al. 2016). All but two of the samples were collected in Aigues-Mortes, the remaining two were collected in Gruissan, France (roughly 100 km South-West of Aigues-Mortes). The line represents a 2nd-degree polynomial local regression (LOESS) fitting.

Figure 3. General prevalence patterns of *A. rigaudi* (A & B) and *E. artemiae* (C & D) parasites in *A. parthenogenetica* (A & C) and *A. franciscana* (B & D) hosts. Solid dots are samples; the area of the dot represents the number of individuals in the sample. Overlapping dots shade to black. Solid line: predictions of the general model, represented by the marginal mean (obtained here by averaging over the predictions for all random effects). Using the marginal mean was necessary to compensate for the high variability between samples. Dashed line with open circles:

mean prevalence across samples. There is no prevalence data for *A. parthenogenetica* in January and February because this host is absent in winter.

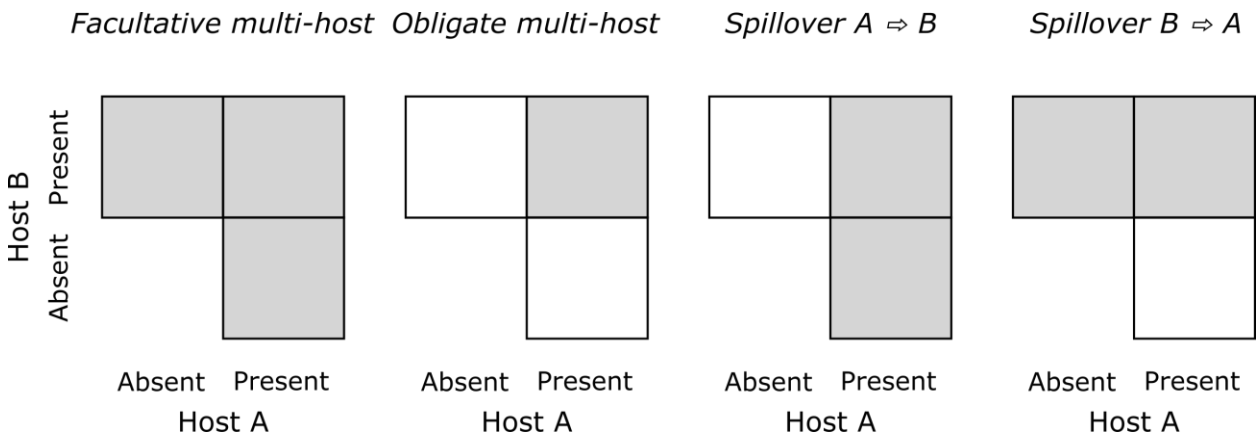
Figure 4. Decomposition of Fig. 2B: prevalence of *A. rigaudi* in *A. franciscana* when *A. parthenogenetica* is present (A) or absent (B). Solid dots are samples; the area of the dot represents the number of individuals in the sample. Overlapping dots shade to black. Solid line: predictions of the best sample-specific model, represented by the marginal mean (obtained here by averaging over the predictions for all random effects). Using the marginal mean was necessary to compensate for the high variability between samples. Dashed line with open circles: mean prevalence across samples.

Figure 5. Infectivity of *A. rigaudi* (A) and *E. artemiae* (B) as a function of temperature and host type (experiment 1). Individuals were sacrificed and tested after six days of exposure and incubation at 15°C or 25°C. Species/Genotypes were *A. franciscana* (F) and *A. parthenogenetica* isofemale lines P6, P7 and P9. Vertical lines represent the 95% confidence intervals.

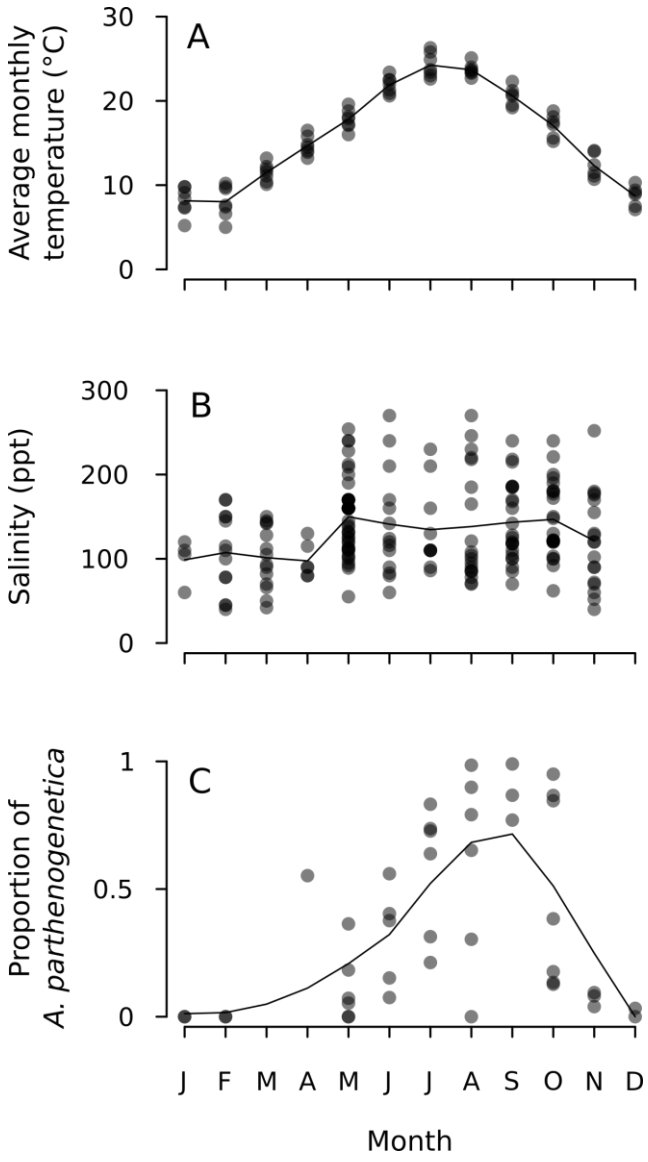
Figure 6. Detection of *A. rigaudi* infections in *A. franciscana* as a function of temperature (experiment 2). Groups of individuals were exposed to *A. rigaudi* and maintained at 15°C or 25°C for six or twelve days, after which they were sacrificed and tested. Two groups were exposed and maintained at 15°C for the first six days, and then moved to 25°C for the remaining six days. Vertical lines represent the 95% confidence intervals.

FIGURES

Figure 1.



687 **Figure 2.**



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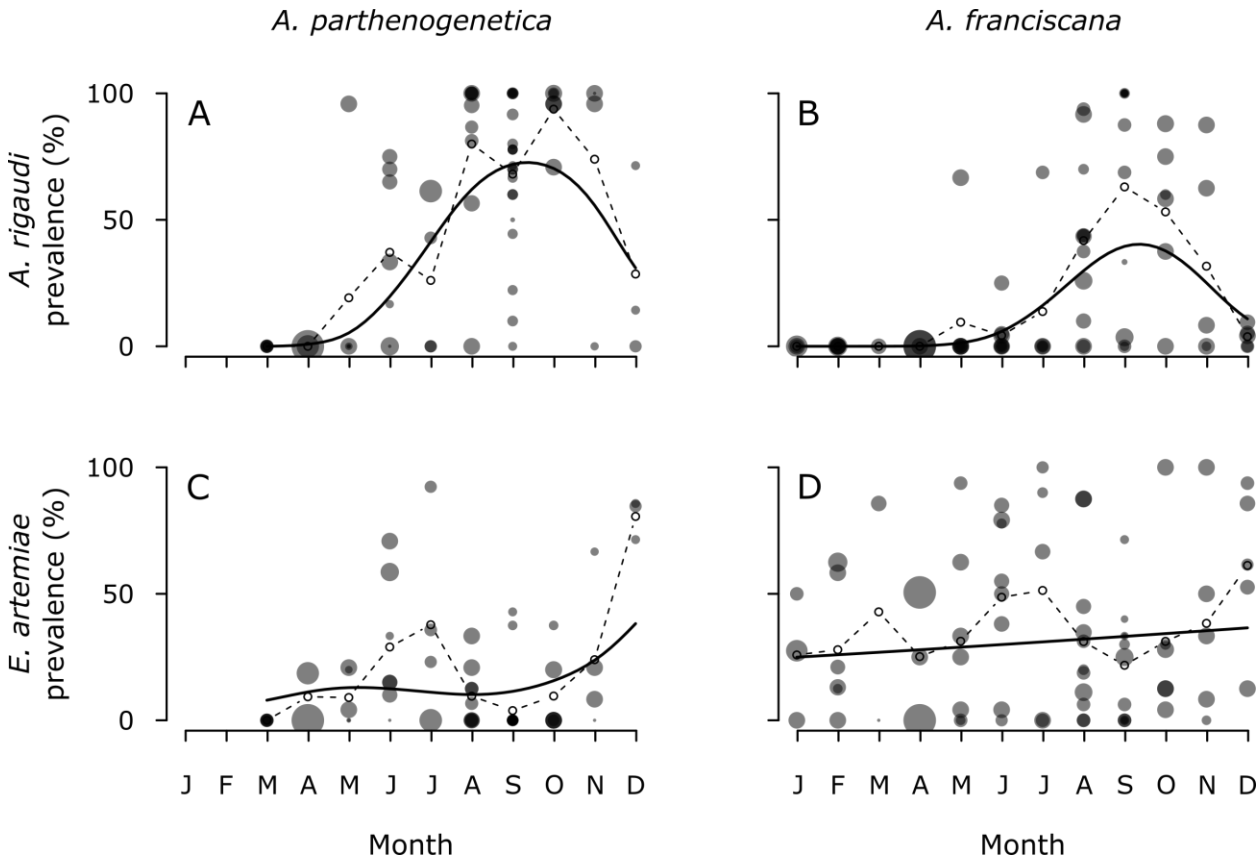
689 **Figure 3.**



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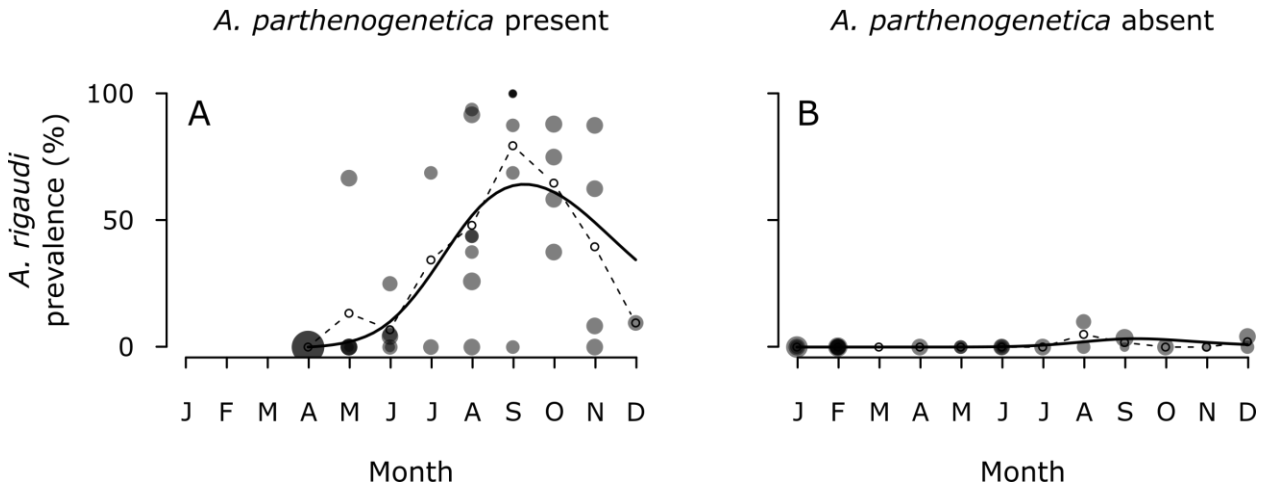
691 **Figure 4.**



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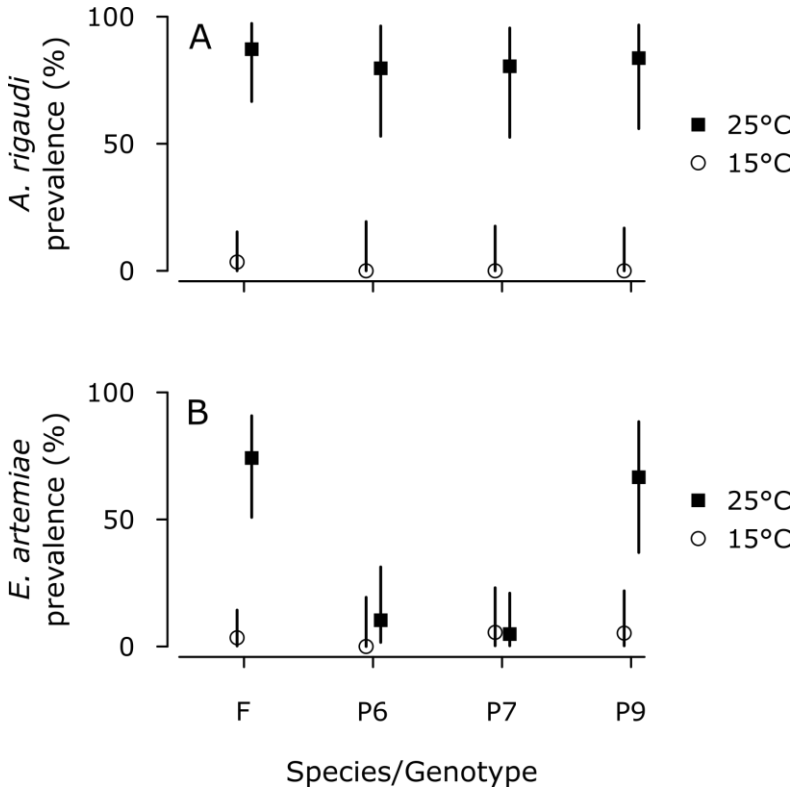

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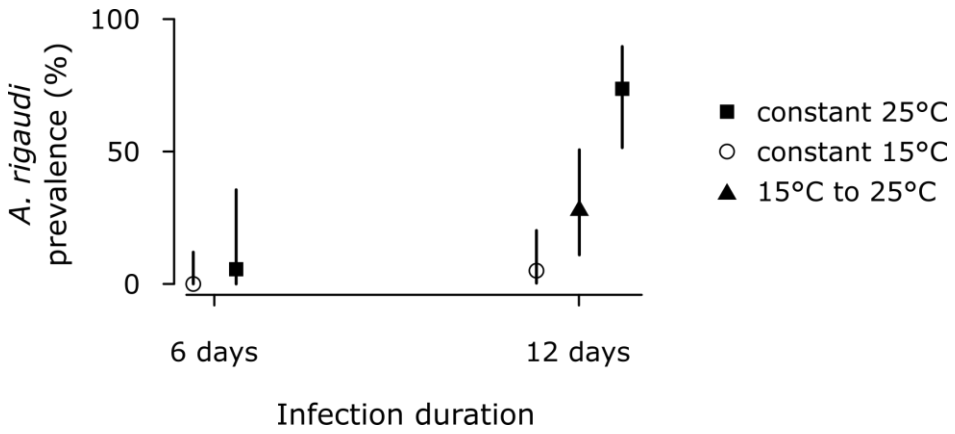
695 **Figure 6.**



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APPENDICES

Supplementary Table 1. Results of the overall models for *A. rigaudi* and *E. artemiae* infecting both *Artemia* hosts, before the addition of sample-specific variables. The full models included a smoothing function of *Month*, *Host species*, and their interaction. All models also included *Sample* as an individual-level random effect, to control for pseudoreplication. Models were compared using the difference in corrected AIC (ΔAICc); also provided are the degrees of freedom used (df) and the Akaike weights (*w*).

Model	Fixed effects			AICc	ΔAICc	df	*w*
A. rigaudi							
1	s(Month)	+ Host species		493.1	0	5	1.00
2 (full model)	s(Month)	+ Host species	+ s(Month) : Host species	506.9	13.8	7	0.00
3		Host species		545.8	52.7	3	0.00
4	s(Month)			649.3	156.2	4	0.00
5	-			707.4	214.3	2	0.00
E. artemiae							
1 (full model)	s(Month)	+ Host species	+ s(Month) : Host species	594.4	0	7	0.90
2		Host species		599.2	4.8	3	0.08
3	s(Month)	+ Host species		602.3	7.9	5	0.02
4	-			722.9	128.5	2	0.00
5	s(Month)			723.9	129.5	4	0.00

706 **Supplementary Table 2.** Results of the sample-specific models for *A. rigaudi* and *E. artemiae*
707 infecting *A. franciscana*. The full models included the fixed factors of the general model (only
708 s(*Month*); *Host species* could not be included because we only used *A. franciscana*), *Presence of*
709 A. parthenogenetica, *Relative salinity*, and their interactions with the smoothing function of
710 *Month*. All models also included *Sample* as an individual-level random effect, to control for
711 overdispersion. Models were compared using the difference in corrected AIC (ΔAICc); also
712 provided are the degrees of freedom used (df) and the the Akaike weights (*w*). Apart from the
713 general mode, only the models that fell within a cut-off value of ΔAICc = 4 are shown.

Model	Fixed effects			AICc	ΔAICc	df	*w*
A. rigaudi							
1	s(Month)	+ Presence of *A. p.*	+ Relative salinity	204.6	0	7	0.61
2	s(Month)	+ Presence of *A. p.*		206.7	2.1	5	0.21
…	…			…	…	…	…
15 (general model)	s(Month)			224.5	20.0	4	0.00
…	…			…	…	…	…
E. artemiae							
1 (general model)	s(Month)			347.7	0	4	0.64
2	s(Month)	+ Presence of *A. p.*		349.7	2.1	5	0.23
…	…			…	…	…	…

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