Morph-dependent nematode infection and its effect on host movement in the land snail *Cepaea nemoralis* (Mollusca, Gastropoda)

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Abstract

Host behaviour can be influenced by parasitic risk and infection through a variety of direct and indirect mechanisms. We can expect individuals expressing different phenotypes to also differ in the ways their behaviour is altered by parasites. We used the land snail *Cepaea nemoralis*, a species with various shell colour morphs differing in behaviour and physiology, as a model to study the link between parasite response and individual behaviour variation. We analysed metazoan parasite abundance and its relation to behaviour (movement and food intake) in snails from three shell morphs (from light unbanded to darker five-banded) and from two neighbouring populations from contrasted environments. Snails were parasitized by mites, trematodes and nematodes, from rarest to most frequent. We confirm that terrestrial gastropods can defend against infection by trapping parasitic nematodes in their shell. We show that nematode encapsulated in shells can uncover past infections even when a snail population is currently nematode-free, possibly due to parasite seasonality. We present new observations suggesting that not only nematodes but also mites might be encapsulated in shells. Infection levels varied between morphs, with darker snails harbouring fewer nematodes. Behaviour (movement and food intake) was dependent on nematode, but not trematode infection. Individuals with higher nematode load ate less, irrespective of morph and population. The most-infected morph (unbanded snails) showed reduced activity in the nematode-infected population compared to the one that was nematode-free at sampling time. As a result, parasites may cancel previously described behavioural differences between morphs. We discuss the possible mechanisms explaining morph-dependent responses to parasites, and how parasite risk may be an unseen force shaping *C. nemoralis* morph variation in space and time. We conclude on the possible ecological consequences of the link, mediated by shell colour, between thermal and immune responses.

Keywords: activity; *Brachylaima*; food intake; *Riccardoella*; shell colour polymorphism; snail host-parasite interactions

Introduction

Parasites and the behaviour of their hosts are interlinked in complex ways: parasites change the environmental context in which potential hosts express their behaviour, and directly alter actual hosts' internal phenotypic state; host behaviour may favour or hinder parasite spread. Focusing on movement-related behaviours (Binning, Shaw & Roche, 2017 for a review), potential hosts may react to the perceived risk of infection by moving to avoid contact with parasites (e.g. Davies & Knowles, 2001). Costs of infection may force infected individuals to limit or delay movements (e.g. Debeffe *et al.*, 2014; O'Dwyer, Kamiya & Poulin, 2014), possibly reshaping the distribution of infection risk in space. All these responses may be shaped by pre-existing behavioural variation, leading to feedbacks between parasite dynamics and the behaviour of uninfected and infected individuals (Ezenwa *et al.*, 2016). To untangle these dynamics, and understand their consequences, we need to map the existing relationships between parasite infection and behavioural variation.

Land snails and slugs host a diverse array of metazoan parasites and parasitoids, including flies, mites, cestodes, trematodes and nematodes (Barker, 2004; Morley & Lewis, 2008; Gracenea & Gállego, 2017; Pieterse, Malan & Ross, 2017; Gérard *et al.*, 2020). Depending on species, parasites use terrestrial gastropods as their only hosts, or as intermediate hosts with definitive hosts often being birds and mammals (Barker, 2004). Some of these parasites can cause diseases (e.g., strongylosis) in domesticated animals and humans (Meerburg, Singleton & Kijlstra, 2009; Butcher, 2016; Giannelli *et al.*, 2016).

Terrestrial gastropods can defend against metazoan parasites in several ways. They may alter their behaviour to avoid parasite-infected microhabitats (Wynne, Morris & Rae, 2016). If parasites manage to enter snails, they can be neutralized through several physiological mechanisms, including cellular and non-cellular immune responses (Furuta & Yamaguchi, 2001; Coaglio *et al.*, 2018). Snails can also trap nematodes directly in the inner layer of their shell (Rae, 2017). As in insects (González-Santoyo & Córdoba-Aguilar, 2012; San-Jose & Roulin, 2018), melanin and the phenoloxidase pathway involved in melanisation play a key role in snail defences against parasites (Coaglio *et al.*, 2018). This association between immunity and

65 pigment-related pathways could be especially relevant in land snails. Indeed, many species
66 exhibit shell colour polymorphism (Williams, 2017), which affects behaviour, thermal responses
67 and vulnerability to predators (e.g. Jones, Leith & Rawlings, 1977; Rosin *et al.*, 2011; Surmacki,
68 Ożarowska-Nowicka & Rosin, 2013; Köhler *et al.*, 2021).

69 The grove snail *Cepaea nemoralis* has a long history as an iconic model in evolutionary biology
70 (Jones *et al.*, 1977; Ożgo, 2009). Individuals can differ in background shell color (yellow, pink or
71 brown) as well as in the number of dark bands (from zero to five). Decades of research have
72 aimed to disentangle the roles of thermal selection, predation risk and neutral processes in
73 driving genetic variation in morph frequencies (Cain & Sheppard, 1954; Lamotte, 1959; Jones *et*
74 *al.*, 1977; Schilthuizen, 2013; Ożgo *et al.*, 2017). These color differences are associated with
75 behavioural and physiological differences (e.g. Kavaliers, 1992; Dahirel, Gaudu & Ansart, 2021),
76 leading to potentially adaptive phenotypic syndromes.

77 If susceptibility and response to parasite infection are shaped by host behaviour and physiology,
78 this means that (i) *C. nemoralis* morphs might consistently differ in their infection levels, (ii)
79 variation in infection between populations may alter host behaviour in a morph-dependent
80 way, (iii) infection and behaviour may also covary independently of morph. In this context, we
81 compared natural infections by metazoan parasites across two habitats differing in microclimate
82 conditions. We examined whether infections varied with morph and potentially morph-
83 dependent behaviours.

84 Based on the link between pigments and immune defence outlined above, we expected snails
85 with more dark bands hosted fewer parasites. Since darker snails are less mobile (Dahirel *et al.*,
86 2021), this is in line with some predictions from the pace-of-life syndrome hypothesis, namely
87 that fast active individuals have lower immune responses (Réale *et al.*, 2010; but see Royauté *et*
88 *al.*, 2018). We also hypothesized that metazoan parasites would have notable negative effects
89 on snail performance (McElroy & de Buron, 2014), leading to slower behaviours and changed
90 food intake in more infected snails (both at the morph level and within morphs). We expected
91 these effects on behaviour to be stronger in morph(s) more vulnerable to parasite infection.

Methods

Snail collection and maintenance

We collected snails in early May 2019 (i.e. at most ≈ 1.5 month after hibernation) from two contrasted sites in and near the village of Arçais, France. From previous studies, we know that snail parasites are present, albeit in a different species (*Cornu aspersum*, Gérard *et al.*, 2020), and that environmental differences between the sites are linked to differences in the frequency of darker *Cepaea nemoralis* morphs (Dahirel *et al.*, 2021).

- The "open habitat" population is in a garden with little to no trees and thus exposed to the sun throughout the year (≈ 46°17'50"N, 0°41'30" W).

- The "shaded habitat" population (≈ 46°18'01" N, 0°42'56" W) is in a small humid woodlot which is not directly exposed to the sun during snail activity months, and occasionally experiences partial winter flooding. The substrate is poorer in calcite than in the open habitat (Dupuis *et al.*, 1983; BRGM, 2005). Five-banded snails are more frequent there, in agreement with hypotheses on shell colour and thermal selection (Dahirel *et al.*, 2021).

We sampled 90 adult individuals per population, divided equally into unbanded, three-banded, and five-banded snails. Adults were recognizable by the presence of a reflected "lip" around the shell opening. We only sampled snails with yellow background shells, leaving aside the rarer brown and pink-shelled morphs. Sampling was random with respect to other shell features (e.g. band fusion).

We kept snails under standard conditions (20 ± 1°C, L:D 16:8) in 30 × 45 × 8 cm^3 plastic crates lined with 1-2 cm of soil kept humid and regularly renewed, with a lid made of a fine plastic mesh. Snails were grouped by morph × population of origin. Food (vegetal flour supplemented with calcium, Hélinove, Saint Paul en Pareds, France) was available *ad libitum* in Petri dishes. Four days before the experiments, we marked snails with a unique identifier on the side of their shell (using Posca pens, uni Mitsubishi Pencil, Tokyo, Japan). At the same time, we measured snail size (greater shell diameter) to the nearest 0.1 mm.

119 **Movement behaviour and food intake**

120 Behavioural experiments began two weeks after snail sampling. We tested each snail twice for
121 each trait, to separate repeatable among-individual variation from within-individual variability.
122 To ensure a manageable workload, we split the 180 snails in three groups of 60, each starting
123 the experimental sequence on a different day, with each group containing snails from all band
124 number × population combinations in equal proportions.

125 We first tested snails for movement behaviour. We stimulated snail activity by placing them in a
126 Petri dish containing ≈ 1 mm of water until they were out of their shell. We then placed each
127 snail individually on a clean lab bench, and used the time snails took to move more than 10 cm
128 in a straight line from their starting point as a measure of movement (longer times implying
129 slower individuals). Importantly, we only counted time after snails moved more than 2 cm
130 (i.e. about one shell length) from their starting point, to ensure all observed snails were truly
131 active. Some snails stayed inactive and the relevant observations were thus excluded (11
132 individuals missed one of the two tests, 6 individuals missed both). We stopped observation 20
133 min after placing snails on the lab bench (not after the start of activity).

134 Movement tests were immediately followed by food intake tests. We kept all snails individually
135 in 10 × 7 × 6 cm^3 boxes for 18 hours (including 8 hours of darkness) and provided them with 1.5
136 g of dry food (a non-limiting quantity, large enough to feed one snail for at least three days,
137 based on preliminary tests). Afterwards, we recovered, dried (60°C for 60 hours) and weighed
138 the remaining food (to the nearest 0.1 mg) to estimate food intake.

139 Snails were then placed back in their original box. The second test for each behaviour was done
140 one week later.

141 **Parasitological research**

142 After freezing at -20°C and thawing, we dissected each snail under a binocular microscope. After
143 removing the body from the shell, we looked for and counted metazoan parasites in various
144 locations: space between shell and soft body, lung, heart, kidney, body cavity and digestive
145 gland, as in Gérard et al. (2020). We found nematodes, larval trematodes and parasitic mites

146 (Acari). Trematodes were identified to the genus level as in Gérard et al. (2020), and mites using
147 Fain (2004). That level of taxonomic precision could not be reached for nematodes. However,
148 nematodes from different families consistently use/infect different organs and reach different
149 body sizes within snails (Morand, Wilson & Glen, 2004; Wilson, 2012). We assigned nematodes
150 to size classes (<1 mm, 1-3 mm, 3-5 mm, >5mm) using graph paper, and used nematode
151 location and size to infer the most likely families infecting our snails. We additionally observed
152 shells and shell fragments under brighter light to detect encapsulated parasites (Rae, 2017). By
153 simplicity, we use "live" as a shorthand to designate parasites found free within the body or
154 between the body and the shell during dissection, and thus presumably still living before snails
155 were sacrificed, by opposition with "trapped" or "encapsulated" parasites found within shells.

156 **Statistical analyses**

157 We analysed our data in a Bayesian framework, using R (version 4.2.0, R Core Team, 2022) and
158 the brms R package (Bürkner, 2017) as frontends for the Stan language (Carpenter *et al.*, 2017).
159 We used the tidybayes, bayesplot, patchwork packages for data preparation, model evaluation
160 and plotting, as well as the tidyverse suite of packages (Gabry *et al.*, 2019; Kay, 2019; Wickham
161 *et al.*, 2019; Pedersen, 2020). One snail (out of 180) died during the experiments and was
162 excluded from statistical analyses, but was used for natural history information (see **Results**).

163 We ran two models, which both included at least effects of population of origin, number of shell
164 bands, as well as population × banding interactions as categorical explanatory variables
165 (hereafter "morph and population effects").

166 We first used a Gaussian linear model to analyse how snail shell diameter (mean centred and
167 scaled to unit 1 SD), varied with morph and population effects.

168 We then ran a multivariate generalized mixed/multilevel model to examine how behaviour and
169 parasite infections varied between morph and populations, and whether these traits were
170 correlated at the individual level. Trematodes and nematodes could not be identified to species,
171 so we used the total numbers of trematodes and nematodes per host snail in our analyses (as in

e.g. Debeffe *et al.*, 2014). Mite infections were too rare to be analysed statistically (**Results**). This multivariate model included four submodels:

- live trematode abundance was modelled as a function of morph and population effects, as well as shell size, using a Poisson model. This model included random effects of test group and individual identity. The latter acts as an observation-level random effect and accounts for overdispersion (Harrison, 2014); it also allows us to build an individual-level correlation matrix connecting all four traits.
- live nematode abundance was modeled as trematode abundance, with one change. As no live nematodes were found in the shaded population, we only fit this submodel to data from the open population and removed the population fixed effect. Importantly, keeping all data instead does not change our conclusions (see **Data availability**).
- food consumption (mean-centred and scaled to unit 1SD) was analysed using a Gaussian model including the same fixed and random effects as trematode abundance. Although food consumption data are proportions (of total food), and so are well-suited to Beta models (Douma & Weedon, 2019), the Gaussian model behaved much better regarding posterior predictive checks (**Data availability**). As individuals were measured twice, the individual random effect here allows separation between within- and among individual variation.
- movement behaviour (latency to move more than 10 cm from the starting point) was inverse-transformed, mean-centred and scaled to unit 1SD, and then analysed using a Gaussian model including the same fixed and random effects as above. We initially attempted to analyse untransformed latencies using lognormal or Gamma models, but the Gaussian model performed better regarding posterior predictive checks (**Data availability**).

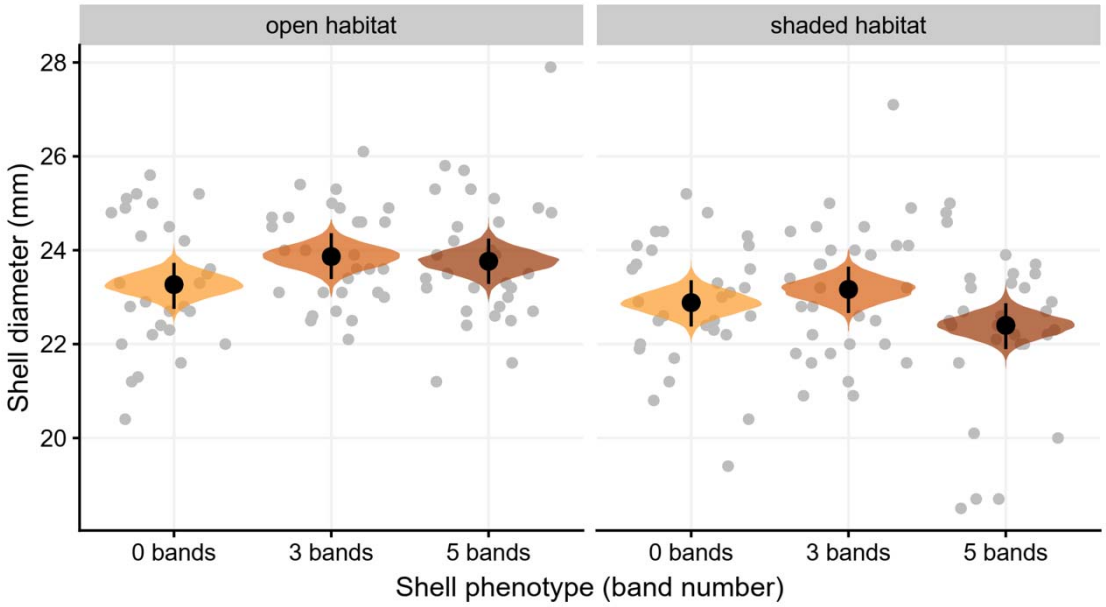
In both models we used weakly informative priors (McElreath, 2020): $\mathrm{Normal}(0,1)$ priors for fixed effects, $\mathrm{half-Normal}(0,1)$ priors for standard deviations (random effect and residual), and a $\mathrm{LKJ}(2)$ prior for the correlation matrix in the multivariate model. See **Supporting Information S1** for detailed model descriptions. We ran four chains per model for 4000

200 iterations each, with the first half of each chain used as warmup. We checked convergence and
201 mixing graphically and using the updated statistic (Vehtari *et al.*, 2021). All parameters had
202 satisfactory effective sample sizes (Vehtari *et al.*, 2021). Posteriors are summarised in text and
203 figures as means [95% Highest Posterior Density Intervals].

204 We also looked at the proportion of snails with encapsulated nematodes as a function of shell
205 morph and population. Here we did not run statistical models, and simply present these
206 proportions as qualitative information about past parasite exposure in both populations. We
207 explicitly refrained from analysing the prevalence or abundance of encapsulated nematodes at
208 the individual level within populations, as it is likely linked to cumulative exposure time/age,
209 and snail age is not known (and cannot be approximated by e.g. size, given *Cepaea nemoralis*
210 exhibits determinate growth).

211 **Results**

212 **Snail size**



213

214 **Figure 1**. Snail shell diameter as a function of band number and population of origin. Grey dots are
215 observed values, displayed along the posterior distributions of the average sizes (black dots and
216 segments are the means and 95% CI of the posteriors).

217 Snails from the shaded population were on average smaller than those from the open habitat if
218 they were three-banded ($\Delta_{[\text{shaded}-\text{open}]}$= -0.70 mm [-1.38; -0.04], **Fig. 1**) or five-banded
219 ($\Delta_{[\text{shaded}-\text{open}]}$= -1.37mm [-2.07; -0.71], **Fig. 1**); unbanded snails were however of similar size in
220 both habitats ($\Delta_{[\text{shaded}-\text{open}]}$= -0.39 mm [-1.03; 0.33], **Fig. 1**).

221

222 **Parasitological information**

223 Snails from both populations and all morphs were infected by metazoan parasites (**Figs. 2-3**, see
224 **Supporting Information S2** for detailed descriptive statistics about prevalence and infection
225 intensity). All trematodes found were live *Brachylaima* sp. metacercariae in snails' kidneys; no
226 live sporocysts were recorded and no trematodes of any stage were found trapped in shells.
227 Among the organs we examined, we detected live nematodes only within the lung and in the
228 space between the body and the shell; most of these nematodes were in the 1-3 mm size class
229 (297/371; 211/269 excluding the snail that died during the experiments). Based on this, we can
230 tentatively assume that most nematodes found belonged to the Rhabditidae family (Morand *et*
231 *al.*, 2004; Wilson, 2012). In addition we frequently (**Fig. 3**) found nematodes encapsulated in
232 shells. No live nematodes were found in individuals from the shaded population (**Fig.3**), by
233 contrast to the open population where 65.6% of snails were infected (59/90, including the dead
234 snail). However, a high number of snails contained encapsulated nematodes in their shells, in
235 both populations (**Figs 2, 3**), indicating past exposure to nematodes even in the shaded
236 population. Only one snail (unbanded from the shaded habitat) harboured live mites (*N* = 93
237 mites), identified as *Riccardoella* sp. Three other snails (all five-banded from the shaded habitat)
238 had (unidentified) mites trapped in their shell (*N* = 1, 2 and 15 mites, **Fig. 2**). As mentioned in
239 **Methods**, one snail among 180 died during the experiments; it harboured 102 live nematodes,
240 more than 4 times the number of the most infected surviving snail (**Fig. 3**).

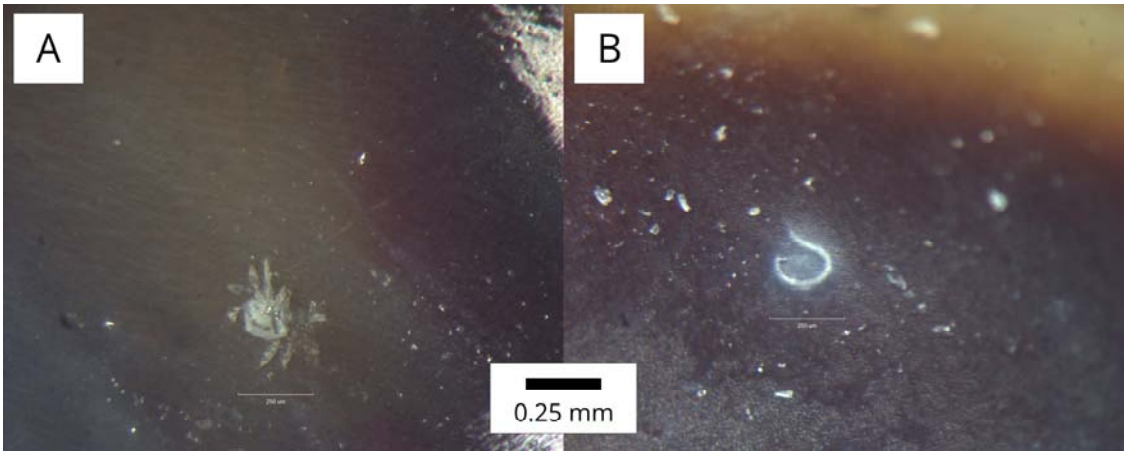


Figure 2. Examples of encapsulated parasites in *Cepaea nemoralis* shells. (A) mite; (B) nematode. Original images, including other images of encapsulated parasites, are made available with the rest of the data (see **Data availability**).

In the open population where live nematodes were found, nematode abundance was morph-dependent: five-banded snails were on average infected by about a third of the nematodes infecting other snails (= 0.29 [0.11; 0.52], = 0.34 [0.12; 0.62]; **Table 1**, **Fig. 3**), mostly due to differences in prevalence between morphs (**Supporting Information S2**). Five-banded snails also contained fewer trematodes, but only when compared to three-banded snails and only clearly in the shaded habitat (= 0.19 [0.00; 0.57]; **Fig. 3**, **Supporting Information S3**). Parasite abundance (trematode or nematode) was not linked to shell size (**Table 1**).

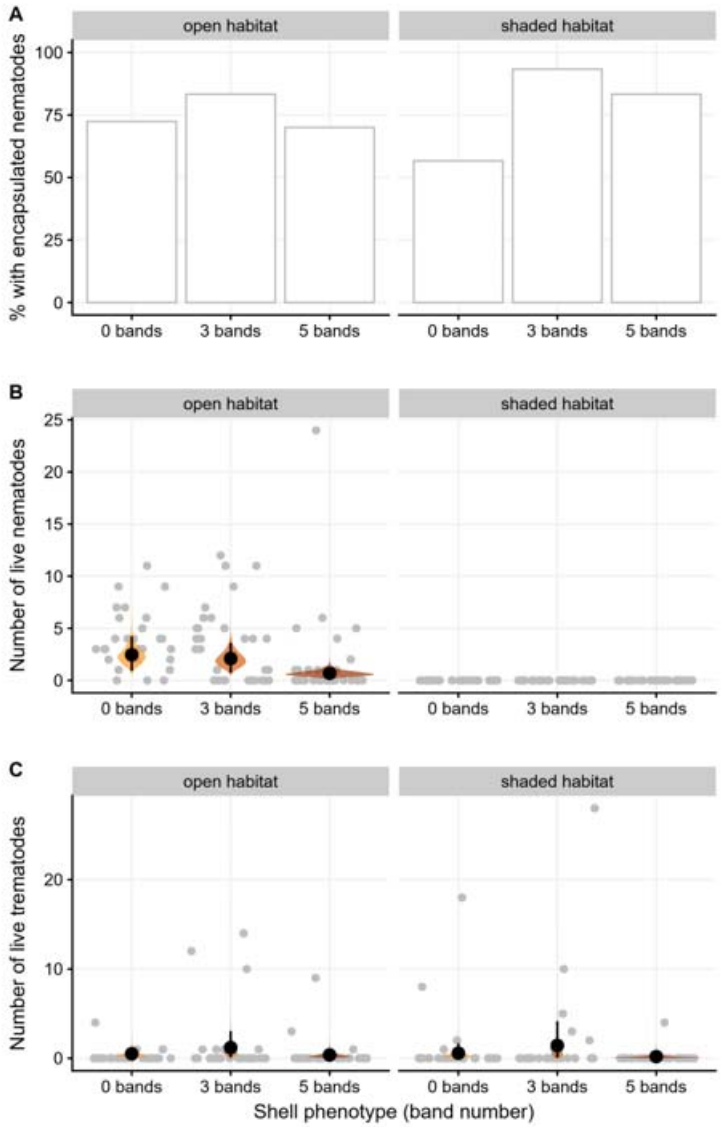


Figure 3. Parasite-related variables as a function of morph and population of origin (*N* = 179, one dead snail was excluded). (A) Proportion of snails having at least one nematode trapped in their shells; (B) Live nematode abundance; (C) Live *Brachylaima* sp. trematode abundance (metacercariae). For (B) and (C): grey dots are observed values, displayed along the posterior distributions of the average abundances based on the model described **Table 1** (black dots and segments are the means and 95% CI of the posteriors). Posterior predictions are based on fixed effects only, and the effects of shell size are averaged out. See **Supporting Information S3** for pairwise comparisons.

Table 1. Summary of posterior model coefficients for the parasite and behaviour multivariate model. Individual-level correlations between traits are presented in **Table 2**. See **Methods** for model details, and **Supporting Information S3** for pairwise comparisons among morph × population groups. Fixed effects of population and size that are different from 0, based on 95% intervals are in bold.

	Nematode abundance*	Trematode abundance	Movement	Food intake
Fixed effects				
Intercept$_{[0\ bands]}$	0.84 [0.09; 1.52]	-0.94 [-2.44; 0.48]	-0.26 [-0.66; 0.16]	-0.22 [-0.61; 0.19]
Intercept$_{[3\ bands]}$	0.67 [-0.08; 1.38]	-0.10 [-1.54; 1.33]	0.16 [-0.24; 0.56]	-0.09 [-0.50; 0.31]
Intercept$_{[5\ bands]}$	-0.47 [-1.31; 0.32]	-1.26 [-2.72; 0.23]	-0.09 [-0.50; 0.31]	-0.04 [-0.45; 0.37]
Population = "shaded"$_{[0\ bands]}$	-–	-0.02 [-1.42; 1.39]	**0.71 [0.31; 1.08]**	**0.42 [0.04; 0.83]**
Population = "shaded"$_{[3\ bands]}$	-–	0.06 [-1.31; 1.34]	-0.23 [-0.61; 0.13]	0.22 [-0.18; 0.63]
Population = "shaded"$_{[5\ bands]}$	-–	-0.88 [-2.44; 0.66]	-0.08 [-0.47; 0.32]	0.13 [-0.26; 0.55]
Shell size (standardised)	-0.19 [-0.53; 0.13]	0.06 [-0.57; 0.72]	0.09 [-0.03; 0.21]	**0.23 [0.10; 0.35]**
Random effects SDs				
Test group SD	0.53 [0.00; 1.23]	1.95 [0.94; 3.19]	0.23 [0.00; 0.70]	0.21 [0.00; 0.67]
Individual-level SD	1.14 [0.85; 1.46]	2.57 [1.99; 3.21]	0.32 [0.04; 0.54]	0.56 [0.43; 0.70]
Residual SD	-–	-–	0.92 [0.82; 1.02]	0.8 [0.72; 0.88]

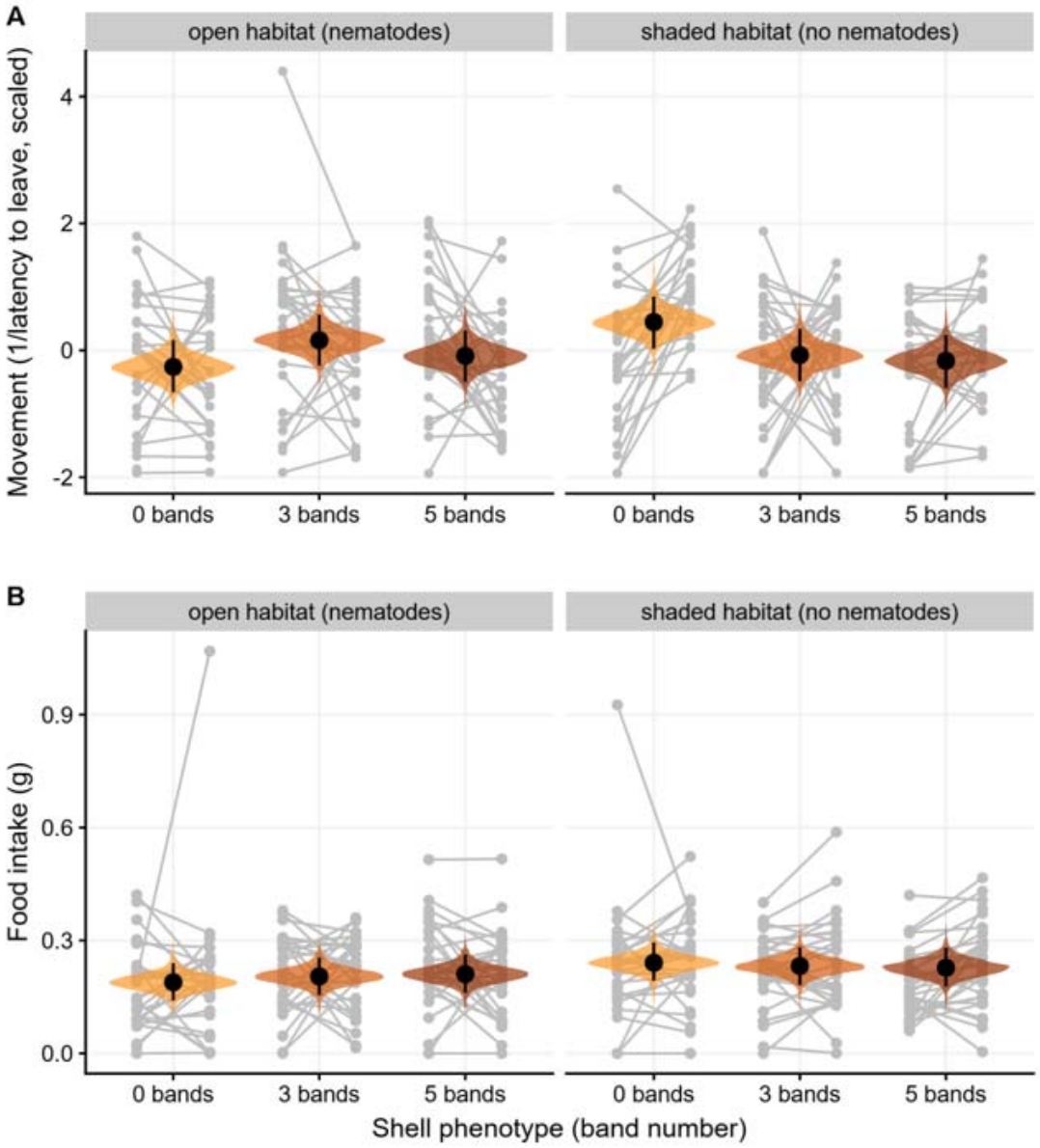
* The nematode model is only estimated for the "sun-exposed" population, since no live nematodes were recorded in the "shaded" population

Snail behaviour

Both movement and food intake were repeatable at the individual level (see individual-level SDs **Table 1**), although adjusted repeatability (sensu Nakagawa & Schielzeth, 2010) was weaker and closer to 0 for movement ($r_{[\text{movement}]}$ = 0.11 [0.00; 0.23]; $r_{[\text{food intake}]}$ = 0.29 [0.15; 0.42]; **Supporting Information S4**).

Movement depended on both population of origin and shell band number (**Table 1**, **Fig. 4**, **Supporting Information S3**). In the shaded population, five-banded snails were on average less mobile than unbanded snails ($\Delta_{[5\text{bands}-0\text{bands}]}$ = -0.61 SDs [-1.00; -0.21]). In snails from the open habitat however, there was no such difference between morphs ($\Delta_{[5\text{bands}-0\text{bands}]}$ = 0.17 SDs [-0.19; 0.59]). This was due to unbanded snails being less mobile when coming from the open habitat than when coming from the shaded population ($\Delta_{[\text{shaded}-\text{open}]}$ = 0.71 SDs [0.31; 1.08]), while there was no clear between-population difference for five-banded snails ($\Delta_{[\text{shaded}-\text{open}]}$ = -0.08 SDs [-0.47; 0.32])(**Table 1**, **Fig. 4**). In any case, there was no detectable link between movement and shell size (**Table 1**).

Larger snails tended to eat more (**Table 1**). Source population had no effect on food intake if snails were three- ($\Delta_{[\text{shaded}-\text{open}]}$= 0.03 g [-0.02; 0.08], **Fig. 4**) or five-banded ($\Delta_{[\text{shaded}-\text{open}]}$= 0.02 g [-0.03; 0.07], **Fig. 4**), but similarly to movement, unbanded snails coming from the shaded population ate more on average than their counterparts from the open habitat ($\Delta_{[\text{shaded}-\text{open}]}$= 0.05 g [0.00; 0.10], **Fig. 4**).



290

291 **Figure 4**. Behaviours as a function of morph and population of origin. (A) Movement metric (1/latency to
292 move beyond 10 cm, centered and scaled to unit 1SD); (B) Food intake. Grey dots are observed values,
293 displayed along the posterior distributions of the average abundances based on the model described in
294 **Table 1** (black dots and segments are the means and 95% CI of the posteriors). Observed values from the
295 same individual are connected by a line. Posterior predictions are based on fixed effects only, and the
296 effects of shell size are averaged out. See **Supporting Information S3** for pairwise comparisons.

297

298 Looking at the individual-level correlation matrix between traits (i.e. once fixed effects of shell
299 morph, size and population of origin are accounted for), food intake and nematode abundance
300 are negatively correlated, while all other correlations (including between nematode and
301 trematode abundance) are not markedly different from zero (**Table 2**). The food-nematode
302 correlation persists if we refit the nematode model to include data from the shaded population
303 (**-0.58 [-0.87; -0.29]**).

304

305 **Table 2**. Individual-level correlations between parasite abundances and behavioural traits. Correlations
306 involving nematode abundance are only based on individuals from the open population (see Methods);
307 results are not qualitatively different if the model is refitted to include all observations (see **Data**
308 **availability**). Correlations that are different from 0 (based on 95% intervals) are in bold.

	Nematode abundance	Trematode abundance	Movement
Trematode abundance	0.01 [-0.35; 0.37]	-–	-–
Movement	-0.24 [-0.73; 0.25]	-0.17 [-0.68; 0.28]	-–
Food intake	**-0.60 [-0.87; -0.30]**	-0.12 [-0.46; 0.19]	0.24 [-0.23; 0.71]

309

310 ## Discussion

311 To understand how individual variation in behaviour and infection interact, we studied
312 movement, food intake and natural parasite infection across two populations and three morphs
313 of the land snail *Cepaea nemoralis*. We found evidence of morph-dependent responses to
314 parasites, as well as links between parasite infection and behaviour. We additionally present
315 evidence of land snails encapsulating parasitic arthropods (mites) in their shell.

316 Snails of the darker morphs were smaller in the shaded population (**Fig. 1**). While the two
317 habitats differ along various abiotic dimensions (calcium availability in substrate,
318 microclimate…, see **Methods**), their documented effect on shell size are highly species- and
319 study-dependent, and there is no consistent evidence for or against their role in driving the

320 differences we observe (Goodfriend, 1986). Differences in parasite infection between the two
321 habitats might also influence body size, for instance if high parasite risk drives resource
322 allocation away from growth and towards immune defence (Rantala & Roff, 2005). In any case,
323 our models conditioned on size, meaning that any effect of morph/population we discuss below
324 is "size being equal". In addition, we did not find any effect of size itself on parasites or
325 movement (**Table 1**), meaning that the effects of morph/population we found are likely not
326 mediated by size differences.

327 One snail was found with live *Riccardoella* mites, hemolymph feeders with negative effects on
328 mollusc life-history and behavior (e.g. Schüpbach & Baur, 2008a). They are transmitted
329 horizontally during mating, but may find new hosts by leaving their host and following snail
330 mucus trails (Schüpbach & Baur, 2008b). However, their limited prevalence in our sample
331 prevented us from investigating their effects. Intriguingly, we found encapsulated mites in three
332 other snails (**Fig. 2**). Many land snails can defend themselves against nematode infection by
333 trapping them in their shell (Rae, 2017), including *Cepaea nemoralis* (this study, Williams & Rae,
334 2016; Rae, 2017). Based on extensive surveys, Rae (2017) wrote that encapsulation in the shell
335 is a specific response against nematode infection; our observations might contradict this
336 affirmation. *Riccardoella* infection is highly variable across populations or species, with reported
337 prevalences of 0% or near 0% being frequently observed (this study; Russo & Madec, 2013; Baur
338 & Baur, 2005; Segade *et al.,* 2013), which may explain why encapsulated mites were not
339 previously found. We need further studies to determine with certainty whether shell
340 encapsulation is a more general response to metazoan parasites or if it is a nematode-specific
341 response, as posited by Rae (2017), but occasionally and anecdotally catches other foreign
342 bodies.

343 A majority of snails in the open population were hosting nematodes, the majority of which were
344 likely Rhabditidae based on size and organ location (Morand *et al.*, 2004; Wilson, 2012).
345 Interactions between Rhabditidae and terrestrial molluscs are complex, including
346 phoresy/endozoochory, facultative parasitism, necromeny (feeding and reproducing on the host
347 snail's body after its death), and obligate larval parasites that actively infect snails by
348 e.g. entering the lung cavity (Morand *et al.*, 2004; Pieterse *et al.*, 2017; Sudhaus, 2018).

349 Delimiting these interaction types is complicated because even when they have no effects on
350 adult snails, these nematodes can have dramatically negative effects on juveniles (Grannell,
351 Cutler & Rae, 2021). By contrast to the open population, we found no live nematodes in any
352 snail from the shaded population (**Fig. 3**; note that our methods were not designed to detect
353 nematodes embedded in e.g. snail foot tissue). Thanks to nematodes trapped in shells (**Fig. 3**),
354 which integrate infections over time, we know parasitic nematodes are not truly absent from
355 the shaded population; they were simply not infecting snails at the time of sampling. The
356 "shaded" woodlot habitat is sheltered from the sun, more humid and likely colder than the
357 open one. Infections are often dependent on abiotic parameters, including temperature and
358 humidity, leading to seasonality (Altizer *et al.*, 2006). Differences in nematode infection
359 between the habitats may then be explained, at least partly, by differences in microclimate.
360 First, the different microclimates experienced by the two populations may favour different
361 nematode species within the soil community, differing in their tendency to parasitism. Second,
362 even if nematode communities are similar, conditions favourable to nematode activity,
363 development or infection may be reached only later in the year in the shaded habitat. For
364 instance, *Deroceras reticulatum* slugs were more negatively impacted by *Phasmarhabditis*
365 *hermaphrodita* infection when conditions were artificially warmed during winter (El-Danasoury
366 & Iglesias-Piñeiro, 2017), indicating that warmer conditions may favour nematode activity. In
367 any case, these results mean that any between-population differences in snail behaviour in the
368 present study need to be interpreted not only in relation with microhabitat and microclimate
369 differences, but also in terms of average infection levels.

370 In the open population where active nematodes were actually found, morph differences in
371 infection seem to validate our initial hypothesis of increased immune defence in darker snails
372 (**Fig. 3**). By contrast, no significant morph difference in immune defence was found in the closely
373 related *Cepaea hortensis* (Scheil *et al.*, 2013, 2014), but their endpoints were different
374 (phenoloxidase activity and mortality). In addition, our behavioural results (discussed below) are
375 also consistent with the hypothesis of morph-dependent immunity. These results are interesting
376 in the light of recent evidence that melanins may not be the pigments forming the dark bands in
377 *C. nemoralis* (Affenzeller *et al.*, 2020). Indeed, our initial hypothesis when designing this

experiment (in 2019) was based on the oft-seen association between immunity and melanin (San-Jose & Roulin, 2018), including in snails (Coaglio *et al.*, 2018), although other pigments may also have links to immunity (Tan *et al.*, 2020). While it is still likely that melanin-associated pathways play a role in immune defence in *Cepaea* (Scheil *et al.*, 2014), it is probably not as simple as "banded snails constitutively produce more melanin, and thus are better protected". More studies are needed to understand the mechanisms linking shell morph to nematode resistance.

Snails from all morphs and both populations were infected by *Brachylaima* trematodes (**Fig. 3**). We previously documented *Brachylaima* infecting *Cornu aspersum* in the same area (Gérard *et al.*, 2020); finding these parasites a year later and in *C. nemoralis* may indicate that they are well-implanted in local communities. Contrary to nematodes, we found no evidence that morph or population had any influence on the abundance of trematodes (**Fig. 3**). This is consistent with another study analyzing both *C. nemoralis* and *C. hortensis* (Żbikowska *et al.*, 2020). We must acknowledge however that snail infections by *Brachylaima* vary seasonally, and that spring may not be the infection peak (Gracenea & Gállego, 2017). Longitudinal studies, possibly combined with experimental infections or cures, may help confirm whether morphs respond differently to trematode risk.

Nematode and trematode numbers were uncorrelated (**Table 2**). Terrestrial gastropods are commonly infected by both nematodes and trematodes at the same time (e.g. Segade *et al.*, 2013; Antzée-Hyllseth *et al.*, 2020). Our results extend these previous studies, and suggest that despite sharing a host, nematode and trematode niches are sufficiently distinct within snails to limit spatial and trophic competition. We reiterate however that studies more expansive in space and time, including seasons and/or populations with higher prevalence and abundance of trematodes and nematodes, are needed to confirm this. Combined with finer-grained studies (e.g. at the parasite species level; Morand *et al.*, 2004), this would allow us to understand better how parasites with similar or different niches within snails interact.

We found that movement behaviour in *C. nemoralis* was morph-dependent, but that this dependency varied between populations: five-banded morphs were less mobile, but contrary to

406 Dahirel et al. (2021), this was only the case in the shaded population (**Fig. 4**). In the open
407 population, snail morphs are mobile at similarly low levels: five-banded snails do not change
408 behaviour between the two populations, while unbanded snails go down to five-banded snail
409 levels of mobility (**Fig. 4**). One of the main differences between the present study and Dahirel et
410 al. (2021) is the collection date. While here snails were collected several weeks after the onset
411 of spring activity and kept active throughout, in Dahirel et al. (2021) they were collected in late
412 fall and stored at cold temperatures inducing dormancy for months before being tested. This
413 difference may be especially relevant: winter or summer dormancy can lead to sharp decreases
414 in parasite infection in several parasite-snail systems (Solomon, Paperna & Matkovics, 1996;
415 Haeussler *et al.*, 2012; Segade *et al.*, 2013). We can thus reconcile the behavioral results from
416 Dahirel et al. (2021) from those of the present study if we assume that in the former
417 experiment, hibernation reduced parasite load across all morphs and populations, and that in
418 the present study, lower temperatures in the shaded habitat led to longer hibernation. We can
419 then interpret population differences in the present study in the light of their different infection
420 patterns. Five-banded snails' behaviour is almost unchanged between the two populations,
421 while unbanded snails from the "infected" population are slower than in the nematode-free
422 population (**Fig. 4**). This is again consistent with the idea that unbanded snails are more
423 vulnerable to parasites than banded ones. The question then becomes: is this reduction in
424 activity in infected unbanded snails simply the direct consequence of the costs of infection, or
425 does it reflect something more? For instance, infective nematodes may be able to follow mucus
426 trails from active snails (Andrus *et al.*, 2020). Reducing activity and the associated mucus
427 production may then be protective for snails, by limiting the risk of further infections.
428 Experimental manipulations of both snail infection and parasite risk in the environment may
429 help disentangle these two responses. Future studies including species-level identification of
430 nematodes will also be needed, to determine whether nematodes with more negative impacts
431 also trigger stronger behavioural responses, or if snails respond indiscriminately to nematode
432 abundance.

433 Food intake was influenced by nematode infection: highly parasitized snails ate less (**Table 2**).
434 Feeding reduction in nematode-infected individuals has been documented in other terrestrial

435 mollusc species (Antzée-Hyllseth *et al.*, 2020; Saeedizadeh & Niasti, 2020) and is an indication
436 that parasite infection is indeed costly for snails even if they are non-lethal, at least in adults
437 (Wilson *et al.*, 2000; Williams & Rae, 2016; Grannell *et al.*, 2021). By contrast, trematode
438 infection had no effect on behaviour, including feeding behaviour (**Table 2**). We however only
439 found snails infected by *Brachylaima* metacercariae (in the kidney). At the sporocyst stage,
440 *Brachylaima* parasitizes the digestive gland (e.g. Gérard *et al.*, 2020), which would likely have a
441 stronger effect on snail behaviour. Indeed, sporocysts can cause high damage, direct and
442 indirect, to that key organ for digestion and for energy storage (Segade *et al.*, 2011; Żbikowska
443 *et al.*, 2020).

444 The fact that snail response to parasites seems to be morph-dependent for nematodes, but not
445 for trematodes, has interesting implications for host-parasite and snail-predator interactions in
446 a changing world. Trematodes infecting helicid land snails are trophically transmitted to
447 vertebrate definitive hosts including rodents and birds (Gérard *et al.*, 2020; Sitko & Heneberg,
448 2020). Visual selection by thrushes (*Turdus philomelos*) has been advanced as one of the key
449 explanations for the maintenance of *Cepaea* shell polymorphism (Jones *et al.*, 1977), and
450 predation by rodents may also be morph dependent (Rosin *et al.*, 2011; Rosin *et al.*, 2013). If
451 confirmed, our results suggest that morph-dependent predation confers no additional
452 trematode-avoiding advantage. In addition, snail morphs differ in their thermal responses:
453 lighter-coloured morphs are better adapted to heat, and increase in frequency with global
454 warming or urbanization (Jones *et al.*, 1977; Silvertown *et al.*, 2011; Ożgo *et al.*, 2017; Kerstes *et*
455 *al.*, 2019). If lighter morphs are more vulnerable to infection by nematodes, then we need to
456 understand how snail immunity will interact with snail thermal tolerance to better predict how
457 snail and nematode dynamics will be altered in response to climate change (El-Danasoury &
458 Iglesias-Piñeiro, 2017; Aleuy & Kutz, 2020).

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463 ## Data availability

464 Data and R scripts to reproduce all analyses presented in this manuscript are available on Github
465 (https://github.com/mdahirel/cepaea-parasite-behaviour-2019) and archived in Zenodo (DOI:
466 https://doi.org/10.5281/zenodo.6327265). Representative images of parasites trapped in shells
467 are also archived in Zenodo (DOI: https://doi.org/10.5281/zenodo.6326305).

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471 ## References

472 Affenzeller, S., Wolkenstein, K., Frauendorf, H. & Jackson, D.J. (2020). Challenging the concept
473 that eumelanin is the polymorphic brown banded pigment in *Cepaea nemoralis*. *Sci. Rep.* **10**,
474 2442.

475 Aleuy, O.A. & Kutz, S. (2020). Adaptations, life-history traits and ecological mechanisms of
476 parasites to survive extremes and environmental unpredictability in the face of climate change.
477 *Int. J. Parasitol. Parasites Wildl.* **12**, 308–317.

478 Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M. & Rohani, P. (2006). Seasonality and
479 the dynamics of infectious diseases. *Ecol. Lett.* **9**, 467–484.

480 Andrus, P., Ingle, O., Coleman, T. & Rae, R. (2020). Gastropod parasitic nematodes
481 (*Phasmarhabditis* sp.) are attracted to hyaluronic acid in snail mucus by cGMP signalling. *J.*
482 *Helminthol.* **94**, e9.

483 Antzée-Hyllseth, H., Trandem, N., Torp, T. & Haukeland, S. (2020). Prevalence and parasite load
484 of nematodes and trematodes in an invasive slug and its susceptibility to a slug parasitic
485 nematode compared to native gastropods. *J. Invertebr. Pathol.* **173**, 107372.

486 Barker, G.M. (Ed.). (2004). *Natural enemies of terrestrial molluscs*. Wallingford, UK: CABI.

Baur, A. & Baur, B. (2005). Interpopulation variation in the prevalence and intensity of parasitic mite infection in the land snail *Arianta arbustorum*. *Invertebr. Biol.* **124**, 194–201.

Binning, S.A., Shaw, A.K. & Roche, D.G. (2017). Parasites and host performance: incorporating infection into our understanding of animal movement. *Integr. Comp. Biol.* **57**, 267–280.

BRGM. (2005). Geological map at 1:50 000 (BD-CHARM-50), department: Deux-Sèvres (79, France).

Bürkner, P.-C. (2017). brms: an R package for Bayesian multilevel models using Stan. *J. Stat. Softw.* **80**, 1–28.

Butcher, A.R. (2016). Children, snails and worms: the *Brachylaima cribbi* story. *Microbiol. Aust.* **37**, 30–33.

Cain, A.J. & Sheppard, P.M. (1954). Natural selection in *Cepaea*. *Genetics* **39**, 89–116.

Carpenter, B., Gelman, A., Hoffman, M.D., Lee, D., Goodrich, B., Betancourt, M., Brubaker, M., Guo, J., Li, P. & Riddell, A. (2017). Stan: a probabilistic programming language. *J. Stat. Softw.* **76**, 1–32.

Coaglio, A.L., Ferreira, M.A.N.D., dos Santos Lima, W. & de Jesus Pereira, C.A. (2018). Identification of a phenoloxidase- and melanin-dependent defence mechanism in *Achatina fulica* infected with *Angiostrongylus vasorum*. *Parasites Vectors* **11**, 113.

Dahirel, M., Gaudu, V. & Ansart, A. (2021). Boldness and exploration vary between shell morphs but not environmental contexts in the snail *Cepaea nemoralis*. *Ethology* 13.

Davies, M.S. & Knowles, A.J. (2001). Effects of trematode parasitism on the behaviour and ecology of a common marine snail (*Littorina littorea* (L.)). *J. Exp. Mar. Biol. Ecol.* **260**, 155–167.

Debeffe, L., Morellet, N., Verheyden-Tixier, H., Hoste, H., Gaillard, J.-M., Cargnelutti, B., Picot, D., Sevila, J. & Hewison, A.J.M. (2014). Parasite abundance contributes to condition-dependent dispersal in a wild population of large herbivore. *Oikos* **123**, 1121–1125.

Douma, J.C. & Weedon, J.T. (2019). Analysing continuous proportions in ecology and evolution: a practical introduction to beta and Dirichlet regression. *Methods Ecol. Evol.* **10**, 1412–1430.

Dupuis, J., Cariou, E., Coirier, B. & Ducloux, J. (1983). *Notice explicative, Carte géologique de la France à 1/50 000, feuille Niort (610)*. Orléans, France: BRGM.

El-Danasoury, H. & Iglesias-Piñeiro, J. (2017). Performance of the slug parasitic nematode *Phasmarhabditis hermaphrodita* under predicted conditions of winter warming. *J. Pestic. Science* **42**, 62–66.

Ezenwa, V.O., Archie, E.A., Craft, M.E., Hawley, D.M., Martin, L.B., Moore, J. & White, L. (2016). Host behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. *Proc. Royal Soc. B* **283**, 20153078.

Fain, A. (2004). Mites (Acari) parasitic and predaceous on terrestrial gastropods. In *Natural enemies of terrestrial molluscs*: 505–524. Barker, G.M. (Ed.). Wallingford, UK: CABI.

Furuta, E. & Yamaguchi, K. (2001). Haemolymph: blood cell morphology and function. In *The biology of terrestrial molluscs*: 289–306. Barker, G.M. (Ed.). Wallingford, UK: CABI.

Gabry, J., Simpson, D., Vehtari, A., Betancourt, M. & Gelman, A. (2019). Visualization in Bayesian workflow. *J. Royal Stat. Soc. A* **182**, 389–402.

Gérard, C., Ansart, A., Decanter, N., Martin, M.-C. & Dahirel, M. (2020). *Brachylaima* spp. (Trematoda) parasitizing *Cornu aspersum* (Gastropoda) in France with potential risk of human consumption. *Parasite* **27**, 15.

Giannelli, A., Cantacessi, C., Colella, V., Dantas-Torres, F. & Otranto, D. (2016). Gastropod-borne helminths: a look at the snail–parasite interplay. *Trends Parasitol.* **32**, 255–264.

González-Santoyo, I. & Córdoba-Aguilar, A. (2012). Phenoloxidase: a key component of the insect immune system. *Entomol. Exp. Appl.* **142**, 1–16.

Goodfriend, G.A. (1986). Variation in land-snail shell form and size and its causes: a review. *Syst. Zool.* **35**, 204–223.

536 Gracenea, M. & Gállego, L. (2017). Brachylaimiasis: *Brachylaima* spp. (Digenea: Brachylaimidae)
537 metacercariae parasitizing the edible snail *Cornu aspersum* (Helicidae) in Spanish public
538 marketplaces and health-associated risk factors. *J. Parasitol.* **103**, 440–450.

539 Grannell, A., Cutler, J. & Rae, R. (2021). Size-susceptibility of *Cornu aspersum* exposed to the
540 malacopathogenic nematodes *Phasmarhabditis hermaphrodita* and *P. californica*. *Biocontrol Sci.*
541 *Technol.* **31**, 1149–1160.

542 Haeussler, E.M., Pizá, J., Schmera, D. & Baur, B. (2012). Intensity of parasitic mite infection
543 decreases with hibernation duration of the host snail. *Parasitology* **139**, 1038–1044.

544 Harrison, X.A. (2014). Using observation-level random effects to model overdispersion in count
545 data in ecology and evolution. *PeerJ* **2**, e616.

546 Jones, J.S., Leith, B.H. & Rawlings, P. (1977). Polymorphism in *Cepaea*: a problem with too many
547 solutions? *Annu. Rev. Ecol. Evol. Syst.* **8**, 109–143.

548 Kavaliers, M. (1992). Opioid systems, behavioral thermoregulation and shell polymorphism in
549 the land snail, *Cepaea nemoralis*. *J. Comp. Physiol. B* **162**, 172–178.

550 Kay, M. (2019). *tidybayes: tidy data and geoms for Bayesian models*.
551 https://doi.org/10.5281/zenodo.1308151

552 Kerstes, N.A.G., Breeschoten, T., Kalkman, V.J. & Schilthuizen, M. (2019). Snail shell colour
553 evolution in urban heat islands detected via citizen science. *Commun. Biol.* **2**, 264.

554 Köhler, H.-R., Capowiez, Y., Mazzia, C., Eckstein, H., Kaczmarek, N., Bilton, M.C., Burmester,
555 J.K.Y., Capowiez, L., Chueca, L.J., Favilli, L., Gomila, J.F., Manganelli, G., Mazzuca, S.,
556 Moreno-Rueda, G., Peschke, K., Piro, A., Cardona, J.Q., Sawallich, L., Staikou, A.E., Thomassen,
557 H.A. & Triebskorn, R. (2021). Experimental simulation of environmental warming selects against
558 pigmented morphs of land snails. *Ecol. Evol.* **11**, 1111–1130.

559 Lamotte, M. (1959). Polymorphism of natural populations of *Cepaea nemoralis*. *Cold Spring*
560 *Harb. Symp. Quant. Biol.* **24**, 65–86.

561 McElreath, R. (2020). *Statistical rethinking: a Bayesian course with examples in R and Stan*. 2nd
562 edition. Boca Raton, USA: Chapman and Hall/CRC.

563 McElroy, E.J. & de Buron, I. (2014). Host performance as a target of manipulation by parasites: a
564 meta-analysis. *J. Parasitol.* **100**, 399–410.

565 Meerburg, B.G., Singleton, G.R. & Kijlstra, A. (2009). Rodent-borne diseases and their risks for
566 public health. *Crit. Rev. Microbiol.* **35**, 221–270.

567 Morand, S., Wilson, M.J. & Glen, D.M. (2004). Nematodes (Nematoda) parasitic in terrestrial
568 molluscs. In *Natural enemies of terrestrial molluscs*: 525–557. Barker, G.M. (Ed.). Wallingford,
569 UK: CABI.

570 Morley, N.J. & Lewis, J.W. (2008). The influence of climatic conditions on long-term changes in
571 the helminth fauna of terrestrial molluscs and the implications for parasite transmission in
572 southern England. *J. Helminthol.* **82**, 325–335.

573 Nakagawa, S. & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a
574 practical guide for biologists. *Biol. Rev.* **85**, 935–956.

575 O'Dwyer, K., Kamiya, T. & Poulin, R. (2014). Altered microhabitat use and movement of littorinid
576 gastropods: the effects of parasites. *Mar. Biol.* **161**, 437–445.

577 Ożgo, M. (2009). Current problems in the research of *Cepaea* polymorphism. *Folia Malacol.* **16**,
578 55–60.

579 Ożgo, M., Liew, T.-S., Webster, N.B. & Schilthuizen, M. (2017). Inferring microevolution from
580 museum collections and resampling: lessons learned from *Cepaea*. *PeerJ* **5**, e3938.

581 Pedersen, T.L. (2020). *patchwork: the composer of plots*. https://CRAN.R-
582 project.org/package=patchwork

583 Pieterse, A., Malan, A.P. & Ross, J.L. (2017). Nematodes that associate with terrestrial molluscs
584 as definitive hosts, including *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae) and its
585 development as a biological molluscicide. *J. Helminthol.* **91**, 517–527.

586 R Core Team. (2021). *R: a language and environment for statistical computing*. Vienna, Austria:
587 R Foundation for Statistical Computing.

588 Rae, R. (2017). The gastropod shell has been co-opted to kill parasitic nematodes. *Sci. Rep.* **7**,
589 4745.

590 Rantala, M.J. & Roff, D.A. (2005). An analysis of trade-offs in immune function, body size and
591 development time in the Mediterranean Field Cricket, *Gryllus bimaculatus*. *Funct. Ecol.* **19**, 323–
592 330.

593 Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V. & Montiglio, P.-O. (2010).
594 Personality and the emergence of the pace-of-life syndrome concept at the population level.
595 *Phil. Trans. R. Soc. B* **365**, 4051–4063.

596 Rosin, Z.M., Kobak, J., Lesicki, A. & Tryjanowski, P. (2013). Differential shell strength of *Cepaea*
597 *nemoralis* colour morphs—implications for their anti-predator defence. *Naturwissenschaften*
598 **100**, 843–851.

599 Rosin, Z.M., Olborska, P., Surmacki, A. & Tryjanowski, P. (2011). Differences in predatory
600 pressure on terrestrial snails by birds and mammals. *J. Biosci.* **36**, 691–699.

601 Royauté, R., Berdal, M.A., Garrison, C.R. & Dochtermann, N.A. (2018). Paceless life? A meta-
602 analysis of the pace-of-life syndrome hypothesis. *Behav. Ecol. Sociobiol.* **72**, 64.

603 Russo, J. & Madec, L. (2013). Linking immune patterns and life history shows two distinct
604 defense strategies in land snails (Gastropoda, Pulmonata). *Physiol. Biochem. Zool.* **86**, 193–204.

605 Saeedizadeh, A. & Niasti, F. (2020). Response of grey slug to entomopathogenic nematodes.
606 *Bragantia* **79**, 447–456.

607 San-Jose, L.M. & Roulin, A. (2018). Toward understanding the repeated occurrence of
608 associations between melanin-based coloration and multiple phenotypes. *Am. Nat.* **192**, 111–
609 130.

610 Scheil, A.E., Hilsmann, S., Triebskorn, R. & Köhler, H.-R. (2013). Shell colour polymorphism,
611 injuries and immune defense in three helicid snail species, *Cepaea hortensis*, *Theba pisana* and
612 *Cornu aspersum maximum*. *Results Immunol.* **3**, 73–78.

613 Scheil, A.E., Hilsmann, S., Triebskorn, R. & Köhler, H.-R. (2014). Shell colouration and parasite
614 tolerance in two helicoid snail species. *J. Invertebr. Pathol.* **117**, 1–8.

615 Schilthuizen, M. (2013). Rapid, habitat-related evolution of land snail colour morphs on
616 reclaimed land. *Heredity* **110**, 247–252.

617 Schüpbach, H.U. & Baur, B. (2008a). Parasitic mites influence fitness components of their host,
618 the land snail *Arianta arbustorum*. *Invertebr. Biol.* **127**, 350–356.

619 Schüpbach, H.U. & Baur, B. (2008b). Experimental evidence for a new transmission route in a
620 parasitic mite and its mucus-dependent orientation towards the host snail. *Parasitology* **135**,
621 1679–1684.

622 Segade, P., Crespo, C., García, N., García-Estévez, J.M., Arias, C. & Iglesias, R. (2011).
623 *Brachylaima aspersae* n. sp. (Digenea: Brachylaimidae) infecting farmed snails in NW Spain:
624 Morphology, life cycle, pathology, and implications for heliciculture. *Vet. Parasitol.* **175**, 273–
625 286.

626 Segade, P., García-Estévez, J., Arias, C. & Iglesias, R. (2013). Parasitic infections in mixed system-
627 based heliciculture farms: dynamics and key epidemiological factors. *Parasitology* **140**, 482–497.

628 Silvertown, J., Cook, L., Cameron, R., Dodd, M., McConway, K., Worthington, J., Skelton, P.,
629 Anton, C., Bossdorf, O., Baur, B., Schilthuizen, M., Fontaine, B., Sattmann, H., Bertorelle, G.,
630 Correia, M., Oliveira, C., Pokryszko, B., Ożgo, M., Stalažs, A., Gill, E., Rammul, Ü., Sólymos, P.,
631 Féher, Z. & Juan, X. (2011). Citizen science reveals unexpected continental-scale evolutionary
632 change in a model organism. *PLOS ONE* **6**, e18927.

633 Sitko, J. & Heneberg, P. (2020). Emerging helminthiases of song thrush (*Turdus philomelos*) in
634 Central Europe. *Parasitol Res.* **119**, 4123–4134.

Solomon, A., Paperna, I. & Matkovics, A. (1996). The influence of aestivation in land snails on the larval development of *Muellerius* cf. *capillaris* (Metastrongyloidea: Protostrongylidae). *Int. J. Parasitol.* **26**, 363–367.

Sudhaus, W. (2018). Dispersion of nematodes (Rhabditida) in the guts of slugs and snails. *Soil Org.* **90**, 101–114.

Surmacki, A., Ożarowska-Nowicka, A. & Rosin, Z.M. (2013). Color polymorphism in a land snail *Cepaea nemoralis* (Pulmonata: Helicidae) as viewed by potential avian predators. *Naturwissenschaften* **100**, 533–540.

Tan, K., Zhang, H., Lim, L.-S., Ma, H., Li, S. & Zheng, H. (2020). Roles of carotenoids in invertebrate immunology. *Front. Immunol.* **10**.

Vehtari, A., Gelman, A., Simpson, D., Carpenter, B. & Bürkner, P.-C. (2021). Rank-normalization, folding, and localization: an improved $\hat{R}$ for assessing convergence of MCMC (with discussion). *Bayesian Anal.* **16**, 667–718.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K. & Yutani, H. (2019). Welcome to the Tidyverse. *J. Open Source Softw.* **4**, 1686.

Williams, A. & Rae, R. (2016). *Cepaea nemoralis* (Linnaeus, 1758) uses its shell as a defence mechanism to trap and kill parasitic nematodes. *J. Molluscan Stud.* **82**, 349–350.

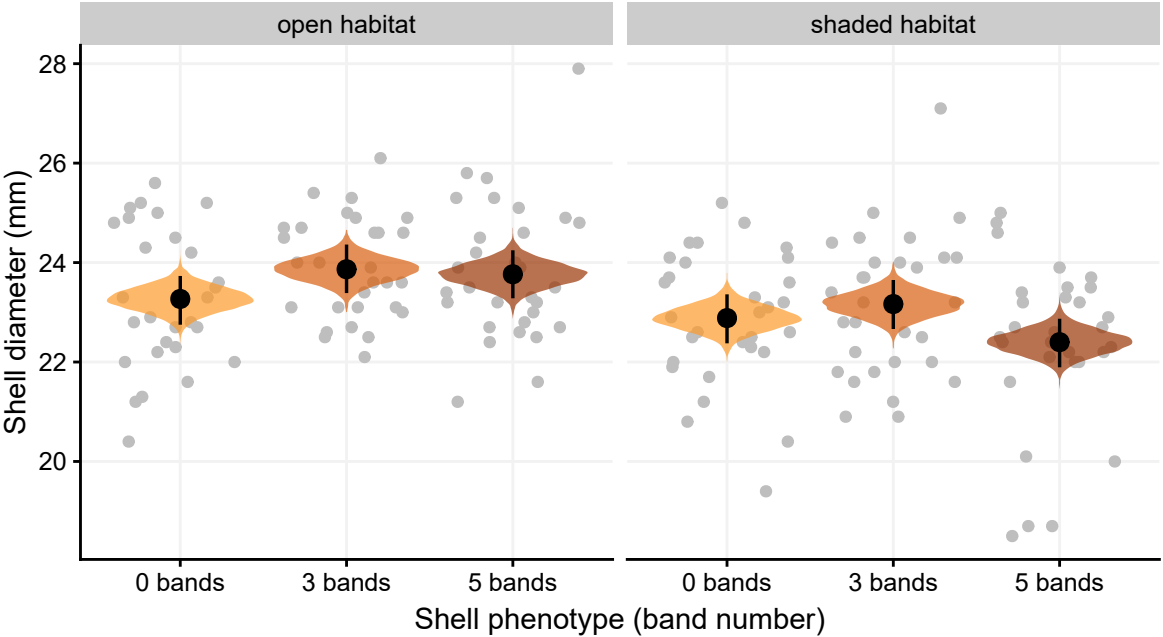
Williams, S.T. (2017). Molluscan shell colour. *Biol. Rev.* **92**, 1039–1058.

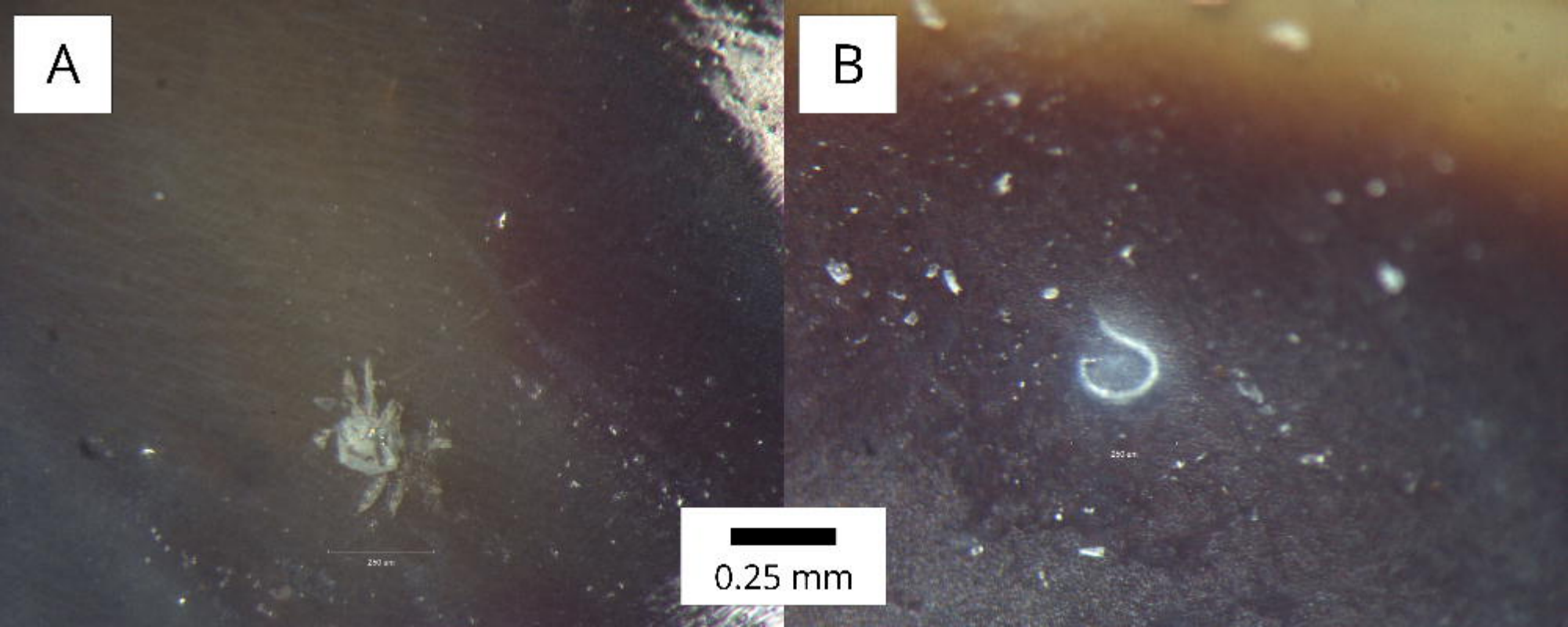
Wilson, M.J. (2012). Pathogens and parasites of terrestrial molluscs. In *Manual of techniques in invertebrate pathology*: 427–439. Lacey, L.A. (Ed.). Oxford; New York: Academic Press.

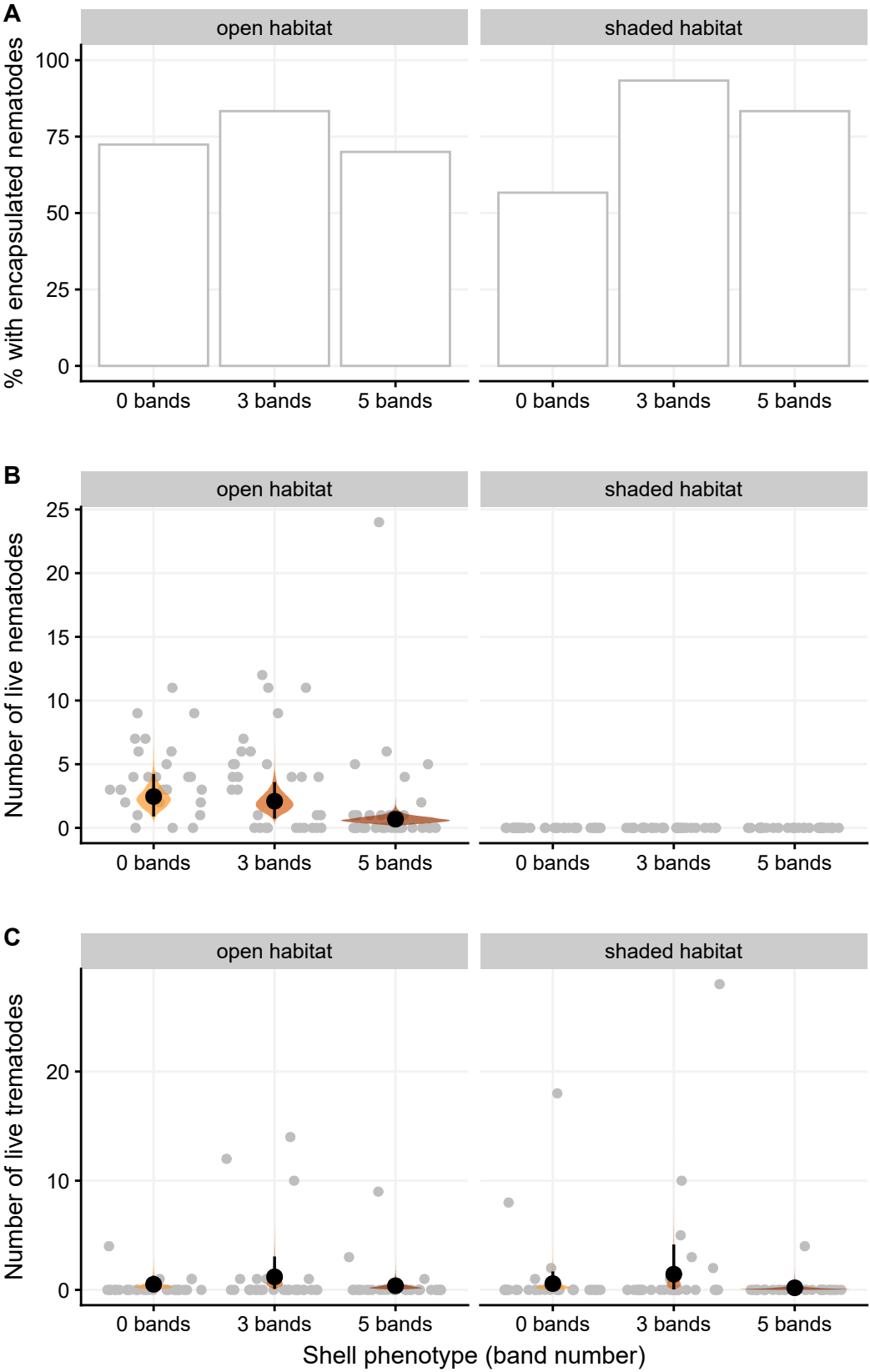
Wilson, M.J., Hughes, L.A., Hamacher, G.M. & Glen, D.M. (2000). Effects of *Phasmarhabditis hermaphrodita* on non-target molluscs. *Pest. Manag. Sci.* **56**, 711–716.

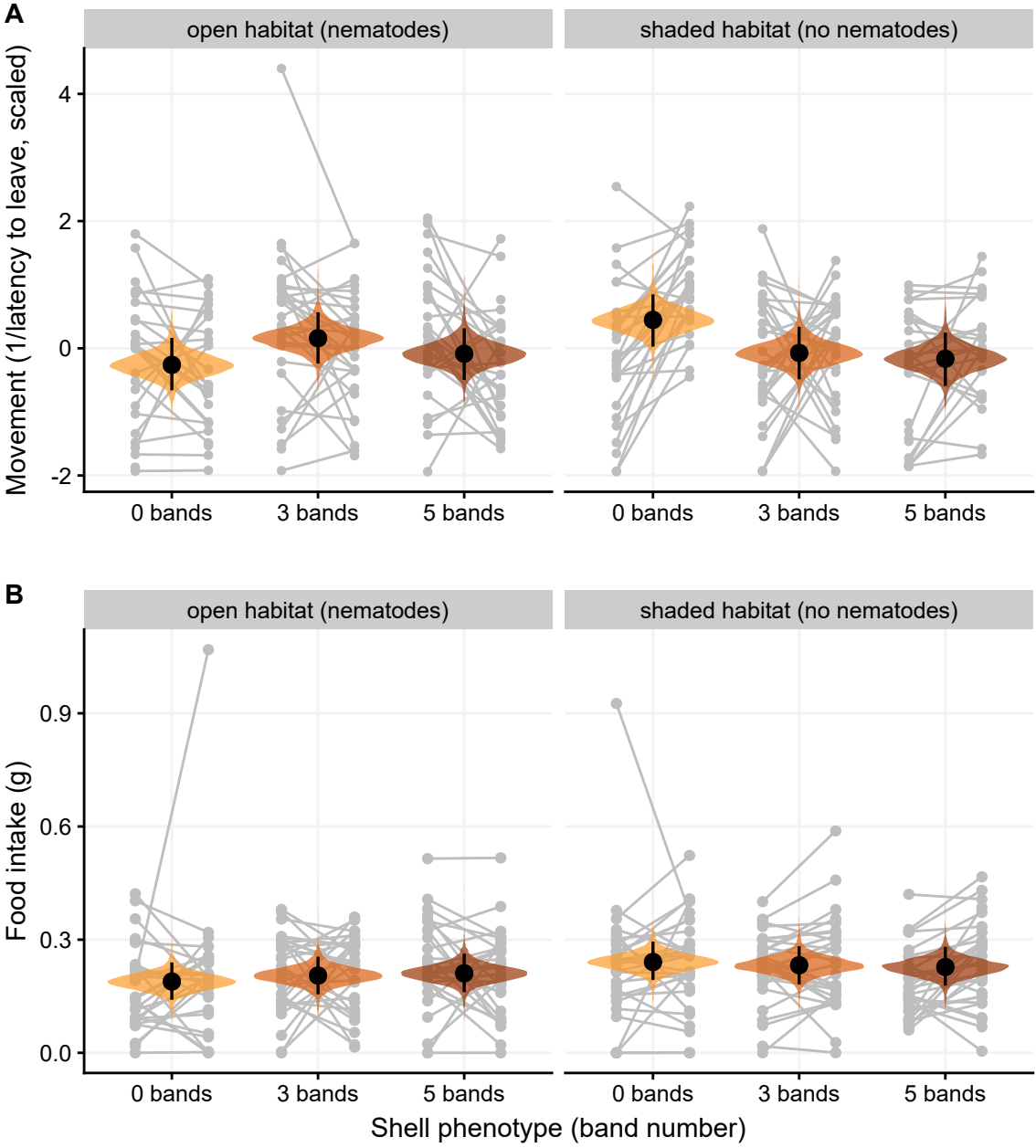
Wynne, R., Morris, A. & Rae, R. (2016). Behavioural avoidance by slugs and snails of the parasitic nematode *Phasmarhabditis hermaphrodita*. *Biocontrol Sci. Technol.* **26**, 1129–1138.

Żbikowska, E., Marszewska, A., Cichy, A., Templin, J., Smorąg, A. & Strzała, T. (2020). *Cepaea* spp. as a source of *Brachylaima mesostoma* (Digenea: Brachylaimidae) and *Brachylecithum* sp. (Digenea: Dicrocoeliidae) larvae in Poland. *Parasitol. Res.* **119**, 145–152.


open habitat
shaded habitat
Shell diameter (mm)
28
26
24
22
20
0 bands
3 bands
5 bands
0 bands
3 bands
5 bands
Shell phenotype (band number)


A
B
0.25 mm


A
open habitat
shaded habitat
% with encapsulated nematodes
100
75
50
25
0
0 bands
3 bands
5 bands
B
Number of live nematodes
25
20
15
10
5
C
Number of live trematodes
Shell phenotype (band number)


A
open habitat (nematodes)
shaded habitat (no nematodes)
Movement (1/latency to leave, scaled)
4
2
0
-2
0 bands
3 bands
5 bands
0 bands
3 bands
5 bands
B
open habitat (nematodes)
shaded habitat (no nematodes)
Food intake (g)
0.9
0.6
0.3
0.0
0 bands
3 bands
5 bands
0 bands
3 bands
5 bands
Shell phenotype (band number)