Morph-dependent nematode infection and its association with 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 linked to 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). Infection costs 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). Parasite infection is often highly variable between populations (Baur & Baur, 2005; Żbikowska *et al.*, 2020).

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

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

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

Based on the link between pigments and immune defence outlined above, we expected snails with more dark bands hosted fewer parasites. Since darker snails are less mobile (Dahirel *et al.*, 2021), this is in line with some predictions from the pace-of-life syndrome hypothesis, namely that fast active individuals have lower immune responses (Réale *et al.*, 2010; but see Royauté *et al.*, 2018). We also hypothesized that metazoan parasites would have notable negative effects on snail performance (McElroy & de Buron, 2014), leading to slower behaviours and changed

food intake in more infected snails (both at the morph level and within morphs). We expected 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 another 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 (22.3% of individuals, compared to 13.5% in the open habitat; 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.

Movement behaviour and food intake

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

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

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

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

143 **Parasitological research**

144 After freezing at -20°C and thawing, we dissected each snail under a binocular microscope. After
145 removing the body from the shell, we looked for and counted metazoan parasites in various
146 locations: space between shell and soft body, lung, heart, kidney, body cavity and digestive
147 gland, as in Gérard et al. (2020). We found nematodes, larval trematodes and parasitic mites
148 (Acari). Trematodes were identified to the genus level as in Gérard et al. (2020), and mites using
149 Fain (2004). That level of taxonomic precision could not be reached for nematodes. However,
150 nematodes from different families consistently use/infect different organs and reach different
151 body sizes within snails (Morand, Wilson & Glen, 2004; Wilson, 2012). We assigned nematodes
152 to size classes (<1 mm, 1-3 mm, 3-5 mm, >5mm) using graph paper, and used nematode
153 location and size to infer the most likely families infecting our snails. We additionally observed
154 shells and shell fragments under brighter light to detect encapsulated parasites (Rae, 2017). By
155 simplicity, we use "live" as a shorthand to designate parasites found free within the body or
156 between the body and the shell during dissection, and thus presumably still living before snails
157 were sacrificed, by opposition with "trapped" or "encapsulated" parasites found within shells.

158 **Statistical analyses**

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

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

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

170 We then ran a multivariate generalized mixed/multilevel model to examine how behaviour and
171 parasite infections varied between morph and populations, and whether these traits were
172 correlated at the individual level. Trematodes and nematodes could not be identified to species,
173 so we used the total numbers of trematodes and nematodes per host snail in our analyses (as in
174 e.g. Debeffe *et al.*, 2014). Mite infections were too rare to be analysed statistically (**Results**).
175 This multivariate model included four submodels:

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

198 In both models we used weakly informative priors (McElreath, 2020): $\mathrm{Normal}(0,1)$ priors for
199 fixed effects, $\mathrm{half-Normal}(0,1)$ priors for standard deviations (random effect and residual),
200 and a $\mathrm{LKJ}(2)$ prior for the correlation matrix in the multivariate model. See **Supporting**
201 **Information S1** for detailed model descriptions. We ran four chains per model for 4000
202 iterations each, with the first half of each chain used as warmup. We checked convergence and
203 mixing following Vehtari *et al.* (2021). All parameters had satisfactory effective sample sizes
204 (Vehtari *et al.*, 2021). Posteriors are summarised in text and figures as means [95% Highest
205 Posterior Density Intervals].

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

213 Results

214 Snail size

215 Snails from the shaded population were on average smaller than those from the open habitat if
216 they were three-banded ($\Delta_{[\mathrm{shaded-open}]}$= -0.70 mm [-1.38; -0.04], **Fig. 1**) or five-banded
217 ($\Delta_{[\mathrm{shaded-open}]}$= -1.37mm [-2.07; -0.71], **Fig. 1**). For unbanded snails, there was no clear
218 difference in size between habitats ($\Delta_{[\mathrm{shaded-open}]}$= -0.39 mm [-1.03; 0.33], **Fig. 1**).

219

220 Parasitological information

221 Snails from both populations and all morphs were infected by metazoan parasites (**Figs. 2-3**, see
222 **Supporting Information S2** for detailed descriptive statistics about prevalence and infection
223 intensity). All trematodes found were live *Brachylaima* sp. metacercariae in snails' kidneys; no

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

239

240 In the open population where live nematodes were found, nematode abundance was morph-
241 dependent: five-banded snails were on average infected by about a third of the nematodes
242 infecting other snails ($N_{[5\text{bands}]}/N_{[0\text{bands}]}$ = 0.29 [0.11; 0.52], $N_{[5\text{bands}]}/N_{[3\text{bands}]}$ = 0.34 [0.12;
243 0.62]; **Table 1**, **Fig. 3**), mostly due to differences in prevalence between morphs (**Supporting**
244 **Information S2**). Five-banded snails also contained fewer trematodes, but only when compared
245 to three-banded snails and only clearly in the shaded habitat ($N_{[5\text{bands}]}/N_{[3\text{bands}]}$ = 0.19 [0.00;
246 0.57]; **Fig. 3**, **Supporting Information S3**). Parasite abundance (trematode or nematode) was not
247 linked to shell size (**Table 1**).

248

249 **Snail behaviour**

250 Both movement and food intake were repeatable at the individual level (see individual-level SDs
251 **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**).

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

Discussion

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

Snails of the darker morphs were smaller in the shaded population (**Fig. 1**). While the two habitats differ along various abiotic dimensions (calcium availability in substrate, microclimate…, see **Methods**), their documented effect on shell size are highly species- and study-dependent, and there is no consistent evidence for or against their role in driving the differences we observe (Goodfriend, 1986). Differences in parasite infection between the two habitats might also influence body size, for instance if high parasite risk drives resource allocation away from growth and towards immune defence (Rantala & Roff, 2005). In any case, our models conditioned on size, meaning that any effect of morph/population we discuss below is "size being equal". In addition, we did not find any effect of size itself on parasites or movement (**Table 1**), meaning that the effects of morph/population we found are likely not mediated by size differences.

One snail was found with live *Riccardoella* mites, hemolymph feeders with negative effects on mollusc life-history and behavior (e.g. Schüpbach & Baur, 2008a). They are transmitted horizontally during mating, but may find new hosts by leaving their host and following snail mucus trails (Schüpbach & Baur, 2008b). However, their limited prevalence in our sample prevented us from investigating their effects. Intriguingly, we found encapsulated mites in three other snails (**Fig. 2**). Many land snails can defend themselves against nematode infection by trapping them in their shell (Rae, 2017), including *Cepaea nemoralis* (this study, Williams & Rae, 2016; Rae, 2017). Based on extensive surveys, Rae (2017) wrote that encapsulation in the shell is a specific response against nematode infection; our observations might contradict this

302 affirmation. *Riccardoella* infection is highly variable across populations or species, with reported
303 prevalences of 0% or near 0% being frequently observed (this study; Russo & Madec, 2013; Baur
304 & Baur, 2005; Segade *et al.,* 2013), which may explain why encapsulated mites were not
305 previously found. We need further studies to determine with certainty whether shell
306 encapsulation is a more general response to metazoan parasites or if it is a nematode-specific
307 response, as posited by Rae (2017), but occasionally and anecdotally catches other foreign
308 bodies.

309 A majority of snails in the open population were hosting nematodes, most of which were likely
310 Rhabditidae based on size and organ location (Morand *et al.*, 2004; Wilson, 2012). Interactions
311 between Rhabditidae and terrestrial molluscs are complex, including phoresy/endozoochory,
312 facultative parasitism, necromeny (feeding and reproducing on the host's body after its death),
313 and obligate larval parasites that actively infect snails through e.g. the lung cavity (Morand *et*
314 *al.*, 2004; Pieterse *et al.*, 2017; Sudhaus, 2018). Delimiting these interaction types is complicated
315 because even when they have no effects on adults, these nematodes can have dramatically
316 negative effects on juveniles (Grannell, Cutler & Rae, 2021). By contrast to the open population,
317 we found no live nematodes in any snail from the shaded population (**Fig. 3**; note that our
318 methods were not designed to detect nematodes embedded in e.g. snail foot tissue). Thanks to
319 nematodes trapped in shells (**Fig. 3**), which integrate infections over time, we know parasitic
320 nematodes are not truly absent from the shaded population; they were simply not infecting
321 snails at the time of sampling. The "shaded" woodlot habitat is sheltered from the sun, more
322 humid and likely colder than the open one. Infections are often dependent on abiotic
323 parameters, including temperature and humidity, leading to seasonality (Altizer *et al.*, 2006).
324 Differences in nematode infection between the habitats may then be explained, at least partly,
325 by differences in microclimate. First, the different microclimates experienced by the two
326 populations may favour different nematode species within the soil community, differing in their
327 tendency to parasitism. Second, even if nematode communities are similar, conditions
328 favourable to nematode activity, development or infection may be reached only later in the
329 year in the shaded habitat. For instance, *Deroceras reticulatum* slugs were more negatively
330 impacted by *Phasmarhabditis hermaphrodita* infection when conditions were artificially

331 warmed during winter (El-Danasoury & Iglesias-Piñeiro, 2017), indicating that warmer
332 conditions may favour nematode activity. In any case, these results mean that any between-
333 population differences in snail behaviour in the present study should be interpreted not only in
334 relation with microhabitat and microclimate differences, but also in terms of average infection
335 levels.

336 In the open population where active nematodes were actually found, morph differences in
337 infection seem to validate our initial hypothesis of increased immune defence in darker snails
338 (**Fig. 3**). By contrast, no significant morph difference in immune defence was found in the
339 related *Cepaea hortensis* (Scheil *et al.*, 2013, 2014), but their endpoints were different
340 (phenoloxidase activity and mortality). In addition, our behavioural results (discussed below) are
341 also consistent with the hypothesis of morph-dependent immunity. These results are interesting
342 in the light of recent evidence that melanins may not be the pigments forming the dark bands in
343 *C. nemoralis* (Affenzeller *et al.*, 2020). Indeed, our initial hypothesis when designing this
344 experiment (in 2019) was based on the oft-seen association between immunity and melanin
345 (San-Jose & Roulin, 2018), including in snails (Coaglio *et al.*, 2018), although other pigments may
346 also have links to immunity (Tan *et al.*, 2020). While it is still likely that melanin-associated
347 pathways play a role in immune defence in *Cepaea* (Scheil *et al.*, 2014), it is probably not as
348 simple as "banded snails constitutively produce more melanin, and thus are better protected".
349 More studies are needed to understand the mechanisms linking shell morph to nematode
350 resistance.

351 Snails from all morphs and both populations were infected by *Brachylaima* trematodes (**Fig. 3**).
352 We previously documented *Brachylaima* infecting *Cornu aspersum* in the same area (Gérard *et*
353 *al.*, 2020); finding these parasites a year later and in *C. nemoralis* may indicate that they are
354 well-implanted in local communities. Contrary to nematodes, we found no evidence that morph
355 or population influenced trematode abundance (**Fig. 3**). This is consistent with another study
356 analyzing both *C. nemoralis* and *C. hortensis* (Żbikowska *et al.*, 2020). We must acknowledge
357 however that snail infections by *Brachylaima* vary seasonally, and that spring may not be the
358 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 Dahirel et al. (2021), this was only the case in the shaded population (**Fig. 4**). In the open population, snail morphs are mobile at similarly low levels: five-banded snails do not change behaviour between the two populations, while unbanded snails go down to five-banded snail levels of mobility (**Fig. 4**). One key difference between the present study and Dahirel et al. (2021) is the collection date. While here snails were collected several weeks after the onset of spring activity and kept active throughout, in Dahirel et al. (2021) they were collected in late fall and stored at cold temperatures inducing dormancy for months before being tested. This difference may be especially relevant: winter or summer dormancy can lead to sharp decreases in parasite infection in several parasite-snail systems (Solomon, Paperna & Matkovics, 1996; Haeussler *et al.*, 2012; Segade *et al.*, 2013). We can thus reconcile the behavioral results from Dahirel et al. (2021) from those of the present study if we assume that in the former experiment, hibernation reduced parasite load across all morphs and populations, and that in the present study, lower temperatures in the shaded habitat led to longer hibernation. We can then interpret population differences in the present study in the light of their different infection patterns. Five-banded snails' behaviour is almost unchanged between the two populations, while unbanded snails from the "infected" population are slower than in the nematode-free

388 population (**Fig. 4**). This is again consistent with the idea that unbanded snails are more
389 vulnerable to parasites than banded ones. The question then becomes: is this reduction in
390 activity in infected or at-risk unbanded snails simply the direct consequence of the costs of
391 infection, or does it reflect something more? For instance, infective nematodes may be able to
392 follow mucus trails from active snails (Andrus *et al.*, 2020). Reducing activity and the associated
393 mucus production may then be protective for snails, by limiting the risk of further infections.
394 Experimental manipulations of both snail infection and parasite risk in the environment may
395 help disentangle these two responses. Future studies including species-level identification of
396 nematodes will also be needed, to determine whether nematodes with more negative impacts
397 also trigger stronger behavioural responses, or if snails respond indiscriminately to nematode
398 abundance.

399 Food intake was influenced by nematode infection: highly parasitized snails ate less (**Table 2**).
400 Feeding reduction in nematode-infected individuals has been documented in other terrestrial
401 molluscs (Antzée-Hyllseth *et al.*, 2020; Saeedizadeh & Niasti, 2020) and is an indication that
402 parasite infection is indeed costly for snails even if they are non-lethal, at least in adults (Wilson
403 *et al.*, 2000; Williams & Rae, 2016; Grannell *et al.*, 2021). By contrast, trematode infection had
404 no effect on behaviour, including feeding behaviour (**Table 2**). We however only found snails
405 infected by *Brachylaima* metacercariae. At the sporocyst stage, *Brachylaima* parasitizes the
406 digestive gland (e.g. Gérard *et al.*, 2020), which would likely have a stronger effect on snail
407 behaviour. Indeed, sporocysts can cause high damage, direct and indirect, to that key organ for
408 digestion and for energy storage (Segade *et al.*, 2011; Żbikowska *et al.*, 2020).

409 The fact that snail response to parasites seems morph-dependent for nematodes, but not
410 trematodes, has interesting implications for host-parasite and snail-predator interactions in a
411 changing world. Trematodes infecting land snails are trophically transmitted to vertebrate
412 definitive hosts including rodents and birds (Gérard *et al.*, 2020; Sitko & Heneberg, 2020). Visual
413 selection by thrushes (*Turdus philomelos*) is held as one of the key explanations for the
414 maintenance of *Cepaea* shell polymorphism (Jones *et al.*, 1977), and predation by rodents may
415 also be morph-dependent (Rosin *et al.*, 2011; Rosin *et al.*, 2013). If confirmed, our results
416 suggest that morph-dependent predation confers no additional trematode-avoiding advantage.

In addition, snail morphs differ in their thermal responses: lighter-coloured morphs are better adapted to heat, becoming more frequent with global warming or urbanization (Jones *et al.*, 1977; Silvertown *et al.*, 2011; Ożgo *et al.*, 2017; Kerstes *et al.*, 2019). If lighter morphs are more vulnerable to infection by nematodes, then we need to understand how snail immunity will interact with thermal tolerance to predict how snail and nematode dynamics will be altered in response to climate change (El-Danasoury & Iglesias-Piñeiro, 2017; Aleuy & Kutz, 2020).

Acknowledgements

We thank participants to the 4th French Conference on Animal Ecophysiology (CEPA4, Rennes, 2019) for interesting discussions. Funding for MD's position was obtained by Elodie Vercken (see Funding).

Data availability

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

Funding

MD was a postdoctoral researcher funded by the French Agence Nationale de la Recherche (PushToiDeLa, ANR-18-CE32-0008), during the course of this research.

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629 **Figure Legends**

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

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

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

644 **Figure 4**. Behaviours as a function of morph and population of origin. (A) Movement metric
645 (1/latency to move beyond 10 cm, centered and scaled to unit 1SD); (B) Food intake. Grey dots
646 are observed values, displayed along the posterior distributions of the average abundances
647 based on the model described in **Table 1** (black dots and segments are the means and 95% CI of
648 the posteriors). Observed values from the same individual are connected by a line. Posterior
649 predictions are based on fixed effects only, and the effects of shell size are averaged out. See
650 **Supporting Information S3** for pairwise comparisons.

651

652

Tables

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.

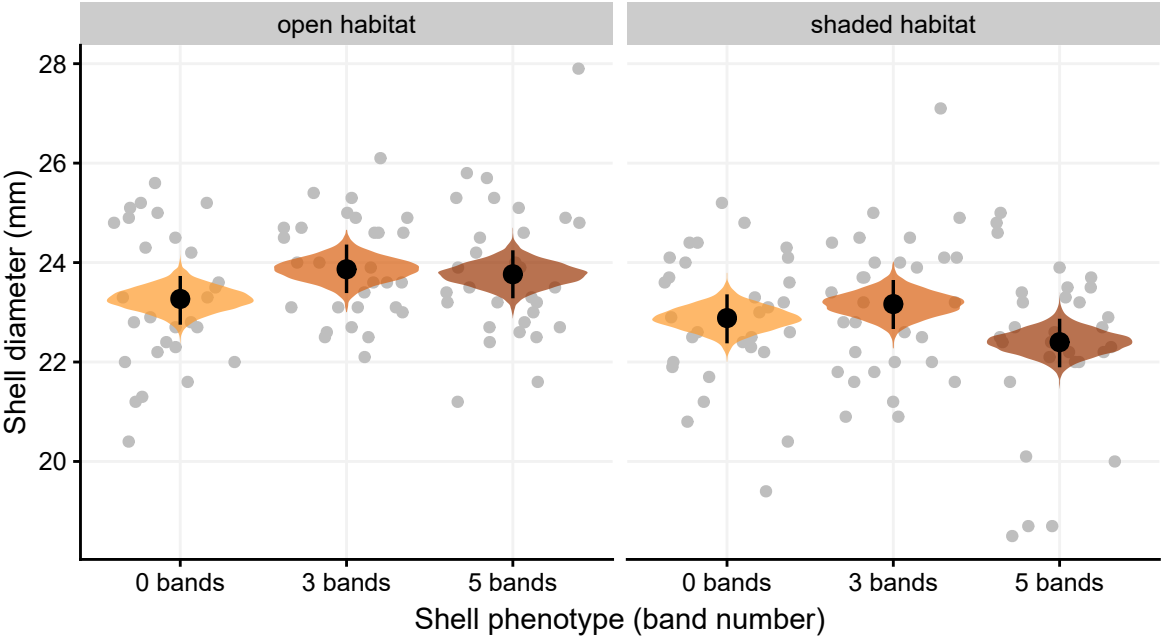
	Nematode abundance*	Trematode abundance	Movement	Food intake
Fixed effects				
Intercept$_{[0\ bands]}$	0.84 [0.09; 1.52]	-0.94 [-2.44; 0.48]	-0.26 [-0.66; 0.16]	-0.22 [-0.61; 0.19]
Intercept$_{[3\ bands]}$	0.67 [-0.08; 1.38]	-0.10 [-1.54; 1.33]	0.16 [-0.24; 0.56]	-0.09 [-0.50; 0.31]
Intercept$_{[5\ bands]}$	-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]

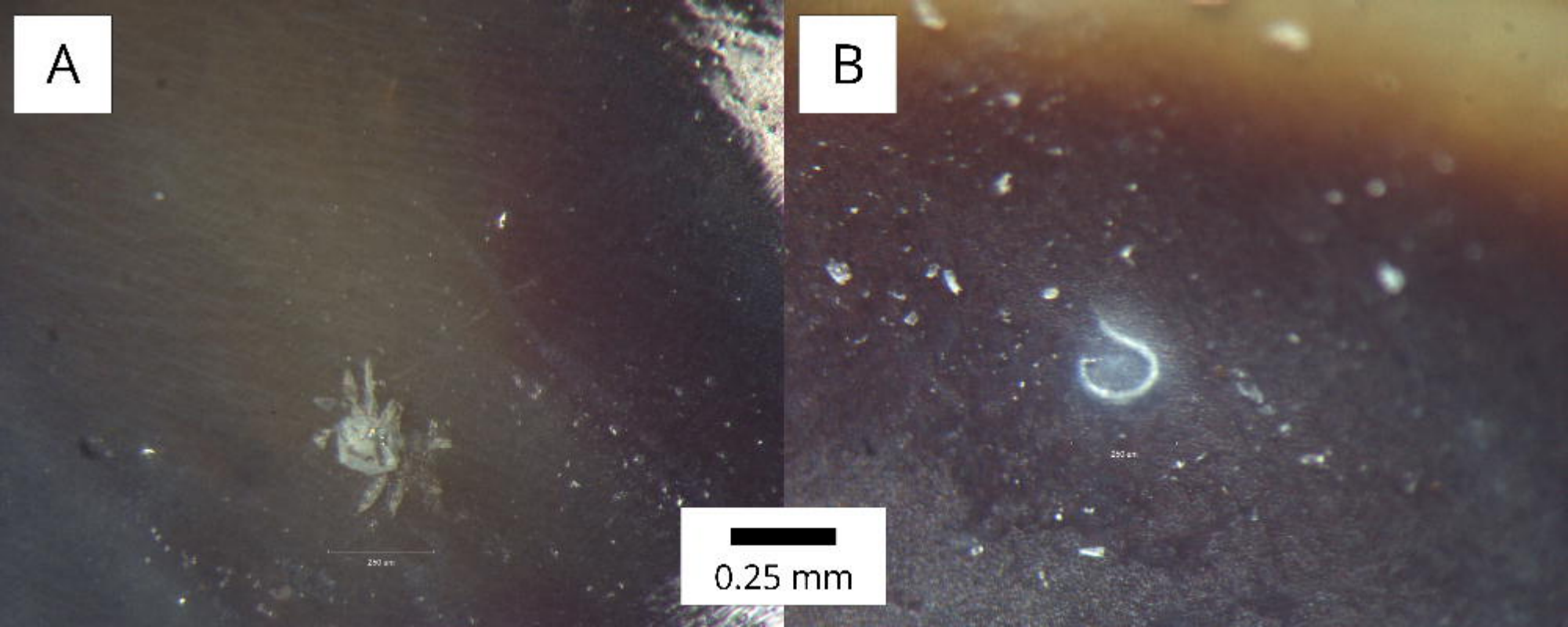
* The nematode model is only estimated for the "sun-exposed" population, since no live nematodes were recorded in the "shaded" population

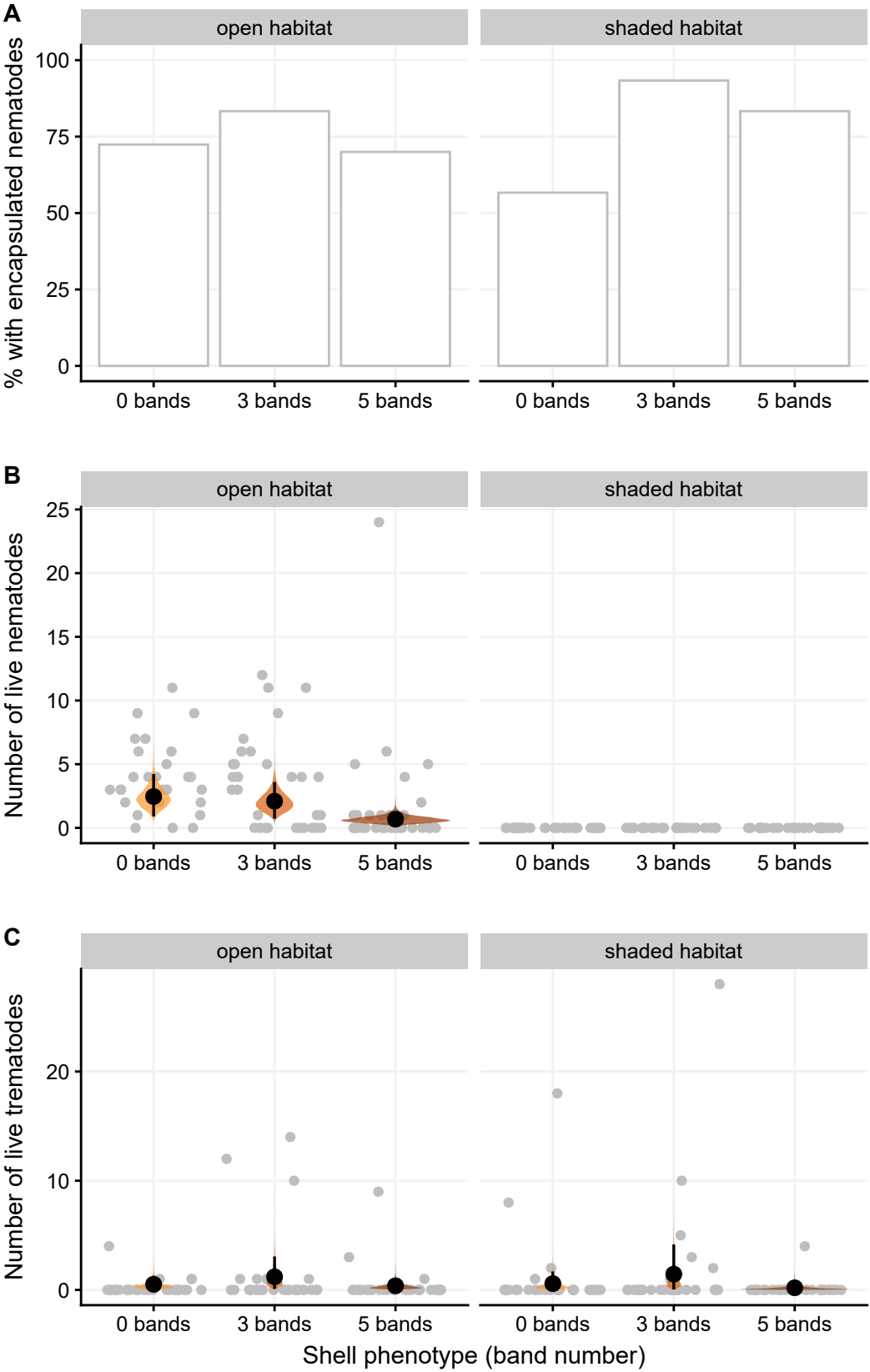
662 **Table 2**. Individual-level correlations between parasite abundances and behavioural traits. Correlations
663 involving nematode abundance are only based on individuals from the open population (see Methods);
664 results are not qualitatively different if the model is refitted to include all observations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

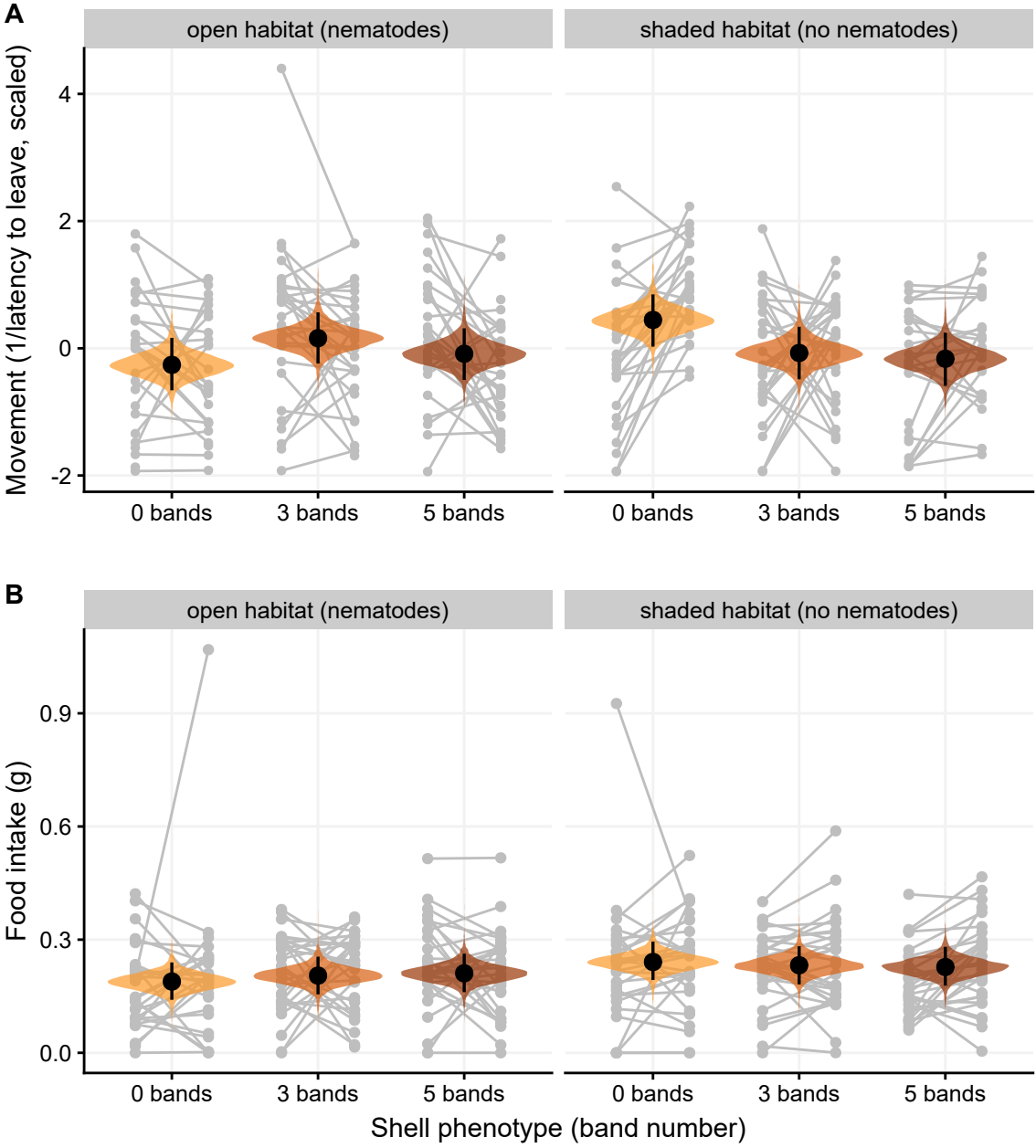
	Nematode abundance	Trematode abundance	Movement
Trematode abundance	0.01 [-0.35; 0.37]	-–	-–
Movement	-0.24 [-0.73; 0.25]	-0.17 [-0.68; 0.28]	-–
Food intake	**-0.60 [-0.87; -0.30]**	-0.12 [-0.46; 0.19]	0.24 [-0.23; 0.71]

666


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)