Are “early birds” bolder? Early daily activity is not correlated with risk-taking behaviour in a major invasive species

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ABSTRACT

Daily behavioural rhythms provide ecological advantages with respect to exploitation of food resources and avoidance of predation and recent studies suggested that timing of activity could form a behavioural syndrome with risk-taking behavior. Behavioural syndromes are often displayed by invasive species but the role of activity rhythms in biological invasions is unknown. Here, we investigated whether early nocturnal activity (the relative amount of locomotor activity displayed early in the night) and risk-taking behaviour (i.e. response to a scare stimulus) form a behavioural syndrome in a major invasive species, the signal crayfish (*Pacifastacus leniusculus*). We first characterized daily rhythms of locomotor activity over five days under controlled laboratory conditions and then scored the response to a scare stimulus across two different contexts (neutral and food) two days apart within the following six days. Crayfish displayed overall daily activity rhythms peaking in the first four hours of darkness. Both early nocturnal activity and risk-taking behaviour showed consistent inter-individual differences with repeatability scores of 0.20 and 0.35, respectively. However, the two behavioural traits were not correlated as in a behavioural syndrome. We argue that in contrast to other behavioural syndromes, a link between early nocturnal activity and risk-taking tendency would not be evolutionary stable as it dramatically increases predictability and therefore predatory pressure to individuals. We suggest that daily activity rhythms and risk-taking behavior can be important traits in understanding the adaptations underlying biological invasions or other processes of contemporary evolution.

34 **Keywords:** crayfish, *Pacifastacus leniusculus*, daily activity rhythms, personality,
35 behavioural syndrome

36

37 INTRODUCTION

38 The behaviour and physiology of almost all animal taxa is characterized by daily activity
39 rhythms (Dunlap and others 2004). Daily activity rhythms are usually synchronized to
40 the day-night cycle, but other environmental or social factors can also play a role
41 (Castillo-Ruiz and others 2012; Hut and others 2012). Daily behavioural rhythms
42 provide ecological advantages with respect to exploitation of food resources and
43 avoidance of predation (Helm and others 2017; Kronfeld-Schor and others 2013;
44 Kronfeld-Schor and Dayan 2003). For example, in the desert golden spiny mouse
45 (*Acomys russatus*) individuals that arrive earlier to a foraging patch gain more food
46 than individuals that arrive later (Levy and others 2012). In zebrafish (*Danio rerio*),
47 individuals displaying higher locomotion early in the activity period were also more
48 risk-taking than individuals that were less active (Tudorache and others 2018). An
49 increase of risk-taking behaviour is usually associated with an increased access to food
50 resources and a decrease of survival towards predators (Lima and Dill 1990; Smith and
51 Blumstein 2008). Therefore, the amount of locomotion displayed early in the activity
52 period could be a fitness-related trait similar to chronotypes (Helm and others 2017).

53 Chronotypes are consistent inter-individual differences in the onset of activity
54 with respect to a reference time point (Helm and others 2017), and they can have
55 fitness consequences. For example, a recent study suggests that razorfish (*Xyrichtys*
56 *novacula*) with early-active chronotypes could experience higher mortality from
57 fisheries than late chronotypes (Martorell-Barceló and others 2018). However, daily
58 activity rhythms may not only differ in the onset (i.e. chronotypes) but also in the
59 relative amount of activity displayed at particular time periods. A recent study on
60 zebrafish (*Danio rerio*) showed consistent inter-group differences in the relative
61 amount of locomotion displayed early in the activity period (Sbragaglia and others
62 2019). Interestingly, elevated locomotion displayed early in the activity period was
63 found to be linked to an increase of risk-taking behaviour in an evolutionary context
64 (Sbragaglia and others 2019), suggesting an adaptive value for such combination of
65 traits.

Animal behaviour plays a paramount role in biological invasions (Holway and Suarez 1999). Personality traits (i.e. consistent inter-individual differences in behaviour; Carere and Maestripietri 2013) such as risk-taking behaviour can determine the success or failure at different stages of biological invasions (Canestrelli and others 2016; Carere and Gherardi 2013; Chapple and others 2012; Juette and others 2015). By contrast, the role of daily activity rhythms in biological invasions is unknown. A key to the understanding of the process of biological invasion could be the finding that successful invaders often display correlation among a suite of consistent behavioural traits (i.e. behavioural syndrome; Chapple and others 2012; Sih and others 2004). For example, a behavioural syndrome between aggression and foraging activity is suggested to enhance the ability of the signal crayfish (*Pacifastacus leniusculus*), a major invasive species, to attain high densities and a negative impact on invaded communities (Pintor and others 2009).

Here, we test the hypothesis that early daily locomotor activity and risk-taking behaviour form a behavioural syndrome in *P. leniusculus*, a commercially harvested crayfish which is one of the most invasive species in Asia and Europe (Gherardi 2017; Larson and others 2012; Lodge and others 2000). We predict that early daily locomotor activity and risk-taking behaviour are consistent behavioural traits and that these traits are positively correlated.

85

86 MATERIALS AND METHODS

87 *Sampling and acclimation*

88 Crayfish were sourced from a commercial supplier (Flowers Farm Lakes, Dorchester, 89 UK). Prior to experiments crayfish were acclimated in communal tanks (60x45x25 cm) 90 in sex-segregated groups of 10-15 individuals. Crayfish were fed a mixed diet of 91 prawns, peas and carrots. The light-darkness cycle was 12-12 hours and water 92 temperature was kept at 15 ± 1 °C.

93 We used a total of 24 male crayfish (cephalothorax length: 36.0 ± 2.7 mm). We 94 conducted a total of three experimental trials from May to July. We ran each trial with 95 8 randomly-selected crayfish in an experimental room where light (light-darkness cycle 96 of 12-12 hours) and water temperature (15 ± 1 C) were under control. Light was 97 provided by fluorescent lamps at an intensity of 0.25 Klux. During the darkness period

98 dim red light (0.01 Klux) was provided by monochromatic LEDs to allow video
99 recording.

100 The experimental setup consisted of 8 separated glass aquaria (45x30x25 cm).
101 We equipped each tank with a recirculation pump (provided with a filter). We glued
102 white opaque tissue at the bottom of the tank to create a suitable surface for the
103 crayfish to walk on and the appropriate background to apply video imaging analysis
104 (see below). We placed a shelter (12 cm long) in each tank, cutting transversally a PVC
105 pipe of a diameter of 8 cm. We covered the sides of each tank with white fabric to
106 eliminate any visual disturbance from neighboring tanks and we placed the tanks
107 behind light proof curtains to eliminate any kind of light contamination.

108

109 ***Experimental trials***

110 We left the crayfish undisturbed during the first stage of the experiment recording a
111 time-lapse video (10 s frequency) that we used to track daily rhythms of locomotor
112 activity (see below). Next, during the second stage we exposed crayfish to a scare
113 stimulus in two different contexts (neutral and food contexts) to evaluate their level of
114 risk-taking behaviour. One of the most important anti-predatory behaviours of crayfish
115 include reduced movement (Stein and Magnuson 1976), therefore we considered the
116 amount of locomotor activity performed 30 min after the scare stimulus as a measure
117 of risk taking behavior (i.e. the less the locomotor activity the less risk-taking
118 behavior). We applied the scare stimulus two days apart within six days moving a
119 plastic black board (15x15 cm) forward and back two times over the surface (see video
120 S1). During the neutral context nothing was changed in the experimental tank, while
121 during the food context a small piece of prawn was added after light-off. The scare
122 stimulus was applied within two hours after light-on only when the crayfish was
123 moving or approaching the food (displacement for more than 1 body length),
124 otherwise the stimulus was not applied and the behavior scored with a 0.

125

126 ***Behavioral tracking***

127 We quantified locomotor activity using automated video image analysis. Four USB
128 webcams (1 webcam for 2 aquaria) were placed behind light proof curtains on the top
129 of the aquaria. We managed frame acquisition using open source camera security

software (ispy: <http://www.ispyconnect.com>). The automated video image analysis was performed in Matlab 7.1 (The MathWorks, Natick, USA), adapting a compilation of a script with the image processing toolbox previously used in several studies with decapods crustaceans (Sbragaglia and others 2013a; Sbragaglia and others 2015a; Sbragaglia and others 2015b). The final output was a time series of the movement of each crayfish (cm) at a frequency of 10 s.

136

137 ***Statistical analysis***

Chi-square periodogram (Sokolove and Bushell 1978) was used to scan for periodicity and the percentage of variance (%V) was reported as a measure of rhythms' robustness (Refinetti 2006). Early nocturnal activity was calculated for each of the five days as the proportion between the percent of locomotor activity within four hours after light-off in relation to the percent of time (more details and examples are provided in Figure 1). This provides a quantitative measure of activity within a defined 4-hour time period respect to the overall activity (see also; Sbragaglia and others 2013b).

Daily values of early nocturnal activity and the scores for risk-taking behaviour in each context were transformed by finding the exponent (lambda), which made the values of the response variable as normally distributed as possible with a simple power transformation. Repeatability was calculated across time (5 days) for early nocturnal activity and across contexts (neutral and food) for risk-taking behaviour (Nakagawa and Schielzeth 2010). Model fitting was examined by checking normality of residuals and plotting theoretical quantiles vs. standardized residuals. The correlation among the average early nocturnal activity and risk-taking behaviour was calculated with the non-parametric Kendall's coefficient of concordance.

Analysis were performed using the software Eltemps (www.el-temps.com) and R 3.3.1 (<https://www.R-project.org/>) with the following additional packages: rcompanion" (<https://CRAN.R-project.org/package=rcompanion>), "rptR" (Stoffel and others 2017). In all cases, we used a 95% confidence interval.

159

160 **RESULTS**

Locomotor activity of crayfish during the first stage of the experiment indicated robust daily activity rhythms (mean robustness $\pm$ standard deviation = 46 ± 12 %; $n = 19$; five crayfish did not show significant periodicity in their activity rhythms) with more activity concentrated during darkness (72 ± 11 %; $n = 19$; Fig. 1a). Early nocturnal activity ranged from 1.26 to 2.58 (i.e. the crayfish displayed a 2.58 times more locomotor activity within 4-hours after light-off with respect to overall activity; fig. 1b). Early nocturnal activity was repeatable over time ($R = 0.20$ [$0.01 - 0.38$]; $p < 0.05$; $n = 19$; Fig. 1d).

During the 30 min after the scaring stimuli, the distance covered by crayfish (risk-taking behavior) ranged from 0 to 760 cm in the neutral context, while it ranged from 0 to 1505 cm in the food context. Two crayfish remained in the shelter (one in the neutral context and one in the food context) during the selected time period (two hours within light-off) in which we applied the stimuli. We scored this behaviour with a 0 because our interpretation is that remaining in the shelter implies a low level of risk-taking behaviour (Jurcak and others 2016). Risk-taking behaviour was significantly repeatable across contexts ($R = 0.35$ [$0.01 - 0.65$]; $p < 0.05$; $n = 24$; Fig. 1e). Finally, early nocturnal activity did not show significant correlations with risk taking behaviour both in the neutral ($r_t = -0.03$; $p = 0.860$; Fig. 2) and food context ($r_t = 0.22$; $p = 0.183$; fig. 2). Finally, early nocturnal activity was not correlated with the central tendency of locomotor activity (mesor; $r_t = -0.04$; $p = 0.806$).

180

181 DISCUSSION

The importance of consistent inter-individual differences in the timing of activity has recently been highlighted with respect to the onset of activity ("chronotypes"; Helm and others 2017). In our study we provide evidence that the relative amount of locomotion displayed early in the activity period can also show consistent inter-individual differences. Daily locomotor activity rhythms have a high degree of plasticity. For example, temporal niche switching (i.e. shifts in the timing of daily locomotor activity) has been suggested as a strategy to maximize survival in response to environmental changes both in endotherms and ectotherms (Chiesa and others 2010; Hut and others 2012; van der Vinne and others 2019). Our results suggest that consistent inter-individual differences could be an additional factor in shaping the ecological significance of biological rhythms. Indeed, behavioural ecologists have

193 already recognized that both consistent inter-individual differences (i.e. personality)
194 and individual plasticity must be considered as complementary aspects of individual
195 phenotypes (Dingemanse and others 2010). It must be noted that the early nocturnal
196 activity measured here does not correlate to the mean level of overall activity (i.e. the
197 central tendency of locomotor activity; see Fig. 1a-c). Therefore, the relative amount of
198 locomotion displayed early in the activity period is a trait that is not correlated to
199 overall activity.

200 Our results provide the first in-depth characterization of daily locomotor
201 activity rhythms of *P. leniusculus*, showing an overall peak of activity in the first hours
202 of darkness (early nocturnal activity) as well as consistency of inter-individual variation.
203 Previous studies already showed that *P. leniusculus* is a nocturnal species (Edmonds
204 and others 2011; Nyström 2005; Thomas and others 2016), but a clear daily activity
205 pattern was never recorded for this major invasive species. The evolution of early
206 nocturnal activity could possibly be related to foraging opportunities as demonstrated
207 in other nocturnal decapod crustaceans such as the Norway lobster, *Nephrops*
208 *norvegicus* (e.g. Aguzzi and others 2015; Katoh and others 2013; Sbragaglia and others
209 2013b). Interestingly, we showed that the consistent inter-individual differences in
210 early nocturnal activity account for 20% of the phenotypic variance across time.
211 Similarly, consistent inter-individual differences in risk-taking behaviour account for
212 35% of the phenotypic variance across contexts. The degree of consistency
213 corresponds to personality traits of other species in a wide range of animal taxa (Bell
214 and others 2009).

215 The main hypothesis of our study must be rejected because we did not find a
216 behavioural syndrome between early nocturnal activity and risk-taking behaviour.
217 Crayfish with a higher early nocturnal activity could have advantages in finding and
218 exploiting food patches; however, higher activity could increase exposure to predators
219 (Lima and Dill 1990). The major predators of adult crayfish are large predatory fish,
220 wading birds and mammals such as raccoons and otters (Jones and others 2016; Jurcak
221 and others 2016). Important anti-predatory behaviours of crayfish include reduced
222 movement (Stein and Magnuson 1976) or seeking cover (Jurcak and others 2016), and
223 crayfish exposed to fish predators spent less time out of the refuge at night than
224 individuals not exposed to predators (Nyström 2005). The trade-off between anti-

225 predatory behaviour and foraging could explain the adaptive value of the lack of a
226 behavioural syndrome between early nocturnal activity and risk-taking behaviour,
227 suggesting that a common architecture (e.g. pleiotropic genes or common neural
228 pathways) between these traits is unlikely. However, previous results on zebrafish
229 indicated an adaptive value of a behavioural syndrome between these two traits
230 (Sbragaglia and others 2019; Tudorache and others 2018). Decapod crustacean are
231 poorly represented in the studies on animal personality and behavioural syndromes in
232 comparison to fishes, birds and mammals (Gherardi and others 2015). Therefore, we
233 lack comparative studies to understand the general ecological significance of such
234 behavioral syndrome in nocturnal crustaceans.

235 Biological invasions are often characterized by contemporary evolution
236 (Colautti and Lau 2015; Reisinger and others 2017). For example, common gardens
237 experiments with the rusty crayfish (*Orconectes rusticus*) indicated trait divergence
238 between native and invaded range populations (Sargent and Lodge 2014). In particular,
239 individuals from the invaded populations had faster growth rates and higher survival
240 than individuals from the native range (Sargent and Lodge 2014). Similarly, the signal
241 crayfish (*P. leniusculus*) show changes in population characteristics at the edge of its
242 invasive range in Europe (Hudina and others 2012). In particular, the population at the
243 invasion front was characterized by a male-biased sex ratio and an increase in relative
244 claw size (Hudina and others 2012). Therefore, our results could be also explained
245 according to an evolutionary scenario associated to the invasion process (Chapple and
246 others 2012). Individuals used in this study were obtained from Dorset (Southern UK)
247 where the presence of *P. leniusculus* was already reported more than 40 years ago
248 (Guan and Wiles 1997; Holdich and others 1999). A behavioural syndrome between
249 early nocturnal activity and risk-taking behaviour could have an adaptive advantage
250 during the first stages of invasion, but may lose its adaptive value once crayfish
251 populations are well established. For example, *P. leniusculus* collected at the invasion
252 front in Croatian rivers showed lower level of intra-specific aggression than individuals
253 from the core population, suggesting that a decrease in aggression is maladaptive once
254 the population is established (Hudina and others 2015). Indeed, the sequential stages
255 of invasion could have different selection landscapes that may erode behavioural
256 syndromes (Chapple and others 2012).

257 A recent study, simulating fisheries-induced evolution, showed that life-history
258 traits, early daily activity and risk-taking behaviour are under selection in population
259 where smaller individuals are selectively harvested (Sbragaglia and others 2019).
260 Although evolutionary adaptations in response to fisheries (Heino and others 2015;
261 Uusi-Heikkilä and others 2015) and biological invasions (Colautti and Lau 2015; Suarez
262 and Tsutsui 2008) are triggering interest among researchers, possible common
263 patterns have seldom been explored. Future studies could benefit from integrating
264 such fields in order to understand the underpinning mechanisms driving contemporary
265 evolution of behavioral syndromes.

266

267 **Ethical statement**

268 Experiments were conducted at the University of Hull and approved by the Ethics
269 committee of the Department of Biological Sciences (approval number U035).

270

271 **Data accessibility**

272 Data are available as supplementary material together with the R code.

273

274 **Authors' contribution**

275 TB and VS conceived the idea; VS run the experiments, analyzed the data and wrote
276 the manuscript with inputs of TB.

277

278 **Competing interest**

279 We declare no competing interests

280

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431 **Figure 1** – Daily activity rhythms and risk-taking behaviour. (a): Average daily
 432 locomotor activity is presented for all individuals together with standard errors
 433 (vertical bars) and the midline estimating statistic of rhythm (i.e. mesor, the central
 434 tendency of locomotor activity; horizontal line). The vertical dashed line and the
 435 double-ended arrow represent the first four hours of darkness where we calculated
 436 the proportion between the percent of locomotor activity in relation to the percent of
 437 time (%AUC/%t). The grey areas represent darkness hours. (b-c): two representative
 438 examples of daily activity rhythms, ID12 (early %AUC/%t = 2.58) and ID11 (early
 439 %AUC/%t = 1.36). (d-e): Repeatability of early activity across five days (n = 19) and risk-
 440 taking behaviour across contexts (n=24). The horizontal dashed line represents a
 441 threshold and values above 1 indicate that locomotor activity is mainly displayed
 442 during the first four hours of darkness.

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Figure 2 – Plot of risk-taking behaviour measured in two different contexts (Nothing and Food) against mean early-active phenotypes (n=19).