

1 The activity of signal crayfish (*Pacifastacus leniusculus*) in relation to  
2 thermal and hydraulic dynamics of an alluvial stream, UK.

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16 Transponder

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18

19 **Abstract**

20 Signal crayfish (*Pacifastacus leniusculus*) are an invasive species of international  
21 significance because of their detrimental impacts on freshwater environments and native  
22 organisms. The movement of signal crayfish was continuously monitored for 150 days  
23 through a 20 m reach of an alluvial stream in the United Kingdom. PIT-tags were attached to  
24 crayfish, allowing their location to be monitored relative to 16 antennae which were buried  
25 beneath the river bed. The activity of crayfish was related to water depth and temperature,  
26 which were continuously monitored within the instrumented reach. Crayfish were highly  
27 nocturnal, with less than 6% of movements recorded during daylight hours. Activity declined  
28 from September and was minimal in November when water temperature was low and flow  
29 depth was high. However, relations between environmental parameters and crayfish activity  
30 had poor explanatory power which may partly reflect biological processes not accounted for  
31 in this study. Water depth and temperature had a limiting relationship with crayfish activity,  
32 quantified using quantile regression. The results extend existing data on signal crayfish  
33 nocturnalism and demonstrate that, although signal crayfish can tolerate a range of flows,  
34 activity becomes limited as water temperature declines seasonally and when water depth  
35 remains high in autumn and winter months.

36

## 37 **Introduction**

38 An understanding of the timing and controls on the movement and other activity of alien  
39 animals is of fundamental importance for understanding their invasions and in attempting to  
40 mitigate detrimental impacts. Crayfish are ecologically dominant in many streams because  
41 they break down organic matter, can occur in high densities, grow to large body size and are  
42 relatively long-lived (Momot, 1995; Nyström *et al.*, 1996; Schofield *et al.*, 2001). As a result,  
43 they can be particularly damaging to populations of other organisms when introduced  
44 outside their native range (Lodge *et al.*, 1998; Gherardi *et al.*, 2006). The signal crayfish  
45 (*Pacifastacus leniusculus*) is native to northwest North America, but, due to introductions by  
46 humans, is now widespread as an invasive species in Europe, Japan and other regions of  
47 North America, including California (Machino and Holdich, 2005). Signal crayfish have had  
48 substantial, deleterious impacts where introduced, including the destruction of macrophyte  
49 stands, the impoverishment of macroinvertebrate fauna and the exclusion of juvenile fish  
50 and other crayfish species through predation and competition (Nyström and Strand 1996;  
51 Guan and Wiles, 1997; Vorburger and Ribic, 1999; Usio *et al.*, 2001; Stenroth and Nyström,  
52 2003; Crawford *et al.*, 2006). Signal crayfish have also spread a disease to which they are  
53 largely immune, but to which the only native crayfish species, the white-clawed crayfish  
54 (*Austropotamobius pallipes*), is highly susceptible (Holdich *et al.*, 1999). As a result of these  
55 impacts, white-clawed crayfish in the UK are being replaced by signal crayfish across their  
56 native range (Almeida *et al.*, 2013) and are therefore listed as endangered and legally  
57 protected. Signal crayfish also have the potential to alter the physical environment of  
58 streambeds through their activity, destabilising river banks and bed sediments (Guan, 1994;  
59 Johnson *et al.*, 2010, 2011; Harvey *et al.*, 2011; in press).

60

61 Despite the significance of signal crayfish, little is known of the temporal pattern of their  
62 activity or of environmental controls on their daily movement. Crayfish activity has been  
63 shown to vary seasonally, with declining movement related to decreases in water

64 temperature. For example, Bubb et al. (2004) found that the movement of radio-tagged  
65 signal crayfish in upland rivers in the UK was significantly correlated with water temperature.  
66 Increases in discharge also impact crayfish movement and other activity. Robinson et al.  
67 (2000) found two out of five radio-tagged white-clawed crayfish (*Austropotemobius pallipes*)  
68 dead after high flow events and others have found crayfish fatalities following floods (Momot,  
69 1966; Royo et al., 2002; Parkyn and Collier, 2004). Light (2003) recorded smaller signal  
70 crayfish populations following spates in upland rivers of the Truckee River catchment,  
71 California, USA. Others have suggested that, although crayfish movements are affected by  
72 high flows, they are capable of finding refuge during an event and re-emerge afterwards. For  
73 example, Bubb *et al.* (2004) noted that signal crayfish stopped moving during high flow  
74 events and resumed moving once flood levels had dropped. Signal crayfish and many other  
75 crayfish species have been found to move preferentially at night (Guan, 1994; Guan and  
76 Wiles, 1998; Gherardi et al., 2000; Nyström, 2005). Much of this research comes from mark-  
77 recapture and baited trapping studies, which are not suited to high resolution (sub-daily)  
78 studies of the temporal activity of animals. More recent studies that have utilised radio-  
79 telemetry also support nocturnalism in crayfish (Robinson *et al.*, 2000; Bubb *et al.*, 2002).

80

81 In this study, radio-telemetry was used to obtain a high temporal resolution record of crayfish  
82 movement in a river reach in the United Kingdom and to relate recorded movement patterns  
83 to environmental characteristics. The aims of the study were to test or confirm the following  
84 hypotheses:

- 85 • Signal crayfish are more active at night than during daylight hours,
- 86 • Signal crayfish are more active in warmer water than colder
- 87 • Signal crayfish activity is limited during high flow events

88

## 89 **Materials and Methods**

### 90 **Site description**

91 Crayfish activity was recorded for 150 days from 26<sup>th</sup> June to 22<sup>nd</sup> November 2009 in the  
92 River Bain, Lincolnshire, UK. The river is a small, lowland, alluvial stream with a  
93 predominantly gravel substrate, with isolated cobbles and a sand-silt matrix. The catchment  
94 upstream of the experimental reach is approximately 63 km<sup>2</sup> and lies over Cretaceous chalk  
95 with surficial deposits of Pleistocene till. Crayfish were tracked in a 20 m long, 4 m wide  
96 meandering reach near Biscathorpe (0° 09' 41" W, 53° 20' 15" N), that is surrounded by  
97 riparian, cattle-grazed grassland with isolated broadleaf deciduous trees. The reach has a  
98 long-established population of signal crayfish, introduced in the 1970s to a pond in the  
99 catchment and now occurring in high densities throughout the River Bain. Densities of  
100 juveniles and adults exceed 10 m<sup>-2</sup> in some parts of the river (pers.com. D. Holdich). A 10 m  
101 long reach of the channel was instrumented for this study. The morphology of this reach is  
102 typical of meander bends in small alluvial rivers. A comparatively steep, straight, coarse-  
103 grained glide flows into a leftward-swinging meander bend, the deep thalweg of which is  
104 closer to the right bank. This outside bank is steep but the adjacent channel bed is complex  
105 due to the slumping of cohesive bank material. Five to eight crayfish burrows were present in  
106 this region for the duration of the study. Crayfish burrows were also evident along the river  
107 length, but crayfish were mostly observed using coarse grains and marginal macrophyte  
108 stands as shelter during the study period. The inner bank is a fine-grained point bar which  
109 grades downstream into an open-framework gravel riffle that crosses the channel and is  
110 succeeded by a rightward swinging meander, where patterns of flow and cross-stream  
111 topography are more or less reversed (Figure 1).

112

### 113 **Environmental variables**

114 Water temperature was recorded continuously using a thermistor located below the lowest  
115 water line on the right bank of the upstream meander bend and marks the downstream limit  
116 of instrumentation. A data logger recorded mean temperature every 10 minutes. A pressure  
117 transducer at the same location recorded water depth every 10 minutes. Depth information  
118 was obtained so that an assessment could be made between crayfish activity and changing

119 river stage. The River Bain is gauged by the Environment Agency of England and Wales  
120 (EA) 5 km downstream from the field site and there are no significant tributaries or  
121 abstractions between the study reach and the gauging station. By relating the gauging  
122 station data with those of the local pressure transducer during the study period, it was  
123 possible to synthesize a longer-term record of flow depth for the study site, making  
124 assumptions that channel geometry has not materially changed. Nocturnalism of crayfish  
125 was studied by relating animal movements to hours of darkness, determined to be those  
126 between sunset and sunrise. By this definition, the hours of darkness change seasonally.

127

### 128 **PIT tagging and data collection**

129 The activity of crayfish was monitored by tracking individual animals using Passive  
130 Integrated Telemetry (PIT) tags. PIT telemetry is a passive form of radio-tagging that is  
131 increasingly used in ecological research because recovery rates are high (95 – 100%), as is  
132 reading accuracy (100%; Gibbins and Andrews 2004). PIT tags are attached to an object or  
133 organism and are located using an antenna (usually within a range of approximately 1 m).  
134 Antennae can be manufactured in a variety of forms depending on the application. In this  
135 study, 16 circular antennae (0.25 m diameter) were buried just beneath the bed surface of  
136 the study reach. Every time a tagged crayfish walked over an antenna, a reading was  
137 logged. Readings consist of a time and date 'stamp', the antenna ID and the ID of the tag  
138 attached to the crayfish. The detection range of the antennae used in this experiment was  
139 approximately 100 mm above the antenna, but only 20 mm from the antenna edge in a  
140 horizontal direction. Consequently, a reading indicates a tagged crayfish was present within  
141 a circle with a maximum diameter 0.29 m centred on the antenna. Antennae were connected  
142 to a multi-point decoder (MPD) that identified any PIT tags within the detection range of each  
143 antenna and logged them. The MPD interrogated the 16 antennae sequentially in a 3  
144 second cycle. This rapid interrogation removed potential issues of interference between  
145 antennae positioned close together. It is unlikely a crayfish could cross an antenna within 3  
146 seconds because of their relatively slow walking speed and, therefore, it is unlikely that the

147 interrogation cycle led to missed contacts. All PIT telemetry and tags were purchased from  
148 Wyremicrodesign Ltd.

149

150 A filter algorithm built into the logging system allowed a distinction to be made between *in*  
151 *situ* and *ex situ* crayfish movements. If a crayfish was recorded consecutively by every 3  
152 second cycle in a 30 second period (i.e. 10 times) the activity was termed *in situ* and  
153 revealed a stationary crayfish or movement within the circumference of the antenna  
154 interrogation area. When crayfish did not trigger the same antenna consecutively, the  
155 reading was considered *ex situ* and indicated that the crayfish had moved across an antenna  
156 without remaining in that area for more than 30 seconds. *Ex situ* activity includes the  
157 possibility that a crayfish moved off, and then back onto the same antenna. Although the  
158 presence of multiple tags should not affect the ability of an antenna to record the presence of  
159 another tagged crayfish, the presence of a large number of tags on the same antennae at  
160 the same moment could lead to missed recordings. To minimise the possibility of  
161 incorporating errors introduced by such events, we only analyse *ex situ* data in this paper.  
162 Moreover, this approach ensures a fairly rigorous definition of activity: although *in situ*  
163 readings indicated that a crayfish had moved out onto the channel bed rather than remaining  
164 in a burrow or other refuge, this action represents significantly less activity than journeys  
165 across the channel bed. The activity of crayfish was therefore parameterised by cumulating  
166 the total number of *ex situ* recordings made by all crayfish in the reach across all antennae  
167 for each day and dividing the total by the number of crayfish that were active within the reach  
168 that day.

169

170 The sixteen antennae were distributed non-uniformly through the study reach in association  
171 with discrete substrate patches because an ancillary aim of this work (not reported here) was  
172 to examine the differential use of different substrate patches by crayfish. Patches were  
173 defined and distinguished by grain-size characteristics, macrophyte presence and flow  
174 conditions (Figure 1). Antennae are not equidistant and, therefore, movements between

175 different pairs of antennae represent displacements of different lengths. Crayfish activity was  
176 therefore also parameterised by distance moved, based on the measured lengths of straight  
177 line paths between consecutively triggered pairs of antennae. Actual journey paths are not  
178 known, but, because the start and end points are defined, minimum displacement distances  
179 can be calculated. An average was again obtained by dividing the total distance moved by  
180 all crayfish each day by the number of crayfish active that day. This provides the average  
181 distance moved by all active crayfish each day.

182

### 183 **Crayfish tagging procedure**

184 Crayfish remained in the instrumented river reach for a mean period of 11 days (S.D. = 9  
185 days), after which, they left the reach and rarely returned. This is consistent with previously  
186 described nomadic behaviour of both signal crayfish (Bubb *et al.*, 2002; 2004, Light, 2003)  
187 and other crayfish species (Gherardi *et al.*, 1998; Schütze *et al.*, 1999; Gherardi *et al.*, 2000;  
188 Robinson *et al.*, 2000). To maintain the stock of PIT-tagged crayfish within the instrumented  
189 reach, animals were tagged and released throughout the tracking period. On average, five  
190 PIT-tagged individuals were tracked in the reach each day (S.D. = 3). Crayfish were caught  
191 within 20 m upstream and downstream of the instrumented reach to reduce disturbance  
192 associated with transport between capture and release and thereby increase the likelihood  
193 that crayfish would remain within range of the antenna network when re-introduced. Crayfish  
194 were not caught within the instrumented reach to avoid disturbing the crayfish being tracked,  
195 their physical environment and the tracking antennae. In total, 65 crayfish were tagged  
196 during the five-month observation period. The size of crayfish that were selected for tagging  
197 was standardised: only those with a carapace length of  $55 \pm 5$  mm were used, because this  
198 represented the mode and mean of caught, adult crayfish in the reach. In addition, a tag  
199 might be a burden to smaller individuals inhibiting their behaviour. Crayfish with obvious  
200 injury, such as the loss of limbs or antennae, were also deselected as this can affect their  
201 exploratory behaviour (Basil and Sandeman, 2000; Koch *et al.*, 2006). No berried females  
202 were caught.



203

204 Crayfish were caught by hand and placed in a plastic handling container. A single glass-  
205 encapsulated PIT tag (12 mm long, 2 mm wide) was attached to the crayfish's cephalothorax  
206 as this causes little upset and results in a large percentage of tags remaining attached (Bubb  
207 *et al.*, 2006). Cyanoacrylate was used to attach tags because it dries in minutes, limiting the  
208 time crayfish needed to be out of water. By minimising stress to the animal, this approach  
209 maximised the likelihood of natural behaviour upon release. Although cyanoacrylate  
210 weakens through time when submerged in water, it successfully attached tags to crayfish in  
211 aquaria experiments until crayfish moulted multiple months later. Given that the average  
212 time crayfish remained in the instrumented reach was 11 days (max. 38 days), the potential  
213 weakening of the adhesive is not seen as a limitation here. However, longer studies may  
214 require alternative strategies or the use of internal tags. Once the adhesive had set, the  
215 crayfish was submerged in a container for 15 minutes to check the tag was properly  
216 attached and the individual had not been adversely affected. Crayfish were then released  
217 into the river over antenna 4, due to its central location in the reach and because the  
218 presence of macrophyte cover prevented undue exposure during daylight hours. Crayfish  
219 are predominately nocturnal so activity during daylight hours on the day of release was  
220 considered likely to be inconsistent with natural behaviour and a direct result of tagging and  
221 release. Consequently, the movement of crayfish on the day of release was removed from  
222 the data-set and all future analysis.

223

224 Robinson *et al.* (2000) described a 'fright response' after release of radiotagged white-  
225 clawed crayfish (*Austropotamobius pallipes*), where individuals moved significantly more in  
226 the two days following release. However, they only quantified long-distance movements and,  
227 consequently, any 'fright response' on the scale observed in that study would have resulted  
228 in crayfish leaving the instrumented reach in this study. Indeed, of the 65 individuals tagged  
229 in this study, seven (11%) left the reach within one day of being caught and released, which  
230 might indicate a 'fright response'. However, because substantial effort was made to minimise

231 the disturbance during tagging, and 89% of tagged crayfish remained in the reach, the loss  
232 of those leaving the study reach is not considered indicative of a major methodological  
233 problem.

234

### 235 **Statistical analysis**

236 Measures of the average distance moved by active crayfish were calculated for hourly and  
237 daily time periods and analysed in SPSS 19.0. Hourly averages were used when exploring  
238 the nocturnalism of crayfish, whereas daily averages were used when relating activity to  
239 environmental conditions. Levene's tests indicated that the assumption of variance  
240 homogeneity was violated for comparisons of hourly and daily data, so Kruskal-Wallis tests  
241 were performed in order to ascertain significance levels. When daily-activity was related to  
242 continuous measures of water temperature and flow depth, regression analysis was used in  
243 SPSS 19.0. All assumptions were met for multiple linear regression; however, crayfish  
244 activity data was heteroscedastic when regressed on water temperature in simple linear  
245 models. Given that linear regression models are only used to demonstrate the lack of clear  
246 mean-based relations between environmental variables and activity, no further action was  
247 taken. In addition, quantile and median regression were performed on the data, providing a  
248 more robust regression analysis which is valid for heteroscedastic data. This was  
249 undertaken using the Quantreg package in R (Koenker, 2012). More information about  
250 quantile regression and its uses can be found in Cade and Noon (2003).

251

## 252 **Results**

### 253 **Environmental variables**

254 The daily-averaged water temperature within the reach ranged from 6.8–17.1°C between  
255 26th June and the 22nd November 2009. The temperature declined steadily from 19th  
256 August to 22nd November 2009, giving a linear trend. During 38 years of gauged flow  
257 recording, the daily-averaged mean flow was  $0.35 \text{ m}^3 \text{ s}^{-1}$ , the 95% exceedance ( $Q_{95}$ ) was

258 0.068 m<sup>3</sup> s<sup>-1</sup> and the 10% exceedance (Q<sub>10</sub>) was 0.729 m<sup>3</sup> s<sup>-1</sup>. In most years, there were  
259 isolated high flow events in the summer and autumn, but these rarely exceeded 2 m<sup>3</sup> s<sup>-1</sup>.  
260 Flow depth during the tracking period was variable (0.25–0.59 m at the pressure transducer),  
261 with a number of isolated high flow events, three of which were clustered in late July/early  
262 August. An extended period of low flow occurred throughout August and September 2009,  
263 producing a minimum recorded depth of 0.21 m over antenna 4 and 0.95 m over antenna 5.  
264 In October and November, the flow depth increased rapidly and remained relatively high  
265 throughout November. This trend in water depth was consistent with those recorded at the  
266 gauging station and is consistent with the 38-year average pattern, which implies that flow  
267 during the tracking period was typical for the river.

268

### 269 **Nocturnalism of signal crayfish**

270 Over the 150-day tracking period, 10884 point locations were registered for 65 tagged  
271 crayfish. Crayfish moved preferentially during the hours of darkness, with less than 6% of all  
272 recorded movements occurring during daylight hours (Figure 2), here defined as occurring  
273 between sunset and sunrise. The nocturnal activity of crayfish is consistent through the  
274 months, with night-time activity always dominant over hours of sunlight. However,  
275 nocturnalism was weaker in the summer months, with significantly more daytime movements  
276 made in July in comparison to other months ( $p = 0.039 - 0.042$ ; Figure 3). Crayfish were  
277 most frequently active between 22:00–23:00 and 02:00–03:00, giving two peaks in night-  
278 time activity, which are statistically significant from both the preceding and subsequent hours  
279 ( $p < 0.01$  in all cases) (Figure 2). The percentage of movements in each hourly interval  
280 demonstrates the broad similarity of this pattern from month to month (Figure 3). However,  
281 the bimodal distribution of night-time activity, with its intervening decline around midnight, is  
282 less distinct in summer months. Male and female crayfish were both highly nocturnal and the  
283 percentage of movements made at night was statistically similar between sexes, equivalent  
284 to 90.6% for males and 89.4% for females.

285

## 286 **Seasonal distribution of crayfish activity**

287 There is a significant difference in crayfish activity levels between some months. Significantly  
288 less activity took place in November in comparison with other months ( $p < 0.001$ ) and  
289 significantly more activity took place in September ( $p = 0.04$ ; Figure 4). Activity levels in  
290 other months were statistically similar ( $p = 0.754$ ). However, there was a great deal of day-  
291 to-day variability in activity throughout the tracking period. Levene's tests indicate that the  
292 variance of daily activity values was significantly different between months. The greatest  
293 range in daily activity occurred in September and the least in November.

294

295 There was no difference in the activity of male and female crayfish during the entire tracking  
296 period (ANOVA;  $p = 0.78$ ) or within individual months, consistent with the findings of others  
297 (Guan and Wiles, 1997; Kirjavainen and Westman, 1999; Bubb *et al.*, 2004). Females were  
298 generally less abundant than males, but a greater number of females were caught in August  
299 and September (47% and 45% females, respectively) in comparison to October and  
300 November (31% and 17%, respectively).

301

302 Time-series of activity data, flow depth and water temperature hint at environmental controls  
303 on crayfish activity (Figure 5) and this is corroborated by the results, given above, which  
304 demonstrate significantly different activity levels between months. The relations between  
305 variables are linear, but there is a lot of scatter in all cases. Simple linear regression of both  
306 the measures of crayfish activity on water depth or water temperature are significant ( $p <$   
307  $0.001$ ). Amongst these, the strongest relations are between average distance moved and  
308 water depth, but in general the simple linear regression models provide poor explanatory  
309 power ( $r^2$  lies between 0.08 and 0.42). Given that several environmental factors are likely to  
310 be simultaneously affecting crayfish behaviour, a more complex analysis was considered  
311 appropriate. Multiple linear regression of average distance moved using temperature and

312 depth as independent variables was statistically significant ( $p < 0.001$ ) but, as with the  
313 simple regression analyses, the model had relatively weak explanatory power ( $R^2 = 0.46$ ).

314

315 To further explore the relations between crayfish activity and water depth and temperature,  
316 the time-series were split into two sub-periods. The division was based on inspection of the  
317 distance moved data and the generation of best fitting least-squares curves for both sub-  
318 periods. This division occurs at the beginning of September and marks the boundary  
319 between summer and autumn months (Figure 5). Autumn is characterised by a clear linear  
320 decline in activity and a decline in water temperature as winter approaches. The regression  
321 of distance moved against temperature in this sub-period has an  $r^2$  of 0.53. In contrast,  
322 during the summer months the regression coefficient is not significant, indicating no temporal  
323 trend (Figure 6).

324

325 Quantile regressions of the 5<sup>th</sup>, 25<sup>th</sup>, 50<sup>th</sup>, 75<sup>th</sup> and 95<sup>th</sup> percentiles are all statistically  
326 significant where water depth is the independent variable ( $p < 0.001$  in all cases). The same  
327 holds true where water temperature is the independent variable, except in the case of the  
328 95<sup>th</sup> percentile, where the regression coefficient is not significant ( $p = 0.06$ ; Figure 7). The  
329 relations are linear in all cases, but general convergence of the regression curves indicates  
330 that crayfish activity became less variable as water-depth increased and temperature  
331 decreased, consistent with the observation that the variance of daily activity was less in  
332 November than September. The regression coefficient of the median (50<sup>th</sup> percentile)  
333 relation between distance moved and depth indicates that crayfish moved, on average, 12.9  
334 m less for every 0.1 m rise in water depth. This model also predicts that crayfish activity  
335 ceased in the River Bain when depth exceeded 0.52 m at the pressure transducer. Quantile  
336 regressions of movement on temperature indicate that, in general, activity increased with  
337 temperature. The median regression suggests that activity ceased when temperature fell  
338 below 5°C and increased with a rise in temperature above this threshold at a rate of 2.5 m  
339 °C<sup>-1</sup>.

340

## 341 **Discussion**

### 342 **Nocturnalism**

343 In the River Bain, peak activity occurred between 21:00 and 23:00 (Figure 3). This is  
344 consistent with other studies; for instance, Nyström (2005) found that signal crayfish were  
345 most active at dusk and Robinson *et al.* (2000) have shown that radio-tagged white-clawed  
346 crayfish were significantly more active between dusk and midnight (21:00–00:00) in  
347 comparison with any other time, including dawn (03:00–06:00). However, the timing of peak  
348 activity changed from one month to another in the present study, occurring in the hour  
349 beginning 23:00 in July, 22:00 in August, September and October, and 21:00 in November  
350 (Figure 3). The sunset time shifted from 21:00 in July to 20:00 in August to 19:00 in  
351 September and 18:00 in October. Consequently, the shift of peak activity from July to  
352 November may reflect the increasingly earlier time of sunset in autumn months, however,  
353 disentangling this from changes in other relevant environmental and ecological/biological  
354 conditions is difficult.

355

356 Unlike previous studies, crayfish in this reach of the River Bain remained active throughout  
357 the night. Guan and Wiles (1998) studied the nocturnal foraging of signal crayfish in the  
358 River Ouse, England, using capture techniques. They found signal crayfish foraged between  
359 17:00 and 01:00 in all seasons, much less between 01:00 and 09:00 and only occasionally  
360 between 09:00 and 17:00. In the present study, crayfish were, cumulatively, more active  
361 between 01:00 and 09:00 than between 17:00 and 01:00, suggesting that, in this small  
362 stream, crayfish only had a weak preference for a particular period during the night when  
363 conducting their activities. In fact, many of the months had two peaks of activity during the  
364 night - at and just after dusk and then again at 02:00. The reasons for this are currently not  
365 known, but it might reflect an initial burst of activity at dusk, perhaps associated with

366 foraging, followed by a secondary burst of activity later, when, perhaps, they had begun to  
367 seek refuge before dawn.

368

369 Crayfish were highly nocturnal, with little daytime activity occurring over the 150 days of  
370 study. They are visual predators, but are nocturnal in their native range due to the threat of  
371 being detected by other visual predators. Where crayfish have invaded, such as in the British  
372 Isles, the threat of predation is likely to be much reduced and, consequently, it might be  
373 beneficial for populations of crayfish to adopt daytime activity. Some authors have identified  
374 invasive crayfish populations as being at least partially active in daylight hours (i.e. Guan  
375 and Wiles, 1998) and the present authors have observed daylight activity in other English  
376 rivers. It may be that the River Bain is characterised by a suite of conditions that make  
377 daytime movement less favourable. For example, it is shallow through most of the  
378 instrumented reach (mean depth of 0.45 m during the tracking period), that crayfish are more  
379 vulnerable to visual terrestrial predators, such as wading birds. However, if this were the  
380 case, it is surprising that nocturnalism was strongest in winter, with more daytime  
381 movements occurring in July and August when, presumably, crayfish are most exposed  
382 because of bright sunlight and low flow depths. Gherardi *et al.* (2000) found that invasive  
383 Red Swamp crayfish (*Procambarus clarkii*) were nocturnal throughout the year, with the  
384 exception of the spring, when they made significantly more daylight movements. Together  
385 with the data presented here, this suggests that nocturnalism in invasive crayfish may be  
386 variable within and between rivers due to the changing hours of darkness and prevailing  
387 environmental conditions.

388

### 389 **Controls on crayfish activity**

390 PIT-tagged signal crayfish were highly active within the instrumented reach during the 150-  
391 day tracking period. However, it is apparent that levels of crayfish activity changed through  
392 time, implying that some periods were favoured by crayfish more than others. It should be

393 noted that 'activity' is defined in this study as a movement greater than 0.29 m, which may  
394 represent foraging for food, escaping a predator or competitor, or exploring the environment  
395 in search of new resources. They might be active in other ways, for instance, feeding or  
396 grooming, but these would not be recorded in this study because two spatially separated  
397 antennae would not be triggered by these comparatively sedentary activities.

398

399 There is a significant difference between crayfish activity levels each month, with less activity  
400 in November and more in September than in other months (Figure 4). As September was  
401 warm with relatively low flow depths (average 12.9°C, 0.30 m) and November was cold with  
402 high flows (8.6°C, 0.49 m), we can hypothesize that these environmental conditions affected  
403 activity. In addition, signal crayfish breed in autumn, with females protecting their eggs by  
404 carrying them under their tails until May. This may explain the increased activity of crayfish in  
405 September and the decline in the number of females caught in later months. Evidence from  
406 other studies supports the hypothesis that crayfish activity is limited by temperature  
407 (Gherardi *et al.*, 2002; Bubb *et al.*, 2002). Bubb *et al.* (2002) found that crayfish stopped  
408 making long-distance movements when water temperature dropped to an average of 4.2°C  
409 (S.D. = 1.3°C). In the River Bain, an extrapolation of the quantile regression model of  
410 median values predicts the complete cessation of movement at 5°C. Previous studies have  
411 also demonstrated that high flows can both displace and cause mortality in several crayfish  
412 species (Momot, 1966; Robinson *et al.*, 2000; Royo *et al.*, 2002), including signal crayfish  
413 (Light 2003). However, Bubb *et al.* (2002; 2004) found, using radio-telemetry, that signal  
414 crayfish were not entrained by high flows because, presumably, they sheltered in burrows or  
415 in stable areas of substrate. Light (2003) suggested that signal crayfish shelter in deep pools  
416 or ponds during storm flows and re-emerge when flow levels recede. Our observations  
417 support these speculations, because crayfish rarely moved during high flow events, but  
418 always re-emerged afterwards.

419



420 There is apparent incongruity between the observed impact of flow characteristics and water  
421 temperature on crayfish activity and the weak levels of explanation given by the simple,  
422 least-squares regression models. This apparent incongruence is not limited to this study. For  
423 example, Bubb *et al.* (2004) found that the daily movement of radiotagged signal crayfish  
424 was significantly correlated with water temperature, but derived a relatively low  $r^2$  of 0.24.  
425 Such low coefficients of determination reflect the heteroscedasticity of the data sets,  
426 specifically the wedge-shaped increase in variance when plotted against temperature and  
427 the decrease in variance when plotted against depth. This suggests that simple models of  
428 this type are not appropriate (Figure 7). Instead, quantile regression models appear to be  
429 more useful here, as they are for other relations between ecological and environmental  
430 variables where there is evidence of limiting conditions (Lancaster and Belyea, 2006). This is  
431 largely because a favourable condition does not necessitate increased activity of an animal  
432 as is implied by least-square regression models but, instead, only provides the opportunity  
433 for increased activity, which animals may or may not decide to undertake based on other  
434 environmental and ecological conditions.

435

### 436 **Nested hierarchy of environmental controls**

437 Variability in crayfish activity is apparent within the data over a large range of time-scales,  
438 from minutes to months. On the basis of these results, it is hypothesised that the activity of  
439 crayfish is controlled by a range of biological and environmental processes that act as a  
440 nested hierarchy, each limiting activity at different time-scales (Figure 8). This is similar to  
441 the spatially nested hierarchy of habitat subsystems in rivers that has been proposed by  
442 Frissell *et al.* (1986). As a result, the significance of an environmental factor to crayfish  
443 activity will be at least partially dependent on the temporal scale at which activity is  
444 measured. Here, it is argued that temperature is of significance at the longest time-scales  
445 (season, year) because there is a clear annual trend in the activity of crayfish and this  
446 broadly parallels the temperature time-series. Therefore, temperature is likely to be noted as

447 significant only when long data-series are recorded, covering many months and, preferably,  
448 several years. In addition, when temperature is relatively constant, changes in activity in  
449 response to small fluctuations in temperature may be undetectable; they may also be  
450 masked by the impact of other factors (e.g. flight because of fright) that influence levels of  
451 activity associated with smaller time-scales. This is reflected in the data, where regression  
452 analyses provide improved predictive models of behaviour in autumn, when temperature  
453 was changing, than in summer, when the temperature was both high and comparatively  
454 constant (Figure 6).

455

456 At smaller time-scales of weeks to days, flow depth appears to be of most significance. This  
457 may also explain why depth provided the strongest relation with activity over the time-scale  
458 studied here. It is clear that when the flow is high, crayfish cease moving even if other  
459 conditions are favourable. This is likely to be the case because changes in water depth in  
460 rivers are likely to manifest over many hours to days, even in rivers with flashy regimes. At  
461 an hourly scale, light levels have the largest impact on activity, with crayfish in the River Bain  
462 generally only moving in darkness. At smaller scales (seconds–hours) where temperature,  
463 depth and light levels are essentially stable, it is likely that conspecific and interspecific  
464 interactions (e.g. fighting, fleeing) and biological imperatives (e.g. feeding) dominate levels of  
465 crayfish activity, although consideration of these effects was not one of the aims of this study  
466 and we did not measure them. These smaller-scale biological and ecological controls are  
467 superimposed on larger scale trends, generating ‘noise’ in the recorded data. In addition,  
468 there are ecological and biological factors that operate across the longer timescales from  
469 years to days (e.g. food availability, predator activity, mating periods) that are also likely to  
470 affect crayfish activity levels. Together with the high frequency noise noted above, this  
471 biological/ecological control is likely to be, at least, partly responsible for the relatively weak  
472 explanatory power of mean-based, least-squares regression models with only environmental  
473 independent variables.

474

475 Further interactions complicate the response of crayfish to environmental changes, making it  
476 yet more difficult to disentangle patterns of cause and effect. So, for example, upper-rung  
477 variables, representing the largest-scale controls on activity, can nullify the influence of  
478 lower-rung variables when they impose conditions that are not conducive of activity (i.e.  
479 when they are limiting). For example, if the temperature is cold enough to limit crayfish  
480 activity, favourable conditions of flow depth do not lure the animals into increased activity.  
481 However, the opposite can obtain. So, for example, the presence of a predator (a lower-rung  
482 variable) is likely to prevent crayfish activity when all other conditions (e.g. temperature and  
483 water-depth) are favourable. The relative position of controlling variables in the hierarchy  
484 may provide useful information about their significance for crayfish activity, such that testing  
485 and extending the conceptual model presented in Figure 8, will provide a useful avenue of  
486 research. It should be noted, however, that the significance of each environmental variable is  
487 likely to be context-dependent and its hierarchical significance may differ from one river to  
488 another.

489

## 490 **Conclusions**

491 The environmental characteristics monitored in this study are shown to have acted as  
492 controls on crayfish activity. They form a nested hierarchy, causing activity to be highly  
493 variable over a range of time-scales and this restricts the value of mean-based regression  
494 models as tools explaining and quantifying the impact of controls on activity. Instead,  
495 quantile regression provided a useful, alternative tool for identifying the conditions  
496 determining and limiting crayfish activity.

497

498 There is evidence that environmental factors affect the extent of nocturnalism in invasive  
499 crayfish, because the proportion of daytime movements was shown to be significantly  
500 greater in summer months than in autumn months. Quantile regression analysis suggests  
501 that crayfish are tolerant of a wide range of flows, but are most active when low flows

502 coincide with periods of high water temperature. Analysis also suggests that signal crayfish  
503 are sensitive to water temperature and activity is shown to decline substantially as water  
504 temperature decreases in autumn. However, given the variability in nocturnalism that has  
505 been reported for different rivers, it is suggested that environmental conditions, such as flow  
506 depth, speed and temperature, may have different impacts both in different rivers and from  
507 reach to reach within the same river, reflecting the variable significance of other factors, such  
508 as the ability to hide from predators. This is important for understanding the invasion of non-  
509 native crayfish and attempting to manage their spread throughout the river network.

510

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517

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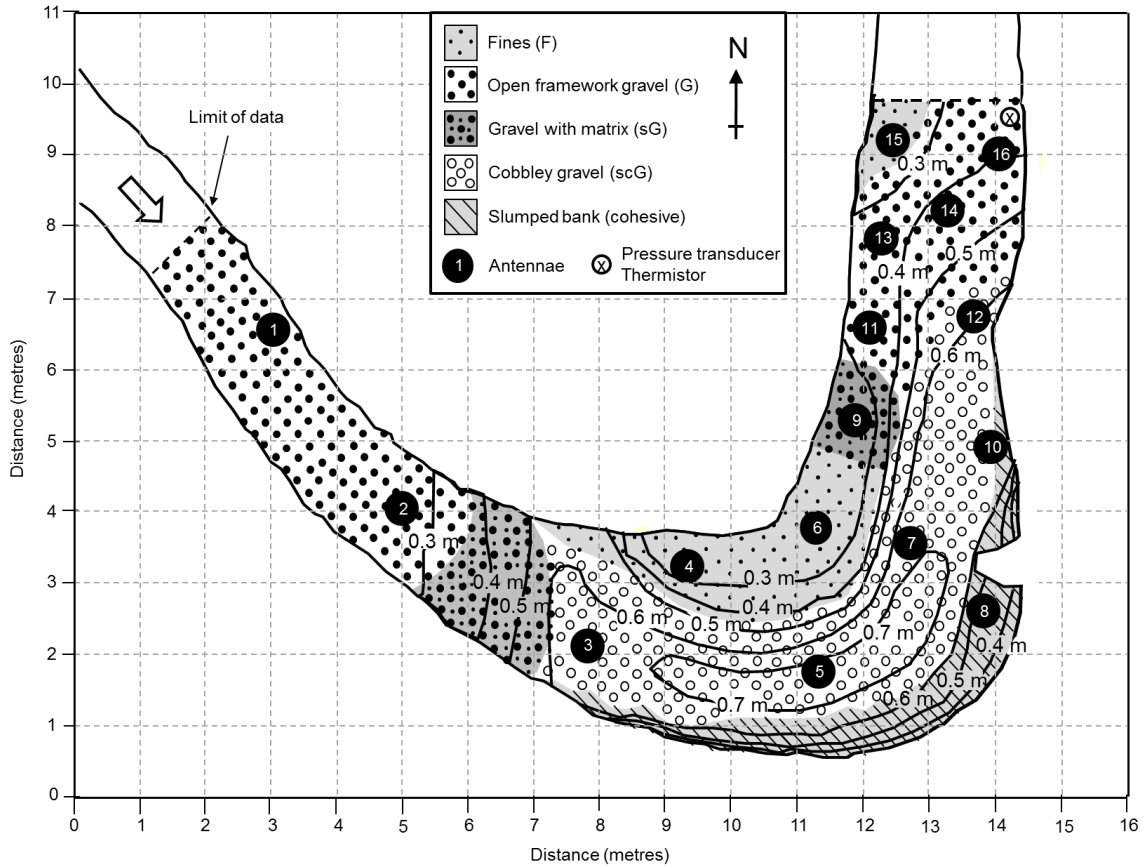
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653 **Figures**

654 **Figure 1:** A map of the instrumented reach of the River Bain, showing antenna locations.

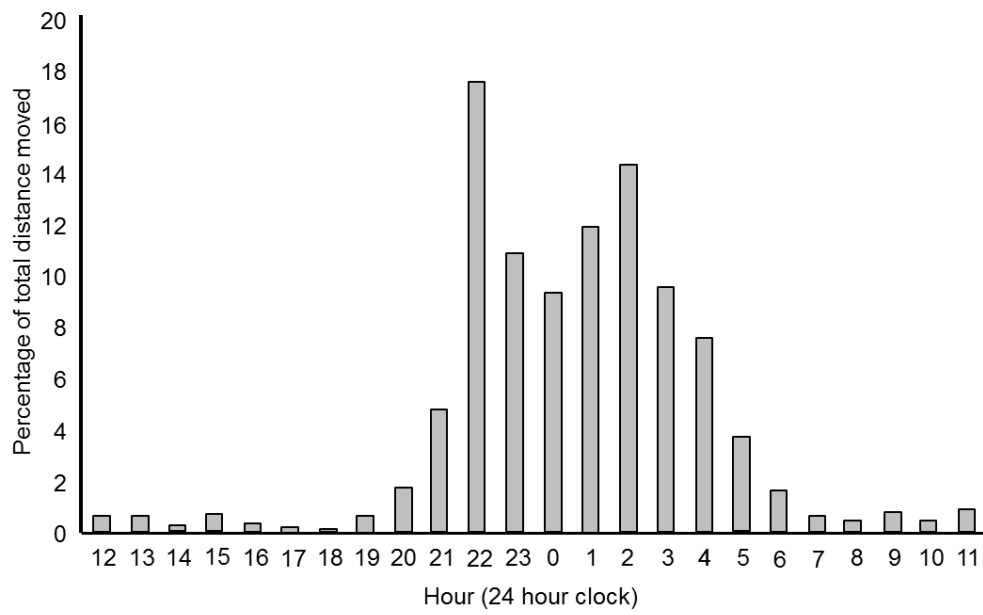
655 Channel-bed contours relate to a local datum.



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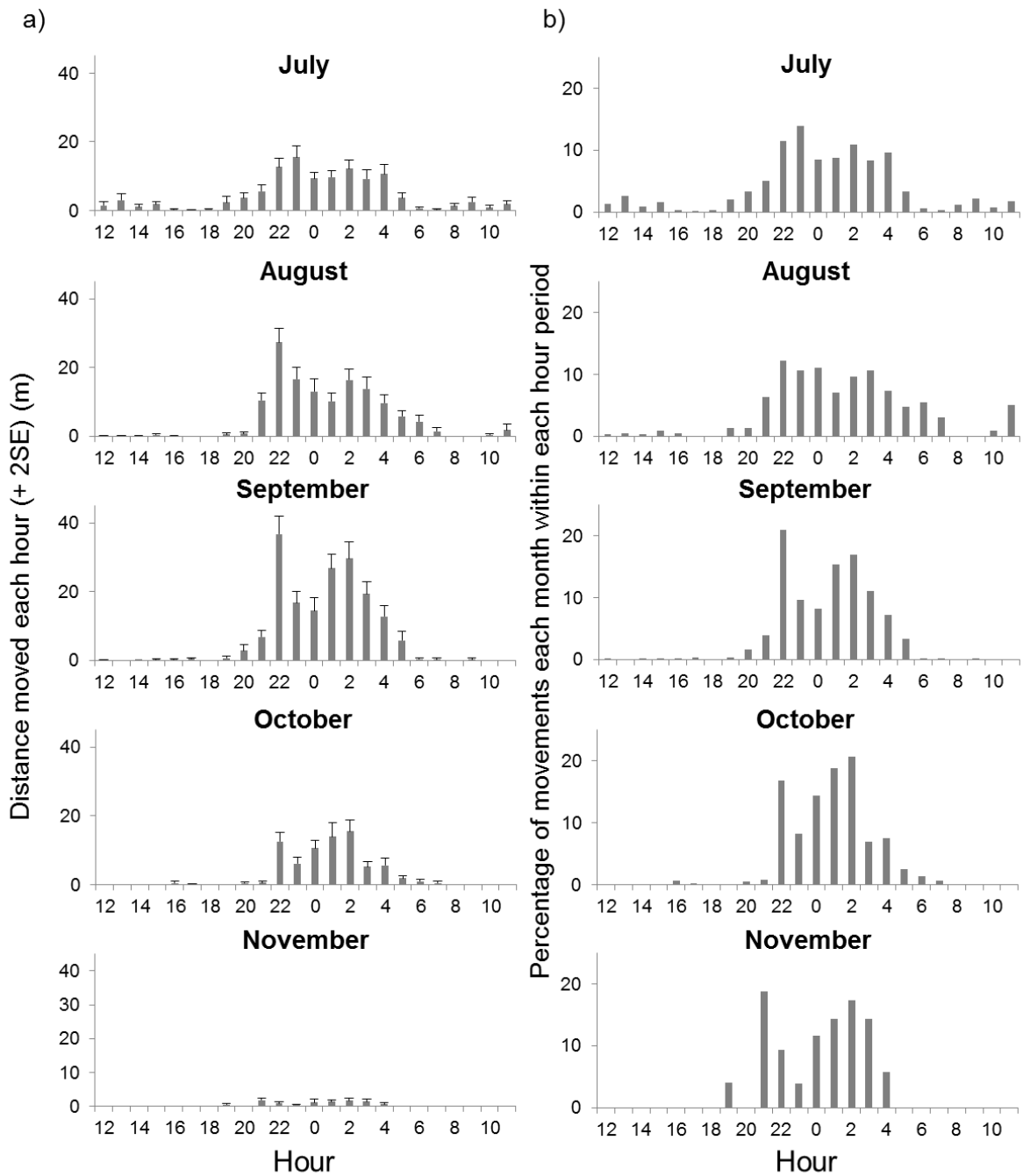
658 **Figure 2:** The percentage of total distance moved by tagged crayfish during each hour of the  
659 day between 22<sup>nd</sup> June and 22<sup>nd</sup> November 2009.



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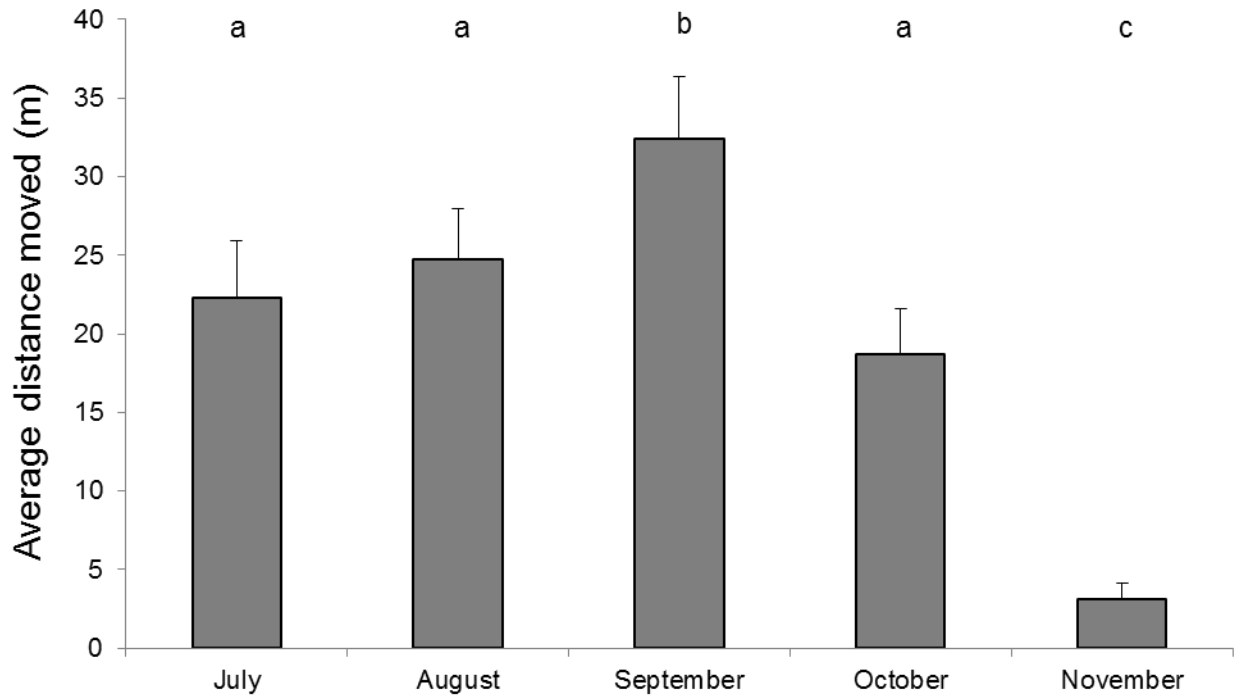
662 **Figure 3:** a) The total distance moved by tagged crayfish and b) the percentage of the total  
 663 distance moved by tagged crayfish in each hour of the day, in each month, July-November  
 664 2009.



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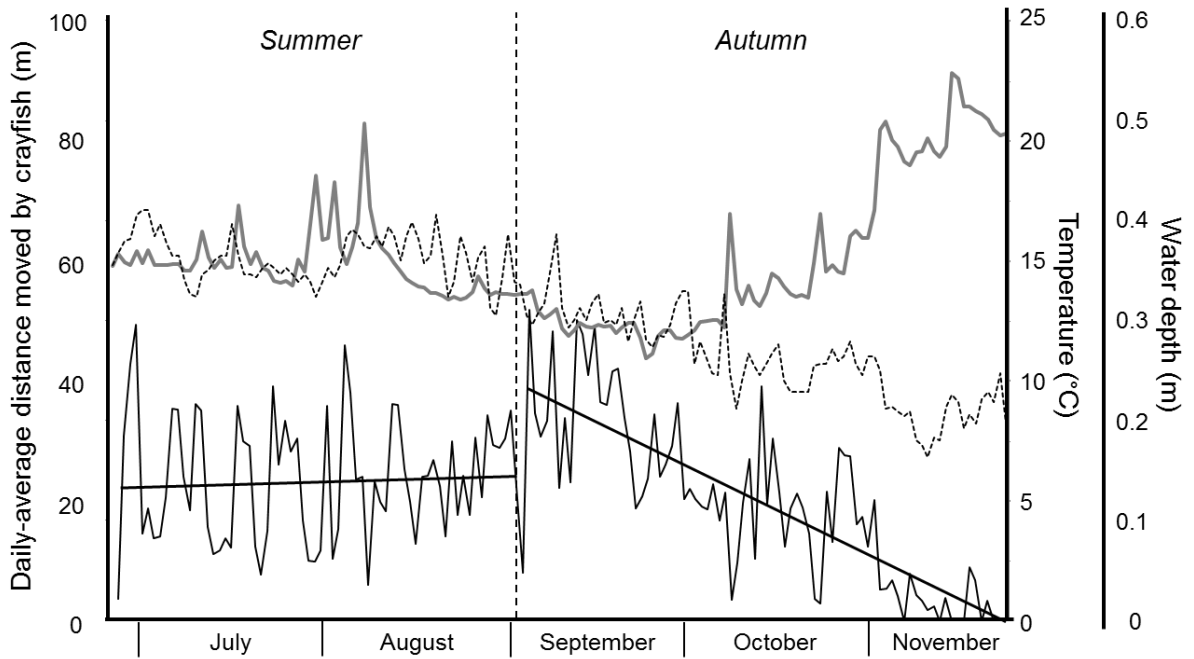
667 **Figure 4:** The average distance (+2 SD) moved by crayfish each month of summer and  
668 autumn 2009. Letters indicate significant statistical groupings based on Kruskal-Wallis with  
669 Bonferroni corrected Mann-Whitney post-hoc tests



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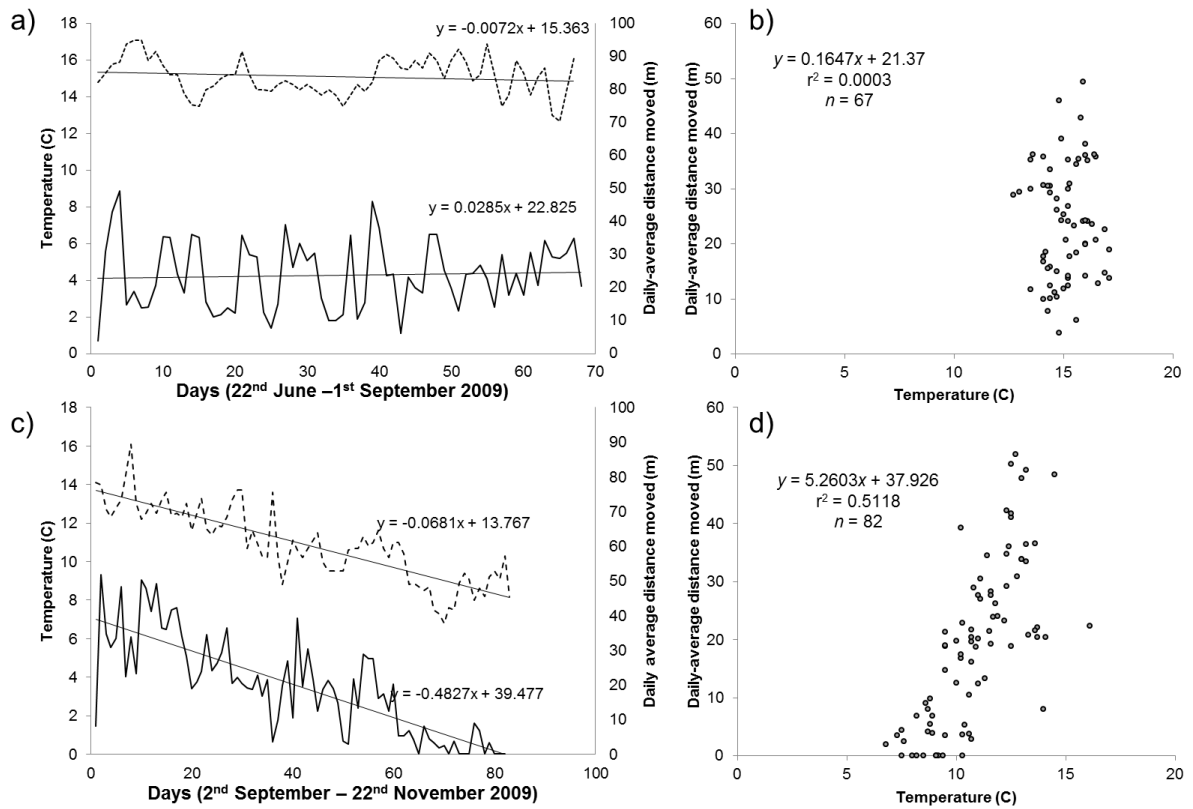
672 **Figure 5:** Time-series of crayfish activity (black line), water temperature (pecked line) and  
673 flow depth (grey line). Vertical pecked line separates the time-series into two broad sub-  
674 periods based on obtaining the best-fit of the two regression lines describing crayfish activity  
675 with time.



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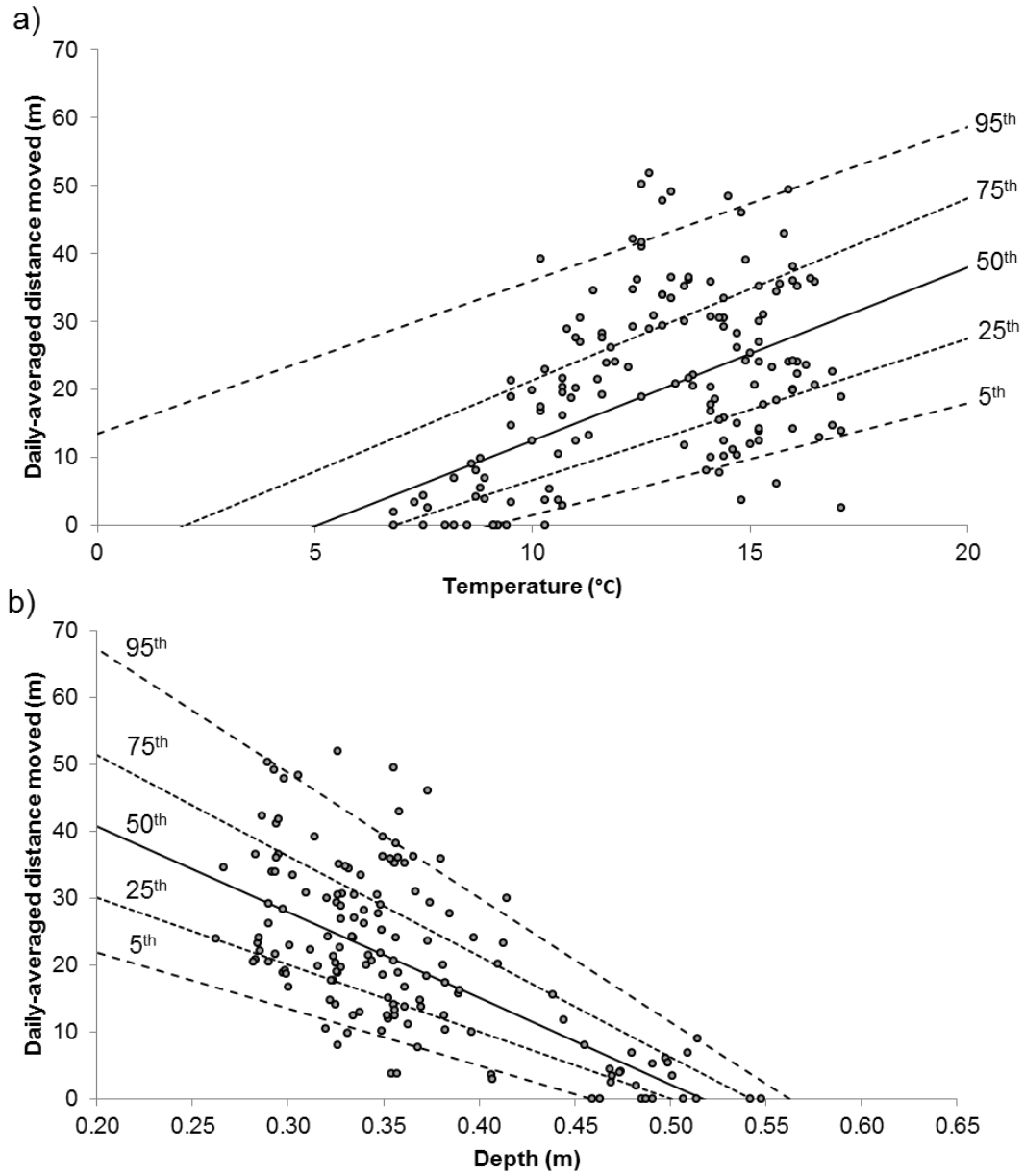
678 **Figure 6:** a) Time-series of the daily-averaged distance moved by crayfish (solid line) and  
 679 the water temperature (pecked line) in (a) summer and (c) autumn. Best-fit lines represent  
 680 the regression of each variable on time. Scatter-plots of daily-averaged distance moved by  
 681 crayfish versus water temperature for (b) summer and (d) winter.



682

683

684 **Figure 7:** Scatterplot of daily-averaged distance moved by crayfish versus (a) water  
685 temperature and (b) water depth with quantile regressions for the 5<sup>th</sup>, 25<sup>th</sup>, 50<sup>th</sup> (median), 75<sup>th</sup>  
686 and 95<sup>th</sup> percentiles.

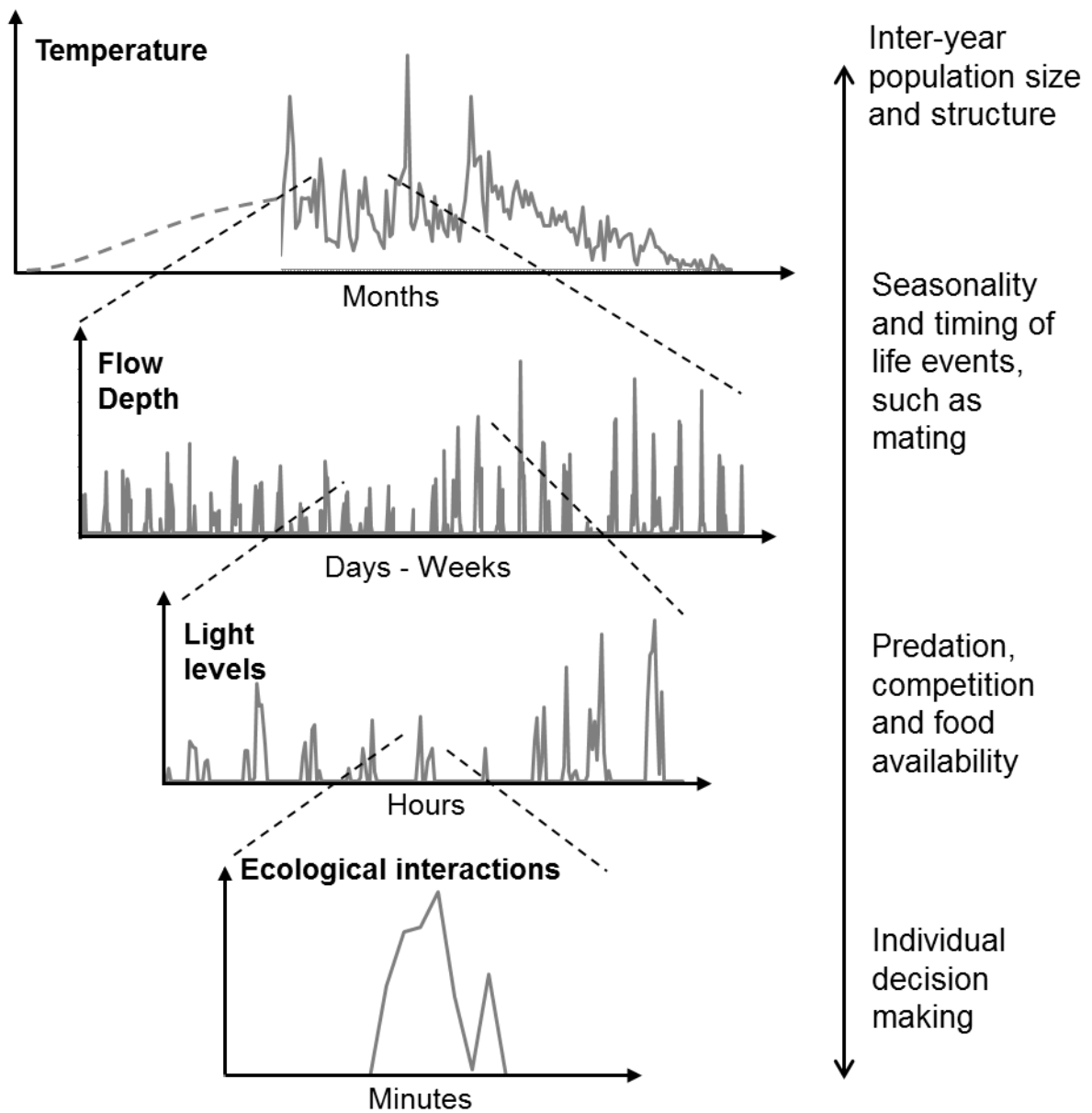


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689 **Figure 8:** A theoretical model of the nested hierarchy of environmental controls on crayfish  
690 activity (y-axis); the significance of each control is dependent on the temporal scale.



691