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Avoidance and aggregation create consistent egg distribution patterns of congeneric caddisflies across spatially variable oviposition landscapes

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22 **Abstract**

23 Amongst oviparous animals the spatial distribution of individuals is often set initially by where females lay
24 eggs, with potential implications for populations and species coexistence. Do the spatial arrangements of
25 oviposition sites or female behaviours determine spatial patterns of eggs? The consequences of spatial
26 patterns may be context independent if strong behaviours drive patterns; context dependent if the local
27 environment dominates. We tested these ideas using a guild of stream-dwelling caddisflies that oviposit on
28 emergent rocks, focussing on genera with contrasting behaviours. In naturally-occurring oviposition
29 landscapes (riffles with emergent rocks) we surveyed the spatial arrangement and environmental
30 characteristics of all emergent rocks, identified and enumerated egg masses on each. Multiple riffles were
31 surveyed to test for spatially-invariant patterns and behaviours. In landscapes, we tested for spatial clumping
32 of oviposition sites exploited by each species and for segregation of congeneric species. At oviposition sites,
33 we characterised the frequency distributions of egg masses and tested for species associations. Genus-specific
34 behaviours produced different spatial patterns of egg masses in the same landscapes. Congregative behaviour
35 of *Ulmerochorema* spp. at landscape scales and an aggregative response at preferred oviposition sites led to
36 clumped patterns, local aggregation and species overlap. In contrast, avoidance behaviours by congeners of
37 *Apsilochorema* resulted in no or weak clumping, and species segregation in some landscapes. Spatial patterns
38 were consistent across riffles that varied in area and oviposition site density. These results suggest that quite
39 different oviposition behaviours may be context-independent, and the consequences of spatial patterns may
40 be spatially invariant also.

41

42 **Key words:** aquatic insects, congregation, rivers, spatial pattern formation, Trichoptera

43

44 **Introduction**

45 A long-standing challenge in ecology is to elucidate the causes and consequences of spatial patterns in
46 ecological systems. The spatial distribution of individuals within populations can have consequences for
47 population dynamics (Morris et al. 1992), predator-prey dynamics (Huffaker 1958) and species coexistence

(Atkinson and Shorrocks 1984; Ives 1988). Mechanistically, spatial aggregation, for example, can exacerbate density-dependent process (e.g. local competition and predation) and density-independent effects (e.g. local disturbance) that potentially reduce fitness. The underlying causes of spatial patterns are also diverse and may include species responses to environmental heterogeneity in resources, how species interact with patchy landscapes (e.g. during movement) and interact with one another. If the consequences of spatial patterns have positive effects on fitness, then heritable variations in behaviour that underpin these patterns should be selected and can result in fixed or intrinsic behaviours (e.g. Vertacnik and Linnen 2017). Although empirical and modelling studies have illustrated the importance of spatial patterns to various ecological processes (references above), less attention is given to whether the behaviours and the spatial patterns they produce are context-dependent.

One challenge for empirical research on the spatial distribution of individuals within populations is to identify useful species and which life cycle stages to examine. Great insights may come from studying life stages where it is possible to quantify survivorship or reproduction, because they influence fitness (Chesson 2008) and spatial patterns of that life stage are likely to influence population dynamics. Oviparous animals, such as insects, can be model systems because egg-laying rate (i.e. birth rate) and the spatial arrangement of eggs at the start of the life cycle may persist through subsequent life stages, and the consequences could also ripple through the life cycle. Females of many species select where they lay eggs and the quality of oviposition sites can have fitness consequences, particularly if all eggs are laid in a single clutch (Refsnider and Janzen 2010). Multiple species can share oviposition sites (e.g. mushrooms, fruits, carcasses), which can lead to competition and mortality among their larvae (Ives 1991; Heard 1998), so behaviours or interactions among females that influence the spatial distribution of eggs could affect population dynamics.

Although theory and evidence suggest significance ecological consequences for the spatial distribution patterns of insect eggs, the behaviours that lead to particular spatial patterns are poorly understood. Similarly, it is unclear whether behaviours and egg distribution patterns differ between landscapes that vary in the density or distribution of oviposition patches that females must locate. This is important because models describing the consequences of spatial patterns (e.g. species coexistence models)

should apply in diverse locations if patterns and the processes of pattern formation are context independent (i.e. do not vary with landscape structure). Models may be more difficult to apply if spatial patterns are context dependent. This could arise if female behaviours vary with environmental characteristics (e.g. movement and search behaviours, Kőrösi et al. 2008; Schultz et al. 2012) such that egg distribution patterns are contingent upon the density or spatial arrangement of oviposition patches (e.g. Doak 2000). Context-dependent distribution patterns could also arise if pattern formation depends on female abundance (supply-side effects), especially if behaviours and interactions are density-dependent. Even simple behaviours could produce egg distribution patterns that are context sensitive. In an aggregative response, for example, eggs may be aggregated if patches vary in attractiveness (quality) or are more easily found (apparency), such that the degree of aggregation depends on the densities of attractive or apparent patches (Kouki 1991; Heard 1998). Alternatively, aggregation can arise from congregative behaviour of females that oviposit on or close to patches where conspecifics have already laid eggs. Congregative behaviour could be fixed if there are strong benefits to females, eggs or larvae, e.g. via predator swamping or increased egg hatch success (Desurmont and Weston 2011), and the degree of aggregation may not vary between landscapes. Of course, females may also avoid one another during oviposition, which could preclude aggregations but also produce context-independent patterns. Critically, testing for context sensitivity in behaviours and spatial patterns requires multiple landscapes encompassing a wide range of environmental characteristics and species abundances, and with natural distributions of patches (Heard 1998) rather than manipulated landscapes or 'garden' experiments. Such tests are rare, yet in their absence it is difficult to confidently identify causes of patterns in natural landscapes.

Much research on oviposition has been conducted on terrestrial insects, especially herbivores that lay eggs on plants that supply food for offspring (Gripenberg et al. 2010) or parasitoids on animal hosts (Hassell and Pacala 1990), but only rarely have such studies included exhaustive and spatially explicit surveys of all egg masses across multiple natural landscapes (for an exception, see Fagan and Folarin 2001). Alternatively, multiple, naturally variable oviposition landscapes with 10s–100s of discrete oviposition patches per landscape occur in rivers and are exploited by multiple co-occurring species of stream insects. Because egg

100 abundance directly affects population dynamics, these are ideal systems to test hypotheses about the causes
101 and consequences of spatial distribution patterns. Species in one diverse guild of aquatic insect lay their eggs
102 as single clutches exclusively on the underside of rocks that emerge above the water's surface, which we call
103 emergent rocks. Emergent rocks are therefore essential resources that are patchily distributed along streams in
104 accordance with geomorphological and hydrological processes within river channels (e.g. emergent rocks are
105 more abundant in riffles than pools). Additionally, larger-scale deviations in geology and fluvial
106 geomorphology produce between-river variations in emergent rock patterns. The density of oviposition sites
107 can constrain population size for insects that lay eggs in this manner, because egg supply can be limited by
108 the supply of oviposition sites (Peckarsky et al. 2000; Lancaster et al. 2010a; Lancaster et al. 2010b), with
109 secondary effects on densities of subsequent larval stages (Lancaster et al. 2011; Encalada and Peckarsky
110 2012; Lancaster and Downes 2014a). Any consequences of spatial patterns are likely to be conspicuous in
111 systems where constraints on population size occur during the life stage involved in the spatial pattern. These
112 species afford an opportunity to test hypotheses about the causes of spatial patterns that can be difficult to test
113 in other systems.

114 We documented patterns of oviposition for co-occurring and potentially competing, stream-dwelling
115 insects (caddisflies, order Trichoptera) in multiple, natural oviposition landscapes. We then tested hypotheses
116 regarding the behavioural processes that generate patterns, and whether patterns varied with landscape
117 characteristics (i.e. context dependency) and female abundance. The aims were to describe the patterns in a
118 way that allowed us to deduce the underlying behavioural mechanisms. Ovipositing caddisflies are difficult to
119 observe because the adults are predominantly nocturnal and lay eggs underwater. The egg masses of many
120 species can be identified in the field (Lancaster and Glaister 2019), however, so we can describe spatial
121 patterns and infer which behaviours influence pattern formation. Our study organisms were congeneric pairs
122 of caddisfly species in the genera *Ulmerochorema* and *Apsilochorema* (family Hydrobiosidae), which co-
123 occur and oviposit contemporaneously on emergent rocks (Reich and Downes 2003a; Reich 2004). There is
124 little overlap in the environmental characteristics of preferred oviposition sites for these genera (Lancaster et
125 al. 2003; Reich and Downes 2003a; Reich 2004), but preliminary observations suggest high overlap between

126 congeneric species. Thus, the behaviours underlying spatial patterns were compared between genera
127 exploiting slightly different resources, and between congeners exploiting very similar resources. Competition
128 for oviposition sites could be high within these genera because population sizes may be constrained by
129 oviposition site densities (Bovill 2013; Lancaster and Downes 2018). For these four species, oviposition
130 landscapes are spatially discrete riffles, where the water is shallow and emergent rocks (i.e. oviposition sites)
131 are more abundant than in the adjacent pools. We surveyed 18 separate riffles spread across three rivers. For
132 each riffle, egg mass distribution patterns were characterized at the landscape (riffle) scale and oviposition
133 site scale, for each species and potentially interacting species pairs. Egg distribution patterns that are
134 contingent upon landscape characteristics could, theoretically, arise through either fixed or flexible
135 behaviours, depending on the nature of the behaviour. However, if behaviours are contingent upon landscape
136 characteristics or female density, then it seems likely that distribution patterns should be context-dependent.
137 The possible outcomes (combinations of egg distribution patterns at these two scales) and pattern variations
138 are numerous so we reserve our interpretations of observed patterns for the Discussion.

139

140 **Methods**

141 **Framework for hypothesis tests and interpretations**

142 The overall objective was to test whether egg mass distribution patterns and the behaviours underlying those
143 patterns differed between oviposition landscapes. In two behavioural contexts (intra- and inter-specific
144 interactions), we posed three kinds of question about the distribution of egg masses, which led to a complex
145 set of data and statistical tests. This overview of the hypothesis framework illustrates how the questions
146 complement one another and how various female behaviours (e.g. avoidance, congregation) may lead to
147 distinct spatial patterns. The first question focussed on species-specific preferences for oviposition habitats
148 according to their local environment. The next two questions about spatial patterns were considered in the
149 same sequence that females follow when deciding where to lay eggs: first at the landscape (riffle) scale and
150 then at the scale of individual oviposition sites (emergent rocks).

151

152 For each species we asked:

153 (Q1a) Do females exploit oviposition sites with particular environmental characteristics?

154 (Q2) How does the spatial arrangement of exploited oviposition sites within a landscape compare to the
155 background arrangement of all suitable oviposition sites?

156 (Q3) What is the frequency distribution of egg masses across suitable oviposition sites?

157 The environmental characteristics of oviposition sites exploited by each species were identified first (Q1a),
158 and this information was used in subsequent questions about spatial patterns. Non-random spatial
159 arrangements of exploited oviposition sites (Q2) may arise from female behaviours during site selection.
160 Exploited oviposition sites that are clumped together may reflect congregation (e.g. oviposition swarms),
161 whereas even spacing might reflect intraspecific avoidance. At the oviposition site scale, Q3 asks similarly
162 whether egg masses are aggregated onto relatively few oviposition sites (e.g. congregation), or under-
163 dispersed across proportionately more sites (e.g. avoidance).

164

165 For potentially interacting pairs of congeneric species we asked:

166 (Q1b) Do congeneric females exploit oviposition sites with similar environmental characteristics?

167 (Q4) Is the spatial arrangement of oviposition sites exploited by one species independent of its congener?

168 (Q5) Do congeners exploit the same oviposition site more or less often than expected by chance?

169 Whether species partition resources based on their environmental characteristics is addressed in Q1b. The
170 next two questions ask whether there is evidence of spatial resource partitioning at the landscape scale (Q4)
171 or oviposition site scale (Q5). Interspecific avoidance might be manifest as spatial segregation of exploited
172 oviposition sites at the riffle scale (e.g. oviposition sites exploited by conspecifics are closer together than
173 expected by chance, ESM-1), or as negative interspecific association at the site scale (e.g. congeners rarely
174 exploit the same oviposition site).

175

176 **Study organisms**

177 Our study organisms were four species of caddisflies in two genera of the family Hydrobiosidae.

Hydrobiosids are common in rivers and streams in Australia and New Zealand (Neboiss 1986), where their aquatic larvae pass through five instars before pupating and emerging as terrestrial, winged adults. This study focussed on two genera, *Ulmerochorema* and *Apsilochorema*, and two species within each genus that co-occurred in our study area: *U. rubiconum* (Neboiss), *U. seona* (Mosely), *A. obliquum* (Mosely), *A. gisbum* (Mosely). Various aspects of oviposition in these genera have been reported previously (Lancaster et al. 2003; Reich and Downes 2003a; Reich and Downes 2003b; Bovill et al. 2013; Bovill et al. 2015), but this is the first study to examine species-level patterns and behavioural interactions across multiple riffles and rivers. All four species have the same mode of oviposition: a gravid female first lands on the surface of an emergent rock, then walks down the side into the water and lays her eggs in a single, gelatinous masses on the underside of the rock. Oviposition on emergent objects other than rocks (e.g. woody debris) is rare in our system. These species lay eggs throughout the year in our study sites, but most frequently in the warm months (Lancaster unpublished data). Typically, eggs hatch within 2-3 weeks in most seasons but may take up to 4 weeks in cold winter months. Larvae of all instars occur in the benthos throughout the year. Neonates and later instar larvae are free-living and able to roam freely, but details of their movement behaviours are largely unknown. Gut contents analyses indicate that all instars are carnivorous (Lancaster unpublished data).

193

194 **Study sites**

Our surveys were carried out during a 3-week period from mid-January to early February 2017, in three rivers in Victoria, Australia. Survey riffles were selected from within a 1-km length of each river: Snobs Creek, Little River and Steavenson River. Similar altitudes, water temperatures and geographical proximity ensured similar climatic conditions and a similar regional species pool (ESM-2). Insect populations within these river lengths were largely isolated by high ridges between river valleys, and this separation of populations facilitates robust hypothesis tests. The study lengths had largely intact riparian vegetation and were broadly similar in size and geomorphological character. However, they did differ in ways that may be relevant to ovipositing insects (ESM-2), so between-river differences in insect behaviour and egg distribution-patterns are possible. Riffles in each river were selected to encompass a wide range of sizes

204 (length and area) and emergent rock densities ($0.40 - 4.32 \text{ m}^{-2}$, ESM-3), in order to capture the natural range
205 of conditions. We could not select riffles according to female abundance, but egg mass densities (a proxy
206 measure of female density), spanned at least an order of magnitude over the sample riffles for each species
207 (ESM-3), suggesting that these riffles encompassed a wide range of female abundances. Riffles were lengths
208 of river channel created by abrupt increases in channel slope, with emergent rocks that would serve as
209 potential oviposition sites, and bounded upstream and downstream by channel lengths with few or no
210 emergent rocks. Thus, they represent fairly discrete units of habitat containing multiple resource patches
211 (emergent rocks) and surrounded by areas with few resource patches.

212

213 **Field survey protocols**

214 Each riffle was surveyed once and in each survey we mapped the spatial coordinates (X, Y) of every potential
215 oviposition site, mapped the riffle perimeter, and calculated riffle area using a total station (Leica TC803).
216 Potential oviposition sites were emergent rocks (ERs) that were $\geq 5 \text{ cm}$ in size (b-axis dimension), in $\geq 5 \text{ cm}$
217 of water and not embedded in fine sediments, because previous work showed that caddisflies rarely lay eggs
218 on small rocks in shallow water (Reich and Downes 2003a; Lancaster et al. 2010b). The egg masses (EMs) of
219 all caddisfly species were counted and identified on every ER; only results for species of *Apsilochorema* and
220 *Ulmerochorema* are reported here. For each ER, we measured several physical variables in a similar manner
221 to previous surveys (Lancaster et al. 2003; Reich and Downes 2003a): water velocity upstream of the rock,
222 rock size (b-axis dimension, Gordon et al. 2004), water depth downstream of the rock, and the height of the
223 rock projecting above the water surface. Velocity was measured near the stream bed using an electromagnetic
224 current meter (Valeport Model 801), fit with a single axis flat sensor, which is suitable for measuring flow in
225 water depths $\geq 6 \text{ cm}$.

226

227 **Numerical analyses**

228 Questions 1–5, posed above, required different forms of data analysis, which are described below. Pattern
229 consistency across riffles was assessed in the context of each question.

230

231 *(Q1a & 1b) Environmental characteristics of oviposition sites.*

232 For each river, we pooled the data over all riffles and used MANOVA with planned contrasts to test, for each
233 species, whether characteristics of emergent rocks (water depth, velocity, rock size and exposed height)
234 differed between those with and without egg masses (Q1a). We also tested whether the characteristics of ERs
235 exploited by species in a congeneric pair differed from one another (Q1b). These analyses defined exploited
236 ERs by the presence of EMs, not the number of EMs. The nature of our survey data deviated from some
237 underlying assumptions of MANOVA but these deviations were minor and did not influence interpretation of
238 the results (ESM-4).

239 Different statistical models were used for the two genera. A two-way orthogonal MANOVA was
240 used for *Apsilochorema* spp., with each ER classified according to two crossed factors: presence *vs* absence
241 of EMs of *A. obliquum*, and of *A. gisbum*. If the interaction term in this test is not significant, then the main
242 effects indicated whether ERs with and without EMs differed in character for each species, and planned
243 contrasts tested whether the congeners selected different kinds of ERs. If the interaction term was significant,
244 then planned contrasts tested whether ERs exploited by each species differed from ERs exploited by neither,
245 and whether congeners selected different kinds of ERs. Note, the interaction term itself is not germane to the
246 hypotheses of interest. Tests for *Ulmerochorema* spp. were different because very few rocks had only EMs of
247 *U. seona*, i.e. exploited ERs either had EMs of *U. rubiconum* only, or both species. Accordingly, these data
248 were analysed with a one-way MANOVA with three treatments (no EMs, EMs of *U. rubiconum* only, and
249 EMs of both species), and planned contrasts were used for all hypothesis tests.

250

251 *(Q2) How does the spatial arrangement of exploited oviposition sites compare to the background of all*
252 *suitable oviposition sites?*

253 The spatial distributions of exploited ERs were compared to random selections of suitable ERs using point
254 process statistics in which ERs are assumed to be points with X–Y coordinates within a polygonal-shaped
255 window bounded by the edges of the riffle. Our numerical analyses were based on the Ripley's $K(t)$ function

and its linear transformation $L(t)$ (Ripley 1976; Besag 1977), which can detect clumping or even spacing of points. Our approach followed the logic used in previous studies for tests using emergent rocks or leaf packs in rivers (Lancaster et al. 2003; Lancaster and Downes 2014b). The null hypothesis was not a random arrangement of exploited ERs in the riffle, because insects can only select ERs that exist and their positions were fixed. Instead the existing spatial arrangement of suitable ERs formed the null or neutral landscape (With and King 1997) and we tested whether observed distributions of exploited ERs were random selections of the suitable ERs (see ESM–5 for definitions and data on suitable ERs). These analyses were restricted to riffles with at least 15 exploited ERs and where at least 50% of all suitable ERs were unexploited. If the number of exploited ERs is too small, it is difficult to establish a clear pattern. If the proportion of suitable but unexploited ERs is too small, permutation tests of statistical significance are not feasible. Analyses were carried out in R (version 3.4.0) (R Core Development Team 2017) and used functions in the package 'spatstat' (version 1.52-1) (Baddeley et al. 2016).

Point pattern description: The second-order $L(t)$ function is the cumulative distribution function of all point-to-point distances in relation to distance t . Patterns were examined over relatively small scales of $t \leq 3$ m (less than half the stream width for most of our riffles), because insect behaviours that influence egg-laying patterns are likely to play out over small scales. The L -function assumes that the point process is first-order stationary, i.e. that the density or intensity of points is spatially homogeneous. The physical processes that determine the arrangement of ERs in rivers result in patterns that are not stationary (Lancaster et al. 2003), so analyses used an inhomogeneous L -function, $LI(t)$ (Baddeley et al. 2000), and the spatial variation in point intensity was estimated as a kernel-smoothed intensity function using the 'density' function in 'spatstat'. Edge correction was not used because our data formed finite point processes in which points cannot exist outside the boundary, i.e. ERs cannot occur beyond the stream banks or ends of the riffle (e.g. Lancaster et al. 2003; Lancaster and Downes 2004).

Statistical tests: For each species we tested whether the pattern of exploited ERs was independent of the underlying pattern of all suitable ERs, i.e. whether exploited ERs were a non-random selection of all suitable ERs. The locations of suitable ERs were fixed and these points were marked according to egg mass

282 presence/absence (a multi-type point pattern). Hypothesis tests involved comparing the LI -function of
283 exploited ERs, $LI(t)_{\text{Obs}}$, with the LI -function of a random selection of the same number of suitable ERs,
284 $LI(t)_{\text{Ran}}$. The null hypothesis (exploited ERs are a random selection of ERs) is indicated if $LI(t)_{\text{Obs}} - LI(t)_{\text{Ran}} =$
285 0 (Lancaster et al. 2003; Lancaster and Downes 2004; Warren-Rhodes et al. 2007). Following recent
286 guidelines (Baddeley et al. 2014), we used a global envelope test (also called a maximum absolute deviation
287 test) to infer statistical significance. This is a conservative test, but avoids some problems interpreting tests
288 using the commonly used pointwise envelope. First, a point-wise 95% confidence interval around the mean
289 difference was calculated from 99 Monte Carlo permutations of presence/absence marks. The most extreme
290 deviation over the entire range of t was used to create a global confidence envelope of constant width around
291 the mean difference. If values of the mean difference and its envelope are positive (i.e. $LI(t)_{\text{Obs}} - LI(t)_{\text{Ran}} > 0$)
292 over **any** values of t , this suggests that exploited ERs are more clumped than the underlying pattern;
293 conversely, negative values of the mean and its envelope suggest an even distribution relative to the
294 underlying pattern.

295

296 *(Q3) What is the frequency distribution of egg masses across suitable oviposition sites?*

297 We used a goodness-of-fit test (G-test) to compare the observed frequency distribution of egg masses per ER
298 with that expected for a random distribution based on a zero-truncated Poisson distribution. Separate tests
299 were carried out for each species and river, with data pooled across all riffles in each river to ensure adequate
300 sample sizes. The zero-truncated Poisson distribution is a parsimonious test that avoids biasing the outcome
301 by including many unsuitable ERs that have no EMs (Lancaster et al. 2010a), which can occur in tests based
302 on the coefficient of dispersion. A significant G-test (EMs are not randomly distributed across ERs) could
303 reflect one of two patterns. Egg masses could be aggregated (a contagious distribution) if ERs with many
304 EMs occur more frequently than expected by chance, such that most EMs occur on just a few ERs, which
305 have very high EM densities. Conversely, EMs could be under-dispersed (a regular distribution) if the
306 number of ERs with only one or a few EMs is greater than expected by chance, such that the majority of all
307 EMs occur on a disproportionately large number of ERs, which have low densities. For significant G-tests,

308 aggregated or under-dispersed patterns were determined by examining whether the majority of EMs occurred
309 on proportionately many vs few ERs.

310

311 *(Q4) Is the spatial arrangement of oviposition sites exploited by one species independent of its congener?*

312 To test whether behavioural interactions between congeners when selecting oviposition sites influenced
313 spatial patterns, we extended our methods of point pattern analyses described in Q2. We used the bivariate L -
314 function, $L_{ij}(t)$, also called the cross-type L -function. Bivariate tests were only possible for *Apsilochorema*
315 spp. because few ERs had egg masses of *Ulmerochorema seona* only. Statistical tests using the bivariate L_{ij} -
316 function were essentially a test of random labelling (Baddeley et al. 2016) and followed procedures
317 analogous to those described above for the univariate patterns (Q2). The null hypothesis is indicated if
318 $L_{ij}(t)_{\text{Obs}} - L_{ij}(t)_{\text{Ran}} = 0$, where $L_{ij}(t)_{\text{Obs}}$ is the bivariate function of observed data, and $L_{ij}(t)_{\text{Ran}}$ is calculated from a
319 random labelling of points. Global envelopes were calculated, as above, to infer statistical significance. If
320 $L_{ij}(t)_{\text{Obs}} - L_{ij}(t)_{\text{Ran}} > 0$ this suggests segregation of the two species, i.e. ERs exploited by the same species were
321 closer together than expected and, conversely, if $L_{ij}(t)_{\text{Obs}} - L_{ij}(t)_{\text{Ran}} < 0$ then ERs exploited by different species
322 were closer together than expected.

323

324 *(Q5) Do congeners exploit the same oviposition site more or less often than expected by chance?*

325 First, the proportions of ERs exploited by both species or by only one species were calculated. Then, we used
326 a modified form of Sørensen's coefficient to describe the proportion of individual egg masses that occurred
327 together (Southwood 1978). This coefficient ranges from -1 (complete negative association) to +1 (complete
328 positive association). A negative association could arise if congeners avoid one another; a positive association
329 could arise if there is attraction (e.g. congregation). This index was calculated for each riffle in each river,
330 with ANOVA to test for differences between rivers and t -tests to determine whether values of the index
331 differed from zero.

332

333 **Results**

334 Rivers differed in the range of environmental characteristics of emergent rocks; minimum values were similar
335 in all rivers and the main differences lay in the high values (ESM–2). The rank order of rivers was Snobs Ck
336 > Little R. > Steavenson R. in terms of the median and maximum values of all four variables: water velocity,
337 water depth, rock size and exposed rock height.

338

339 **(Q1a & 1b) Do species select oviposition sites with particular environmental characteristics? Do**
340 **congeners select different kinds of oviposition sites?**

341 For all four species, MANOVA indicated that ERs with and without egg masses differed in their
342 environmental characteristics in most rivers (Tables 1, 2). One exception, *Apsilochorema gisbum* in Snobs
343 Ck, was marginally non-significant ($P = 0.098$). The two species of *Apsilochorema* did not differ from one
344 another in their choice of oviposition site characteristics in any river, whereas the congeners of
345 *Ulmerochorema* differed in all rivers. Although the collective effects of all environmental variables were
346 tested within a MANOVA, the relative strengths of different variables can be inferred from the univariate F -
347 tests within the MANOVA (ESM–6). Compared to the range of ERs available, both species of
348 *Apsilochorema* generally selected large ERs in deep water that protruded high above the water surface and
349 velocity was a weaker predictor of oviposition site selection (Fig. 1). In contrast, both species of
350 *Ulmerochorema* were strongly influenced by all environmental variables and tended to select large ERs in
351 fast flowing, deep water that protruded high above the water surface (Fig. 1). The two congeners differed
352 from one another in that *U. rubiconum* exploited ERs with a wide range of environmental characteristics
353 whereas *U. seona* was restricted to a subset of the ERs exploited by *U. rubiconum*. Specifically, *U. seona*
354 tended to exploit the largest ERs in the fastest flows and deepest water. There was no correlation between the
355 number of egg masses of *U. rubiconum* and *U. seona* on individual ERs, regardless of whether the tests
356 included all ERs with any EMs of *Ulmerochorema* (Little R.: $r_{242} = 0.115$, $P = 0.073$; Snobs Ck: $r_{105} = 0.154$,
357 $P = 0.113$) or only ERs with EMs of both species (Little R.: $r_{60} = 0.151$, $P = 0.241$; Snobs Ck: $r_{23} = 0.037$, P
358 $= 0.861$). Calculating effect sizes is difficult in a MANOVA, which simultaneously considers multiple

359 dependent variables, but the test outcomes for the hypotheses of interest were generally clear cut (P -values $\leq$
360 0.001) and patterns were consistent across rivers.

361

362 **(Q2) How does the spatial arrangement of exploited oviposition sites compare to the background of all**
363 **suitable oviposition sites?**

364 In most riffles, ERs exploited by congeners of *Ulmerochorema* were spatially clumped relative to the
365 distribution of suitable ERs, whereas ERs exploited by *Apsilochorema* were distributed randomly in most
366 riffles, with a few clumped patterns (Table 3, Fig. 2). *Ulmerochorema seona* was sufficiently abundant for
367 univariate PPA tests in only one riffle, which showed a clumped pattern. Clumping was statistically
368 significant in nine of the 12 riffles for *U. rubiconum*; the remaining three were random patterns. Riffles with
369 clumped patterns had significantly higher egg mass densities ($N\ m^{-2}$) than those with random patterns ($t_4 =$
370 3.34, $P = 0.015$, using log-transformed densities in riffles of the Little R.). These were one-tailed tests
371 because we were only interested in whether clumping might relate to high female abundance. Patterns were
372 generally random for the congeners of *Apsilochorema*, with clumping in one out of eight riffles for *A.*
373 *obliquum*, and two out of seven for *A. gisbum*. As with *Ulmerochorema*, riffles with significantly clumped
374 patterns had higher densities of egg masses ($t_3 = 3.04$, $P = 0.028$; $t_3 = 2.78$, $P = 0.035$, for *A. obliquum* and *A.*
375 *gisbum* respectively, one-tailed t -tests as above). There was no consistency in which rivers showed random or
376 clumped patterns. Plots of the LI -function and the differences between observed and random patterns, $LI_{Obs}(t)$
377 - $LI_{ran}(t)$, all showed a step increase at $t \approx 0.38$ m (Fig. 2b, 2d). This is could be evidence of clumping at very
378 small scales, or an artefact because ERs form a hard core point process in which even tightly packed rocks
379 must be some minimum distance apart (Baddeley et al. 2014). In our riffles, most nearest-neighbour distances
380 were 0.2–0.4 m, coinciding with this step increase at ≈ 0.38 m. Thus, we took a conservative approach and
381 only values of $t \geq 0.5$ m were considered when categorising the spatial pattern of exploited ERs.

382

383 **(Q3) What is the frequency distribution of egg masses across suitable oviposition sites?**

384 Egg masses of *Apsilochorema obliquum* and *A. gisbum* were significantly under-dispersed in one and two
385 rivers, respectively (Table 4). In other words, in these rivers the majority of all EMs occurred on ERs that had
386 low egg mass densities for that species. In the Little R. for example, 72% of the EMs of *A. obliquum* occurred
387 on 90% of the exploited ERs, and those exploited ERs had densities of ≤ 2 egg masses per rock. The
388 apparently random patterns in some rivers may result from low degrees of freedom in the G-test (i.e. low
389 power because few rocks had >2 egg masses). In contrast, both species of *Ulmerochorema* were significantly
390 aggregated such that most EMs occurred on relatively few rocks (Table 4). For example, in the Little R., 70%
391 of egg masses of *U. rubiconum* occurred on only 30% of the exploited ERs, and those exploited ERs had
392 densities of ≥ 25 egg masses per rock.

393

394 **(Q4) Is the spatial arrangement of oviposition sites exploited by one species independent of its**
395 **congener?**

396 Six riffles had adequate numbers of ERs exploited by both species of *Apsilochorema* to carry out bivariate
397 point pattern analysis: four in the Little R. and two in the Steavenson R. Four of these riffles (three in the
398 Little R., one in Steavenson R.) showed evidence of an interaction between the two species, i.e. non-random
399 labelling of points (example in ESM-7), and the remaining two (one in each river) suggested no interaction.
400 The direction of the significant patterns suggests segregation, i.e. the distance between ERs exploited by the
401 same species was smaller than the distance between ERs exploited by different species (ESM-1). As in Q2,
402 we considered only values of $t \geq 0.5$ m when assessing for evidence of interaction.

403

404 **(Q5) Do congeners exploit the same oviposition site more or less often than expected by chance?**

405 Egg masses of both congeners of *Apsilochorema* co-occurred on few ERs, whereas EMs of only *A. obliquum*
406 or only *A. gisbum* occurred on $\geq 40\%$ of ERs each (Fig. 3a). In contrast, EMs of both species of
407 *Ulmerochorema* occurred on $\approx 25\%$ of the exploited ERs, most of the remainder held only *U. rubiconum*, and
408 few had only EMs of *U. seona* (Fig. 3b). Sørensen's coefficient of interspecific association was negative for
409 congeners of *Apsilochorema* in all rivers (Fig. 3c). For congeners of *Ulmerochorema*, there was no evidence

410 of association (Fig. 3d). Egg masses of *U. rubiconum* were far more abundant than those of *U. seona* and this
411 could make it difficult to detect association.

412

413 **Discussion**

414 The behaviours of egg-laying females create patterns in the spatial distribution of individuals at the start of
415 the life cycle and these patterns can have implications for population dynamics. In this study of a guild of co-
416 occurring aquatic insects that lay eggs exclusively on emergent rocks in rivers, we found that female
417 behaviours during oviposition were fixed or inflexible, and the resulting egg distribution patterns were
418 relatively invariant across multiple, natural landscapes. Thus, egg mass distribution patterns were generally
419 consistent across multiple riffles in rivers, despite wide and natural variations in riffle size, and in densities of
420 prospective oviposition sites that varied over an order of magnitude. Patterns were most apparent where
421 gravid females were abundant, but there was no compelling evidence to suggest that behaviours and egg
422 distribution patterns varied with female density. Such invariant or context-independent patterns and fixed
423 oviposition behaviours can arise when there are strong fitness benefits (Vertacnik and Linnen 2017).
424 Furthermore, we found different behaviours (congregation vs avoidance) for congeneric species pairs, which
425 produced different distribution patterns of egg masses in the same landscapes (strong aggregation vs
426 segregation), yet behaviours and patterns were still context-independent. Our ability to detect such patterns
427 and gain insight into their prospective causes was enabled by a spatially-explicit approach with
428 complementary tests addressing different aspects of species' behaviour that manifest at different scales, and
429 by large sample sizes. With fewer landscapes (riffles), it is doubtful that we would have uncovered some
430 patterns and had confidence that they are ecologically meaningful rather than chance events. The use of
431 ecologically relevant neutral landscapes in hypothesis tests was also pivotal in providing ecologically
432 meaningful inferences about behaviours, even though some landscapes were not amenable to all tests.

433 The consistency of egg mass distribution patterns of hydrobiosid caddisflies across multiple
434 landscapes means that models predicting the consequences of egg distribution (e.g. species coexistence
435 models: Ives 1988; Ives 1991) should apply in many locations for this guild. In other circumstances, the way

species interact with one another and their environment can vary with environmental characteristics and species' identity (Menge 1976; McPeck 2017; references above). For example, research on Lepidoptera (a sister order of Trichoptera) that oviposit on food plants suggests that the physical structure of landscapes and the distribution of resources within habitat patches can affect movement behaviours (Schtickzelle et al. 2007; Schultz et al. 2012) and this can lead to landscape-specific patterns in the density and distribution of eggs (Jones 1977; Doak 2000; Körösi et al. 2008). Why egg distribution patterns should be context-dependent for some insects but not others may relate to differences in the nature of oviposition sites (discussed below), but this variation means that the consequences of egg distribution patterns may be more difficult to model or predict.

The characteristics of egg mass distribution patterns and the potential underlying behaviours differed between the genera *Apsilochorema* and *Ulmerochorema*, at both the landscape and site scales. These warrant some consideration because particular behaviours or behavioural syndromes may be common to other species and systems where spatial patterns and consequences are also context-independent. We discuss each species pair in turn and then consider potential ecological consequences of the patterns.

For *Ulmerochorema*, the overall pattern in all landscapes was of highly clumped and aggregated egg masses on selected oviposition sites. The physical characteristics of oviposition sites (emergent rocks, ERs) selected by each species differed from unexploited ERs, differed between the two congeners, and the patterns were consistent across rivers. More interestingly, the ERs exploited by *U. seona* were a near perfect subset of those exploited by *U. rubiconum*, suggesting that *U. seona* has an included niche (sensu Miller 1967). Both species preferred large ERs in fast flowing, deep water, as observed previously for the genus (Lancaster et al. 2003; Reich and Downes 2003a), but *U. seona* showed a stronger preference for the biggest ERs in the fastest and deepest water. Spatially, the exploited ERs were clumped relative to the background pattern of ERs in 75% of landscapes. In a minority of riffles, exploited ERs were random and these landscapes were not systematically different in physical character, but egg mass densities were lower, suggesting low statistical power when females were scarce. At the oviposition site scale (individual ERs), the egg masses of both congeners were strongly aggregated with 10s –100s of egg masses on some ERs, as found previously

462 (Lancaster et al. 2003; Reich and Downes 2003a). We found no evidence of interspecific association (positive
463 or negative), although the numerical dominance of *U. rubiconum* may have made it difficult to detect
464 association at the site scale, and bivariate PPA testing for interactions between the two congeners at the
465 landscape scale was not possible.

466 Congregative behaviour at the landscape (riffle) scale coupled with an aggregative response at the
467 oviposition sites is a parsimonious explanation of the behaviours underlying the egg mass distribution
468 patterns of *Ulmerochorema*. Spatial clumping could arise if females swarm when laying eggs and, although
469 we lack direct evidence of congregation, adults of both congeners are often numerous in light traps (J.
470 Lancaster, pers. obs.). Aquatic insects can occur in multi-species swarms (Haddow and Corbet 1961), which
471 may contribute to the strong co-occurrence of *U. rubiconum* and *U. seona*. Aggregation pheromones and sex
472 pheromones are well known in insects, including caddisflies (Resh and Wood 1985; Löfstedt et al. 1994), and
473 can result in aggregated oviposition (Anderson 2002). For *U. rubiconum*, previous research indicated an
474 aggregative response of ovipositing females to the local environment of ERs (Reich et al. 2011), and adults
475 alight more often on ERs in fast than slow flow, suggesting a capacity to detect suitable rocks from the air
476 (Reich and Downes 2003b). *Ulmerochorema seona* may have a similar aggregative response albeit to a more
477 specific environmental factor or suite of factors, given its more narrow choice of rocks.

478 In contrast, the egg masses of *Apsilochorema* spp. were distributed differently and avoidance
479 behaviours may drive these patterns. The preferred oviposition sites of the congeners had identical physical
480 characteristics in all rivers, and they differed from unexploited ERs. These species preferred large ERs in
481 deep water, as reported previously for the genus (Lancaster et al. 2003; Reich and Downes 2003a). Velocity
482 was a good predictor of preferred oviposition sites for *Apsilochorema* in experiments (Reich and Downes
483 2003b) and, indeed, the soft jelly of these egg masses cannot withstand the shearing forces of fast flows
484 (Bovill et al. 2013). Weak effects of velocity in this study may be attributed to correlations between rock size
485 and velocity in surveys, which can be de-coupled in experiments. At the scale of individual oviposition sites,
486 egg masses of each congener were under-dispersed (most ERs had only one egg mass), suggesting
487 intraspecific avoidance. In oviposition landscapes, the ERs exploited by each congener were distributed

488 randomly relative to background in 12 out of 15 riffles; the remaining three riffles had clumped spatial
489 distributions. There were no obvious differences in the landscape characteristics of riffles with different
490 patterns, but clumped patterns occurred where egg mass densities were the highest, a proxy measure of
491 female density. These clumped patterns at landscape scales are unlikely to be chance events, they are
492 ecologically interesting and could arise from avoidance behaviour at oviposition site scales.

493 What is the mechanistic basis for the intraspecific avoidance of *Apsilochorema* spp.? It is unlikely
494 that adult females encounter and avoid one another during oviposition, because egg masses on a single ER
495 could accumulate over several nights and direct encounters between females may be infrequent. It is more
496 likely that females avoid ovipositing on ERs where egg masses of conspecifics are present. A parsimonious
497 process of avoidance could generate the observed spatial patterns: if a female encounters an egg mass of a
498 conspecific on an ER, she then abandons that ER and subsequently exploits another ER nearby. Observations
499 of another species of Hydrobiosidae, *Ethochorema turbidum*, showed that females can reject oviposition sites
500 and fly to others (Reich 2002). Many insects follow a behavioural sequence of searching and evaluating
501 potential oviposition sites before deciding to accept or reject a particular location, including Lepidoptera
502 (Renwick and Chew 1994). Oviposition-deterrent pheromones in insects can be associated with the eggs
503 directly or with secretions produced during oviposition (Anderson 2002), and can be responsible for under-
504 dispersed distributions of eggs (Schoonhoven et al. 1990; Gabel and Thiéry 1992). Adult caddisflies have
505 diverse and numerous sensory receptors, including chemoreceptors, on the tarsi, antennae and other body
506 parts (Melnitsky and Ivanov 2011; Lancaster and Downes 2013), so contact evaluation of egg masses is
507 plausible. This simple form of intraspecific avoidance (abandoning exploited ERs and flying to the nearest
508 available ER) could lead to clumped arrangements of exploited oviposition sites at landscape scales, which
509 we observed in some riffles. For caddisflies, if the females and egg mass densities are high, it is likely that
510 females will encounter egg masses frequently and landscape-scale patterns will be pronounced and easily
511 detected.

512 Interspecific avoidance by the congeners of *Apsilochorema*, suggested at both oviposition site and
513 landscape scales, could also arise from similar avoidance behaviours. At oviposition sites, egg masses of

514 congeners occurred on the same ERs less often than expected (negative association). This can arise though
515 different oviposition site preferences (Lounibos 1981), but these congeners had identical oviposition site
516 preferences, making avoidance more likely. At the landscape scale, bivariate point pattern analyses suggested
517 spatial segregation and hence inter-specific avoidance of *A. obliquum* and *A. gisbum* in four of the six riffles
518 that we could analyse. Segregation was apparent in riffles with high egg mass densities and random patterns
519 in the minority of riffles may reflect low encounter rates when densities are low. Spatial segregation can be
520 accompanied by clumped spatial arrangements for one or both species (illustrations in ESM-1) and, indeed,
521 the riffles that displayed clumping of single species also displayed segregation of congeners. The intra-
522 specific avoidance behaviour outlined above could also produce inter-specific avoidance if females avoid
523 ERs that have egg masses of either conspecifics or congeners. The egg masses of these congeners are
524 morphologically similar (Lancaster and Glaister 2019) and it is plausible that contact cues elicit similar
525 behavioural responses from congeners and conspecifics. Inter-specific avoidance via oviposition-deterrent
526 pheromones occurs in insects and can generate characteristic distribution patterns of eggs (Schoonhoven et al.
527 1990; Gabel and Thiéry 1992). It is notable that spatial clumping and segregation of *Apsilochorema* spp. was
528 detected at small scales (≤ 3 m). These scales are pertinent to our proposed avoidance behaviour because a
529 female rejecting one oviposition site is likely to select an alternative site nearby and within the same riffle
530 (<10 m), rather than travelling farther ($>>10$ m) to a different riffle and incurring the risks associated with
531 prolonged flight, e.g. interception by aerial predators (Encalada and Peckarsky 2007). Collectively, these
532 results provide compelling evidence of both intra- and inter-specific avoidance for two species of
533 *Apsilochorema* at oviposition sites, which may arise from a simple avoidance behaviour and produce
534 distinctive distribution patterns of egg masses in oviposition landscapes.

535 Contrasting egg distribution patterns and oviposition behaviours of these stream-dwelling caddisflies
536 with other systems requires caution, because spatially explicit research on oviposition in natural landscapes is
537 rare (as noted above) and systems can differ in quite significant ways. The dominant literature on insect
538 oviposition focusses on terrestrial, herbivorous insects, which are biologically very different to hydrobiosid
539 caddisflies. Plants can offer multiple cues to searching females, including plant architecture (Mattingly and

540 Flory 2011) and phytochemicals (Städler 2002), which may be associated with behaviour that is different to
541 caddisflies searching for inert and structurally simple emergent rocks. Additionally, herbivorous insects seek
542 plants that typically provide food for larvae, whereas emergent rocks are not associated with food for
543 predatory larval caddis. Oviposition site choices by hydrobiosids may be associated more strongly with
544 reducing mortality of offspring due to competition or predation, rather than characteristics of the oviposition
545 site itself. Consistent with this notion, reducing predation risks for offspring can influence oviposition site
546 choice by predatory terrestrial insects (Doubt et al. 1998) and pond-dwelling aquatic insects (Reser et al. 2001).
547 However, because stream-dwelling larvae may move long distances from oviposition sites, which
548 themselves may be unrelated to larval habits, it is feasible that pond insects are also not ideal systems for
549 comparison. It is nevertheless intriguing that these caddisflies show such strong oviposition preferences, and
550 fitness consequences seem likely.

551 The spatial patterns observed in this survey suggest intriguing implications for the coexistence of
552 hydrobiosid species. All four study species co-occur in the same riffles, along with several other con-familial
553 species with similar oviposition habits (Lancaster and Glaister 2019) and their larvae form a guild of
554 potentially competing carnivores. Clumped and aggregated distributions of egg masses create aggregations
555 over small areas. In addition, the egg masses of all species that oviposit on ERs will be aggregated relative to
556 total riffle area (e.g. maximum potential habitat for larvae), because ERs are typically clumped within riffles
557 (Lancaster et al. 2003). Egg hatching rates are high for both *Ulmerochorema* and *Apsilochorema* (Reich
558 2004; Bovill et al. 2013) suggesting low egg mortality for these genera (Bovill et al. 2015). Thus, the
559 aggregated distribution of egg masses will result in similarly aggregated groups of neonate larvae comprising
560 100s–1000s of siblings, conspecifics, congenetics and confamilials. Competition for food may be intense
561 because these neonates have virtually no yolk in the gut (Lancaster pers. obs.), so capturing the first prey item
562 may be critical (Hildrew and Wagner 1992). The avoidance behaviour of *Apsilochorema* may reduce
563 competition among neonates and also enhance coexistence between *Apsilochorema* species, e.g. depending
564 on whether inter-specific aggregation of eggs is greater than intra-specific aggregation, which requires
565 consideration of clutch sizes (Ives 1988; Ives 1991). Alternatively, the included niche of *U. seona* may

566 suggest either that *U. seona* is a superior competitor to *U. rubiconum*, or that larvae of both species move
567 rapidly and far away from natal areas upon hatching. Currently, we lack information about the neonate
568 behaviour and further data are needed to elucidate the mechanisms of species coexistence for this intriguing
569 guild of insects.

570 The spatial distribution of individuals is non-random in most ecological systems. This study shows
571 that for some caddisflies the nature of those patterns may not vary between locations that differ naturally in
572 size and the density of resource patches, a marked contrast to the context-dependent patterns suggested for
573 some terrestrial insects. Landscape configuration may influence movement patterns in some systems, but
574 strong preferences for patch quality and strong inter-individual interactions can result in similar spatial
575 distribution patterns of individuals in natural landscapes. Quite different kinds of inter-individual interactions,
576 avoidance and congregation, both created consistent patterns across landscapes in this study. Notably, this
577 suggests that the consequences of particular spatial patterns may be similar in multiple locations, and this
578 now requires specific tests. It is possible that the behaviours driving pattern formation in these caddisflies
579 may be shared by other species and also create context-independent spatial patterns in other systems.

580

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588

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Table 1 *Apsilochorema*: Summary of MANOVA with planned contrasts in each of the three rivers. For non-significant interaction terms (Snobs Ck, Steavenson R.), the presence/absence test for each species indicates whether rocks with and without EMs differ; for a significant interaction term (Little R.) then pairwise contrasts provide this test. In all rivers, planned contrasts indicate whether environmental characteristics of the ERs used for oviposition differed between the two species.

River	Source	df	Pilai's trace	<i>F</i>	<i>P</i>
Snobs Ck	Presence/absence <i>A. obliquum</i>	4, 554	0.025	3.55	0.007
	Presence/absence <i>A. gisbum</i>	4, 554	0.014	1.97	0.098
	Interaction	4, 554	0.010	1.37	0.244
	<i>A. obliquum</i> vs <i>A. gisbum</i>	4, 554	0.013	1.88	0.113
Little R.	Presence/absence <i>A. obliquum</i>	4, 2057	0.034	17.8	<0.001
	Presence/absence <i>A. gisbum</i>	4, 2057	0.020	10.5	<0.001
	Interaction	4, 2057	0.008	4.32	0.002
	<i>A. obliquum</i> vs no eggs	4, 2057	0.050	27.3	<0.001
	<i>A. gisbum</i> vs no eggs	4, 2057	0.029	15.4	<0.001
	<i>A. obliquum</i> vs <i>A. gisbum</i>	4, 2057	0.003	1.70	0.146
Steavenson R.	Presence/absence <i>A. obliquum</i>	4, 1748	0.016	6.88	<0.001
	Presence/absence <i>A. gisbum</i>	4, 1748	0.022	9.99	<0.001
	Interaction	4, 1748	0.000	0.189	0.944
	<i>A. obliquum</i> vs <i>A. gisbum</i>	4, 1748	0.002	0.850	0.494

Table 2 *Ulmerochorema*: Summary of MANOVA in each of the three rivers showing the overall multivariate test and planned contrasts, which compared exploited and unexploited ERs for each species, and compared exploited ERs of the two species.

River	Source	df	Pilai's trace	<i>F</i>	<i>P</i>
Snobs Creek	Multivariate test	8, 1102	0.277	22.2	<0.001
	<i>U. rubiconum</i> vs no eggs	4, 551	0.931	1850	<0.001
	<i>U. seona</i> vs no eggs	4, 551	0.873	948	<0.001
	<i>U. rubiconum</i> vs <i>U. seona</i>	4, 551	0.13	20.6	<0.001
Little River	Multivariate test	8, 4118	0.248	72.8	<0.001
	<i>U. rubiconum</i> vs no eggs	4, 2058	0.928	6664	<0.001
	<i>U. seona</i> vs no eggs	4, 2058	0.875	3606	<0.001
	<i>U. rubiconum</i> vs <i>U. seona</i>	4, 2058	0.101	57.6	<0.001
Steavenson River	Multivariate test	8, 3498	0.284	72.3	<0.001
	<i>U. rubiconum</i> vs no eggs	4, 1748	0.915	4702	<0.001
	<i>U. seona</i> vs no eggs	4, 1748	0.787	1614	<0.001
	<i>U. rubiconum</i> vs <i>U. seona</i>	4, 1748	0.055	25.3	<0.001

Table 3 Summary of univariate point pattern analyses: the number of riffles in each river that provided evidence of random or clumped spatial patterns of exploited ERs for each species, relative to the background pattern of suitable ERs. Even distribution patterns did not occur. Only riffles with enough ERs and EMs were used (ESM–5, see text for explanation). In only one riffle were ERs exploited by *Ulmerochorema seona* sufficiently abundant for these analyses.

Species	River	Random	Clumped
<i>U. rubiconum</i>	Snobs	0	2
	Little	2	4
	Steavenson	1	3
<i>U. seona</i>	Little	0	1
<i>A. obliquum</i>	Little	4	1
	Steavenson	3	0
<i>A. gisbum</i>	Snobs	1	0
	Little	3	1
	Steavenson	1	1

Table 4 For each species and river, summary of G-tests for goodness-of-fit tests of EM frequency data to zero-truncated Poisson distribution. Under-dispersed pattern = more rocks with a single egg mass than expected by chance; aggregated = more rocks with very many egg masses than expected by chance. df varied between tests because some frequency categories had to be combined to ensure expected frequencies >5 for each category, as typically required for goodness-of-fit tests.

Species	Site	G _{adj}	df	P	Pattern
<i>Apsilochorema obliquum</i>	Snobs Ck	3.22	2	0.200	random
	Little R.	17.1	3	<0.001	under-dispersed
	Steavenson R.	1.69	2	0.429	random
<i>Apsilochorema gisbum</i>	Snobs Ck	11.1	3	0.011	under-dispersed
	Little R.	21.6	3	<0.001	under-dispersed
	Steavenson R.	1.58	2	0.454	random
<i>Ulmerochorema rubiconum</i>	Snobs Ck	594	5	<0.001	aggregated
	Little R.	617	5	<0.001	aggregated
	Steavenson R.	235	8	<0.001	aggregated
<i>Ulmerochorema seona</i>	Snobs Ck	100	3	<0.001	aggregated
	Little R.	90.9	4	<0.001	aggregated
	Steavenson R.	6.60	2	0.037	aggregated

FIGURE LEGENDS

Fig. 1. Examples of cumulative frequencies of emergent rocks according to four environmental variables (velocity, water depth, rock size, exposed height of rock) for *Apsilochorema* spp. in the Steavenson River (top panels), and for *Ulmerochorema* spp. in the Little River (bottom panels). Similar patterns were found in all rivers, but have been omitted for brevity. In most cases, the cumulative frequencies of exploited ERs lie to the right of the lines for unexploited ERs. This indicates that the proportions of exploited ERs with low values of each environmental variable were lower than their relative abundance in the set of unexploited ERs

Fig. 2. Examples of maps and point pattern analysis of ERs exploited by (a) and (b) *Ulmerochorema rubiconum* (Little R. riffle 7), and by (c) and (d) *Apsilochorema obliquum* (Little R. riffle 3). Maps in (a) and (c) are drawn to the same scale and oriented with water flowing from right to left. Solid lines represent the wetted channel width, dashed lines the up- and downstream extent of the surveyed riffle. Point pattern analyses illustrating (b) a clumped distribution of ERs exploited by *U. rubiconum* and (d) a random distribution of ERs exploited by *A. obliquum*. Solid line indicates the mean difference between the observed *LI*-function and the *LI*-function of a random selections of suitable rocks, dotted lines indicate 95% global confidence envelope for this difference, dashed lines indicate the null hypothesis of no difference. Patterns at small values of t (within the grey area) were ignored when assessing the kind of distribution patterns (see text for explanation: Results (Q2))

Fig. 3. Co-occurrence of different species' egg masses on oviposition sites: (a) and (b) Proportion of all exploited ERs that had egg masses of only one species or both congeners of (a) *Apsilochorema* and (b) *Ulmerochorema*. Bars represent means $\pm$ SE of the different rivers. (c) and (d) Sørensen's coefficient of association (mean $\pm$ SE) between congeners of (c) *Apsilochorema* and (d) *Ulmerochorema* in each river. Values < 0 suggest negative association; values > 0 suggest positive association. N = number of riffles per river used to calculate the mean. No data for Steavenson River in (d) because *U. seona* were too scarce for meaningful analysis. Statistically,

Sørensen's coefficient did not differ among rivers for either genus (*Apsilochorema*: $F_{2,16} = 1.09$, $P = 0.363$; *Ulmerochorema*: $F_{1,10} = 0.038$, $P = 0.849$). Combining data across rivers, the index was significantly less than zero for *Apsilochorema* ($t_{16} = 4.84$, $P < 0.001$), but did not differ from zero