



Everall, Nicholas C. and Johnson, Matthew F. and Wilby, Robert L. and Bennett, Cyril J. (2015) Detecting phenology change in the mayfly *Ephemera danica*: responses to spatial and temporal water temperature variations. *Ecological Entomology*, 40 (2). pp. 95-105. ISSN 1365-2311

**Access from the University of Nottingham repository:**

<http://eprints.nottingham.ac.uk/45017/1/Everall%20et%20al.%202015.%20Eco%20Ent.pdf>

**Copyright and reuse:**

The Nottingham ePrints service makes this work by researchers of the University of Nottingham available open access under the following conditions.

This article is made available under the University of Nottingham End User licence and may be reused according to the conditions of the licence. For more details see: [http://eprints.nottingham.ac.uk/end\\_user\\_agreement.pdf](http://eprints.nottingham.ac.uk/end_user_agreement.pdf)

**A note on versions:**

The version presented here may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the repository url above for details on accessing the published version and note that access may require a subscription.

For more information, please contact [eprints@nottingham.ac.uk](mailto:eprints@nottingham.ac.uk)

1 Detecting phenology change in the mayfly *Ephemera danica*:  
2 Responses to spatial and temporal water temperature variations

3

4 Nicholas C. Everall,<sup>1</sup> Matthew F. Johnson<sup>2</sup>, Robert L. Wilby<sup>2</sup> and Cyril J. Bennett<sup>1</sup>

5

6 <sup>1</sup> Aquascience Consultancy, 18 Hawthorne Way, Ashgate, Chesterfield, Derbyshire S42 7JS,  
7 UK. Email: rnaquaconsult@aol.com

8 <sup>2</sup> Department of Geography, Loughborough University, Leicestershire, LE11 3TU, UK.

9

10 Everall NC, Johnson MF, Wilby RL and Bennett CJ. (2015) Detecting phenology change in  
11 the mayfly *Ephemera danica*: Responses to spatial and temporal water temperature variation.  
12 *Ecological Entomology* **40**: 95–105.

13

14 Link: <http://onlinelibrary.wiley.com/doi/10.1111/een.12164/abstract>

15

16 **Abstract**

17 1. Rising water temperatures under climate change are expected to affect the phenology of  
18 aquatic insects, including the mayfly *Ephemera danica* which is widespread throughout  
19 Europe.

20 2. To assess temporal and spatial variability in mayfly emergence, *E. danica* were monitored  
21 at two thermally contrasting reaches in the River Dove, English Peak District over the period  
22 2007 to 2013. Inter-annual variations in growing degree days (GDDs) were modelled for an  
23 upstream site with intermittent spring flow supplementing main channel flow (Beresford Dale)  
24 and downstream site dominated by near constant discharges of cool groundwater (Dovedale).

25 3. A strong association exists between the emergence cycle of *E. danica* and GDDs at each  
26 site. Beresford Dale accumulated on average 374 more GDDs than Dovedale. Following  
27 warm summers *E. danica* emerged after only one year in Beresford Dale but began to revert  
28 to a bi-annual cycle after the particularly wet/cool year of 2012. In Dovedale, *E. danica*  
29 maintained a two-year cycle throughout the monitoring period despite the phenology changes  
30 observed 8 km upstream.

31 4. Data from the present study suggest that habitats near cool groundwater may provide  
32 important refugia for populations of insects, potentially delaying permanent shifts in  
33 phenology under climate change. However, ability to detect changes in the thermal triggers  
34 and phenological response may be hindered by conventional spot sampling protocols.

35

36 **Keywords:** Mayfly; Phenology; Water temperature; Emergence; Thermal refugia

37

## 38 INTRODUCTION

39 Water temperature ( $T_w$ ) affects many aspects of aquatic life including the metabolism of  
40 animals (e.g. Weatherley & Ormerod, 1990) and photosynthesis of plants (Berry & Björkman  
41 1980). Insects are poikilothermic ectotherms so their development and phenology are  
42 regulated by ambient temperatures (Raddum & Fjellheim, 1993; Knispel *et al.*, 2006).  
43 Development ceases when  $T_w$  is outside upper or lower thresholds, and thermal extremes can  
44 cause stress or even mortality (Dallas & Rivers-Moore, 2012). Consequently, cumulative or  
45 growing degree days (GDDs) are often used to relate thermal conditions to organism  
46 development (Neuheimer & Taggart, 2007). GDDs are the number of degrees that exceed a  
47 minimum temperature threshold each day, accumulated over the development period or year.

48 Insect phenology is extremely diverse, often very plastic, and characterised by individuals  
49 progressing through multiple, distinct life-stages. Timing of emergence from aquatic larval  
50 stage to the sexually mature, terrestrial stage is of particular significance because many  
51 insects have only a short window to mate and lay eggs. It is, therefore, important that  
52 emergence coincides with favourable weather and is synchronous, both as a population  
53 defence against predation and as a means of maximising potential genetic spread (Watanabe  
54 *et al.*, 1999; Sparks *et al.*, 2010).

55 River temperature is spatially heterogeneous and temporally variable (Webb *et al.* 2008).  
56 Water temperatures usually have strong diel and seasonal cycles related to solar forcing, and  
57 typically increases with distance downstream. Tributaries and groundwater can interrupt this  
58 pattern and create locally distinct thermal regions. For example, phreatic groundwater usually  
59 has a relatively constant temperature that reduces diel and seasonal temperature ranges  
60 (Constantz, 1998; O'Driscoll & DeWalle, 2006).

61 There is evidence that river Tw is changing in response to climate change (van Vliet *et al.*,  
62 2011; Isaak *et al.*, 2012; Orr *et al.* 2014). In addition, land drainage, alteration of river courses  
63 and ponding by weirs, can alter the thermal dynamics of rivers (Caissie, 2006), as can  
64 removal of riparian vegetation (e.g. Broadmeadow *et al.*, 2011). Aquatic organisms respond  
65 to changing thermal conditions in complex ways (Ward & Stanford, 1982). Given the  
66 dependence of phenology on heat accumulation, emergence of insects is particularly  
67 susceptible to changing temperature and can have adverse effects on freshwater insects  
68 populations (Harper & Peckarsky, 2006; Durance and Ormerod, 2007; Thackeray *et al.*,  
69 2010). In this paper, we describe spatial and temporal variability in mayfly emergence in the  
70 River Dove, English Peak District. We then assess links between varying Tw and mayfly  
71 phenology, and discuss whether conventional monitoring protocols are adequate for detecting  
72 changes in the thermal driver and ecological response.

73

## 74 **MATERIALS AND METHODS**

### 75 *Study organism*

76 *Ephemera danica* Muller, 1764 (Ephemeroptera) is one of the largest mayflies found in the  
77 British Isles with some females reaching over 30 mm. The larvae are burrowing animals and  
78 are often found where silt accumulates in rivers. Traditionally *E. danica* has been reported to  
79 emerge after two years in an aquatic nymphal stage and is referred to as a semivoltine species  
80 (Wright *et al.*, 1981; Tokeshi, 1985; Elliott *et al.*, 1988). The adult emergence period is  
81 normally in late-May and early-June. Tokeshi (1985) found that male *E. danica* have a  
82 minimum growth threshold of 2.6 °C and require at least 3398 annual GDDs to emerge.  
83 Females have a higher minimum growth threshold (3.1 °C) and hence require more GDDs for

84 emergence (3631). Differing growth thresholds for males and females is common in mayfly  
85 populations (Svensson 1977; Wright *et al.* 1981).

86 In controlled laboratory experiments, Bennett (2007) noted that both male and female *E.*  
87 *danica* can reach maturity in a single year with larvae reaching up to 19 mm within four  
88 months from hatching when Tw averages 20 °C. Bennett (2007) also noted that *E. danica* in  
89 the North Wey, Surrey, UK reached maturity in a single year between 1995 and 2002. The  
90 current study builds on these observations by examining the life cycle of *E. danica* from 2007  
91 to 2013 at two contrasting sites in the River Dove, UK.

92

### 93 *River Dove and sampling sites*

94 The River Dove rises on Axe Edge from moorland springs and runs southward for 73 km  
95 through the Peak District National Park before joining the River Trent at Newton Solney. The  
96 catchment area upstream of monitoring sites is 131 km<sup>2</sup> and elevation ranges from 450 m at  
97 source to 155 m in Dovedale. Land-use is predominately grazed pasture with isolated stands  
98 of deciduous woodland covering 5% of the catchment. Annual precipitation exceeds 1000  
99 mm. The Dove flows parallel to a Carboniferous limestone outcrop with phraetic springs,  
100 which it intersects at Beresford.

101 Invertebrate and Tw monitoring sites are located in Beresford Dale and Dovedale (Figure 1).  
102 Beresford Dale is 20 km from the source of the Dove, situated at the upstream end of a  
103 limestone gorge. Here, intermittent springs discharge water of relatively constant temperature  
104 (9–14 °C) during autumn and winter. Dovedale is 9.7 km downstream of Beresford Dale in a  
105 limestone gorge with Ash (*Fraxinus excelsior*) woodland. Here, groundwater discharges into  
106 the river all year-round at ~8.5 °C. The stretch between Beresford and Dovedale is affected

107 by over 100 weirs (< 0.5 m high) installed over a century ago to increase the feeding area for  
108 trout. The Environment Agency of England and Wales (EA) have recorded daily discharge at  
109 a gauging station in Dovedale since 1969 (Figure 1).

110

#### 111 *Water temperature monitoring and analysis*

112 The Loughborough University TEMperature Network (LUTEN) consists of 36 monitoring  
113 sites in the Rivers Dove and Manifold. At each site, Gemini Aquatic 2 *Tinytag* thermistors  
114 record the maximum, mean and minimum air and  $T_w$  every 15-minutes since March 2011.  
115 *Tinytag* thermistors have a quoted accuracy of 0.2 °C, which has been confirmed under  
116 experimental conditions (Johnson & Wilby, 2013). There are eight LUTEN sites between  
117 invertebrate monitoring sites (Figure 1). Sites D16 (Beresford Dale) and D24 (Dovedale) are  
118 the closest records. However, D24 has an incomplete record due to sensor failure and as such,  
119 D23 is used herein, which records almost identical temperatures to D24 (maximum difference  
120 in daily temperature was 0.62 °C during model calibration and validation period). Full details  
121 of LUTEN, the monitoring strategy and data validation can be found in Wilby *et al.* (2012)  
122 and Johnson *et al.* (2014).

123 The EA takes monthly spot measurements for routine monitoring. To test the ability of this  
124 sampling strategy to detect thermal changes one value per month was randomly selected from  
125 the 15 minute LUTEN data during typical EA sampling hours (08:00 to 18:00) from June  
126 2011 to May 2012. These 12 values were then used to estimate the annual mean at site D16.  
127 This was repeated 1000 times allowing estimation of the variance in the mean due to daylight  
128 sample times, when compared with the ‘true’ estimate based on the full LUTEN-record.

129 Water temperatures for the years 2007 to 2013 were hindcast from air temperatures measured  
130 at Buxton, Derbyshire (~20 km from study sites and significantly correlated ( $r^2 = 0.8$ ) to 3-  
131 years of monitored air temperature at each site). Air and water temperature are not directly  
132 related but because both are ultimately driven by solar radiation, the latter can be predicted  
133 from air temperatures using regression analysis (Stefan and Preud'homme, 1993; Mohseni *et*  
134 *al.*, 1998). We deploy logistic regression models built previously for LUTEN sites and tested  
135 under contrasting weather conditions (Johnson *et al.* 2014; Wilby *et al.*, 2014). These models  
136 explained 85% of the variance in Tw at D16 and 83% at D23 (Table 1) and have the form:

$$137 \quad Tw = \frac{\alpha}{(1 + \exp^{\gamma(\beta - Ta)}} \quad (1)$$

138

139 where  $\alpha$  is the model asymptote,  $\beta$  is the model inflection point and  $\gamma$  is the model gradient at  
140  $\beta$ . Using the same models, a Tw record was constructed for 2005–2013 in order to calculate  
141 GDDs using the thresholds of Tokeshi (1985). GDDs were accumulated from 1 June to the 31  
142 May each year to match the normal development period of *E. danica* from egg-laying.

143

#### 144 *Invertebrate sampling*

145 Invertebrate monitoring sites were approximately 40 m long, 7–8 m wide, and located in  
146 riffles. Two sites in Beresford Dale were 100 m downstream of Hartington bridge and  
147 approximately 100 m apart. The substrate consists of clean, coarse-gravels (median grain size  
148  $[D_{50}] = 48$  mm) and sparse stands of water crowfoot (*Ranunculus* spp.) with very occasional  
149 starwort (*Callitriche* spp.). The Dovedale site also comprises of clean, coarse-gravels ( $D_{50} = 41$   
150 mm) with sparse weed-beds of water crowfoot (*Ranunculus* spp.).



151 All study reaches hold good populations of *E. danica* based on data collected from previous  
152 surveys (Everall, 2010; 2012). *E. danica* samples were taken from exposed gravels and fine-  
153 sediment beneath weed-beds using a 0.1 m<sup>2</sup> Surber net sampler fitted with a 2 cm deep steel  
154 curtain. Sampling was undertaken in Aprils 2007, 2010, 2011, 2012 and 2013 following the  
155 life-stage techniques of Bennett (2007). The number of *E. danica* individuals and their body  
156 length was recorded by site and year. Mayflies larger than 7 mm were also sexed; this was  
157 not always possible for smaller individuals.

158 In a two-year cycle it is expected that there will be a tri-modal size distribution with separate  
159 peaks for male and female adults about to emerge in June, and a third peak of smaller  
160 mayflies that require an additional year of growth. Male and female mayflies usually form  
161 distinct size classes because of the differing GDDs required for development. In a one year  
162 cycle, mayfly samples are expected to have a uni-modal size distribution with the majority  
163 about to emerge in June plus a few smaller, over-wintering individuals (Figure 2). Hence, the  
164 number of small, unsexed mayfly present each year, and the size difference between males  
165 and females, are indicators of the presence of the two-year emergence cycle.

166

### 167 *Statistical analysis*

168 SPSS 19.0 was used for all regression analysis. Statistical comparison between the total  
169 length of male and female mayflies between and within years was undertaken with Mann-  
170 Whitney U tests in SPSS 19.0.

171

## 172 **RESULTS**

### 173 *Variations in water temperature*

174 Daily-mean Tw has clear seasonality in Beresford Dale (Figure 3). Year 2006 was the  
175 warmest in the monitoring period and amongst the hottest on record in the UK. Hindcast  
176 annual mean Tw varied by no more than 1.0 °C between consecutive years, but GDDs fell  
177 between 2006 and 2010 before rising in 2011 and 2012 (Table 2). However, summer GDD  
178 increased between 2008 and 2011. Overcast conditions and high discharge resulted in  
179 markedly lower summer Tw in 2011–2012 even though the annual GDDs were the second  
180 highest. Observed Tw were under-predicted in summer 2011 but over-predicted by the model  
181 in 2012, because of the changing significance of spring flow contributions which are not fully  
182 replicated by the model (Figure 3). At Dovedale, annual mean Tw was less variable between  
183 years and the seasonal range is less than at Beresford due to groundwater inflows (Table 2).  
184 Consequently, summer temperatures are relatively cool and there were on average, 260 fewer  
185 GDDs each year between 2006 and 2011 (Figure 3). The contrast between Beresford Dale  
186 and Dovedale was greatest in the summer. For example, in 2012-2013 there were 266 more  
187 GDDs in Beresford Dale of which 231 were due to higher summer temperatures (Table 2).

188 The EA spot sampling yields Tw mean  $10.3 \pm 1.1$  °C ( $n=12$ ) compared with the resampled  
189 LUTEN Tw mean  $9.5 \pm 0.01$  °C in Beresford Dale (Figure 4), whereas the entire LUTEN  
190 record yields Tw mean  $9.7 \pm 0.02$  °C ( $n=32160$ ).

191

### 192 *Temporal variations in emergence*

193 In 2007, *E. danica* at Beresford Dale had a tri-modal size distribution, with larger males and  
194 females about to emerge plus a third group of smaller mayflies (Figure 5a). Monitoring in  
195 2010 revealed a uni-modal size distribution indicating a single year cohort (Figure 5b).  
196 Mayfly with body length >22 mm may be a remnant cohort of second year females. However,  
197 the lack of a second generation indicated by smaller, unsexed individuals, and lack of size

198 distinction between the sexes, suggest emergence mostly within one year of hatching. This  
199 pattern continued in 2011 and 2012 with few small (<10 mm) mayflies (Figure 5b).

200 In 2013, the nymph populations in Beresford Dale appeared to be reverting back to a two  
201 year, tri-modal size distribution (Figure 5b). The presence of small individuals (<8 mm)  
202 suggests over-wintering mayflies and the greater distinctiveness between males and females  
203 is indicative of a two year cohort (Table 3). This apparent reversion back to a two-year cycle  
204 coincides with the cool summer and low GDDs of the previous year (Figure 6a).

205 In 2011, adult male and female mayflies were on average 11.1 mm and 11.5 mm long,  
206 respectively (compared with 18.0 mm and 23.7 mm in 2007). This is statistically different in  
207 both cases (Mann-Whitney U;  $p < 0.001$ ) (Figure 6). The distribution of mayfly sizes was  
208 also significantly different between years, indicated by Levene's tests ( $p < 0.001$  for both  
209 males and females), with 2007 populations of male and female being less varied than in 2010  
210 and 2011. In Beresford Dale, females were significantly larger than males within all years,  
211 except 2011 when sexes were statistically similar (see Table 3 for  $p$ -values). In Dovedale,  
212 females were statistically distinct from males in both years.

213 The one year cohorts between 2010 and 2012 coincided with warmer summers and the return  
214 to a bi-annual cohort in 2013 with the unusually cool summer of 2012. As expected, the  
215 average size depends on the number of GDDs over the preceding year with mayfly larger  
216 when emerging after a warm year (Figure 6). In addition, mayflies developing in one year are  
217 generally smaller than those with a two year generation because more GDDs are accumulated  
218 over two moderately warm years than one very warm year. However, the relatively warm  
219 year 2011 produced large mayfly, despite the fact that the population had a one year  
220 generation period (Figure 6).

221

## 222 *Spatial variations in emergence*

223 The *E. danica* population in Dovedale is unlike that in Beresford Dale during the same year  
224 (Figure 7). Populations in Dovedale retained a large number of small, unsexed mayfly in  
225 2012 and 2013, in comparison to none (2012) and six (2013) in Beresford. Males and females  
226 also formed distinct size classes, in contrast to Beresford Dale where there was substantial  
227 overlap (although in 2013 there was some divergence between size of males and females).

228 *E. danica* sampled in Dovedale were generally larger than those in Beresford even though  
229 male mayflies in Dovedale were exposed on average to 374 fewer GDDs between 2005 and  
230 2011. However, the contrast in thermal regime between the two sites was reduced in 2012–  
231 2013 when Dovedale had 266 fewer GDDs (Table 2). It should also be noted that because  
232 mayflies in Dovedale retained two year generations, they actually accumulated substantially  
233 more GDDs than those in the warmer sites of Beresford.

## 234 **DISCUSSION**

### 235 *Plasticity in phenology*

236 In 2007 at Beresford Dale, *E. danica* nymphs entering a second year largely accounted for  
237 good recruitment the following year but, by 2010–2012 the mayfly population appeared to be  
238 supplemented by nymphs reaching maturity in a single year. This is consistent with studies of  
239 *E. danica* in southern England chalk streams showing that both males and females can reach  
240 maturity in a single year depending on Tw (Bennett, 2007). Field data from the River Dove  
241 suggests that summer maximum temperatures above 15 °C result in *E. danica* moving  
242 towards a one year cohort. These results are consistent with temperature thresholds in both  
243 field and laboratory studies (Bennett, 2007). Thermal dynamics in the River Dove were  
244 related to changing river flow during the monitoring period with high summer temperatures

245 in 2011 associated with drought conditions and low temperatures in 2012 with exceptionally  
246 wet conditions (Parry et al. 2013). Other studies have tested the phenological response of  
247 mayfly (*Baetis bicaudatus*) to both temperature and discharge and found that temperature  
248 accelerated emergence but flow had no impact (Harper & Peckarsky 2006).

249 *E. danica* phenology in the River Dove appears to be plastic, changing temporally from year-  
250 to-year. GDDs proved more useful in generalising thermal regimes than annual mean  
251 temperature. River temperatures in preceding summer and autumn strongly determine  
252 subsequent emergence patterns. Bennett (2007) also found that summer-autumn was the  
253 critical period influencing *E. danica* development and summer temperatures have been  
254 identified as significant to other mayfly species (*Ephoron shigae*, Watanabe *et al.*, 1999) and  
255 insect groups including stonefly, caddisfly and beetles (Haidekker & Hering 2008; Li *et al.*,  
256 2011). As far as the authors are aware, the apparent change in *E. danica* populations in the  
257 River Dove from a one year cycle in 2012 back to two year cycle in 2013 is the first  
258 documented evidence of a reversal in mayfly phenology related to Tw. This reversal was  
259 associated with cooler summer (and annual) Tw in Beresford Dale. In other words, a short-  
260 term reduction in thermal exposure coincided with a phenology reversal in *E. danica*.

261 Mayflies grew larger, more were caught, and there was greater distinctiveness in size  
262 between the sexes when exposed to higher GDDs. Conversely, mayflies were smaller, less  
263 numerous and the size of males and females were more alike when developing over fewer  
264 GDDs. However, this relationship was complicated because fewer GDDs were accumulated  
265 when developing over a single hot year in comparison to two cooler years. Consequently,  
266 mayflies emerging in a one-year cycle, associated with warm years, were smaller than those  
267 emerging after two relatively cool years, consistent with the findings of Bennett (2007).

268

269 *Implications of changing mayfly phenology*

270 The phenology of many insect species has been related to temperature, in terms of timing of  
271 emergence, size of emerging individuals, and generation period (see Thackeray et al., 2010).  
272 Phenology changes could be of significance because populations with single year cycle are  
273 potentially more vulnerable to adverse weather when the majority of the population is in  
274 terrestrial, adult form (Bennett, 2007). For example, prolonged high winds and heavy rain  
275 during the main emergent period in 2000 prevented female *E. danica* with a mainly one-year  
276 generation on the River Wey, Surrey from returning to the water to lay eggs (Bennett &  
277 Gilchrist, 2010). As a result, the larval population was much reduced the following year,  
278 whereas a large population remained in the River Test in Hampshire where larvae had  
279 maintained a predominately two year cycle (Bennett & Gilchrist, 2010). In other words,  
280 populations with a two year cycle have a reserve of over-wintering individuals that  
281 supplement emergence in the following year.

282 Water temperature has also been related to the size and fecundity of adults in a number of  
283 species (see reviews in Honêk 1993; Blanckenhorn, 2005). Consequently, altering the growth,  
284 development and size of insects may impact population dynamics by affecting reproductive  
285 success. Hence, smaller mayflies emerging from a one year cycle in the Dove are likely to  
286 have less reproductive success than larger mayflies emerging after two years development.  
287 This has been confirmed in *E. danica* by Bennett (1996) who found smaller females produced  
288 fewer eggs than larger individuals: ~6000 in 24 mm females, compared with ~3000 in 18 mm  
289 females. Consequently, mayflies in the River Dove at Beresford in 2010 and 2011 are likely  
290 to have produced fewer than half the eggs of mayfly in Dovedale over the same time period.

291 Given the dependence of insect development on heat accumulation through larval stages, it is  
292 likely that phenology changes have occurred during warm periods in the climate record.

293 However, the negative connotations associated with one-year emergence cycles combined  
294 with long-term warming of freshwaters (particularly in summer) suggests that anthropogenic  
295 climate change could have adverse impacts on mayfly phenology. In addition, the differing  
296 size and abundance of mayfly between years could affect trophic relationships, altering food  
297 availability and food-web dynamics. However, the spatial heterogeneity in mayfly phenology  
298 in the Dove might act to buffer against mayfly shortages at a site because predatory birds and  
299 fish could move to other sites where mayfly populations may be larger.

300

### 301 *Management of thermal refugia*

302 *E. danica* populations at sites A and B (separated by 300 m) in Beresford Dale were very  
303 similar. However, the phenology of mayflies from Beresford Dale and Dovedale (separated  
304 by 8.4 km) were substantially different. Dovedale is fed by considerable groundwater inflows,  
305 which lower mean Tw with dampened seasonal and diel cycles (Johnson et al. 2014). The  
306 cooler water of the River Dove (especially in summer) appears to have provided a thermal  
307 refuge where *E. danica* phenology has remained unchanged compared to Beresford Dale.  
308 Such areas could be of critical ecological significance in the context of climate change  
309 because they could delay changes in insect phenology. Conversely, warming reaches could  
310 experience substantial changes in phenology.

311 Given the implications of rising Tw for insect populations, it is important to understand and  
312 attempt to manage river temperatures. Spot sampling of daytime Tw 12-times a year,  
313 following the sampling strategy of the EA, over-estimated annual mean Tw relative to  
314 continuous monitoring and the standard error of the EA estimate (1.1 °C) is comparable to  
315 the difference between warmest and coolest years in the LUTEN hindcast series (1.6 °C). It is  
316 further recognised that any creep in spot sampling time, for example, from early-morning to

317 midday, could artificially increase Tw estimates (Toone et al. 2011). Moreover, annual Tw  
318 were poor indicators of thermal regime relevant to *E. danica*, as summer Tw was of greater  
319 importance. Consequently, spot sampling would have been insufficient to resolve differences  
320 in thermal regime between years or sites. Higher resolution sampling is needed to relate  
321 ecological changes to Tw, at least accounting for seasonal variations in temperature and  
322 preferably including sub-daily temperature changes which may be relevant to nocturnal fauna  
323 (Wilby et al., 2014). Reliable, sub-daily resolution temperatures can now be obtained via  
324 robust, field-deployable thermistors.

325 Regulatory bio-monitoring in the UK typically involves identifying invertebrates to family  
326 level for water quality and ecological assessment (Murray-Bligh, 1999; Environment Agency,  
327 2009). Given the species-specificity of insect-temperature relationships it is important that  
328 finer resolution information is obtained when evaluating the impacts of changing thermal  
329 regime on invertebrates. Furthermore, monitoring schemes usually focus on invertebrate  
330 community composition (Paisley *et al.*, 2007). Whilst Tw may alter community composition,  
331 this is likely to be preceded by shifts in the growth, development and phenology of insects.  
332 Consequently, consideration of insect size and sex ratios within species would allow the  
333 identification of thermal effects before the loss of species from the community pool. Such  
334 metrics could be used for early climate change detection.

335

## 336 **CONCLUSIONS**

337 The phenology of *E. danica* was found to be highly plastic in the River Dove due to  
338 variations in summer Tw over the years 2007-2013. In addition, *E. danica* phenology varied  
339 between sites with and without permanent groundwater inflows. Changes in phenology can  
340 be detrimental to insect populations because of reduced fecundity and increased vulnerability



341 to adverse weather. Annual mean Tw can mask important thermal characteristics, particularly  
342 increasing summer temperatures, which correlate with *E. danica* emergence. In addition,  
343 high-resolution invertebrate monitoring (size of individuals within an individual species) was  
344 required to identify the impacts of changing Tw on *E. danica* populations. Routine, coarse-  
345 scale biomonitoring is unlikely to have detected such trends. An unusually cool summer in  
346 2012 returned mayfly phenology to a two-year cycle. Consequently, the protection of thermal  
347 refugia (such as those areas where phraetic groundwater or riparian shade buffers against  
348 solar forcing) could buffer against phonological change in insects subject to rising Tw under  
349 climate change. In addition, artificial creation or enhancement of thermal refugia by riparian  
350 shade management could delay, or even reverse, changes to the phenology of some species  
351 otherwise impacted by higher water temperatures.

352

## 353 **ACKNOWLEDGEMENTS**

354 The authors wish to thank The Wild Trout Trust, Beresford Fishery, Derbyshire County  
355 Angling Club, Peak District National Park Authority and Staffordshire Wildlife Trust for  
356 part-funding this work. The opinions expressed by the authors do not necessarily reflect those  
357 of the aforementioned organisations. The manuscript was improved in response to the helpful  
358 comments of two anonymous reviewers.

359

### 360 *Contribution of authors*

361 Everall and Johnson designed the project. Everall and Bennett collected and analysed  
362 invertebrate data. Johnson and Wilby collected and analysed temperature data. All authors  
363 contributed to paper writing.

364

365 **REFERENCES**

366 Bennett CJ. (1996) The ecology of mayflies (Ephemeroptera) in the upper reaches of the  
367 River Wey in Surrey. PhD thesis, University of London, UK (unpublished).

368 Bennett CJ. (2007) The ecology of a seven year study of the life cycle of the mayfly  
369 *Ephemera danica*. *Freshwater Forum* **27**: 3–14.

370 Bennett CJ, Gilchrist W. (2010) Riverflies. In: Maclean, N. (ed) *Silent Summer*. Cambridge  
371 University Press, UK. pp. 401–414.

372 Berry J, Bjorkman O. (1980) Photosynthetic response and adaptation to temperature in higher  
373 plants. *Annual Review of Plant Physiology* **31**: 491–543.

374 Blanckenhorn WU. (2005) Behavioral causes and consequences of sexual dimorphism.  
375 *Ethology* **111**: 977–1016.

376 Broadmeadow SB, Jones JG, Langford TEL, Shaw PJ, Nisbet TR. (2011) Influence of  
377 riparian shade on lowland stream water temperatures in southern England and their viability  
378 for brown trout. *River Research and Applications* **27**: 226–237.

379 Caissie D. (2006) The thermal regime of rivers: a review. *Freshwater Biology* **51**: 1389–1406.

380 Constantz J. (1998) Interaction between stream temperature, streamflow, and groundwater  
381 exchanges in alpine streams. *Water Resources Research* **34**: 1609–1615.

382 Dallas HF, Rivers-Moore NA. (2012) Critical thermal maxima of aquatic macroinvertebrates:  
383 towards identifying bioindicators of thermal alteration. *Hydrobiologia* **679**: 61–76.

384 Durance I, Ormerod SJ. (2007) Climate change effects on upland stream macroinvertebrates  
385 over a 25-year period. *Global Change Biology* **13**: 942–957.

386 Elliott JM, Humpesch UH, Macan TT. (1988) *Larvae of the British Ephemeroptera. A key*  
387 *with ecological notes*. Scientific Publication No. 49. Freshwater Biological Association.  
388 Ambleside.

389 Environment Agency (2009) *Freshwater Macro-invertebrate Analysis of Riverine Samples*.  
390 Operational Instruction 024\_08. Environment Agency, UK.

391 Everall NC. (2010) The aquatic ecological status of the rivers of the Upper Dove Catchment  
392 in 2009. *Natural England Commissioned Report NECR046*. Natural England: Sheffield.

393 Everall NC, Farmer A, Heath AF, Jacklin TE, Wilby RL. (2012). Ecological benefits of  
394 creating messy rivers. *Area* **44**: 470–478.

395 Gurnell A, Tockner K, Edwards P, Petts G. (2005) Effects of deposited wood on  
396 biocomplexity of river corridors. *Frontiers in Ecology and Environment* **3**: 377–382.

397 Haidekker A, Herring D. (2008) Relationship between benthic insects (Ephemeroptera,  
398 Plecoptera, Coleoptera, Trichoptera) and temperature in small and medium-sized streams in  
399 Germany: A multivariate study. *Aquatic Ecology* **42**: 463–481.

400 Harper MP, Perckarsky BL. (2006) Emergence cues of a mayfly in a high-altitude stream  
401 ecosystem: Potential response to climate change. *Ecological Applications* **16**: 612–621.

402 Honêk A. (1993) Intraspecific variation in body size and fecundity in insects: a general  
403 relationship. *Oikos* **66**: 483–492.

404 Imholt C, Gibbins CN, Malcolm LA, Langan S, Soulsby C. (2010) Influence of riparian  
405 cover on stream temperatures and the growth of the mayfly *Baetis rhodani* in an upland  
406 stream. *Aquatic Ecology* **44**: 669–678.

407 Isaak DJ, Wollrab S, Horan D, Changler G. (2012) Climate change effects on stream and  
408 river temperatures across the northwest U.S. from 1980–2009 and implications for  
409 salmonid fishes. *Climate Change* **113**: 499–524.

410 Johnson MF, Wilby RL. (2013) Shield or not to shield: Effects of solar radiation on water  
411 temperature sensor accuracy. *Water* **5**: 1622–1637.

412 Johnson MF, Wilby RL, Toone JA. (2014) Inferring air-water temperature relationships  
413 from river and catchment properties. *Hydrological Processes* **28**: 2912–2928.

414 Knispel S, Sartori M, Brittain JE. (2006) Egg development in the mayflies of a Swiss glacial  
415 floodplain. *Journal North American Benthological Society* **25**: 2, 430–443.

416 Li JL, Johnson SL, Sobota JB. (2011) Three responses to small changes in stream  
417 temperature by autumn-emerging aquatic insects. *Journal of the North American*  
418 *Benthological Society* **30**: 474–484.

419 Mohensi O, Stefan HG, Erickson TR. (1998) A nonlinear regression model for weekly stream  
420 temperatures. *Water Resources Research* **34**: 2685–2692.

421 Murray-Bligh JAD. (1999) *Procedure for collecting and analysing macro-invertebrate*  
422 *samples*. Quality Management Systems for Environmental Biology: Biological Techniques,  
423 BT001 Version 2.0. Bristol: Environment Agency.

424 Neuheimer AB, Taggart CT. (2007) The growing degree-day and fish size-at-age: the  
425 overlooked metric. *Canadian Journal of Fisheries and Aquatic Science* **64**: 375–385.

426 O'Driscoll MA, DeWalle DR. (2006) Stream-air temperature relations to classify stream-  
427 ground water interactions in a karst setting, central Pennsylvania, USA. *Journal of Hydrology*  
428 **329**: 140–153.

429 Paisley MF, Trigg DJ, Walley WJ. (2007) Revision and Testing of BMWP scores. Final  
430 report SNIFFER Project WFD72a. Edinburgh, UK.

431 Parry S, Marsh T, Kendon M. (2013) 2012: from drought to floods in England and Wales.  
432 *Weather* **68**: 268–274.

433 Raddum GG, Fjellheim A. (1993) Life-cycle and production of Baetis-Rhodani in a regulated  
434 river in Western Norway-comparison of pre-regulation and post-regulation conditions.  
435 *Regulatory Rivers Reservoir Management* **8**: 49–61.

436 Sparks TH, Preston CD, Roy DB. (2010) Climate Change. In: Silent Summer The State of  
437 Wildlife in Britain and Ireland. Ed. N. Maclean. Cambridge University Press, 765pp.

438 Stefan HG, Preud'homme EB. (1993) Stream temperature estimation from air temperature.  
439 *Water Resource Research* **29**: 27–45.

440 Svensson B. (1977) Life cycle, energy fluctuations and sexual differentiation in Ephemera  
441 Danica (Ephemeroptera), a stream-living mayfly. *Oikos* **29**: 78–86.

442 Thackeray SJ, Sparks TH, Frederiksen M, Burthes S, Bacon PJ, Bell JR, Botham MS,  
443 Brereton TM, Bright PW, Carvalho L, Clutton-Brock T, Dawsons A, Edwards M, Elliott M,  
444 Harrington R, Johns D, Jones ID, Jones JT, Leech DI, Roy DB, Scott WA, Smith M,  
445 Smithers RI, Winfield IJ, Wanless S. (2010) Trophic level asynchrony in rates of  
446 phonological change for marine, freshwater and terrestrial environments. *Global Change*  
447 *Biology* **16**: 3304–3313.

448 Tokeshi M. (1985) Life-cycle and production of the burrowing mayfly, *Ephemera danica*: a  
449 new method of estimating degree-days for growth. *Journal of Animal Ecology* **54**: 919–930.

450 Toone JA, Wilby RL, Rice SP. (2011) Surface-water temperature variations and river  
451 corridor properties. *Water Quality: Current Trends and Expected Climate Change Impacts*  
452 Proceedings of symposium H04 held during IUGG2011 in Melbourne, Australia, July 2011.  
453 IAHS Publication **348**; 129–134.

454 van Vliet MTH, Ludwig F, Zwolsman JJG, Weedon GP, Kabat P. (2011) Global river  
455 temperatures and sensitivity to atmospheric warming and changes in river flow. *Water*  
456 *Resources Research* **47**: 10.1029/2010WR009198.

457 Ward JV, Stanford JA. (1982) Thermal responses in the evolutionary ecology of aquatic  
458 insects. *Annual Review of Entomology* **27**: 97–117.

459 Watanabe NC, Mori I, Yoshitaka I. (1999). Effect of water temperature on the mass  
460 emergence of the mayfly, *Ephoron shigae*, in a Japanese river (Ephemeroptera:  
461 Polymitaarcyidae). *Freshwater Biology* **41**: 537–541.

462 Weatherly NS, Ormerod SJ. (1990). Forest temperatures of upland streams in Wales: a  
463 modelling exploration of the biological effects. *Freshwater Biology* **24**: 109–122.

464 Webb BW, Hannah DM, Moore RD, Brown LE, Nobilis F. (2008) Recent advances in stream  
465 and river temperature research. *Hydrological Processes* **22**: 902–918.

466 Wilby RL, Orr H, Watts G, Battarbee RW, Berry PM, Chadd R, Dugdale SJ, Dunbar MJ,  
467 Elliott JA, Extence C, Hannah DM, Holmes N, Johnson AC, Knights B, Milner NJ, Ormerod  
468 SJ, Solomon D, Timlett R, Whitehead PJ, Wood PJ. (2010). Evidence needed to manage

469 freshwater ecosystems in a changing climate: Turning adaptation principles into practice.  
470 *Science of the Total Environment* **408**: 4150-4164.

471 Wilby RL, Johnson MF, Toone JA. (2012) The Loughborough University Temperature  
472 Network (LUTEN): Rationale and analysis of stream temperature variations. *Proceedings of*  
473 *Earth Systems Engineering 2012: Systems Engineering for Sustainable Adaptation to Global*  
474 *Change*. Newcastle, UK.

475 Wilby RL, Johnson MF, Toone JA. (2014) Nocturnal river water temperatures: Spatial and  
476 temporal variations. *Science of the Total Environment* **482-483**: 157–173..

477 Wright JF, Hiley PD, Berrie AD. (1981). A nine-year study of the life cycle of *Ephemera*  
478 *danica* Müller. (Ephemeridae: Ephemeroptera) in the river Lambourn, England. *Ecological*  
479 *Entomology* **6**: 321–331.

480

481 **Table 1** Logistic regression model parameters ( $\alpha$ ,  $\beta$  and  $\gamma$ ), the amount of explained variance  
 482 ( $r^2$ ) and the standard error of the estimate (SE) in both calibration and validation periods,  
 483 where SE is the RMS of error about the model, giving an estimate of the difference between  
 484 observed and modelled values.

<i>Site</i>	Calibration (2011)					Validation (2012)	
	$\alpha$ °C	$\beta$ °C	$\gamma$ °C	$r^2$	SE °C	$r^2$	SE °C
Beresford	23.4	15.0	0.1	0.85	1.3	0.83	1.9
Dovedale	13.5	4.5	0.1	0.83	0.8	0.80	1.5

485

486



487 **Table 2** Mean water temperature and GDDs in Beresford Dale and Dovedale. Annual (1 June  
 488 to 31 May each year) and seasonal GDDs are also shown for summer (June to July); autumn  
 489 (September to November); winter (December to February); and spring (March to May).

Year	Beresford Dale						Dovedale					
	Mean (°C)	Cumulative degree days					Mean (°C)	Cumulative degree days				
		ANN	JJA	SON	DJF	MAM		ANN	JJA	SON	DJF	MAM
2005/06	10.0	2697	1017	735	352	593	8.9	2305	749	616	398	542
2006/07	10.9	3038	1105	783	446	704	9.4	2495	781	643	461	610
2007/08	10.1	2749	967	692	455	634	9.0	2369	733	601	468	568
2008/09	9.8	2642	983	653	325	682	8.9	2293	738	578	377	599
2009/10	9.8	2615	1004	708	265	638	8.8	2260	745	610	332	572
2010/11	10.1	2705	998	647	329	731	8.9	2311	744	570	376	621
2011/12	10.3	2796	966	769	403	658	9.1	2483	731	668	432	682
2012/13	9.3	2426	958	614	334	520	8.4	2160	727	557	379	498

490

491

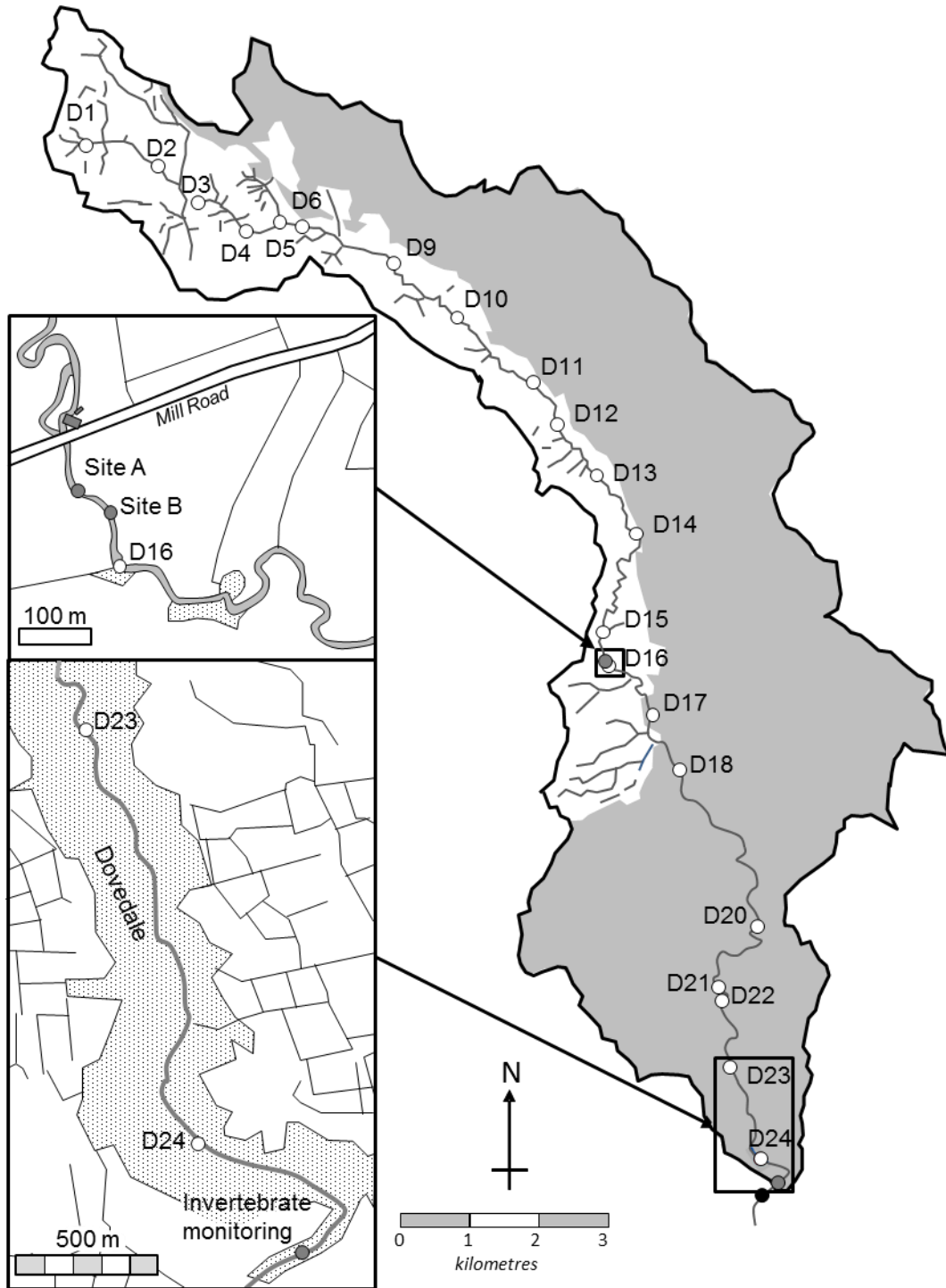
492 **Table 3** The number (n) of male, female and small, unsexed mayfly and the percentage total  
 493 each constitutes each year. The mean, median, maximum and minimum total lengths for male,  
 494 female and unsexed individuals are included along with *p*-values from Mann-Whitney U tests  
 495 between male and female sizes. The number of GDD preceding emergence over 1 and 2 years  
 496 are quoted, along with the expected number of years emerging mayfly have developed over

		Beresford Dale						Dovedale		
		2007	2008	2009	2010	2011	2012	2013	2012	2013
Male	<i>n</i>	70			48	61	279	101	53	56
	%	20			32	27	48	47	27	19
	Mean	18.0			14.4	11.1	14.7	10.8	18.7	16.0
	Max	22			18.5	17	20	19	20.5	19
	min	14.5			9	8	9	8.5	15.5	14
Female	<i>n</i>	132			101	163	308	78	60	120
	%	37			68	73	52	37	30	41
	Mean	23.7			18.1	11.5	20.5	13.8	24.3	22.4
	Max	28			27	17.5	28	28	26.5	26
	min	19.5			9.5	6.5	13	11.5	21.5	19.5
<i>p</i> -value		<0.01			<0.01	0.496	<0.01	<0.01	<0.01	<0.01
Unsexed	<i>n</i>	156			0	0	0	34	86	115
	%	44			0	0	0	16	43	40
	Mean	3.6			0	0	0	7.5	4.3	6.5
	Max	5.5			0	0	0	8	7.5	10.5
	min	1			0	0	0	6	1	2.5
1-year GDD		3038	2749	2642	2615	2705	2796	2426	2483	2160
2-years GDD		5735	5787	5391	5257	5320	5501	5222	4794	4643
Expected life-cycle		2			1	1	1	2	2	2

497

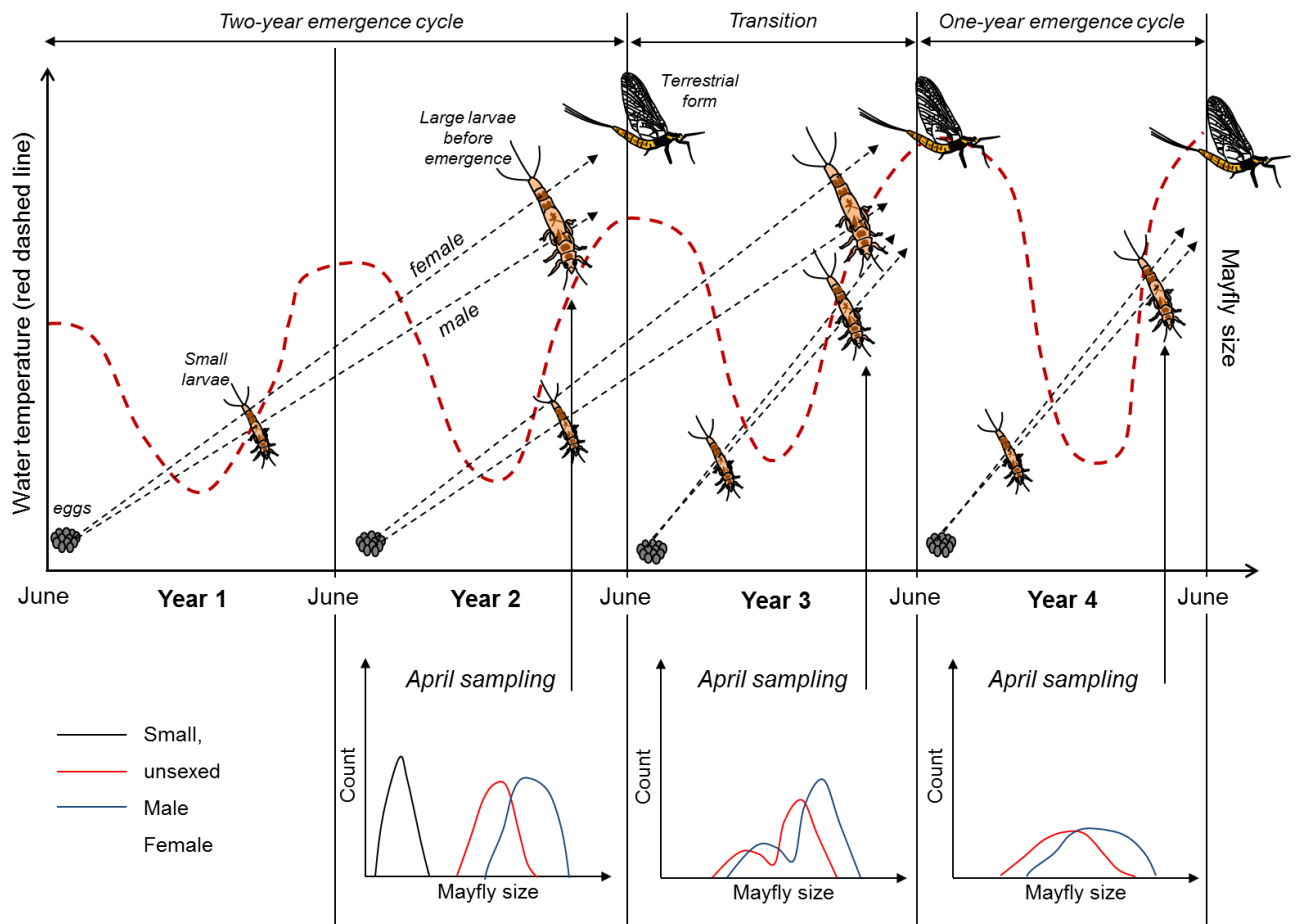
498

499 **Figure 1** The River Dove catchment showing invertebrate (grey circles), LUTEN (white circles) and Environment Agency river gauge (black circle) sites. Grey indicates limestone  
 500 outcrop; unshaded is millstone grit. Insets show Beresford Dale and Dovedale invertebrate  
 501 sampling areas and closest LUTEN sites. Dotted areas indicate woodland.



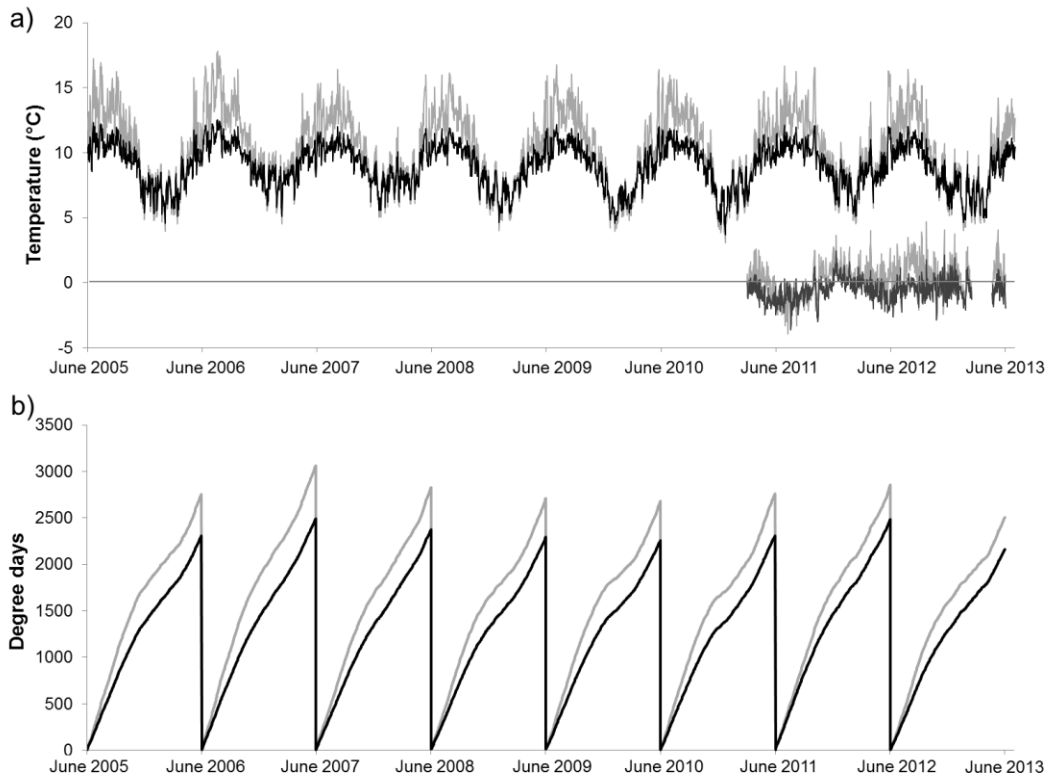
503

504 **Figure 2** Schematic of the *E. danica* life-cycle. From hatching, larvae develop as they  
 505 accumulate heat during their aquatic stage. Females grow larger than males over the same  
 506 period and this variance widens as heat accumulates. Emergence occurs in early-June. Year 2  
 507 shows two cohorts with distinct male and female size distributions in one, and a group of  
 508 small mayfly in their first year of growth. Year 4 shows a single year cohort, with mayflies  
 509 about to emerge all of moderate size and males and females of similar dimensions. The  
 510 transition between one and two year cycles (Year 3) also has no peak in small mayfly.



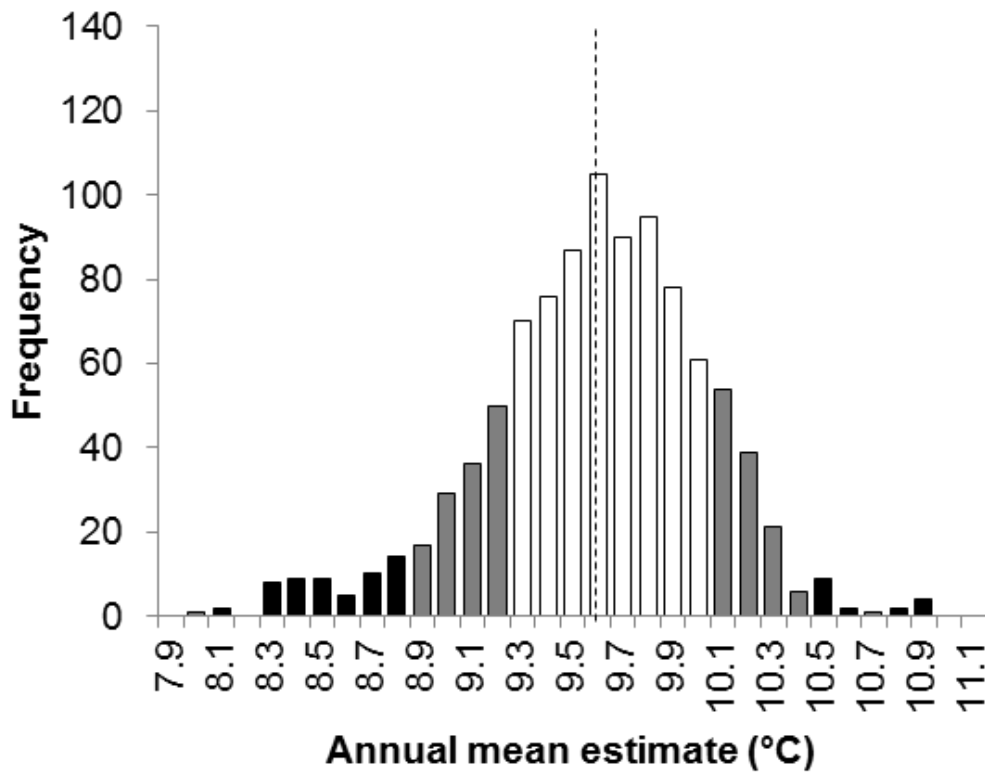
511  
 512

513 **Figure 3a)** Hind cast daily-mean Tw at Beresford Dale (grey) and Dovedale (black) for June  
514 2005 to May 2013. Model residuals are shown below for Beresford Dale (light grey) and  
515 Dovedale (dark grey) for the period with LUTEN data (March 2011 to May 2013). b) GDDs  
516 for years beginning in June 2005 to May 2013 at Beresford Dale (grey) and Dovedale (black)  
517 assuming minimum growth threshold for male *E. danica* (2.6 °C).



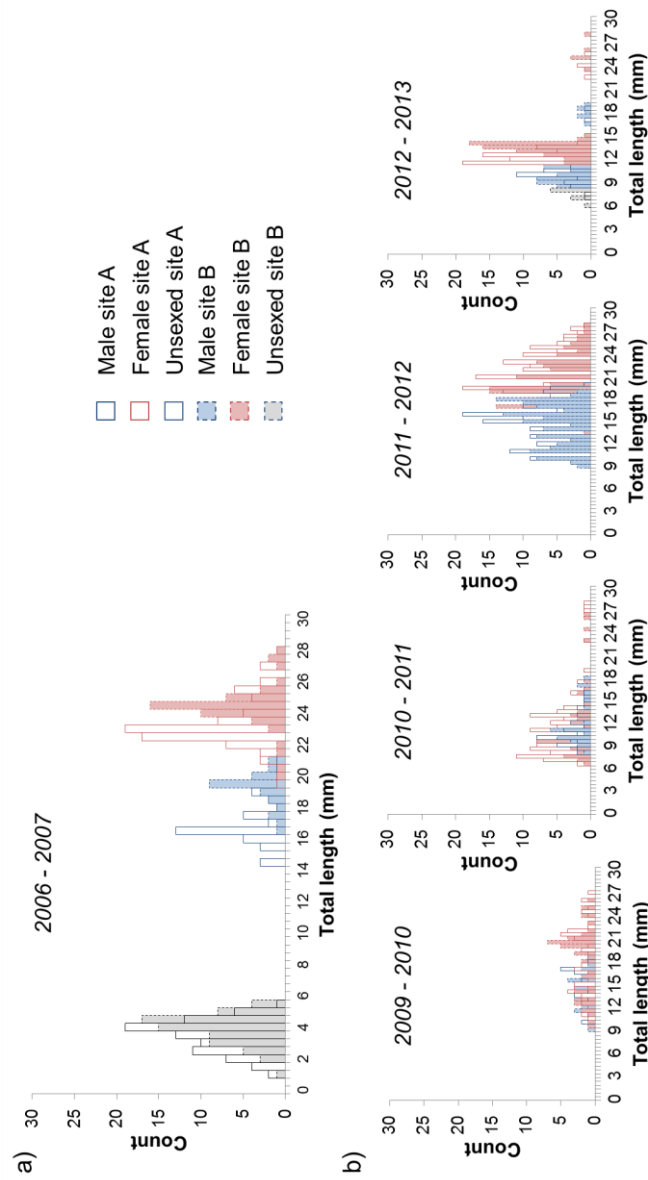
518

519 **Figure 4** Estimated annual mean temperatures at D16 (Beresford) based on 1000 replicates of  
520 one sample per month drawn from 15-minute LUTEN data for the hours 08:00 to 18:00. The  
521 overall LUTEN mean is shown by the vertical line. Shading denotes standard deviations from  
522 the mean.



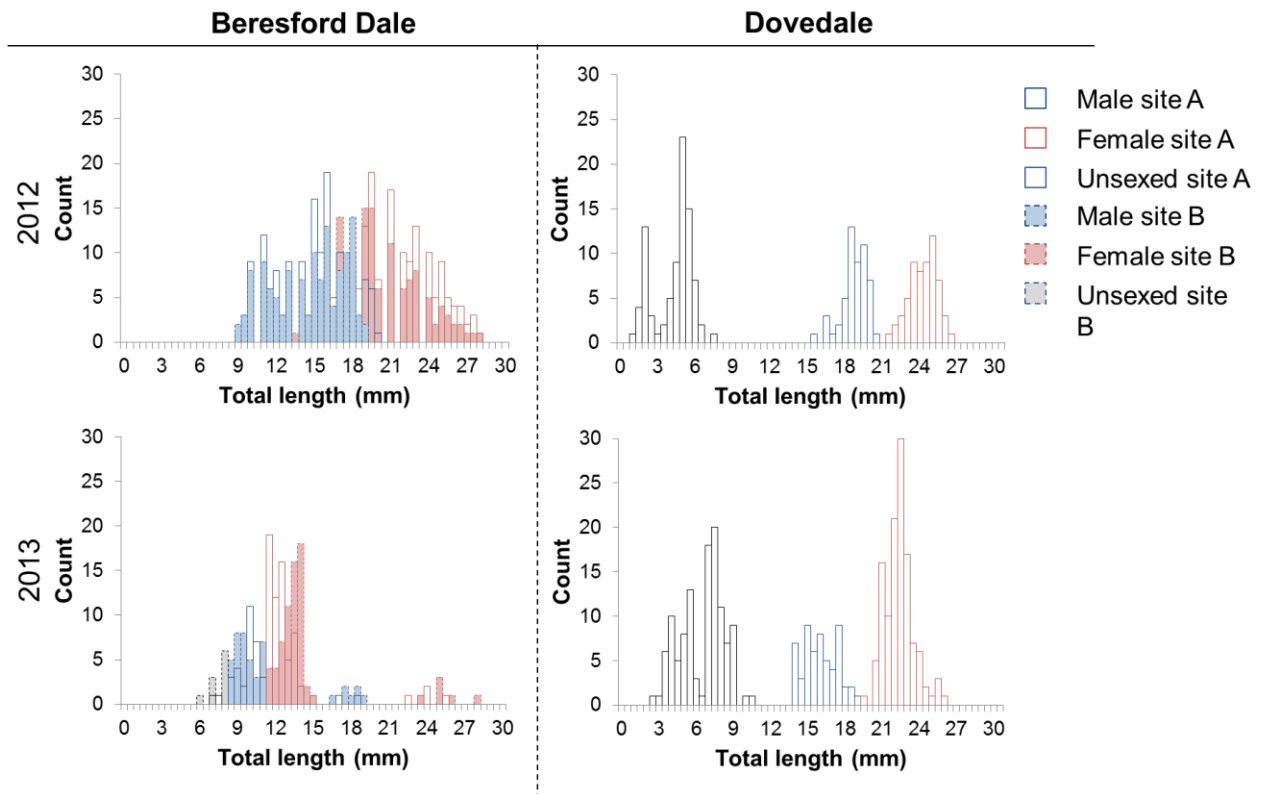
523

524 **Figure 5** Number and size of mayflies caught in Beresford Dale in a) April 2007 and b) each  
 525 April from 2010 to 2013.



526

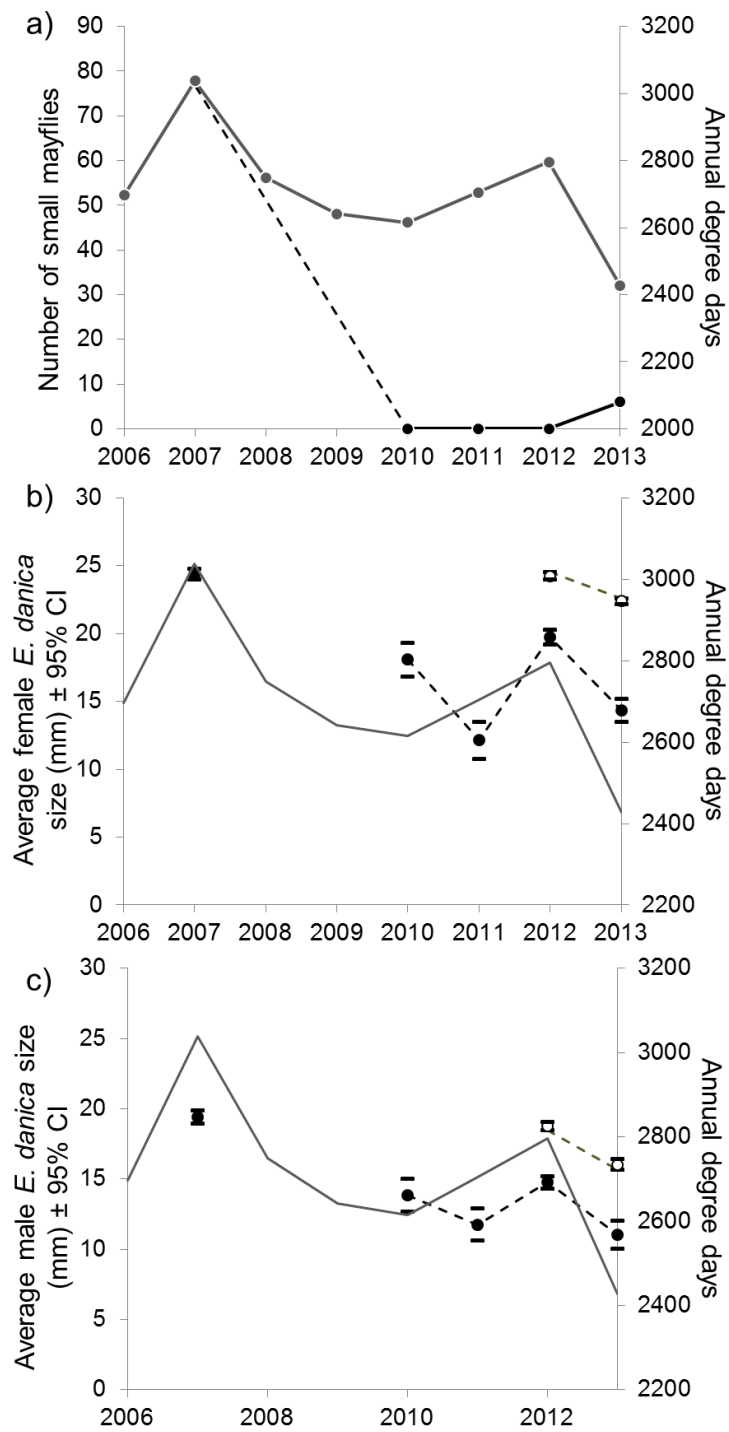
527 **Figure 6a)** Annual GDDs for growth threshold 2.6 °C (grey line) and number of small  
 528 mayfly (<7 mm) sampled each year (black line) in Beresford Dale. b) Average length of  
 529 female *E. danica* with 95% confidence intervals sampled in Beresford Dale (filled circles)  
 530 and Dovedale (open circles). The grey line indicates GDDs at Beresford for growth threshold  
 531 of 2.6 °C. c) As above for male *E. danica*. All years were statistically distinct for females.



532  
 533



534 **Figure 7** Number and length of *E. danica* caught in Beresford Dale (left) and Dovedale (right)  
 535 in 2012 and 2013.



536