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Citation: EVERALL, N.C. ... et al, 2015. Detecting phenology change in the mayfly Ephemera danica: responses to spatial and temporal water temperature variations. Ecological Entomology, 40 (2), pp.95-105.

Additional Information:

• This is the peer reviewed version of the article, which has been published in final form at http://dx.doi.org/10.1111/een.12164. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Metadata Record: https://dspace.lboro.ac.uk/2134/17672

Version: Accepted for publication

Publisher: Wiley Publishing / © The Royal Entomological Society

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

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Accepted for publication: *Ecological Entomology*

Date of acceptance: 16 September 2014
Abstract

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

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

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

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

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

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

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

River temperature is spatially heterogeneous and temporally variable (Webb et al. 2008). Water temperatures usually have strong diel and seasonal cycles related to solar forcing, and typically increases with distance downstream. Tributaries and groundwater can interrupt this pattern and create locally distinct thermal regions. For example, phreatic groundwater usually has a relatively constant temperature that reduces diel and seasonal temperature ranges (Constantz, 1998; O’Driscoll & DeWalle, 2006).
There is evidence that river Tw is changing in response to climate change (van Vliet et al., 2011; Isaak et al., 2012; Orr et al. 2014). In addition, land drainage, alteration of river courses and ponding by weirs, can alter the thermal dynamics of rivers (Caissie, 2006), as can removal of riparian vegetation (e.g. Broadmeadow et al., 2011). Aquatic organisms respond to changing thermal conditions in complex ways (Ward & Stanford, 1982). Given the dependence of phenology on heat accumulation, emergence of insects is particularly susceptible to changing temperature and can have adverse effects on freshwater insect populations (Harper & Peckarsky, 2006; Durance and Ormerod, 2007; Thackeray et al., 2010). In this paper, we describe spatial and temporal variability in mayfly emergence in the River Dove, English Peak District. We then assess links between varying Tw and mayfly phenology, and discuss whether conventional monitoring protocols are adequate for detecting changes in the thermal driver and ecological response.

MATERIALS AND METHODS

Study organism

*Ephemera danica* Muller, 1764 (Ephemeroptera) is one of the largest mayflies found in the British Isles with some females reaching over 30 mm. The larvae are burrowing animals and are often found where silt accumulates in rivers. Traditionally *E. danica* has been reported to emerge after two years in an aquatic nymphal stage and is referred to as a semivoltine species (Wright et al., 1981; Tokeshi, 1985; Elliott et al., 1988). The adult emergence period is normally in late-May and early-June. Tokeshi (1985) found that male *E. danica* have a minimum growth threshold of 2.6 °C and require at least 3398 annual GDDs to emerge. Females have a higher minimum growth threshold (3.1 °C) and hence require more GDDs for
emergence (3631). Differing growth thresholds for males and females is common in mayfly populations (Svensson 1977; Wright et al. 1981).

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

**River Dove and sampling sites**

The River Dove rises on Axe Edge from moorland springs and runs southward for 73 km through the Peak District National Park before joining the River Trent at Newton Solney. The catchment area upstream of monitoring sites is 131 km² and elevation ranges from 450 m at source to 155 m in Dovedale. Land-use is predominately grazed pasture with isolated stands of deciduous woodland covering 5% of the catchment. Annual precipitation exceeds 1000 mm. The Dove flows parallel to a Carboniferous limestone outcrop with phreatic springs, which it intersects at Beresford.

Invertebrate and Tw monitoring sites are located in Beresford Dale and Dovedale (Figure 1). Beresford Dale is 20 km from the source of the Dove, situated at the upstream end of a limestone gorge. Here, intermittent springs discharge water of relatively constant temperature (9–14 °C) during autumn and winter. Dovedale is 9.7 km downstream of Beresford Dale in a limestone gorge with Ash (*Fraxinus excelsior*) woodland. Here, groundwater discharges into the river all year-round at ~8.5 °C. The stretch between Beresford and Dovedale is affected
by over 100 weirs (< 0.5 m high) installed over a century ago to increase the feeding area for trout. The Environment Agency of England and Wales (EA) have recorded daily discharge at a gauging station in Dovedale since 1969 (Figure 1).

Water temperature monitoring and analysis

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

The EA takes monthly spot measurements for routine monitoring. To test the ability of this sampling strategy to detect thermal changes one value per month was randomly selected from the 15 minute LUTEN data during typical EA sampling hours (08:00 to 18:00) from June 2011 to May 2012. These 12 values were then used to estimate the annual mean at site D16. This was repeated 1000 times allowing estimation of the variance in the mean due to daylight sample times, when compared with the ‘true’ estimate based on the full LUTEN-record.
Water temperatures for the years 2007 to 2013 were hindcast from air temperatures measured at Buxton, Derbyshire (~20 km from study sites and significantly correlated ($r^2 = 0.8$) to 3-years of monitored air temperature at each site). Air and water temperature are not directly related but because both are ultimately driven by solar radiation, the latter can be predicted from air temperatures using regression analysis (Stefan and Preud’homme, 1993; Mohseni et al., 1998). We deploy logistic regression models built previously for LUTEN sites and tested under contrasting weather conditions (Johnson et al. 2014; Wilby et al., 2014). These models explained 85% of the variance in Tw at D16 and 83% at D23 (Table 1) and have the form:

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

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

**Invertebrate sampling**

Invertebrate monitoring sites were approximately 40 m long, 7–8 m wide, and located in riffles. Two sites in Beresford Dale were 100 m downstream of Hartington bridge and approximately 100 m apart. The substrate consists of clean, coarse-gravels ($D_{50}$=48 mm) and sparse stands of water crowfoot (*Ranunculus* spp.) with very occasional starwort (*Callitriche* spp.). The Dovedale site also comprises of clean, coarse-gravels ($D_{50}$=41 mm) with sparse weed-beds of water crowfoot (*Ranunculus* spp.).
All study reaches hold good populations of *E. danica* based on data collected from previous surveys (Everall, 2010; 2012). *E. danica* samples were taken from exposed gravels and fine-sediment beneath weed-beds using a 0.1 m\(^2\) Surber net sampler fitted with a 2 cm deep steel curtain. Sampling was undertaken in Aprils 2007, 2010, 2011, 2012 and 2013 following the life-stage techniques of Bennett (2007). The number of *E. danica* individuals and their body length was recorded by site and year. Mayflies larger than 7 mm were also sexed; this was not always possible for smaller individuals.

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

**Statistical analysis**

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

**RESULTS**

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

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

Temporal variations in emergence

In 2007, *E. danica* at Beresford Dale had a tri-modal size distribution, with larger males and females about to emerge plus a third group of smaller mayflies (Figure 5a). Monitoring in 2010 revealed a uni-modal size distribution indicating a single year cohort (Figure 5b). Mayfly with body length >22 mm may be a remnant cohort of second year females. However, the lack of a second generation indicated by smaller, unsexed individuals, and lack of size
distinction between the sexes, suggest emergence mostly within one year of hatching. This pattern continued in 2011 and 2012 with few small (<10 mm) mayflies (Figure 5b).

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

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

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

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

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

DISCUSSION

Plasticity in phenology

In 2007 at Beresford Dale, *E. danica* nymphs entering a second year largely accounted for good recruitment the following year but, by 2010–2012 the mayfly population appeared to be supplemented by nymphs reaching maturity in a single year. This is consistent with studies of *E. danica* in southern England chalk streams showing that both males and females can reach maturity in a single year depending on Tw (Bennett, 2007). Field data from the River Dove suggests that summer maximum temperatures above 15 °C result in *E. danica* moving towards a one year cohort. These results are consistent with temperature thresholds in both field and laboratory studies (Bennett, 2007). Thermal dynamics in the River Dove were related to changing river flow during the monitoring period with high summer temperatures.
in 2011 associated with drought conditions and low temperatures in 2012 with exceptionally wet conditions (Parry et al. 2013). Other studies have tested the phenological response of mayfly (*Baetis bicaudatus*) to both temperature and discharge and found that temperature accelerated emergence but flow had no impact (Harper & Peckarsky 2006).

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

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

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

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

Given the dependence of insect development on heat accumulation through larval stages, it is likely that phenology changes have occurred during warm periods in the climate record.
However, the negative connotations associated with one-year emergence cycles combined with long-term warming of freshwaters (particularly in summer) suggests that anthropogenic climate change could have adverse impacts on mayfly phenology. In addition, the differing size and abundance of mayfly between years could affect trophic relationships, altering food availability and food-web dynamics. However, the spatial heterogeneity in mayfly phenology in the Dove might act to buffer against mayfly shortages at a site because predatory birds and fish could move to other sites where mayfly populations may be larger.

Management of thermal refugia

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

Given the implications of rising Tw for insect populations, it is important to understand and attempt to manage river temperatures. Spot sampling of daytime Tw 12-times a year, following the sampling strategy of the EA, over-estimated annual mean Tw relative to continuous monitoring and the standard error of the EA estimate (1.1 °C) is comparable to the difference between warmest and coolest years in the LUTEN hindcast series (1.6 °C). It is further recognised that any creep in spot sampling time, for example, from early-morning to
midday, could artificially increase Tw estimates (Toone et al. 2011). Moreover, annual Tw were poor indicators of thermal regime relevant to E. danica, as summer Tw was of greater importance. Consequently, spot sampling would have been insufficient to resolve differences in thermal regime between years or sites. Higher resolution sampling is needed to relate ecological changes to Tw, at least accounting for seasonal variations in temperature and preferably including sub-daily temperature changes which may be relevant to nocturnal fauna (Wilby et al., 2014). Reliable, sub-daily resolution temperatures can now be obtained via robust, field-deployable thermistors.

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

**CONCLUSIONS**

The phenology of E. danica was found to be highly plastic in the River Dove due to variations in summer Tw over the years 2007-2013. In addition, E. danica phenology varied between sites with and without permanent groundwater inflows. Changes in phenology can be detrimental to insect populations because of reduced fecundity and increased vulnerability
to adverse weather. Annual mean Tw can mask important thermal characteristics, particularly increasing summer temperatures, which correlate with *E. danica* emergence. In addition, high-resolution invertebrate monitoring (size of individuals within an individual species) was required to identify the impacts of changing Tw on *E. danica* populations. Routine, coarse-scale biomonitoring is unlikely to have detected such trends. An unusually cool summer in 2012 returned mayfly phenology to a two-year cycle. Consequently, the protection of thermal refugia (such as those areas where phreatic groundwater or riparian shade buffers against solar forcing) could buffer against phonological change in insects subject to rising Tw under climate change. In addition, artificial creation or enhancement of thermal refugia by riparian shade management could delay, or even reverse, changes to the phenology of some species otherwise impacted by higher water temperatures.

**ACKNOWLEDGEMENTS**

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

**Contribution of authors**

Everall and Johnson designed the project. Everall and Bennett collected and analysed invertebrate data. Johnson and Wilby collected and analysed temperature data. All authors contributed to paper writing.
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Table 1 Logistic regression model parameters ($\alpha$, $\beta$ and $\gamma$), the amount of explained variance ($r^2$) and the standard error of the estimate (SE) in both calibration and validation periods, where SE is the RMS of error about the model, giving an estimate of the difference between observed and modelled values.

<table>
<thead>
<tr>
<th>Site</th>
<th>Calibration (2011)</th>
<th>Validation (2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha$ °C</td>
<td>$\beta$ °C</td>
</tr>
<tr>
<td>Beresford</td>
<td>23.4</td>
<td>15.0</td>
</tr>
<tr>
<td>Dovedale</td>
<td>13.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>
Table 2 Mean water temperature and GDDs in Beresford Dale and Dovedale. Annual (1 June to 31 May each year) and seasonal GDDs are also shown for summer (June to July); autumn (September to November); winter (December to February); and spring (March to May).

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean (°C)</th>
<th>Beresford Dale</th>
<th>Mean (°C)</th>
<th>Dovedale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cumulative degree days</td>
<td>Cumulative degree days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ANN</td>
<td>JJA</td>
<td>SON</td>
<td>DJF</td>
</tr>
<tr>
<td>2005/06</td>
<td>10.0</td>
<td>2697</td>
<td>1017</td>
<td>735</td>
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<td>10.9</td>
<td>3038</td>
<td>1105</td>
<td>783</td>
</tr>
<tr>
<td>2007/08</td>
<td>10.1</td>
<td>2749</td>
<td>967</td>
<td>692</td>
</tr>
<tr>
<td>2008/09</td>
<td>9.8</td>
<td>2642</td>
<td>983</td>
<td>653</td>
</tr>
<tr>
<td>2009/10</td>
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<td>1004</td>
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<td>10.3</td>
<td>2796</td>
<td>966</td>
<td>769</td>
</tr>
<tr>
<td>2012/13</td>
<td>9.3</td>
<td>2426</td>
<td>958</td>
<td>614</td>
</tr>
</tbody>
</table>
Table 3 The number (n) of male, female and small, unsexed mayfly and the percentage total each constitutes each year. The mean, median, maximum and minimum total lengths for male, female and unsexed individuals are included along with p-values from Mann-Whitney U tests between male and female sizes. The number of GDD preceding emergence over 1 and 2 years are quoted, along with the expected number of years emerging mayfly have developed over

<table>
<thead>
<tr>
<th></th>
<th>Beresford Dale</th>
<th>Dovedale</th>
</tr>
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<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>18.0</td>
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</tr>
<tr>
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<tr>
<td>min</td>
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</tr>
<tr>
<td>Female</td>
<td></td>
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</tr>
<tr>
<td>n</td>
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<td></td>
</tr>
<tr>
<td>%</td>
<td>37</td>
<td>68</td>
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<tr>
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<td>23.7</td>
<td>18.1</td>
</tr>
<tr>
<td>Max</td>
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<td>27</td>
</tr>
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<td>9.5</td>
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Figure 1 The River Dove catchment showing invertebrate (green circles), LUTEN (red circles) and Environment Agency river gauge (black circle) sites. Grey indicates limestone outcrop; unshaded is millstone grit. Insets show Beresford Dale and Dovedale invertebrate sampling areas and closest LUTEN sites.
Figure 2 Schematic of the *E. danica* life-cycle. From hatching, larvae develop as they accumulate heat during their aquatic stage. Females grow larger than males over the same period and this variance widens as heat accumulates. Emergence occurs in early-June. Year 2 shows two cohorts with distinct male and female size distributions in one, and a group of small mayfly in their first year of growth. Year 4 shows a single year cohort, with mayflies about to emerge all of moderate size and males and females of similar dimensions. The transition between one and two year cycles (Year 3) also has no peak in small mayfly.
Figure 3a) Hind cast daily-mean Tw at Beresford Dale (red) and Dovedale (black) for June 2005 to May 2013. Model residuals are shown for Beresford Dale (pink) and Dovedale (grey) for the period with LUTEN data (March 2011 to May 2013). b) GDDs for years beginning in June 2005 to May 2013 at Beresford Dale (red) and Dovedale (black) assuming minimum growth threshold for male *E. danica* (2.6 °C).
Figure 4 Estimated annual mean temperatures at D16 (Beresford) based on 1000 replicates of one sample per month drawn from 15-minute LUTEN data for the hours 08:00 to 18:00. The overall LUTEN mean is shown by the vertical line. Shading denotes standard deviations from the mean.
Figure 5 Number and size of mayflies caught in Beresford Dale in a) April 2007 and b) each April from 2010 to 2013.
**Figure 6a)** Annual GDDs for growth threshold 2.6 °C (red line) and number of small mayfly (<7 mm) sampled each year (purple line) in Beresford Dale. **b)** Average length of female (left) and male (right) *E. danica* with 95% confidence intervals sampled in Beresford Dale (blue) and Dovedale (green). The red line indicates GDDs at Beresford for growth threshold of 2.6 °C. All years were statistically distinct for females.
Figure 7 Number and length of *E. danica* caught in Beresford Dale (left) and Dovedale (right) in 2012 and 2013.