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Variations in fecundity over catchment scales: implications for caddisfly populations spanning a thermal gradient

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Summary

1. Population sizes of stream insects depend on the number of eggs that enter the benthos. Densities of oviposited eggs have been linked to densities of oviposition habitat, but variability in fecundity has not been explored. Fecundity may differ between sites where females experience different thermal environments during development, because insects generally grow larger and produce more eggs in cold places (e.g. high elevation and latitude). We predicted that because the fecundity of emerging females should increase with elevation then the numbers of eggs in oviposited egg masses (clutch sizes) should also increase with elevation. Alternatively, individuals may routinely disperse along channels or between rivers, mixing individuals that have grown and developed in different thermal environments, in which case clutch sizes may not vary with elevation but could vary greatly within sites.
2. We tested these hypotheses across an elevation gradient of 250 – 1080 mASL, in seven streams in southeast Australia. We surveyed the fecundity of three species of caddisflies (Trichoptera: Hydrobiosidae), in 2009 (7 sites) and 2010 (17 sites). Female hydrobiosids attach their entire clutch of eggs to an emergent stream boulder, in a single egg mass. At each site we censused the number of egg masses in two random 50 m stretches, and photographed a sample of egg masses. Clutch sizes were quantified by counting eggs from the photographs. From numbers of egg masses and mean clutch size per site, we estimated the number of individual eggs per site, to determine whether spatial differences in clutch size affect patterns of egg supply. Potential trade-offs between egg number and size were also examined.

3. Strong thermal gradients were observed in both years. Sites at lowest elevations were 4.8 °C (2009) and 3.6 °C (2010) warmer than sites at highest elevations, and accumulated 392 (2009) and 361 (2010) more degree days (> 0°C) over a period of 88 days. Clutch sizes of each species varied markedly within and between species but did not differ along the thermal gradient. Within species, 83.7 - 93.7 % of variation in clutch size was expressed within sites. Relatively little variation occurred between sites or rivers. Within species, counts of egg masses adequately described relative egg supply to each site, because clutch sizes were similar between sites and rivers. Comparing egg supply between species, however, required including clutch sizes to estimate supply. Egg size differed within but not between species. Significant but small variations in egg size were trivial compared to much larger variations in clutch size.
4. Our results suggest that any spatial differences in fecundity caused by temperature may be erased by dispersal, such that females from multiple locations mix between sites prior to oviposition. A warming global climate may reduce the fecundity of ectotherms like aquatic insects but consequences for populations are difficult to predict. Moreover, climate-related effects on fecundity may not be apparent when dispersal by individuals between elevations mixes any effects of climate change on fecundity.

Introduction

"Supply-side" ecology identifies that, for species with spatially patchy populations connected by dispersal, recruitment numbers via birth and immigration can have lasting effects on the size and spatial distributions of populations (Caley, Carr, Hixon *et al.*, 1996, Connell, 1985, Hughes, Baird, Dinsdale *et al.*, 2000). The relevance of supply-side ecology to freshwater species was identified two decades ago (e.g. Downes and Keough, 1998, Palmer, Allan and Butman, 1996) and subsequent studies have demonstrated that variation in egg supply (oviposition success) can determine the population sizes and spatial distributions of stream insects in the benthos. For some mayflies and caddisflies, the number and spatial distribution of oviposited egg masses is influenced strongly by the amount and spatial distribution of oviposition habitat (Alp, Indermaur and Robinson, 2013, Encalada and Peckarsky, 2012, Lancaster and Downes, 2014, Lancaster, Downes and Arnold, 2010, Lancaster, Downes and Arnold, 2011, Macqueen and Downes, 2015, Peckarsky, Taylor and Caudill, 2000). Numbers

or densities of even mid- and late-instar larvae can be explained by the distribution of oviposition habitat for some species (Encalada and Peckarsky, 2012, Lancaster and Downes, 2014, Lancaster *et al.*, 2011), suggesting that factors affecting oviposition may have a greater effect on spatial distribution patterns than post-recruitment factors such as competition, predation and disturbance (Encalada and Peckarsky, 2011).

Spatial variation in population sizes could also arise from differences in the numbers of eggs produced by females (fertility) or ultimately laid by females ('clutch size' – a measure of fecundity). This hypothesis has not yet been tested for aquatic insects, but is plausible because variations in fecundity within species can be large. For example, females of the mayfly *Siphlonurus aestivalis* (Siphlonuridae) may produce from 933 to 2678 eggs (Degrange, 1960). The baetid *Baetis rhodani* has an even larger range in fecundity, producing from 200 to 4500 eggs per female (Benech, 1972). Fecundity ranges are less extreme for caddisflies, but can still be large. *Oecetis lacustris* (Leptoceriedae) produces some 35 to 60 eggs per female, a twofold difference, and *Notidobia ciliaris* (Sericostomatidae) produces 92 to 323 eggs Hanna (1961). If fecundity differences of this magnitude are expressed between sites, rivers or catchments, they have the potential to cause large spatial differences in the recruitment of benthic eggs and larvae, with knock-on effects for population sizes. The purpose of this study is to test whether such spatial differences in fecundity occur.

What might drive spatial variations in clutch size within species? Fecundity varies strongly with body size in insects (Honek, 1993, Roff, 2001). In turn, insect body-size (and therefore fecundity) is influenced strongly by both nutritional uptake and the thermal environment during development. Relationships between nutrition and clutch size can nevertheless be complex. The quantity and quality of nutrition, and the timing of nutritional uptake can all affect growth and egg production (Hinton, 1981, Wheeler, 1996). Further, when nutrition is in short supply there may be trade-offs in allocation between abdominal (reproductive) tissues and soma (thoracic tissues and wings, affecting longevity and dispersal capacity). Such tradeoffs are likely to maintain fecundity in species with a short lived adult phase (e.g. *Odontocerum albicorne*, and most other aquatic insects), but could favour longevity at the expense of fecundity for species with long lived adults (e.g. *Glyphotaelius pellucidus*) (Stevens, Hansell, Freel *et al.*, 1999, Stevens, Hansell and Monaghan, 2000).

On the other hand, relationships between temperature and clutch size are straightforward. Body size in ectotherms generally increases at cooler temperatures (the Temperature-Size Rule (TSR)) because slowed rates of development allow individuals more time to feed and grow before reaching maturity (Arendt, 2011, Atkinson, 1994). Therefore, thermal gradients may cause differences in fecundity across spatial scales (elevation, latitude) and temporal cycles (seasons) (Sweeney and Vannote, 1978). As temperature falls below a thermal optimum, the very coldest parts of a species' range may produce smaller individuals with lower fecundity (Hinton, 1981, Sweeney & Vannote, 1978), but such cold environments typically occur near the lower limit of the species thermal range and negative relations between temperature and fecundity and body size/fecundity are seldom observed. Indeed, a review by Atkinson (1995) upheld the TSR for 22 of 26 species of freshwater insects. The remaining four species showed the reverse pattern so that in every case the temperature environment during development had strong effects on physiology of aquatic insects. This may offer a parsimonious explanation for the large variability in the fecundity of aquatic insects.

Upland streams provide an ideal system to test temperature/fecundity relationships because they flow across thermal gradients from high (cold) to low (warm) elevations, allowing sites with a wide range of temperatures to be sampled within an area that is sufficiently small to fall within the geographical range of target species. Nonetheless, if thermal gradients generate predictable differences in fecundity, the spatial scale at which these differences can be detected after ovioposition (i.e. clutch size) depends on whether individuals disperse frequently between sites at high and low elevations. If there is very little dispersal, then average clutch sizes should vary systematically with elevation, but if dispersal is frequent and widespread, then any effects of temperature will be well-mixed along elevational gradients. Although genetic similarities between stream channels suggest some overland dispersal by aquatic insects (Hughes, 2007), little is known about dispersal frequencies and hence the spatial scales over which populations of aquatic insects extend, e.g. whether populations encompass whole catchments or are restricted to individual channels, etc. (Downes and Reich, 2008).

In this study we tested whether the clutch sizes of egg masses laid by stream insects differ across temperature (elevation) gradients. We compared clutch sizes within and between three species of caddisflies (Family: Hydrobiosidae) across elevation/temperature gradients in

seven streams. Between species, differences in mean clutch sizes could not be predicted *a-priori* because females of the study species grow to a similar average size, and it is unknown whether other factors (e.g. tradeoffs between egg number vs egg size or fecundity vs soma) affect the clutch sizes of these species. Within species, we predicted strong differences in clutch sizes between sites at different elevations, with larger clutches in cold compared with warm locations (H_{ii} Table 1). If correct, this finding further implies that individuals do not disperse far from natal sites - i.e. individuals hatch, develop and emerge as adults having experienced a relatively consistent thermal regime. Alternatively, if clutch sizes vary between rivers, but not within rivers, this suggests that individuals disperse frequently along channels (hence temperature-related effects of elevation on individuals are mixed) and that rivers vary in factors affecting fecundity (H_i Table 1). Finally, if caddisflies disperse widely, then any effects of nutrition and temperature will be well-mixed at all spatial scales. Most variation will be between egg masses at individual sites (H_{iii} Table 1).

The numbers of eggs laid at sites (and therefore prospective larval recruitment) is a function of the number of oviposited egg masses coupled with clutch sizes. We compared patterns in the supply of egg masses vs individual eggs, to determine whether clutch size differences affect patterns of recruitment between species, or between sites (within species). Finally, we compare egg sizes between females and species to determine whether trade-offs occur between egg size vs egg number that might further affect the spatial supply and fitness of recruits.

Methods

Our primary hypothesis was that spatial differences in fecundity, across a temperature gradient, cause spatial differences in the supply of eggs. To test this hypothesis required a series of analyses conducted in the following logical sequence: (1) establish the presence and magnitude of the elevation/temperature gradient; (2) identify any systematic differences in clutch sizes, across the temperature gradient and at scales within and between sites, rivers, years and species; (3) determine whether spatial patterns in clutch sizes affect spatial patterns in the supply of individual eggs (a test of the primary hypothesis); (4) determine whether potential tradeoffs between egg size vs clutch size influence the spatial distribution of eggs. Data were collected and analysed in this order, and our Methods and Results are presented in this order.

Study taxa

We surveyed the clutch sizes of three species of caddisflies *Ulmerochorema rubiconum*, *Apsilochorema gisbum* and *Apsilochorema obliquum* (family: Hydrobiosidae). Egg masses of these species are described by Reich (2004) and Lancaster and Glaister (2018). All three species oviposit exclusively on 'emergent rocks' (rocks that protrude from the water) making it easy to locate egg masses (Reich, 2002). Each female lays her entire clutch of eggs in a single egg mass and, in the absence of hydraulic disturbance, the eggs of these species hatch with near 100% success in the field (Bovill, Downes and Lancaster, 2013, Bovill, Downes and Lancaster, 2015, Reich, 2004), meaning that estimates of egg numbers likely represent recruitment numbers of neonate larvae that enter the benthos. Based on measures of forewing length (a surrogate for body size), average body size of females is similar for all species (*A. obliquum* 8 – 9.5 mm; *A. gisbum* 8.5 – 10 mm; *U. rubiconum* 8 – 10 mm, Neboiss, 1986).

Study sites and the elevation/temperature gradient

Clutch sizes and egg mass abundance were surveyed in seven streams of the Goulburn River catchment (southeast Victoria, Australia), at eight sites in 2009 and 17 sites in 2010 (total 18 sites, Figure 1). Each site comprised a randomly located 250 m stretch of river, of which two random 50 m stretches were surveyed for egg mass abundance and clutch size. All rivers occur within a narrow latitudinal range, are fed by rainwater runoff, and were sampled during summer base-flow periods. Therefore, we did not expect any systematic differences in the temperature profiles of rivers. Sites were restricted to upland reaches surrounded by native forest (predominantly wet sclerophyll forest), and ranged in elevation from 250 - 1080 mASL. Although these elevations are low on a global scale, they are typical in Australia, which lacks many very tall mountains. Sites were not available at higher elevations within the study region.

Temperature loggers (HOBO Pendant® Temperature/Light Data Logger, UA-002-64, Onset Computer Corporation, Massachusetts, USA) were deployed at seven sites (250 – 1080 mASL) in 2009 and nine sites (250 – 775 mASL) in 2010 to measure water temperatures across the elevation gradient (Figure 1). Some loggers were relocated between years, so that temperature data was collected from 12 of the 18 sites. Water temperature (°C) was logged at 30-minute intervals, from September 2008 to April 2009 and from January to June in 2010. These periods capture the peak summer oviposition period for our study species (January to

March) but may not describe the temperatures experienced by females during larval growth. Nevertheless, the temperature data demonstrate consistent rank differences in water temperature between sites along the elevation gradient in both years (see Results).

Measuring temperature in Degree Days, implications for growth and clutch size

Insects are ectothermic and individuals must accumulate a certain amount of heat (constant within species) to complete the life cycle or part thereof. The species-specific amount of accumulated heat required to complete the life cycle is termed the 'thermal constant', and can be expressed in accumulated degree-days (Lancaster and Downes, 2013). Degree days (DD) are calculated by summing mean daily temperatures over a period of time, and the period of time may vary depending on ambient temperatures (for example, a thermal constant of 100 DD may be accumulated in 20 days at 5ºC, or 10 days at 10ºC). within species, individuals in warm places accumulate heat quickly and reach the thermal constant quickly, so have less time to grow and mature at smaller sizes than individuals in cold places.

To compare the rates at which sites at high vs low elevations accumulated heat, we calculated degree-days by summing mean daily temperatures (> 0º C) across an 88-day period of overlapping temperature records (January 22 – April 19 of 2009 and 2010). We also calculated the number of days required for sites to accumulate 1000 DD. Values of the thermal constant are unknown for our species, but 1000 DD is likely to represent a significant portion of the heat required to complete development (see Discussion).

Survey protocol

At each site (250 m long), egg masses were surveyed by inspecting all manipulable emergent rocks within the two random 50 m reaches. Only emergent rocks larger than 5 cm b-axis (the shortest axis of the maximum projection plane, Gordon, McMahon, Finlayson *et al.*, 2004) were surveyed, because hydrobiosids rarely oviposit on smaller rocks in our study system (Reich and Downes, 2003). In 2010 the total numbers of egg masses were censused at each site and these data combined with clutch sizes to estimate numbers of individual eggs per site (method below).

It is not feasible to count clutch sizes *in situ*, so egg masses were photographed for later clutch size analysis using the 'super-macro' setting of a 3 megapixel waterproof digital camera (Olympus µ720SW). Sample sizes varied with local species abundances, but typically

a minimum of 10 egg masses of each target species (mean 27 egg masses per species) were photographed per site. Some species were rare (< 10 egg masses) at particular sites, but excluding these data from analyses did not affect the outcomes of statistical tests, so we used data from all sites.

Incomplete egg masses

Variations in clutch sizes that are related to fecundity can arise if females do not successfully lay all of their eggs in one mass, or if some eggs are eaten prior to being surveyed. The hydroptilid *Orthotrichia armata* is an egg predator in this system, but seldom eats the eggs of our study species (Bovill *et al.*, 2015). We did observe some incomplete egg masses suggesting that females were sometimes interrupted during oviposition, perhaps being preyed upon or dislodged by flow, but such egg masses are uncommon and visually distinct and were not included in our clutch size analysis.

Estimating clutch size

Photographs of egg masses were analysed for clutch size using one of two methods: (1) For high quality images (good colour contrast between eggs and background), clutch size was estimated using the 'Analyze Particles' function in ImageJ image processing and analysis software (National Institutes of Health Research Services Branch, U.S. Department of Health and Human Services). Particle analysis identifies and counts foreground objects (in this case, eggs), against a contrasting background. (2) For poorer quality images (low colour contrast) clutch size was counted manually. To verify the accuracy of automated estimates, the clutch sizes of 24 egg masses (*A. gisbum* and *A. obliquum*) were estimated using both methods, and the estimates compared by linear regression. Automated clutch size estimates agreed strongly with manual counts, with a slope approximating 1 ($y = 0.986 \times x + 3.704$; $F_{1, 23} = 1072$, $P = < 0.001$, $R^2 = 0.979$).

Estimating numbers of eggs per site

Numbers of individual eggs were estimated for each species in 2010 only, because densities of egg masses were not estimated in 2009. For each species in 2010, numbers of individual eggs were estimated by multiplying the number of egg masses at a site by the mean clutch size at that site. Clutch size data were not available for five species × site combinations so we substituted the mean clutch size of egg masses from a nearby site in the same river. Because

clutch sizes of conspecifics did not vary within rivers (see Results), substituting clutch size values from adjacent sites is unlikely to affect our estimates of egg numbers.

Egg size

Reproductive fitness may be expressed in the size instead of (or as well as) the number of eggs. To determine whether differences in clutch sizes may be offset by differences in egg size, we compared the sizes of individual eggs within and between species. Egg size was measured for 70 eggs of each species (10 eggs per mass, 7 egg masses per species). Egg masses were collected during clutch size surveys in 2010, and supplemented by samples collected from the Little R. and Taggerty R. in March 2016. Egg shape is tri-axial ellipsoid. We measured the three perpendicular axes of symmetry (a, b and c axes) to the nearest 0.01 mm with an eyepiece graticule at 100× magnification (Olympus BX40 compound microscope) and calculated egg size as the volume of an ellipsoid. Only 'eyed' eggs were measured to ensure that eggs were measured in a comparable state of development. Eyed eggs contain embryos with eyespots on the head capsule, indicating an advanced stage of development, and were larger and shaped differently (laterally compressed ellipsoid) compared to newly laid eggs (ellipsoid).

Statistical analyses

1. Temperature gradient and relation to clutch size: Temperature vs elevation relationships were examined with linear regression. Because temperature was strongly described by elevation (see Results) we used elevation as a temperature proxy in regressions of elevation (temperature) vs clutch size for each species. Although we predicted negative relationships between temperature and clutch sizes, linear, positive and parabolic relationships are possible if the range of sites surveyed includes very cold places (Hinton, 1981). Therefore, we used linear and quadratic regressions to test for a range of relationships.

2. Spatial differences in clutch sizes: To test for differences in clutch sizes between rivers and sites, for each species in each year, we used 2-factor nested ANOVA with sites nested within rivers. Rivers with data from fewer than two sites were excluded from this analysis. Pairwise comparisons (post-hoc Tukey tests, $P < 0.05$) identified rivers where mean clutch sizes differed statistically. For each species we also report the percentage of variation in clutch sizes that accounted for differences between rivers, sites, and within-sites. These values are effect sizes and help with interpretation of significant results.

335
336 3. Differences in clutch sizes between species: We tested for differences between species
337 using a two-way ANOVA, with 'species' and 'site' as fixed factors. This analysis was
338 conducted using data from two sites at similar elevation (Little River, 325 mASL; Snobs
339 Creek, 250 mASL), to eliminate any potential confounding effects caused by site differences
340 in temperature.
341
342 4. Spatial patterns in egg supply and egg size: To determine whether site differences in clutch
343 size affect spatial patterns of egg supply, we compared numbers of egg masses vs individual
344 eggs per site using linear regression. Egg size (mm^3) was compared between species and
345 between egg masses (nested within species) with a 2-factor nested ANOVA.
346
347
348 **Results**
349 *Temperature gradients*
350 Mean temperatures per site related negatively with elevation in both years (2009: $F_{1, 6}$ =
351 42.67, P = < 0.001, R^2 = 0.895; 2010: $F_{1, 8}$ = 45.00, P = < 0.001, R^2 = 0.865) and average
352 temperatures differed between high and low elevations by 4.8 °C in 2009 and 3.6 °C in 2010.
353 Mean daily water temperatures showed similar temporal fluctuations at high and low
354 elevation sites, but sites at high elevations were consistently cooler than sites at low
355 elevations (Figure 2). Linear regression confirmed strong negative relationships between
356 elevation and measures of water temperature relevant to larval development (DD > 0ºC)
357 (Figure 3a). Sites at lowest elevations accumulated up to 392 (2009) and 361 (2010) DD
358 more than sites at highest elevations in just 88 days, and accumulated 1000 DD much faster
359 than sites at highest elevations (39 days faster in 2009; 25 days faster in 2010) (Figure 3b).
360
361 *Spatial variations in clutch size*
362 Collectively, the results reported below are consistent with patterns predicted by H_{iii} (Table
363 1). For each species, there were either no or weak differences in clutch size between sites and
364 rivers, and large differences between egg masses laid at the same site. Thus, despite strong
365 effects of elevation on temperature, linear and quadratic regression revealed no significant
366 relationship between elevation and clutch-size for any species in either year (Figure 4).
367

Most of the variation in clutch sizes (83.7 – 98.5 %) occurred within sites for all species (residual error terms, Table 2). We cannot separate clutch size differences from actual errors (e.g. mistakes in counting clutch sizes) captured by the error term, but counting eggs is straightforward and miscounts are likely to account for only a small minority of variation. Further, for each species the variation (CV) in clutch sizes within sites was similar to variation across all sites pooled (site values withheld for brevity, see Table 3 for pooled values).

Clutch sizes were not statistically different between sites within rivers except in one instance, and partitioning of variance components likewise suggested weak effects in contrast with the strong differences expected under H_{ii} (Table 2). Although there was a marginally significant effect of Site on the clutch size of *A. gisbum* in 2009, this accounted for only 7.3 % of variation in clutch size, and a Tukey test failed to locate any significant pairwise differences between sites.

Between rivers, clutch sizes differed significantly for both species of *Apsilochorema*, but not for *U. rubiconum* (Table 2). Mean clutch sizes differed between rivers by up to 57 eggs per mass for *A. gisbum* in 2010, and by up to 59 and 70 eggs per mass for *A. obliquum* in 2009 and 2010, respectively. Clutch sizes were largest on Taggerty River and smallest on Jerusalem Creek for both species of *Apsilochorema* in 2010 (Table 2) and these differences correspond with temperature differences between streams. Average temperatures were warmer at a temperature-logging site in Jerusalem Creek (350 mASL, mean temperature 13.7 ºC, 1840 accumulated DD in 88 days) than at a temperature logging site at similar elevation on the Taggerty River (375 mASL, mean temperature 12.7 ºC, 1734 Accumulated DD in 88 days).

Differences in clutch size between species

Clutch sizes did not differ between species at similar elevations (2009: $F_{1, 124} = 2.17$, $P = 0.143$; 2010: $F_{1, 254} = 1.15$, $P = 0.284$) and there were no significant species × site interactions (2009: $F_{2, 124} = 0.43$, $P = 0.651$; 2010: $F_{2, 254} = 2.53$, $P = 0.082$), meaning that clutch size data could be pooled across sites within species to compare clutch sizes between species. Clutch sizes differed between species in both years (2009: $F_{2, 124} = 104$, $P = < 0.001$; 2010: $F_{2, 254} = 218$, $P = < 0.001$). The largest clutches were observed for *A. obliquum*, which

consistently had clutch sizes about twice as large as the clutches of *A. gisbum* and *U. rubiconum* (Table 3).

Supply of egg masses and individual eggs to sites

Because clutch size did not differ between sites (Table 2), numbers of egg masses strongly described the number of individual eggs per site, for all species (linear regression: *U. rubiconum*: $F_{1, 16} = 2452.9$, $P = < 0.001$, $R^2 = 0.994$; *A. gisbum*: $F_{1, 16} = 240.9$, $P = < 0.001$, $R^2 = 0.941$; *A. obliquum*: $F_{1, 16} = 1027.9$, $P = < 0.001$, $R^2 = 0.986$). Densities of egg masses are, therefore, a good proxy for estimating the numbers of individual eggs and relative rates of recruitment per site for each species.

Despite having a relatively small clutch size, recruitment of *U. rubiconum* eggs was higher than for both *Apsilochorema* congeners because *U. rubiconum* laid such high densities of egg masses (Table 4). However, recruitment comparisons between *Apsilochorema* congeners must incorporate both egg mass density and clutch size. Both species laid similar densities of egg masses, but recruitment of eggs was twice as high for *A. obliquum* (Table 4) due to the larger clutch sizes of this species (Table 3).

Egg size

Mean sizes of eggs (mm^3) did not differ between species ($F_{2, 18} = 2.941$, $P = 0.078$) but differed between egg masses produced by females of the same species ($F_{18, 189} = 0.001$, $P = < 0.001$). Differences in egg size were small compared with the large intraspecific differences in clutch sizes (Table 3) and are not consistent with tradeoff strategies between egg size and number.

Discussion

Warmer locations should produce insects with lower fecundity and, if insects do not disperse far from natal sites, then there should be strong differences in the average clutch sizes of egg masses between sites at different elevations. We tested this hypothesis for three species of caddisfly by surveying fecundity across a thermal/elevation gradient in streams. Sites at high elevations were colder and accumulated Degree Days (DD) more slowly than sites at low elevations, however, we detected no difference in mean clutch sizes for any species across the temperature gradient. Instead, most of the variation in clutch size occurred within sites, with

436 less variation between rivers and years. This pattern is consistent with our dispersal
437 hypothesis (H_{iii}, Table 1), in which females grow to different sizes at different (e.g. warm vs
438 cold) sites, but movements by larvae or adults prior to oviposition erase any spatial patterns
439 in fecundity caused by environmental conditions during development. Such a pattern has
440 precedent: clutch sizes for the mayfly *Baetis bicaudatus* did not correspond to the body sizes
441 of pre-emergent larvae (a surrogate for adult size) at seven of nine sites, suggesting that
442 oviposition at these sites was dominated by migrant females (Peckarsky *et al.*, 2000). For *A.*
443 *gisbum* and *A. obliquum* our data suggest that dispersal may be strongest along rather than
444 between river corridors, because significant clutch size differences were detected between
445 rivers for these species. Clutch size differences between rivers were consistent with
446 temperature differences between rivers, suggesting that temperature effects on clutch sizes
447 may be detectable at scales where dispersal connections break down. Our replication is too
448 low, however, to draw such conclusions definitively.
449
450 An alternative explanation for the pattern we observed is that temperature has no effect on
451 growth or fecundity of hydrobiosid caddisflies, but this contradicts a wealth of knowledge of
452 insect development. The DD differences among our sites should be adequate to create large
453 differences in body sizes and fecundity. Few studies describe thermal constants for
454 caddisflies, but the scraper *Glossosoma nigrior* (Glossosomatidae) requires approximately
455 1500 DD to complete development (Georgian and Wallace, 1983, Jin and Ward, 2007), and
456 the predator *Himalopsyche japonica* (Rhyacophilidae) may require some 1900 DD
457 (Tsuruishi, 2006). If thermal constants for Hydrobiosidae are within a similar range, the large
458 differences in physiological temperature (DD) across our thermal gradient are likely to cause
459 large differences in development rates and body size between sites at high vs low elevations.
460 Indeed, sites at high elevations required about one month longer to accumulate 1000 DD than
461 sites at low elevations, suggesting that larvae at cold, high elevation sites may take up to
462 several months longer to reach maturity, providing more time to feed and grow.
463
464 *Clutch size and the fitness of individuals*
465 Because egg size varied little within species, variations in female reproductive fitness were
466 expressed primarily by the number and not the size of eggs produced. This may be a common
467 pattern for many aquatic insects, given that clutch sizes were more variable than egg sizes for
468 29 out of 33 (88 %) species of caddisflies and mayflies in studies from Europe, North
469 America, Australia and the British Isles (Benech, 1972, Degrange, 1960, Hanna, 1961,

Peckarsky *et al.*, 1993, Reich, 2004). Intriguingly, because egg sizes did not vary between our species, the larger clutches of *A. obliquum* must contain approximately twice the volume of eggs as *A. gisbum* and *U. rubiconum*. However, available measures of body size (see Methods) suggest no commensurate increase in the size of *A. obliquum* females to accommodate such a difference in egg numbers. It is possible that a larger range of body sizes (or forewing lengths) is required to detect between-species patterns in clutch size and body size (Lancaster and Glaister, 2018).

Large differences in clutch sizes have obvious implications for the reproductive fitness of individual adults. Large clutches confer high reproductive output, but may also benefit egg survival and hatching rates. For example, Bovill *et al.* (2015) observed that egg predators (*Orthotrichia armata*, Hydroptilidae) were sated or unable to consume all eggs from large masses prior to hatching, whereas small egg masses were sometimes consumed entirely. Clutch sizes might also affect the survival of neonates, and these effects may be positive or negative. Benefits may include reduced predation (dilution effects) when neonates hatch synchronously from large egg masses (Reich and Downes, 2004), or even cooperation with kin. For example, hatchlings of the caddisfly *Plectrocnemia conspersa* (Polycentropodidae) cooperate to provide shelter and obtain a first meal (Hildrew and Wagner, 1992). On the other hand, neonates hatching from large egg masses may face density-dependent competition or even cannibalism (Willis and Hendricks, 1992), and may suffer increased mortality or be forced to disperse from natal sites. These types of interactions are known for many terrestrial insects, but a dearth of research on the early life stages of aquatic insects means that we can only speculate about the likelihood that such interactions occur, and the potential knock-on effects for recruitment densities and distributions of benthic larvae.

Quantifying recruitment of eggs and larvae

A common way to estimate recruitment of aquatic insects is to count the number of egg masses laid at sites. This approach may be valid when comparing species that lay very different numbers of egg masses (e.g. *U. rubiconum* vs *Apsilochorema* species), or when comparing conspecifics with clutch sizes that do not vary much between sites. In these two situations, any variation in clutch sizes are unlikely to offset the large differences in prospective recruitment created by differences in egg mass numbers. However, when species lay similar numbers of egg masses per site, clutch size variation becomes important. Thus,

503 the larger clutch sizes of *A. obliquum* compared to *A. gisbum* meant that, on average, *A.*
504 *obliquum* laid about twice as many eggs per site as its congener.
505
506 Whether differences in densities of eggs have implications for population densities depends
507 on larval mortality rates. Densities of eggs (m^{-2}) were high compared with densities of 1st
508 instar larvae recorded in the same system at a similar time of year (Reich and Downes, 2004),
509 which suggests larval mortality could be substantial, but these effects depend on whether the
510 mortality is density dependent or not. Density-independent mortality will on average maintain
511 density differences between species set by oviposition, whereas density-dependent mortality
512 can erase such differences. For example, Reich and Downes (2004) observed mean densities
513 of larvae (all instars) across sites in the Acheron River that were higher for *A. obliquum*
514 (mean ± 1 SE: 50 ± 11 m^{-2}) than *U. rubiconum* (18.25 ± 4.75 m^{-2}), but we observed densities
515 of eggs that were an order of magnitude higher for *U. rubiconum* (361 ±131 eggs m^{-2}) than *A.*
516 *obliquum* (20.6 ±4.1 m^{-2}). Of course, there are ~10 years between these different studies, but
517 it is feasible that species with high densities of eggs suffer disproportionately higher larval
518 mortality. Additionally, dispersal from natal sites may create a discrepancy between densities
519 of eggs and larvae within riffles. Reich and Downes (2004) recorded similar densities of
520 larvae in reaches with and without oviposition habitat for *A. obliquum* and *U. rubiconum*,
521 suggesting that some movement or dispersal probably occurs. Disentangling the fate of
522 larvae, particularly early instars is required before we can understand whether different
523 densities of oviposited eggs have persistent effects on population densities (Lancaster and
524 Downes, 2014).
525
526
527 Finally, all temperature-related aspects of development and growth are likely to be affected
528 by global climate change but the considerations above illustrate the difficulties of predicting
529 effects on populations. Thus, while some studies of aquatic insects focus on losses of species
530 (e.g. Giersch, Hotaling, Kovach *et al.*, 2017) or range shifts (e.g. Domisch, Araujo, Bonada *et*
531 *al.*, 2013, Nukazawa, Arai, Kazama *et al.*, 2018), changes to fecundities may have more
532 complex effects on populations. Even a simplistic prediction that population sizes will be
533 reduced by warmer average temperatures that reduce body sizes and hence fecundities
534 depends critically on the fate of larvae. For example, if larval mortality is density-dependent
535 then larval survivorship could increase if egg densities drop, which may mean that population
536 densities are largely unchanged. Another difficult problem is that temperature extremes may

have more critical effects on fecundity than average temperatures (Buckley and Kingsolver, 2012). Moreover, if individuals disperse between high and low elevation locations rather than being restricted to particular elevations, which is consistent with the results of this study, then changes to fecundities will be spread over potentially large geographical areas and difficult to detect. Australia is a particularly flat continent compared to others, and hence temperature effects on fecundity that will be mixed across different temperature regimes may be more relevant in Australia than elsewhere (although see Jourdan, O'Hara, Bottarin *et al.*, 2018). Nevertheless, these results and others that focus on populations (e.g. Bowler, Haase, Hof *et al.,* 2017) emphasize that climate change may act more subtly than range shifts of cold-adapted species.

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Conflict of Interest

The authors declare no conflicts of interest

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Tables

Table 1. Three alternative hypotheses ($H_{i\text{-}iii}$) that predict different spatial patterns in fecundity along with possible underlying mechanisms, and the scale (within sites, between sites within rivers, between rivers) at which fecundity is likely to be most variable. The hypotheses assume that species are at the same latitude and rivers have broadly similar temperature regimes (e.g. do not contrast spring-fed vs snow-melt rivers). H_i and H_{ii}, respectively, predict outcomes for fecundity mediated by nutritional differences, and thermal differences when dispersal is negligible. H_{iii} is the predicted outcome if widespread dispersal between sites, or sites and rivers, mixes females with different fecundities that have developed at different sites.

Hypotheses		Largest variation in clutch sizes
H_i:	**Clutch size differences increase with spatial separation of individuals** Mechanism: River-level differences in temperature, nutrition or other factors that can affect fecundity (e.g. genetics).	Between rivers
H_{ii}:	**Clutch size varies across temperature/elevation gradients** Mechanism: Temperature differences associated with elevation	Between sites within rivers
H_{iii}:	**Dispersal mixes females from different sites and/or rivers.** Mechanism: Temperature and/or nutrition cause variation in clutch sizes. Subsequent dispersal mixes females from different sites and/or rivers at all spatial scales	Within sites

Table 2 Results of two-way nested ANOVA testing for differences in clutch size between rivers and between sites nested within rivers in 2009 and 2010 (significant results in bold). Only rivers with data for ≥ 2 sites were included in this analysis. Results of variance partitioning describe the percentage of variation in clutch sizes explained by rivers, sites, or residual error; the latter term includes real variation between egg masses within sites plus errors of measurement and any other unquantified factors. Post-hoc Tukey comparisons identify rivers with statistically different mean clutch sizes (vertical lines denote homogeneous subsets).

	Tukey comparisons (between rivers)

		df	MS	F	P	Percentage of variation	Rivers (rank order)	Mean Clutch size
A. gisbum								
2009	River	2	13713	0.82	0.514	0.0		
	Site (River)	3	17470	2.74	**0.046**	7.3		
	Error	146	6412			92.7		
2010	River	3	29883	12.8	< **0.001**	16.3	Taggerty R.	253
	Site (River)	8	2161	0.71	0.681	0.0	Snobs Ck.	196
	Error	190	3038			83.7	Acheron R.	194
							Jerusalem Ck.	186
A. obliquum								
2009	River	2	72688	8.37	**0.049**	15.5	Royston R.	480
	Site (River)	3	8743	1.11	0.346	0.4	Taggerty R.	421
	Error	130	7853			84.0	Snobs Ck.	408
2010	River	3	34856	5.02	**0.015**	6.3	Taggerty R.	439
	Site (River)	8	6796	0.90	0.514	0.0	Acheron R.	406
	Error	227	7522			93.7	Snobs Ck.	369
							Jerusalem Ck.	369
U. rubiconum								
2009	River	3	7964	1.99	0.247	3.0		
	Site (River)	4	4138	1.76	0.138	2.8		
	Error	202	2349			94.2		
2010	River	3	7691	2.66	0.095	1.5		
	Site (River)	7	2809	0.89	0.518	0.0		
	Error	422	3173			98.5		

Table 3 Clutch size and egg size (mm^3) statistics for each study species. Data pooled across sites. Sample size *n* represents numbers of egg masses (for estimates of clutch size) and individual eggs (for estimates of egg size).

	Year	*n*	Mean (± SE)	Range	CV
Clutch size					
A. gisbum	2009	180	261 (19)	77 – 587	32
	2010	293	210 (12)	52 – 384	30
A. obliquum	2009	169	437 (34)	200 – 680	21
	2010	337	394 (21)	153 – 636	22
U. rubiconum	2009	210	163 (11)	22 – 321	31
	2010	609	186 (8)	37 – 406	33
Egg size					
A. gisbum	2016	70	0.13 (0.005)	0.10 - 0.16	10.6
A. obliquum	2016	70	0.12 (0.003)	0.11 - 0.14	5.7
U. rubiconum	2016	70	0.12 (0.002)	0.11 - 0.13	4.4

Table 4 Mean (± SE) recruitment densities of egg masses and individual eggs, averaged across all sites pooled.

Species	Egg masses / m^2	Eggs / m^2
A. gisbum	0.06 (0.01)	12 (2)
A. obliquum	0.05 (0.01)	21 (4)
U. rubiconum	1.89 (0.67)	357 (129)

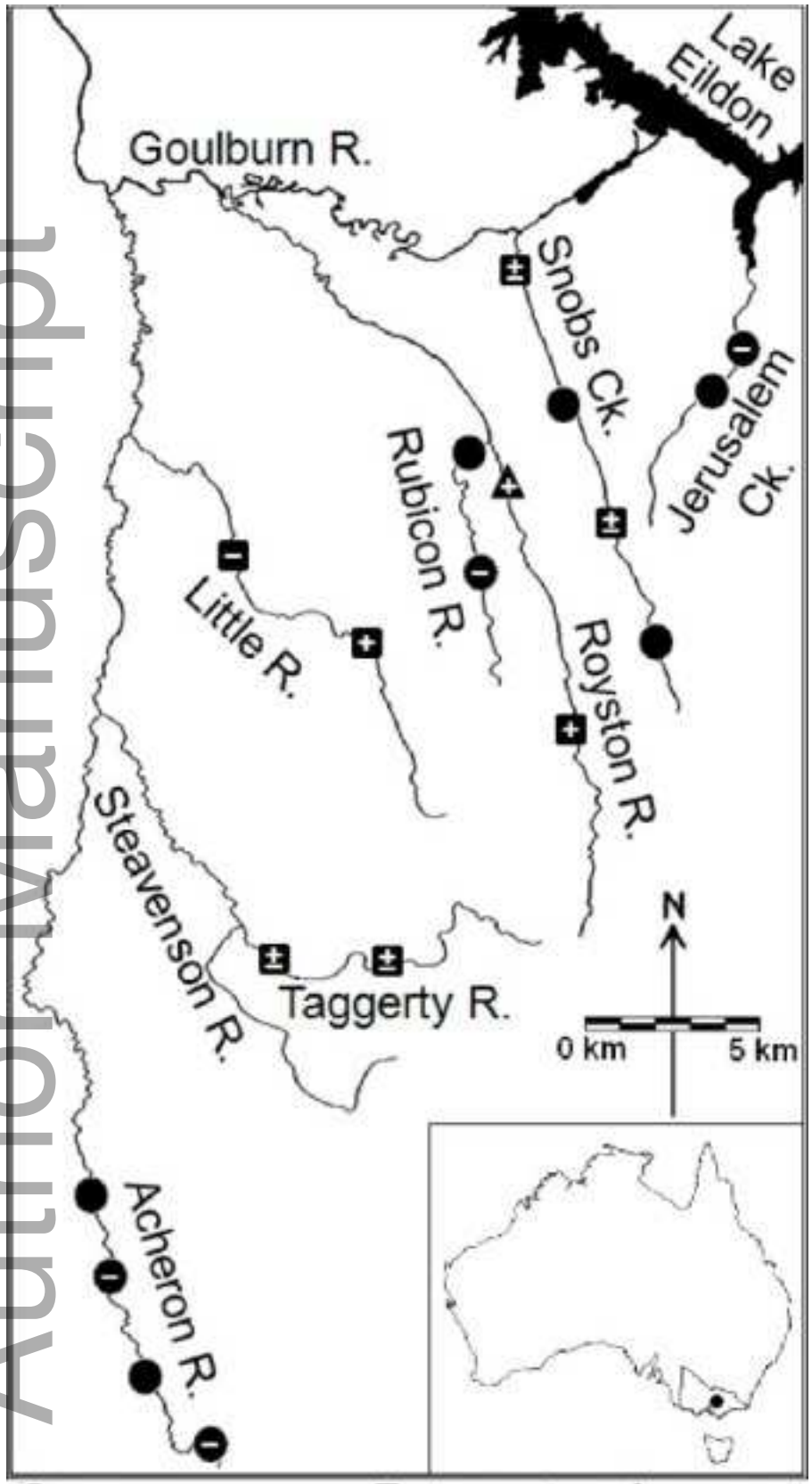
Figure captions

Fig. 1 The seven streams and 18 sites surveyed in the Acheron and Goulburn River catchments in southeast Victoria, Australia. Clutch size was surveyed at eight sites in 2009 and 17 sites in 2010. Temperature was recorded at seven sites in 2009 and nine sites in 2010.

Fig. 2 Mean daily temperatures in (a) 2008-2009 and (b) 2010, showing peak annual temperatures during summer (December – March). For clarity, data are shown only for one low, medium and high elevation site per year, but similar temporal fluctuations in temperature were recorded at all sites and mean site temperatures were ranked by elevation. Dotted lines indicate the overlapping calendar period of temperature records between years (January 22 – April 19, 88 days). We used data from this period to calculate accumulated degree days (Figure 4).

Fig. 3 Summary of temperature data collected during the period January 21-April 19 in 2009 (closed circles, solid lines) and 2010 (open circles, dashed lines). Across an elevation gradient we detected systematic variations in: (a) the number of accumulated DD per site (2009: $F_{1,6}$ = 32.17**, R^2 = 0.865; 2010: $F_{1,8}$ = 38.80***, R^2 = 0.847) and (b) the time (days) required to accumulate 1000 DD (2009: $F_{1,6}$ = 25.05**, R^2 = 0.834; 2010: $F_{1,8}$ = 34.93***, R^2 = 0.833).

Fig. 4 Clutch size (mean ±1 SE) across the elevation gradient for: *A. gisbum* in (a) 2009 and (b) 2010; *A. obliquum* in (c) 2009 and (d) 2010; *U. rubiconum* in (e) 2009 and (f) 2010. Note differences in the scale of the y-axis for each species. *F*-values are provided for linear regression of clutch size vs elevation; 'ns' indicates $P > 0.05$ in all cases.



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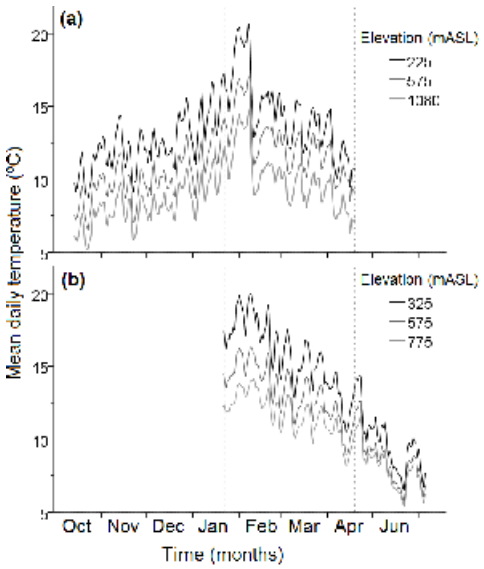

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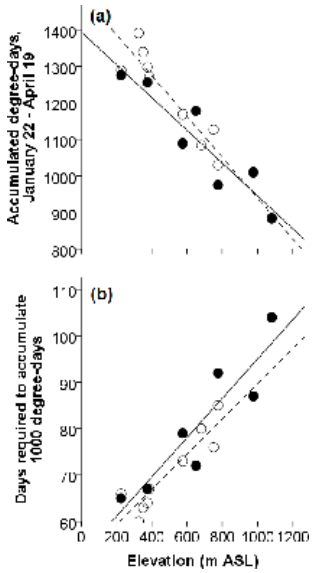

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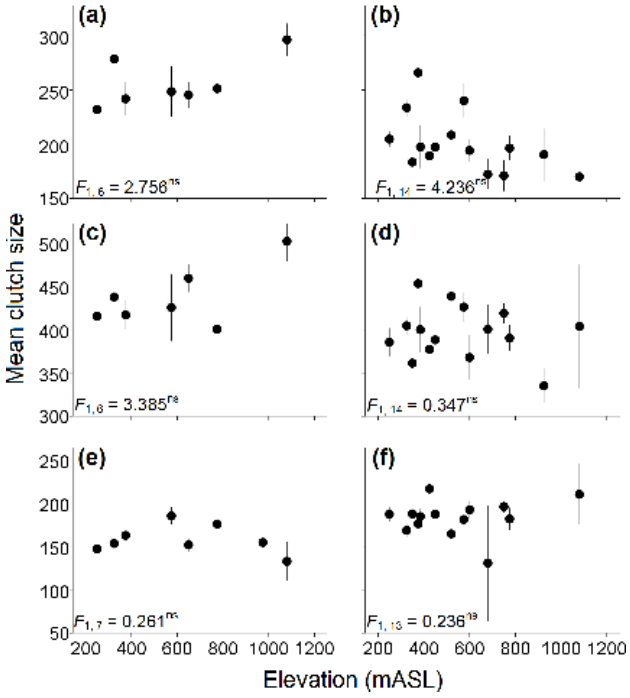

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