Aestivation provides flexible mechanisms for survival of stream drying in a larval trichopteran (Leptoceridae)

S. Wickson[A,C], *E. T. Chester*[B] *and B. J. Robson*[B]

[A]School of Life and Environmental Sciences, Deakin University, PO Box 423, Warrnambool,
  Vic. 3280, Australia.
[B]School of Environmental Sciences, Murdoch University, 90 South Street, Murdoch,
  WA 6150, Australia.
[C]Corresponding author. Email: sjwi@deakin.edu.au

Abstract.   Some freshwater species aestivate to resist drying; however, little is known about factors affecting post-aestivation survival. Climate change prolongs drying and may make short bursts of flow more frequent in southern Australian streams, thereby affecting aestivation success. The tolerance of larval *Lectrides varians* (Mosley) to drying was tested by inducing aestivation in dry or moist sediment and then re-immersing larvae and measuring survival and activity. Survival did not differ between individuals that were continually immersed (78%) or aestivating on moist sediment (70.5%) after 16 weeks. Survival was significantly lower on dry sediment (29.3%). Furthermore, some larvae showed delayed responses to re-immersion; 65% of individuals showed activity within 4.5 h, whereas over 30% of larvae did not become active until 72 h after re-immersion. *L. varians* can survive extended periods (112 days) without surface water, showing a bimodal response to re-immersion that increases the likelihood of population persistence by enabling some larvae to remain aestivating during short-lived bursts of stream flow. *L. varians* populations will therefore be more robust to prolonged stream drying and short-lived flow events than are some other insect taxa, although as the duration of aestivation increases larval survivorship decreases, suggesting that there are limits to the flexibility of aestivation traits.

Introduction

The relationship between environmental constraints and life-history traits has long been studied in ecology (Statzner *et al.* 2001) and has recently achieved greater urgency, with the need to predict the outcome of climate change for species (Steffen *et al.* 2009; Robson *et al.* 2011). Hydrology is fundamental in shaping the ecology of seasonally dry streams (Stanley *et al.* 1997), with the biota displaying behavioural and/or physical adaptations that provide resistance and resilience to their communities (Humphries and Baldwin 2003). In relatively intact ecosystems, natural disturbances are a normal component of the system and some organisms have evolved to survive and even exploit them (Lytle and Poff 2004). Organisms that are exposed to frequent disturbance (such as seasonal drying) may evolve life-history strategies that tend to be synchronised with the long-term average dynamics of a system rather than allowing organisms to survive particular spates or droughts (Masaki 1980; Lytle and Poff 2004; Diaz *et al.* 2008). Migration, use of refuges and dormancy (diapause and aestivation) are strategies commonly used by insects, as well as other taxa to avoid inhospitable environments (Hynes 1970, 1976; Boulton 1989; Lancaster and Hildrew 1993; Robson *et al.*

2011). However, stream flow regimes are changing across southern Australia, with prolonged frequency and duration of droughts and the impact of these changes on freshwater invertebrates will be related to their life-history traits (Chessman 2009; Robson *et al.* 2011). Little is known of the flexibility of drought-survival traits in freshwater invertebrates and, in particular, of whether traits such as the ability to aestivate can adjust to drier conditions.

Several aquatic insect taxa are known to employ summer dormancy (diapause and aestivation) that is synchronised with seasonal drying in streams to avoid desiccation-related mortality (Hynes 1970, 1976). However, little is known about the levels of recovery and tolerance to drying, especially where the timing of drying and/or rewetting is not highly predictable. Whereas diapause is a period of greatly reduced metabolism, aestivation is a period of dormancy entered into during hot or dry periods and is analogous to hibernation. This is generally considered to be a less extreme process than diapause (Allaby 1998). Aestivation is most common in insects during the adult or pupal life stages (Masaki 1980). Dormancy may be for long periods, with butterfly pupae being capable of remaining dormant for up to 3 months (Pörschmann and Speith 2011), and some mosquito

species having adult dormant phases lasting up to 8 months (Adamou *et al.* 2011).

The ability of invertebrates to aestivate often requires particular morphological characteristics, such as having a highly sclerotised cuticle (Masaki 1980), or a shell (e.g. bivalve molluscs) or behaviours, such as the ability to burrow into the hyporheic zone (Stubbington *et al.* 2009). For stream insects, particularly some Trichopteran families, shelter during dormancy may be provided by their external case, constructed from either plant material or sediment particles by using silk (Wiggins 2004). Trichopteran species require particularly long lead times to prepare for drying disturbance as the larvae obtain dissolved oxygen via gills and the aquatic pupal stage can last for several weeks (Wiggins 1973). In spite of this, several Trichopteran families are known to inhabit temporary waters in the northern hemisphere (Wiggins 1973) and adaptations to drying can be seen in all life stages. Summer diapause has been observed in adult caddisflies in the northern hemisphere, which results in delay of the reproductive phase until flows return in autumn or winter (Masaki 1980). Aestivation is used by Trichopteran pupae (Wiggins 1973), adults (Colburn 1984; Salavert *et al.* 2011) and eggs (Towns 1983) to avoid unfavourable conditions during summer. Larvae of some species can leave temporary pools in summer and aestivate in their unsealed cases under leaf litter (Flint 1958) and some leptocerids have been observed within leaf litter in drying streams and under gravel (St Clair 1993). In Australia, previous authors have reported late instar leptocerid larvae alive in dried pools and under rocks on damp substrate (Boulton 1989; St Clair 1993; Chester and Robson 2011). However, the extent and length of drying that these species can tolerate is largely unknown and will be important in determining the species response to climate change.

During the present study, live specimens of late instar *Lectrides varians* (Leptoceridae) larvae were found on dry, sandy river beds under sparse leaf litter at several sites in streams in the Victoria Range in the Grampians National Park (GNP). Some of these sites were known to have been dry for several weeks before the discovery of larvae. This suggested that *L. varians* larvae were adapted to tolerate the seasonal dry period without standing water (at least 12 weeks), allowing them to exploit habitats not available to other taxa and become one of the most abundant macroinvertebrates in these temporary streams (Robson *et al.* 2005, 2011). This abundance and the hydrological history of the streams suggested that individuals might withstand an 'average' summer drying period of ~12 weeks without surface flow, providing that they find shelter under rocks or leaf litter. However, the proportion of individuals able to survive stream drying for extended periods was unknown. The role played by sediment moisture was also unknown, although both are relevant to survival under the prolonged drying expected as climate change progresses. Therefore, we used a laboratory experiment to determine the length of time and degree of drying that aestivating larval *L. varians* could survive in two hydrological regimes observed in the field, namely, damp substrata and completely dry substrata, and compared survival under these regimes with larval survival during continuous inundation (the control). The hypothesis tested was that larval survival rates would differ between each treatment and the control because aestivation

would entail some mortality and dampness would be less physiologically costly than complete dryness, affecting the survival rate.

Materials and methods

Study species

Lectrides varians Mosely is a case-forming caddisfly from the family Leptoceridae. Leptocerid caddisflies are among the most abundant stream macroinvertebrates in the Victoria Range, with the following four species known: *Lectrides varians*, *Triplectides truncatus* Neboiss, *T. proximus* Neboiss and *T. similus* Mosely (Doeg 1996; Robson *et al.* 2005; Chester and Robson 2011). All four species are shredders and are important processors of coarse particulate organic matter. *L. varians* occurs in the widest range of habitats of any Australian leptocerid caddisfly, from lakes and streams, alpine and lowland areas, in warm and cool climates and temporary or permanent waters. *L. varians* is found throughout Australia, from southern Queensland to Tasmania (Towns 1983; St Clair 1994). Aggregations of living *L. varians* larvae were observed in field surveys during February and March (late summer to early autumn) in 2007 in dried pools under stones and leaf litter. These observations were made at six sites across three streams (Hut Creek, Deep Creek and Cultivation Creek). At each site, numbers ranged from two to more than 50 individuals per aggregation. Surveys completed during the present study, and Chester and Robson (2011) and by Doeg (1996) found *L. varians* to be the most widely distributed and populous macroinvertebrate shredder in the Grampians National Park, present in numbers up to an order of magnitude greater than other taxa.

Eggs are laid terrestrially in gelatinous masses on hard substrates, 10–20 cm above the waterline around permanent pools. Oviposition occurs in late spring and early summer, with eggs developing rapidly with no diapause, and hatching 4–8 weeks later (Towns 1983). Larvae develop rapidly, being able to maximise production during seasonal low-flow periods and have been observed to grow to fifth instar from eggs in 135 days after hatching in captivity (Towns 1991). Larvae are shredders of allochthonous detritus and aggregate on leaf-litter accumulations. They are closely associated with detritus in backwater sections of streams and show low mobility (Towns 1983; St Clair 1994). The pupal case is formed by modifying the larval case with an oblique silk closure at the anterior and attaches to a hard substrate (Jackson 1985). Pupae have been observed in the field attached to hard substrate; however, little is known about the ecology of the pupal and adult stages.

Larvae for the experiment were taken from three sites within a single stream, Deep Creek, because this stream contained the high numbers of larvae required. Three hundred late-instar individuals of *L. varians* were collected in early autumn from large pools before the stream had recommenced flow. Larvae were actively moving around and feeding on leaf litter in the pools.

Study sites

Deep Creek is located in the Victoria Range, which is the western-most of a series of mountain ranges running north–south in the Grampians National Park, Victoria, Australia. (N142.258165, E37.278612). The region is classified as

Mediterranean to semiarid (400–600-mm average annual rainfall) and a maximum altitude of 979 m (Chester and Robson 2011). Streams in the region range from flowing permanently, to having seasonal or intermittent flow. The flow regimes of several streams are affected by regulation and water abstraction and all streams had reduced inflows as a result of drought during the study (for further description of Victoria Range streams and their flow regimes see Robson *et al.* 2005, 2008; Chester and Robson 2011).

The upper reaches of Deep Creek feature sandstone boulders and cobbles with areas of bedrock, descending to an alluvial plain where the channel becomes deeply incised and consists of a sandy bed. The channel ranges from 1 to 4 m wide and is less than 2 m deep throughout. Permanent surface water is mostly restricted to the upper sections of the stream with boulder and bedrock substrates, whereas the lower alluvial sections tended to dry out completely in the summer months, with the exception of a few pools, less than a metre deep and 4 m wide. Vegetation is dry sclerophyll woodland and forest, dominated by *Eucalyptus* species. Flow is mostly derived from precipitation, but is supplemented by low-pressure springs. Deep Creek is best described as a seasonal stream that dries to disconnected pools over summer and autumn.

Experimental design

The following three treatments were used to simulate the rate and degree of drying in small sandy pools: saturated substrata exposed to air (moist treatment), completely dry substrata exposed to air (dry treatment) and a control (substrata completely submerged with 5 cm of free water for duration of experiment). Forty-five microcosms consisting of 1-L polyethylene jars, with holes drilled around the base to allow water drainage, were used to hold larvae during the experiment. Each microcosm was filled with 250 mL of washed river sand and 7 g of dried conditioned leaf litter collected from Deep Creek. At the start of the experiment, all 45 microcosms were submerged in 10 cm of water within a single 750-L tank (filled with water from town supply and allowed to stand for 2 weeks before the experiment). Microcosms were kept under ambient conditions (with similar light and temperature regimes to those found in the field) and supplied with conditioned leaf litter from Deep Creek for 14 days to acclimate to laboratory conditions. After 2 weeks of acclimation, each 1-L microcosm received five randomly selected larvae, and then 15 microcosms were randomly allocated to each treatment ($n = 45$).

Water levels in the two drying treatments were reduced at a rate of 8 mm per day for 10 days. This was achieved by gradually raising jars until the surface of the sand was above the water level. Microcosms for the moist treatment were left partially submerged to allow sand to remain moist, but with no water above the sand surface, whereas microcosms for the dry treatment were raised entirely above the water level on Day 10 to allow the sand to dry naturally. Control microcosms remained submerged at all times.

Commencing after the first 10 days of the experiment, three microcosms from each treatment were removed from the tank at each of five time periods (1, 2, 4, 8, 16 weeks) and were not returned to the tank. The size and number of surviving animals was recorded in each microcosm. Therefore, there were three independent replicates for each treatment at each sampling time because each microcosm was sampled only once. Individuals were placed in clean trays with 20 mm of water and observed every 15 min for 8 h, then every 8 h thereafter for the following 7 days. We had no reason to expect that individuals would become active immediately after rewetting, so we observed them over a long period to ensure that we were not overestimating mortality. Individuals were deemed alive after rewetting if any part of the antennae, legs or head could be seen protruding from the case, or if the individual was moving. Alive individuals were counted and removed from the tray at each time period. Animals that could not be found within each microcosm were considered dead. No individuals pupated during the experiment.

Eighteen additional microcosms were used to measure the moisture content of sand in the moist treatment. These additional microcosms were treated exactly the same as those in the dry treatment, except they had no larvae or leaf litter. This prevented disturbing the aestivating larvae to measure the declining moisture content of the dry treatment during the experiment. At each time period, the sand was removed from three randomly selected microcosms, and wet weights were recorded using a Metter BB1200 digital balance (0.01-g precision). Moisture content of the sediment was determined after drying the sand at 100°C for 48 h and determining the dry weight through re-weighing of samples. Microcosms in the dry treatment contained 16% moisture after removal from submersion (Day 1 of the experiment), declining to 10% by Week 2 and less than 1% moisture by Week 4 of the experiment. Moisture content of the moist treatment remained 100% throughout the experiment.

Data analysis

Probit analysis is a regression technique commonly used in toxicity testing to model mortality response over time (Ramsey and Schafer 1997), as the proportion of survivors declines in a population. This analysis (Probit function, SYSTAT version 13; Systat Software Inc., Chicago, IL) was performed to test the null hypothesis that there was no difference in survivorship between treatments, as indicated by the slopes for lines fitted to each treatment type. The probit function $(\mathrm{Probit}(\pi(x)) = a + \beta x$, where $a = Y$ intercept, $\beta =$ slope (estimate) and $x =$ time (weeks) expresses the probability of survival (or mortality) for each treatment at each time period. This, therefore, estimates the likelihood of mortality, and the period for effectively total mortality (%loss) was then able to be estimated for each treatment.

Results

Larval survivorship

Once surface water was lost, all signs of activity (movement, feeding) quickly ceased. Most animals had retreated under leaf litter, whereas in the controls, such activity continued for the duration of the experiment. There was a significant decline (Fig. 1) in larval survival over time, across all treatments ($\beta = -0.137$, $z = -4.682$, $P < 0.001$). There was also sufficient evidence to reject the null hypothesis of no difference in slopes between the moist and dry treatments because there was a significantly lower rate of survivorship in the dry treatment
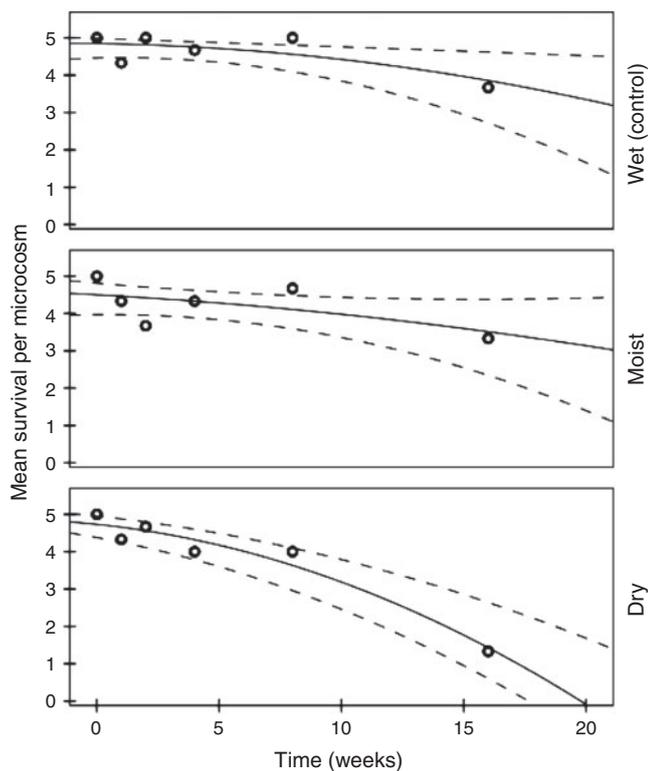
Fig. 1. Survival estimates from Probit regression (3 levels) over the experiment's duration (from 0 to 16 weeks) for each treatment (solid lines), with 95% confidence intervals (dashed lines) based on three replicate microcosms per treatment per time ($n = 54$). Mean counts for microcosms are also plotted (open circles).

Fig. 2. Histogram showing the percentage of surviving *Lectrides varians* larvae that responded to rewetting of sediments from the moist and dry treatment over a period of 3 days.

period, the probability of survival is more than halved for animals in treatments without access to moisture.

Larval response times after re-immersion

L. varians showed a bimodal response to rewetting after aestivation in both the moist and dry treatments (Fig. 2). From the moist treatment, 62% of surviving individuals responded within 3 h of rewetting, with another 31% responding after 72 h. A small percentage (7%) responded between 24 and 50 h. From the dry treatment, 77% of surviving individuals responded within 4 h of rewetting, with another 18% responding after 72 h. A small percentage (5%) responded at 24 h. All animals from the control microcosms were active immediately on removal from microcosms.

Discussion

The experiment showed that ~80% of larval *L. varians* can survive at least 4 months with no surface water, and that many will survive even when the substratum is dry. However, survivorship was reduced over time, particularly on dry sediment. The period of time that *L. varians* individuals were able to withstand drying in the laboratory (16 weeks) is at the longer end of the dry period expected in Grampians streams (Robson *et al.* 2008; Chester and Robson 2011). This confirms previous field observations (St Clair 1994) and shows a life-history adaptation that may allow individuals to survive the entire summer low-flow period without water, contributing to the persistence of *L. varians* in high numbers within Grampians National Park streams during a 10-year drought (Bond *et al.* 2008). This has important ramifications for management because it shows that although this organism is successfully adapted to periods of drying, its chance of survival decreases with time under completely dry conditions.

During the most severe drought, even subsurface water will dry out, leading to a hyporheic drought (Boulton 2003). Although *L. varians* does not enter the hyporheos for refuge from drought, this experiment does suggest that its survival during drought is dependent on the presence of water in the sediment beneath it. This water may be hyporheic or arise from local seeps or springs that are sufficient to keep the sediment damp. Although this caddisfly is adapted to periods of drying, its chance of survival decreases with time under completely dry

(faster rate of loss in latter) only ($\beta = 0.090$, $z = -2.240$, $P = 0.025$; Fig. 1). Therefore, the analysis suggested that a separate model was appropriate for the dry treatment. Otherwise, although the survivorship was lower on moist substrate, the rate of loss was not statistically distinguishable from that of the fully submerged larvae.

While there were mortalities in the control microcosms (estimated 22% loss at 16 weeks); the predicted probit for survival decreased only slowly ($\beta = -0.070$; 95% C.I. -0.134 to -0.007, $z = -2.178$, $P = 0.029$). At this rate, 99% mortality would occur at 60.02 weeks, although this duration is unrealistic because surviving larvae would pupate well before that time. The estimated loss at the end of the experiment for the moist treatment was 29.5%; the slope of the probit for survival was not distinguishable from that of the control (and in fact was not statistically different from zero: $\beta = -0.047$; 95% C.I. -0.101 to 0.007, $z = -1.702$, $P = 0.089$), predicting 99% mortality at 77.17 weeks. This compares with an increased rate of change in survival for animals on dry substrate in microcosms; the slope of the probit function was strongly negative ($\beta = -0.137$; 95% C.I. -0.194 to -0.079, $z = -4.682$, $P < 0.001$), with an estimated loss of 70.7% of individuals by the end of the experiment, and leading to 99% mortality at 29.02 weeks. Such is the difference in rates of change in survival that at 12 weeks, which is equivalent to the average length of a summer dry
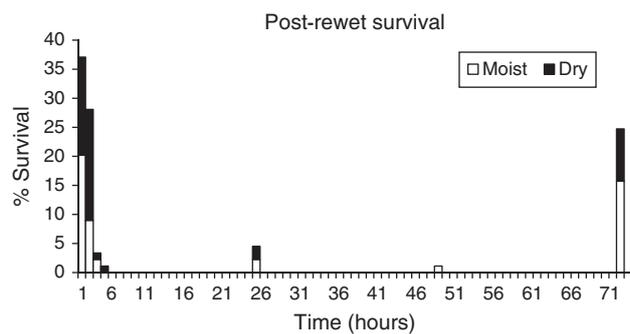
conditions, suggesting that in regulated streams, flow management should avoid creating completely dry sediment in pools. Prolongation of dry periods and increased temperatures, especially over multiple years (as can occur with water extraction), are likely to cause local extinctions of this species in streams that lack perennial pools or reaches with perennial flows. However, it also suggests that flow releases from extraction weirs could be used to maintain damp sediment and assist populations by increasing aestivation survival rates.

The experiment also showed a bimodal response to rewetting in *L. varians*, with 98% of individuals responding either within 5 h of rewetting or at 72 h post-rewetting. Seasonal polymorphism within population cycles is not uncommon and can be used as a survival or 'bet-hedging' strategy in unpredictable environments (Stearns 1976; Southwood 1977; Winterbourn *et al.* 1981). Other taxa such as mayflies, damselflies and moths have been shown to display life-history plasticity when under environmental and biotic stress (Waldbauer and Sternburg 1973; Boulton 2003; De Block *et al.* 2008; Robson *et al.* 2011). Boulton (2003) showed that aquatic habitats may experience several 'false starts' at the end of drying periods, where they become wetted, then dry out again within a few days. False starts can also arise from flow regulation, where the first flows from winter rains flow downstream, but after a few days, weir gates are closed and flows are captured, so that no flows occur downstream of weirs. The bimodal response of *L. varians* may offer some protection from these false starts because there are physiological costs to both entering and leaving aestivation and it is not known whether a larva is capable of entering aestivation more than once in its life. The bimodal response at least allows some of the later 'awakening' group to remain dormant until flows have resumed for a period greater than 72 h.

Diapause by some members of a metapopulation and not others leads to polymodal emergence of adults, increasing the likelihood that some individuals are present during optimum environmental conditions, and providing insurance against unpredictability in fluctuating environments (Masaki 1980; Lytle 2008). Caddisfly populations with multiple cohorts arising from asynchronous life-history development has been shown to be advantageous in regions with unpredictable disturbance (González and Graca 2003). This strategy would allow a population to hedge its bets, with some individuals reacting quickly to wetting and taking advantage of available resources, whereas others remain in stasis, waiting for persistent water. This may be the case in *L. varians*, with the early responders in a state of aestivation and the late responders in a true diapause, although this is unlikely because the individuals were responding to a rapid external stimulus, rather than seasonal cues (Masaki 1980). It is more likely that the first cohort is responding to the initial flush of water and taking advantage of available resources immediately, whereas the second cohort is responding to the longer duration of inundation.

These adaptations for surviving drying will become more important to populations of *L. varians* if water resources are placed under more pressure through increased extraction, increased temperatures and reduced runoff, leading to prolonged dry periods and an increased frequency of 'false-start' flows in streams. The findings from this experiment could be further strengthened by replicating the treatments with

individuals collected from several streams, both intermittent and permanent, and from multiple populations. Whereas the results here show that individuals from the *L. varians* population in Deep Creek can withstand prolonged drought and completely dry substrata, it remains uncertain whether populations from perennial streams have this capacity. As climate change progresses and perennial streams increasingly become intermittent, it will be important to know whether all populations of *L. varians* can survive drying. Although *L. varians* is an abundant species and an important shredder in Victoria Range streams, such information is urgently required for a much wider range of freshwater invertebrates so that their likely response to drier flow regimes can be predicted (Chessman 2009; Robson *et al.* 2011). Chessman (2009) pointed out that species that occur mainly in fast-flowing streams are most likely to be negatively affected by water extraction and increased drying due to climate change. These alterations to flow regimes may significantly alter macroinvertebrate assemblages in intermittent streams, with assemblages becoming less diverse over time (Williams 1996; Lake 2003; Sponseller *et al.* 2010; Chester and Robson 2011), although this is not always the case (Chessman *et al.* 2010). This experiment has showed that at least one population of one species of leptocerid caddisfly has some degree of flexibility in both the duration of dry periods that it can withstand and the proportion of the population that responds to short-lived bursts of streamflow (days). This should confer robustness to increased drying and flashiness in stream-flow regimes in the Victoria Range. It remains to be seen whether this flexibility will be sufficient to ensure survival in the future; however, it shows that human interventions, such as flow releases, could be used to improve aestivation survival rates and support population persistence of this species.

Acknowledgements

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