Life History Adaptations by a Leptocerid Caddisfly in a Drying Environment

Steve Wickson, BEnvSci (Hons)

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Deakin University
February, 2013
I am the author of the thesis entitled

submitted for the degree of

This thesis may be made available for consultation, loan and limited copying in accordance with the Copyright Act 1968.

'I certify that I am the student named below and that the information provided in the form is correct'

Full Name: ..............Steven John Wickson ..................................................
(Please Print)

Signed: [Signature Redacted by Library] ..........................................................

Date: ...........28/08/2014.............................................................................
I certify the following about the thesis entitled (10 word maximum)

**Life History Adaptations by a Leptocerid Caddisfly in a Drying Environment**

submitted for the degree of __Doctor of Philosophy__________________

a. I am the creator of all or part of the whole work(s) (including content and layout) and that where reference is made to the work of others, due acknowledgment is given.

b. The work(s) are not in any way a violation or infringement of any copyright, trademark, patent, or other rights whatsoever of any person.

c. That if the work(s) have been commissioned, sponsored or supported by any organisation, I have fulfilled all of the obligations required by such contract or agreement.

I also certify that any material in the thesis which has been accepted for a degree or diploma by any university or institution is identified in the text.

'I certify that I am the student named below and that the information provided in the form is correct'

**Full Name:** ............Steven John Wickson .................................................................

(Please Print)

**Signed:**  

[Signature Redacted by Library]

**Date:** ............28/08/2014.................................................................
## AUTHORSHIP STATEMENT

### 1. Details of publication and executive author

<table>
<thead>
<tr>
<th>Title of Publication</th>
<th>Publication details</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>School/Institute/Division if based at Deakin; Organisation and address if non-Deakin</th>
<th>Email or phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steven Wickeon</td>
<td>Life &amp; Environmental Science</td>
<td><a href="mailto:sjwi@deakin.edu.au">sjwi@deakin.edu.au</a></td>
</tr>
</tbody>
</table>

### 2. Inclusion of publication in a thesis

Is it intended to include this publication in a higher degree by research (HDR) thesis? | Yes | If Yes, please complete Section 3. If No, go straight to Section 4. |

### 3. HDR thesis author's declaration

<table>
<thead>
<tr>
<th>Name of HDR thesis author if different from above, (if the same, write &quot;as above&quot;)</th>
<th>School/Institute/Division if based at Deakin</th>
<th>Thesis title</th>
</tr>
</thead>
<tbody>
<tr>
<td>As above</td>
<td>As above</td>
<td>Life History Adaptations by a Leptocerid Caddisfly in a Drying Environment</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author's contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

I undertook project inception, the review of the current literature, development of experimental design, execution and data collection and analysis and the drafting and review of the manuscript.

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

Signature and date: 29/08/14

### 4. Description of all author contributions

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Ed Chester</td>
<td>assisted in the development of experimental design, editing the manuscript and data analysis</td>
</tr>
<tr>
<td>Assoc. Prof. Belinda Robson</td>
<td>assisted in the development of experimental design, editing the manuscript and data analysis</td>
</tr>
</tbody>
</table>

Signature Redacted by Library
5. Author Declarations
I agree to be named as one of the authors of this work, and confirm:
i. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
ii. that there are no other authors according to these criteria,
iii. that the description in Section 4 of my contribution(s) to this publication is accurate,
iv. that the data on which these findings are based are stored as set out in Section 7 below.

If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further
v. consent to the incorporation of the publication into the candidate’s HDR thesis submitted to Deakin
University and, if the higher degree is awarded, the subsequent publication of the thesis by the
university (subject to relevant Copyright provisions).

<table>
<thead>
<tr>
<th>Name of author</th>
<th>Signature*</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Ed Chester</td>
<td>Signature Redacted by Library</td>
<td>29/8/14</td>
</tr>
<tr>
<td>Assoc. Prof. Belinda Robson</td>
<td>Signature Redacted by Library</td>
<td>29/8/14</td>
</tr>
</tbody>
</table>

6. Other contributor declarations
I agree to be named as a non-author contributor to this work.

<table>
<thead>
<tr>
<th>Name and affiliation of contributor</th>
<th>Contribution</th>
<th>Signature* and date</th>
</tr>
</thead>
</table>

* If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to suggest that the person would object to being named as author.

7. Data storage
The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

<table>
<thead>
<tr>
<th>Data format</th>
<th>Storage Location</th>
<th>Date lodged</th>
<th>Name of custodian if other than the executive author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsoft Word, Microsoft Excel</td>
<td>Deakin University Server</td>
<td>December 2012</td>
<td>Dr Ty Matthews</td>
</tr>
</tbody>
</table>

This form must be retained by the executive author, within the school or institute in which they are based.
If the publication is to be included as part of an HDR thesis, a copy of this form must be included in the thesis with the publication.
Acknowledgements

Individuals who have made specific contributions to each piece of research have been thanked within the acknowledgements of each chapter. More generally I would like to thank each of my supervisors, both past and present (in alphabetical order) for initially giving me the opportunity to undertake this research as well as sharing their time and knowledge and pushing me on when I felt I couldn’t continue: Prof Chris Austin, Dr Alecia Bellgrove, Dr Edwin Chester, Dr Rebecca Lester, Dr Ty Matthews, Dr Adam Miller & Associate Professor Belinda Robson. I also would like to thank Dr Agnes Lautenschlager for reviewing and editing my thesis. I would like to thank the three examiners, Professor Vincent Resh, Professor Alan Hildrew and a third anonymous examiner for their helpful comments and suggestions.

Thanks also to the academic staff and my cohort of postgraduate students of the School of Life and Environmental Sciences at Deakin University Warrnambool campus for support, guidance and inspiration.

Final acknowledgement is needed for my family who have supported me for the last seven years. Particularly my wife Lisa and daughters Eve and Lola, for standing by me as I worked through nights, weekends and holidays to complete this thesis.
Statement of contribution

As the thesis was prepared as a series of manuscripts, it contains some repetition, particularly in the introductory and methods sections. Dr Ty Matthews and Dr Rebecca Lester, as my primary supervisors, provided conceptual and methodological input throughout the writing of the thesis. Dr Belinda Robson and Dr Ed Chester, as my initial primary supervisors assisted in project design and framing of my study.

Chapter 2 has been submitted to *Journal of Invertebrate Systematics*. It was co-authored by my supervisors, Dr Ed Chester, Dr Rebecca Lester, Dr Ty Matthews and Dr Adam Miller who assisted in conceptual and methodological guidance and assistance in data analysis and drafting. Dr Isabel Valenzuela and Mr Bryce Halliday assisted in the confirmation of molecular and morphological differences in *Lectrides* sp. nov. and the processing and sequencing of genetic samples. I undertook all field data collection, initial genetic and morphological analyses, identified species delineation and prepared manuscript drafts.

Chapter 3 was accepted for publication to *Marine and Freshwater Research* (a reprint of this paper is included at the end of this thesis). It was co-authored by Dr Edwin Chester and Dr Belinda Robson who assisted in the development of ideas, editing the manuscript and data analysis. I was the primary author and undertook the review of the current literature, experimental design, execution and the writing of the manuscript.

Chapter 4 will be submitted for publication to *Journal of Animal Ecology*. The manuscript was co-authored by my supervisors Dr Edwin Chester and Dr Rebecca Lester, who provided conceptual and methodological guidance, helped develop the data analysis technique and code and edited the paper. I undertook the literature review, data collection, analyses and wrote the manuscript.
Abstract ............................................................................................................................................ 5

CHAPTER 1 INTRODUCTION ......................................................................................... 8
Disturbance, flow regimes and refuges in aquatic ecosystems ......................................................... 8
The impact of stream permanence on response to disturbance ..................................................... 13
Life history traits in response to drying disturbance ....................................................................... 17
Mechanisms and importance of dispersal for aquatic organisms .................................................... 20
Main objectives of study and thesis structure ................................................................................ 25
Summary of chapter content .......................................................................................................... 26

CHAPTER 2 - POPULATION GENETIC STUDY OF LECTRIDES VARIANS
MOSELY (TRICHOPTERA: LEPTOCERIDAE) UNCOVERS CRYPTIC
SPECIATION ...................................................................................................................... 28

ABSTRACT ......................................................................................................................... 28

Introduction ................................................................................................................................... 29
Hypothesis ......................................................................................................................................... 32

Methods ......................................................................................................................................... 32
Larval sampling ................................................................................................................................. 32
DNA extraction and sequencing....................................................................................................... 32
Population differentiation ................................................................................................................ 33
Phylogenetic analysis ....................................................................................................................... 34
Morphological analysis .................................................................................................................. 35

Results ............................................................................................................................................ 36
CHAPTER 3 AESTIVATION PROVIDES FLEXIBLE MECHANISMS FOR SURVIVAL OF STREAM DRYING IN A LARVAL TRICHOPTERAN (LEPTOCERIDAE) .......................................................... 55

Abstract .......................................................................................................................................... 55

Introduction ................................................................................................................................... 56

Materials and Methods .................................................................................................................. 59
  Study species ................................................................................................................................. 59
  Study sites ..................................................................................................................................... 60
  Experimental design ..................................................................................................................... 61
  Data analysis ............................................................................................................................... 63

Results ............................................................................................................................................ 64
  Larval survivorship ....................................................................................................................... 64
  Larval response times after re-immersion ................................................................................... 65

Discussion ....................................................................................................................................... 65

Acknowledgements ........................................................................................................................ 45

Tables ............................................................................................................................................. 45

Figure captions ............................................................................................................................... 48
CHAPTER 4 PUPAL SITE SELECTION BY A LEPTOCERID CADDISFLY IN STREAMS WITH VARIABLE FLOW REGIMES

Abstract

Introduction

Hypotheses

Methods

Study species

Study sites

Sampling methods

Data analysis

Results

Relationship between rock size and the presence & absence of pupae

Correlation between rock size and water depth and the numbers of pupae found on a given rock

The spatial distribution of pupae and micro-aggregations

Discussion

Micro-aggregation of pupae

Effect of depth and rock size

Implications of life history

Landscape effects

Acknowledgements

Tables

Figure captions

Appendices
CHAPTER 5 GENERAL DISCUSSION AND CONCLUSIONS .................. 113

Summary of findings ................................................................. 113
  Population genetics and phylogeny ........................................... 116
  Aestivation limits ................................................................. 118
  Pupal site selection ............................................................. 120

Implications of findings for other freshwater taxa .................. 122

Further work ............................................................................ 126

Management implications ...................................................... 127

Conclusion ............................................................................... 130

REFERENCES ......................................................................... 133
ABSTRACT

From 1995 to 2009 much of Eastern Australia was under severe drought conditions. This is considered to be the longest and most extensive drought since European settlement in Australia and placed immense stress on aquatic systems. Biotic surveys in the Grampians National Park have shown a sharp reduction in aquatic macroinvertebrate biodiversity over this period. The most likely explanation for this reduction is a reduced permanence of water supply and loss of habitat due to decreased rainfall and increased water abstraction. Despite such losses, it has been found that trichopterans from the family Leptoceridae (in particular Lectrides varians) were still relatively widespread and abundant. In order to understand their persistence while other species declined, a series of investigations were undertaken to improve the understanding of the environmental requirements and life history strategies of L. varians. This thesis provides a broader understanding of the ecology of benthic invertebrates in temporary streams, including life-history traits that facilitate persistence of larval stages in seasonal streams as they become dry.

Through the analysis of mitochondrial DNA it was found that this taxon has no genetic population structure across western Victoria, suggesting that the adults are long lived and are have strong dispersal capabilities. This would afford L. varians greater opportunity to locate suitable habitat for oviposition in a dynamic and drying landscape. Genetic sampling also uncovered a likely species complex and cryptic speciation of L. varians. While larvae of both genotypes were identifiable as L. varians using the current morphological key, further investigation showed this genetic difference expressed as two distinct morphotypes (light and dark). These morphs where consistently matched to the two genotypes across all sampled populations. The potential new species of Lectrides appears to be unique to western Victoria.
The morphological differences are catalogued and used to develop an alternative morphological key for larvae. As I found at least two distinct and sympatric species of *L. varians* within a small extent of its range, it could be reasonably expected that this taxa is, in fact, a complex of multiple species.

A second study used a novel method of testing the tolerance limits of benthic biota to stream drying. During the course of field sampling, *L. varians* larvae were found on dry streambeds, under leaf litter and large rocks. While these streambeds were known to be dry for a number of weeks, some individuals became mobile after rewetting. A laboratory experiment was devised to measure the extent and degree of drying that *L. varians* larvae can withstand. This method simulated extended stream drying with moist and dry substrates. It was found that larval populations showed no significant decline in survivorship, compared to fully submerged populations, for up to 16 weeks on a moist substrate, but that survival was significantly lower where the substrate was allowed to dry completely. Larvae also showed a bimodal response to rewetting, with individuals either becoming active within a few hours, or a number of days after rewetting. This represented the first quantification of the aestivation potential of this species, which had previously been suggested by other authors. The technique developed in this chapter has also been adopted by other authors to test the aestivation limits of a range of other macroinvertebrate taxa.

Finally, a third study investigated the potential for active site selection by *L. varians* larvae for pupation. While sampling, *L. varians* pupa were consistently found clustered on the underside of large rocks at the deepest point of a pool, in close proximity to other clusters. A field sampling program mapping the presence of *L. varians* pupa was developed to determine whether these patterns
were consistent and quantifiable. A close association was found between size of the rock that pupa were attached to, the depth at which they were found and the presence and number of pupa. Despite this, no consistent spatial patterns were uncovered across streams. Selecting pupation sites on large rocks in deep water would potentially allow pupa to avoid predators and afford protection against flooding and drying disturbance. This work reinforces the need to maintain heterogeneity in river substrates and bed profile and extends current knowledge of the relationship between the distribution of larvae and habitat in seasonal streams.

These studies investigated adaptations to drying (both morphological/physical and behavioural/life history) at three life history stages. These results can potentially be used to explain why *L. varians* has maintained robust populations throughout the Grampians National Park during a period of extended and severe drought, while almost all other aquatic macroinvertebrate taxa had reduced populations and ranges. This autoecological information for a resilient species such as *L. varians* is important to further our understanding of the drivers of persistence despite disturbance and forms the basis of broader population ecology and management practices.
CHAPTER 1 INTRODUCTION

DISTURBANCE, FLOW REGIMES AND REFUGES IN AQUATIC ECOSYSTEMS

The structure of most ecological communities, in particular stream benthic communities, is driven largely by natural disturbances (Resh et al. 1988). Disturbance can be defined as a discrete event which disrupts ecosystem function, community or population structure through alterations to resource availability or to the physical environment (Resh et al. 1988). There are many types of natural and anthropogenic disturbances that affect stream ecosystems. These disturbances are varied and some of the most commonly-studied disturbances include drought, flooding, chemical pollution, dredging, channelization, habitat removal, eutrophication, sedimentation and catchment modification and can lead to profound effects on stream ecosystems (Niemi et al. 1990; Boulton 2003; Humphries & Baldwin 2003; Lake 2003; 2011).

Flood and drought are often considered among the most important abiotic disturbances in intermittent streams (Fritz & Dodds 2004). Drought is a key disturbance in Australian waterways, but its impacts are not as well understood as other disturbances (Lake 2003; Boulton & Lake 2008; Lake 2011). However, it is likely that the increased frequency and severity of extended droughts, as predicted in climate-change models for south eastern Australia and many other parts of the world, will have an increasing and enduring impact on stream ecosystems, perhaps greater than other types of disturbance (Steffen et al. 2009).
Broadly speaking, disturbances can be described as press, pulse or ramp events (Lake 2003). Pulse disturbances often result in relatively immediate changes in conditions when compared to press or ramp events, after which the abiotic (and sometimes the biotic) systems may revert back to a previous equilibrium state (e.g. a return to base flows following a high-flow event and gradual re-establishment of biota) (Death 2008). A press disturbance is a sustained change in environmental conditions which often leads to local extinctions and new community composition. Ramp disturbances, however, are more gradual, increasing in strength and spatial extent over time (Lake 2003). For example, seasonal droughts can lead to local extinctions (Bender et al. 1984), which could be considered to be a press disturbance.

In relatively intact ecosystems (which are, for the most part, unmodified), natural disturbances such as flood and drought are a normal component of the system, with some organisms able to survive, and even exploit, these disturbances (Lytle & Poff 2004). Flow events at either end of the hydrological spectrum (i.e. periods of extremely high flow or no flow) can greatly influence the distribution, abundance and diversity of lotic species. For example, spates or floods are considered to be pulse disturbances that often lead to altered community structure and localised population losses (Resh et al. 1988; Niemi et al. 1990; Lancaster & Hildrew 1993). These changes to community structure arise partly because floods form a major source of physical disturbance in rivers. They may shift or bury substrata and hence cause crushing or displacement of biota. This loss of biota can subsequently lead to a release of space and resources (Biggs 1996; Peterson 1996; Downes et al. 1998).
During flood events, organisms susceptible to increased flow are most likely to seek refuge. Use of refuges may be pronounced in groups of species that are poorly adapted to running water (Lancaster & Hildrew 1993). Benthic communities will often respond to spates quickly, through the use of in-stream refuges to avoid high flow (e.g. low flow patches and interstitial spaces, Hildrew et al. 1991; Lancaster & Hildrew 1993). For example, experiments have shown that the micro-distribution of benthic invertebrates can shift among hydraulic patch types that provide refuge during high-flow events (Lancaster & Hildrew 1993).

The concept of ecological refuges in rivers was originally developed in studies of flooding (Lancaster & Hildrew 1993; Lancaster & Belyea 1997), but has since been developed to apply to other types of disturbance (Lake 2011; Robson et al., 2012). Here, I follow the definition of Keppel et al. (2012) where the term ‘refuges’ applies to locations that protect organisms from disturbances on ecological time scales and ‘refugia’ are locations that protect species at geological time scales. Refuges are defined as places that protect populations of plants and animals from disturbances at ecological scales such as flooding, fire and drought (Robson et al. 2012). This distinction has been recognised in the literature, not just Keppel et al. (2012) and Robson et al. (2012), but also Bennett & Provan (2008), Rull (2009), Ashcroft (2010), Temunović et al. (2013) and Lake (2011).

Human pressures may reduce the condition and occurrence of refuges either directly, for example, due to extraction of water from refuge pools, or via other processes that contribute to pollutant levels or sedimentation (Boulton & Lake 2008). Furthermore, the increasing human demand on streams for water supply through impoundment, irrigation and groundwater abstraction is leading to increased drying of streams worldwide (Stanley et al. 1997). These issues are
exacerbated in Australia because of extravagant water use, with Australians having among the highest *per capita* water consumption in the world (Lake & Bond 2007). Due to this excessive use, changes in natural flow regimes as a result of water extraction and supply are likely to form a considerable press disturbance and place significant, ongoing stress on many Australian aquatic systems.

Low-flow periods associated with drought can also greatly affect stream biota (Lake 2003). Such periods can be seasonally-predictable drying events such as those experienced in wet-dry tropical or Mediterranean environments (Boulton 2003). Seasonal droughts tend to be predictable presses, whereas supra-seasonal droughts, resulting from extended decreased rainfall, are often considered as ramp disturbances (Lake 2003). Droughts often cause distinct changes to ecosystem processes and community structure (Boulton 2003; Humphries & Baldwin 2003), but changes to biological communities can also be non-linear and tend to be stepped, as different habitats become disconnected then lost as hydrological thresholds are crossed (Bender *et al.* 1984; Lake 2003). The most likely cause of invertebrate mortality during drought is associated with desiccation stress associated with water and habitat loss, but can also be a result of decreases in food availability and deteriorating water quality (Lake 2003). The impact of drought and drying can differ between aquatic environments and can be influenced by the regularity, duration and intensity of the drying period, as well as the availability of refuges (Boulton 2003; Chester & Robson 2011; Robson *et al.* 2011; Mackie *et al.* 2012; Robson *et al.* 2012).

Since climate change is predicted to extend the frequency and duration of these events beyond natural patterns, it should be considered as a driver of disturbance (including press, pulse and ramp disturbances) for stream fauna in coming decades. Climate change models predict more extreme weather events,
less available water (Moss 2010) and prolonged and more frequent droughts for southern Australia (Steffen et al. 2009). In general, air temperatures are expected to rise between 0.4–2.0°C by 2030 and 1.0–6.0°C by 2070 relative to 1990 levels (Pittock 2003; Collins et al. 2013). In south-eastern Australia, changes in rainfall between –10% to +5% (by 2030) and –35% to +10% (by 2070) are also predicted (Lake & Bond 2007). Further to this, it is likely that mid-latitude and subtropical arid and semi-arid regions will receive less precipitation and many moist mid-latitude regions will likely experience more precipitation by the end of this century under the RCP8.5 scenario (Collins et al. 2013). Short-duration precipitation events are also expected to become more intense storm events with fewer weak storms likely as temperatures rise (Collins et al. 2013). This is expected to reduce the quality and quantity of pool refuges in these landscapes.

The proximity and quality of refuges as sources for recolonisation are particularly important for facilitating the survival of invertebrates during disturbance such as extended drought periods (Boulton 2003). A number of key drought refuges for stream macroinvertebrates have been identified by previous authors, including: rotting wood and bark (Boulton 1989), moist leaf packs (Boulton 1989; St Clair 1994; Wickson et al. 2012), crayfish burrows (Boulton 1989; Williams 2006), the hyporheic zone (Harper & Hynes 1970; Gray & Fisher 1981) and the ability for invertebrates to become dormant (i.e. diapause) in dry sediment (Towns 1983; Miller & Golladay 1996). Chester & Robson (2011) found that the most important drought refuges in the Grampians National Park (NP), Australia, are pools and perennially flowing reaches. In the arid zone of Australia, Sheldon et al. (2010) also found perennial waterholes to be a crucial refuge in dryland rivers. Pool refuges are often unpredictable because they may dry at varying rates irrespective of their size (Smith & Pearson 1987).
As a result, longer-lived, stable pools tend to have more diverse invertebrate assemblages than those which dry more quickly (Smith & Pearson 1987; Sheldon et al. 2010). It is expected that the quality and quantity of water within refuge pools and the frequency of occurrence of perennial pools, will decline under predicted climate change (Chester & Robson 2011).

THE IMPACT OF STREAM PERMANENCE ON RESPONSE TO DISTURBANCE

The extent and frequency of drying is also likely to have varying impacts on lotic systems depending on whether they are perennial or temporary. Such impacts include reduced biodiversity, reduced productivity and localised extinctions (Boulton 1989). Temporary streams can be defined as lotic ecosystems that experience a recurrent dry phase (Williams 2006), but they can exhibit an extensive array of hydrological regimes, ranging between those which are generally wet, but sometimes dry, those which are wet for short unpredictable periods and those alternating between being wet and dry intra-annually (Moss 2010). Ephemeral and intermittent streams characterise many parts of Australia, due to limited runoff and high evapotranspiration rates (Boulton & Brock 1999; Robson et al. 2005). Temporary streams exhibit physical and chemical extremes, such as more frequent and prolonged periods of increased temperature and decreased dissolved oxygen concentrations, to a much greater extent than conditions typically experienced in permanent waters (Williams 2006).

It has been predicted that the number and length of temporary rivers will decline as pressure from water abstractions increases and these systems experience increased drying related to climate change (Larned et al. 2010; Sabater & Tockner 2010). Despite their importance and relative high occurrence within the landscape (compared to permanent systems), temporary streams...
have historically attracted little investigation compared with perennial systems, particularly investigating the pressures, limits on and adaptations of intermittent stream biota (Boulton & Suter 1986; Larned et al. 2010). Also, the effects of disturbance from periodical drying has received far less attention from ecologists than those of floods or low flows (Sheldon 2005; Datry et al. 2007; Arscott et al. 2010; Datry et al. 2011). Consequently, our understanding of the biota inhabiting temporary rivers remains sparse compared to those of permanent streams. However, the number of ecological studies that have focussed on temporary systems has increased during the last decade with on average of 20–25 papers published each year on temporary rivers over the last 4–5 years, (Datry et al. 2011).

Studies in intermittent waterways cover a range of topics, for example:

**Community structure**, which is taxonomically and functionally altered in response to changes in permanence, depth and flow of water and associated physiochemical changes (Williams et al. 2004; Boix et al. 2010; Porst et al. 2012; Vidal-Abarca et al. 2013). Community response varies among sites, as a result of small-scale variability in habitat heterogeneity, among other factors (Boulton 2003; Pace et al. 2013). Intermittent habitats tend to show lower species richness than perennial systems (Robson et al. 2005; Bogan et al. 2013), particularly after periods of increased aridity (Feminella 1996).

**Food webs and production**, as drying has been found to be a more important determinant than habitat in macroinvertebrate production (Chadwick & Huryn 2007; Power et al. 2008). Ecosystem metabolic rates are known to be profoundly affected by stream intermittency (Acuna et al. 2005). Increased frequency of stream drying can lead to lowered species richness, which can then result in low food web complexity with predators being especially vulnerable to
localised extinction (Woodward et al. 2012). Furthermore, allochthonous detritus (particularly from Eucalyptus spp.) is the primary energy source for benthic macroinvertebrates in intermittent streams, leading to communities dominated by detritivores (Reid et al. 2008; Bishop & Kelaher 2013).

**Biotic and community recovery from drying.** The importance of resilience and/or resistance traits for the persistence of stream biota has been discussed by a number of authors (Miller & Golladay 1996; Acuna et al. 2005; Lytle et al. 2008). Desiccation resistance in aquatic macroinvertebrates has also been shown to be a key factor in recolonisation, with drought resistant taxa likely to colonise re-wetted areas first, followed by active dispersers (Boulton 2003; Porst et al. 2012).

**The role of habitat and refuges,** where several authors have noted the importance and reliability of refuges (such as permanent pools and moist leaf litter) in intermittent systems for the maintenance of community structure and the persistence of robust populations (Hershkovitz & Gasith 2013; Store & Quinn 2013). Marked differences have been detected in macroinvertebrate assemblages among patches of similar habitat that differ in refuge reliability, with significantly lower species numbers in those streams without refuges (Collinson et al. 1995; Beche et al. 2006; Bogan et al. 2013).

This thesis explores morphological/physical and behavioural/life history adaptations to drying of a highly disturbance resilient taxon at three life history stages. The aim of the thesis was to explain key attributes that may allow *L. varians* to persist in the face of habitat fragmentation and extended and severe drought. Autoecological studies such as this are uncommon, however this information is crucial to the understanding of ecosystem function, population
ecology and can guide more effective management of natural systems in the face of increasing disturbance.

Changes in the presence (or absence) of refuges can lead to rapid changes in macroinvertebrate communities (McManamay et al. 2013; Morrison et al. 2013). Furthermore, post-flooding recruitment is greater in areas with drought refuges as individuals emerge from refuges to a more diverse range of microhabitats (Rayner et al. 2009; García-Roger et al. 2011). However, there is still much to learn about how aquatic invertebrate diversity and assemblage composition is influenced by flow intermittency (Hershkovitz & Gasith 2013) and, in particular, there is a distinct lack of autoecological studies in this realm (which this thesis addresses, in part). To better understand the workings of biotic assemblages and communities, an understanding of the conditions necessary for survival of individual taxa and their associated limits, is required. In particular, tolerance limits to desiccation or hydration, refuge-seeking behaviour and refuge habitat characteristics are important (Datry et al. 2011, Robson et al. 2011, 2012). Such work can lead to the development of quantitative relationships and predictive models which can be used to estimate biological and biogeochemical responses to increased frequency and duration of drying (Datry et al. 2007; Arscott et al. 2010).

The processes and physical structures involved in river regulation by human activities can lead to modification or destruction of habitats though the creation of barriers to longitudinal movement of plants and animals, and upstream reduction in water quality, as well as providing conditions more favorable to exotic and unpalatable species such as blue green algae (Ball et al. 2001). Alterations to the timing and increased drying as a result of reduced runoff and water abstraction have been shown to significantly alter macroinvertebrate
assemblages in intermittent streams, with communities becoming less diverse (Sponseller et al. 2010; Chester & Robson 2011; Mackie et al. 2012).

River reaches directly downstream of off-take weirs often exhibit reduced diversity of macroinvertebrates in the Grampians NP relative to comparable habitat in unregulated streams (Mackie et al. 2012). Other longer-term studies (carried out over a number of years) have shown that climate driven changes in hydrology will reduce diversity and population size of invertebrate communities in temperate systems (Beche & Resh 2007; Durance & Omerod 2007; Magalhaes et al. 2007). However, little research has focused on the potential effects of climate change on Mediterranean-climate seasonal streams (Beche & Resh 2007; Robson et al. 2012). While droughts of a duration and intensity within the bounds of long term climatic averages (approximately every 10 to 20 years) tend to have small effects on biotic assemblages of seasonally intermittent streams, the predicted increases in the intensity and period of drying is likely to lead to significant loss of macroinvertebrate biodiversity and local extinctions of sensitive taxa (Durance & Omerod 2007; Magalhaes et al. 2007).

LIFE HISTORY TRAITS IN RESPONSE TO DRYING DISTURBANCE

Variation in hydrological processes and water temperature are two of the most important factors affecting life-history patterns of temporary stream dwellers (Alvarez & Pardo 2005). Arguably the greatest environmental influence on the life history of biota in temporary streams is the degree and extent of drying (Williams 2006). Summer drying has been found to be a major factor structuring intermittent riffle assemblages in North America and Australia, possibly due to impacts on the ability of flow-dependent taxa to complete their life cycles (Miller & Golladay 1996; Boulton & Brock 1999). Therefore, taxa that
reside in systems that are regularly exposed to disturbances such as drying will require one or more life-history adaptations to persist during adverse conditions.

Despite a limited ecological understanding of Australian intermittent streams, the combined consequences of climate change and increased human pressure are likely to be catastrophic (Boulton & Lake 2008). Aquatic macroinvertebrates that inhabit temporary or season systems often possess traits such as aestivation (a state of hibernation over the summer period, which is triggered by environmental cues) and diapause (a state of torpor which involves a more significant physiological response than aestivation, triggered by internal cues) which enhance their resistance to water loss (Imhof & Harrison 1981; Boulton 1989; Williams 1998).

Other macroinvertebrate survival adaptations may correlate with the timing of life-cycle behaviours to seasonal flow events; this however typically requires long-term stability in the dynamics of flow regimes to develop (Lytle & Poff 2004). Highly-variable flow regimes, that include the alternation of flood and low-flow periods, can greatly influence a number of population processes of aquatic macroinvertebrates, including life history traits (Marchant et al. 1984; Stanley et al. 1997), recolonisation pathways (Mackie et al. 2012) and the distribution and abundance of taxa within and between streams. The high flow variability of Australian streams, compared with other regions worldwide, may be the reason for a large number of taxa exhibiting asynchronous life histories compared with taxa elsewhere (Humphries & Baldwin 2003). For example, Australian aquatic insect species often have a range of instars or development stages present throughout the year, which reduces the risk of an entire cohort being eliminated by a disturbance event (St Clair 1993). These types of population-level processes are driven by behavioural and/or physical
adaptations which provide resilience to disturbances associated with drying (Humphries & Baldwin 2003). Therefore, key life-history traits facilitate the survival of stream invertebrates during and following disturbance events, particularly periods of stream drying.

The range of life-history traits shown by aquatic insects are variable from adult emergence at the same time each year, coinciding with the average time for seasonal drying (Lytle 2008), the release of larvae before wet-season peak flows (Hancock & Bunn 1997), egg laying during seasonal low flow (Bunn 1988), and bivoltinism through cohort splitting, which provides an extended period of adult emergence and increasing chances of avoiding unfavourable conditions (Bunn 1988).

Physical adaptations to disturbance are also important. In addition to using refuges as described above, macroinvertebrates may survive periods of drying by possessing behavioural responses (including forms of desiccation resistance) that facilitate survival during extended dry periods (Lake 2003; Williams 2006; Moss 2010; Wickson et al. 2012). The importance of these life-history traits may become increasingly important for some invertebrate taxa since climate change may diminish the quality of refuge pools in the future (Boulton & Lake 2008). Aestivation and summer diapause are commonly used by biota inhabiting seasonally-dry streams (St Clair 1993; Salavert et al. 2008; Wickson et al. 2012). Other taxa have desiccation-resistant life stages (Clifford 1966), while some can emerge at an immature stage of development and enter summer reproductive diapause to avoid drying and high temperatures (Colburn 1984).

Behavioural adaptations or life-history traits that facilitate survival during a disturbance may require a proximal cue such as a change in temperature, light or flow velocity, which indicate the onset of disturbance (Humphries & Baldwin
This disturbance can then be avoided by moving to refuges outside of the stream environment (Lytle 2008). Taxa may be able to track changes in flow to relocate to nearby streams to avoid drying (Lytle 2008). In the absence of appropriate refuges, some taxa have life stages that are resistant to disturbance (Adis & Junk 2002) or they will exhibit rapid development to take advantage of favourable conditions (Gray 1981). Broadly, the adaptations discussed above fall under two categories: response to an external stimulus such as aestivation or accelerated development and seasonal adaptions such the release of larvae before wet-season peak flows or egg laying during seasonal low flow which coincide with the timing of regular changes to habitat conditions (Lytle 2008). Adaptations which coincide with seasonal variation depend on a more stable seasonal system which dries and floods to the same degree at regular intervals (Statzner et al. 2001; Lytle et al. 2008). Long-term persistence in more unpredictable intermittent systems requires adaptations which respond directly to external cues allowing individuals to rapidly take advantage of favorable conditions or withstand or avoid unfavorable ones (Boulton 2003; Lake 2003).

**MECHANISMS AND IMPORTANCE OF DISPERSAL FOR AQUATIC ORGANISMS**

Knowledge of oviposition and pupal site selection processes is required to understand the behavioural ecology of organisms with complex life histories and how dispersal ability is related to distribution among suitable patches of habitat (Downes & Reich 2008). These processes are central to understanding species population dynamics because they ultimately influence the survivorship and spatial distribution of subsequent generations (Lancaster et al. 2011). Random oviposition is often considered to be characteristic of aquatic insects (Hinton 1981), but few empirical studies have been completed to test whether selective oviposition occurs (Lancaster et al. 2011). Holometabolous insects
have attached eggs and pupae that consequently are particularly vulnerable to disturbance and predators (Lucas et al. 2000). Therefore, through natural selection, many taxa have developed morphological (Hinton 1955, Porst et al. 2012), chemical (Edmunds 1974, Attygalle et al. 1993) and behavioural (Hinton 1955) adaptations which enhance the likelihood of survival of individuals and populations by either being able withstand or escape unfavourable conditions.

While life-history traits may facilitate the survival of particular life-cycle stages during a disturbance, the ability to disperse and then recolonise an area is crucial for the persistence of metapopulations and their associated sub-populations once the disturbance has passed. This process is termed a resilient response to drying (Chester & Robson, 2011). Dispersal capability is instrumental in determining the persistence, abundance and dynamics of natural populations (Dieckmann et al. 1999). Some authors suggest that, as opposed to sessile or sedentary invertebrates, highly mobile taxa are likely to recolonise streams more quickly once flow resumes after a period of drying (Boulton 2003). However, measuring dispersal capabilities can be extremely difficult, particularly for small invertebrates that are difficult to tag, so alternative methods such as molecular techniques are required.

The distribution of organisms in space and time is inherently patchy, rather than random or homogeneous, which leads to spatial and temporal heterogeneity (Pringle 2001). The spatial distribution of benthic invertebrates in any aquatic environment at a particular point in time is the result of a series of biological and non-biological events (Poff 1997; Underwood & Keogh 2001). Firstly, suitable habitat must be available for mobile stages to disperse to. Secondly, where aquatic dispersal is by passive means rather than active swimming or walking, dispersing stages must be able to negotiate currents to be able to settle on the substrate. Thirdly, some species may display selective
behaviour, which directs individuals to choose a particular site over others, which will supposedly enhance survivorship and fitness (Downes et al. 2005).

Freshwater invertebrates may disperse via aerial flight by adult stages between water bodies, by passive means such as wind and water flow or via animal vectors (Bilton et al. 2001). Known animal vector pathways include attachment to the feet and feathers of waterbirds (Kew 1893; Johnson & Carlton 1996) or to aquatic insects (Kew 1893; Bohonak 1999), passing through the digestive tract of birds and mammals (Brown 1933; Proctor et al. 1967) or attachment of parasitic larvae on fish (Gaines & Bertness 1992). The location of populations in the stream network is likely to affect connectivity among populations (Hughes et al. 2009). This is because dispersal between suitable habitat patches is limited not only by distance, but also by the size (MacArthur & Pianka, 1966) and spatial arrangement of patches (including hydrological connections and arrangement of drainages), the configuration and slope of the surrounding landscape and the density and height of vegetation (Jackson & Resh 1989). Temporal changes in landscape structure also play a significant role in the location of populations across a metapopulation (Fahrig & Merriam 1994) as well as the hierarchy of reaches, streams, sub-catchments and catchments (Meffe & Vrijenhoek 1988).

Given the difficulties associated with directly measuring dispersal described above, the ability of taxa to migrate through the landscape and the connectedness between populations can be measured or approximated by measuring gene flow. Various models of gene flow have been proposed, including two by Meffe and Vrijenhoek (1988), who used physical landscape structure (i.e. hydrologic or geographic setting) and the biological traits of a number of fish species to interpret spatial genetic patterns. As fish are confined to the water column throughout their life cycle, the models they proposed
(known as the ‘Death Valley’ and ‘Stream Hierarchy’ models) are relevant only to organisms with similarly restricted dispersal. In 2009, Hughes et al. broadened the scope of the models to include a wider range of stream taxa and added two more models to account for the range of dispersal-related traits found in stream organisms. These further two models are the ‘headwater’ and ‘widespread gene flow with isolation by distance’ models (Hughes et al. 2009).

**Death Valley model:** The Death Valley model (DVM) was originally conceived by Meffe and Vrijenhoek (1988) for remnant populations of fully aquatic taxa in small isolated pools with limited hydrological connection. The presence of small isolated populations in those pools is likely to lead to local genetic drift dominating population genetic structure. This dominance of genetic drift is predicted to result in a high level of genetic variation among populations (when compared to other models), with no spatial genetic structure explained by landscape structure (such as drainage boundaries or Euclidian distance between populations) (Hughes et al. 2009).

**Stream Hierarchy Model:** A pattern of population structure occurs for aquatic biota that inhabit branched river systems, with varying degrees of connectedness and gene flow. In this case, populations are only partially isolated from each other. With at least some opportunities for gene flow through periodic or permanent connections between local populations, divergence is less extreme than in the Death Valley Model. This results in genetic structuring which is hierarchical in nature. Higher frequencies of gene flow are expressed as greater similarity among populations. The genetic divergence of populations under the stream hierarchy model is a function of geographic connectedness of habitats (Meffe & Vrijenhoek 1988). Populations that are frequently connected and exchange individuals will have greater genetic similarities than those that are disconnected. For freshwater taxa which are not constrained to particular
habitats, but are restricted to the channel (such as fish), riverine conditions tend to impose a population structure fitting the stream hierarchy model (Hughes et al. 2009).

**Headwater model:** The first model proposed by Hughes *et al.* (2009) is the headwater model, where headwaters are defined as the smallest and uppermost branches of stream networks. This model predicts spatial genetic structure for headwater-specialist species with low capacity for terrestrial dispersal among streams. Significant genetic structure is expected at localised geographic areas with a high density of headwater streams (such as mountain ranges). In contrast to the stream hierarchy model, the headwater specialisation required by this model impedes dispersal throughout the stream network, in effect isolating populations to the upper reaches. However, the potential for some terrestrial movement may allow dispersal and gene flow among nearby headwaters, even if this requires that catchment boundaries are crossed. This model has been illustrated using a range of invertebrate species with low flight ranges such as Simuliidae blackflies (Finn *et al.* 2006), or that are able to crawl across land such as Belosomatidae giant water bugs (Finn *et al.* 2007) and Parastacidae freshwater crayfish (Ponniah & Hughes 2006).

**Widespread gene flow with isolation by distance.** The effect of the hydrological configuration of a landscape on population genetic structure is usually low or non-existent for aquatic species with strong terrestrial dispersal traits. In the genetic isolation by distance model, the spatial pattern expected reflects the Euclidean distance among populations. This pattern may be found in strong-flying insects with stream-dwelling juveniles (Hughes *et al.* 2009). However, large distances between habitats may reduce gene flow and there are several examples of widespread gene flow and broad-scale isolation by distance in aquatic insects with a strong-flying adult stage, including trichopterans
(Geenen et al. 2000; Baker et al. 2003) and odonates (Conrad et al. 1999; Kelly et al. 2002).

Current climate change models predict increased disturbance and greater fragmentation of inland aquatic systems (Steffen et al. 2009). Fragmented populations in highly-disturbed systems have an increased risk of extinction if the fluctuations of local populations relative to each other are not synchronous (Fahrig & Merriam 1994; Hanski & Gilpin 1997). With large and unpredictable inter-annual variation in Mediterranean-climate streams (Bonada et al. 2008), the most successful biota with the greatest chance of sustaining populations will be those that are strong dispersers and can readily colonise new patches (Chester & Robson 2011).

**MAIN OBJECTIVES OF STUDY AND THESIS STRUCTURE**

Both climate change and human activities are exerting increasing pressures on lotic ecosystems and the predicted rates of change associated with climate change may be greater than those experienced in the past. Therefore, it is important to understand those traits that facilitate the persistence of stream biota under current-day disturbance regimes in order to predict how they may respond to future scenarios of altered stream flow regimes, particularly drying. Furthermore, while environmental change is an agent for natural selection, if it is occurring at a rate that is greater than evolutionary processes can maintain, local extinctions may follow (Boulton & Lake 2008). Life-history studies of trichopterans are uncommon in Australia (St Clair 1993) and autecological data relating to the response of common stream taxa to drought and how species recover following the resumption of flow are limited (Boulton & Lake 2008). Therefore, there are three components to this PhD thesis that attempt to provide a greater understanding of how macroinvertebrates persist in a highly
dynamic environment. These three examinations include: 1) dispersal capabilities within a metapopulation; 2) key life-history traits, such as the ability to aestivate and persist in drying streams; and 3) important abiotic factors that might influence site selection for pupation to avoid drying and flooding disturbance and enhance potential for adult emergence. These three broad components are tested using a common leptocerid caddisfly, *Lectrides varians*, within the Grampians NP and form three separate chapters. The hypotheses for each study are outlined in the introduction of each of the following chapters. A brief description of each chapter, is outlined in the following section.

**SUMMARY OF CHAPTER CONTENT**

Chapter 2 investigated dispersal in *L. varians* using genetic markers across the Grampians NP and the phylogenetics of *Lectrides* in Victoria. Molecular testing was carried out using mitochondrial DNA markers (*cytochrome oxidase I, COI*). Previous molecular investigations of large caddisflies have shown the capacity for wide dispersal (Baker *et al.* 2003; Wilcock *et al.* 2005), but have also resulted in the discovery of species complexes (Hogg *et al.* 2009; Malm & Johanson 2004).

Chapter 3 focuses on the ability of *L. varians* to aestivate. The scientific literature indicated that a number of species of Limnephilidae larvae in the USA (MacKay 1977; Colburn & Garretson Clapp 2006) and the United Kingdom (Hickin 1964) as well as Brachycentridae in the USA can enter a stage of diapause or aestivation. In Australia, larvae of Leptoceridae caddisflies have been reported to withstand periods of drying in intermittent streams (St Clair 1993). This was reinforced by observations garnered on field investigations by the author of this theses. Therefore, a laboratory experiment was devised to test
the extent and degree of drying that *L. varians* larvae are able to withstand. This chapter is published in Marine and Freshwater Research (Wickson et al. 2012).

Chapter 4 examines the physical habitat characteristics that are associated with pupation sites. Previous authors have shown that freshwater macroinvertebrates actively select preferred microhabitats for oviposition and feeding (Lancaster & Hildrew 1993; Lancaster et al. 2003). Field observations suggested that accurate predictions could be made as to where clusters of pupae of *L. varians* would be found within a section of a stream. A survey was conducted across a number of streams in the Grampians NP to determine whether *L. varians* pupae were associated with particular habitats (i.e. different water depths and stone sizes).

A general discussion (i.e. Chapter 5) provides a synthesis of results from the three data chapters and relates this new information to studies elsewhere in Australia and overseas, together with consideration of how aquatic biota may respond to altered stream flows associated with climate change and other anthropogenic disturbances.
CHAPTER 2 - POPULATION GENETIC STUDY OF *Lectrides varians* Mosely (Trichoptera: Leptoceridae) UNCOVERS CRYPTIC SPECIATION

**ABSTRACT**

Species complexes have the potential to mask diversity in life-history strategies and adaptations in what were once thought to be widespread, common species. The detection of complexes has become more common with the increased use of molecular taxonomic techniques, but many taxa remain unexamined. *Lectrides varians* (Mosely) is a large, ecologically-important caddisfly found in perennial and intermittent streams throughout much of eastern Australia. We investigate the dispersal potential of *L. varians* by conducting a population genetic study, building on previous works that have assessed life-history traits associated with drought resistance. Genetic analyses of *L. varians* from the Grampians region of Victoria, based on mitochondrial DNA sequence data, revealed extensive gene flow and a lack of genetic structure across the sample range ($\Phi_{ST} = 0.03$). This suggests that the species is an effective disperser and is likely to be resilient to increased drying and habitat fragmentation under climate change considering other known resistance traits. However, during this study, two divergent genotypes were identified, indicating a potential species complex. A comprehensive phylogenetic analysis of *L. varians* across its current range was subsequently performed, confirming the species is indeed paraphyletic, consists of one lineage that is restricted to the Grampians National Park and the other being widespread throughout south-eastern Australia.
Further analyses revealed consistent morphological differences between these lineages supporting the notion that *L. varians* is a species complex. We discuss the implications of these findings with regard to conservation and taxonomy of this important invertebrate group.

**INTRODUCTION**

Worldwide, natural ecosystems are increasingly facing threat of climate change and anthropogenic disturbances (Vörösmarty *et al.* 2010). This is particularly true for stream ecosystems as climate change is predicted to generally lead to more frequent drought events that will consequently reduce flows and habitat availability for aquatic biota (Poff *et al.* 2010). Climate change impacts are likely to be exacerbated by increasing demands for water by humans, with impoundments, irrigation and groundwater abstraction from waterways expected to further reduce flows and water quality (Stanley *et al.* 1997; Poff *et al.* 2010). Consequently, shifts in aquatic assemblages and ecosystem function are expected (Vörösmarty *et al.* 2010). However, predicting the impacts of environmental stress on stream biodiversity, including that associated with climate change, is impeded by our current lack of knowledge on the ecology, biology and genetics of organisms inhabiting inland waterways.

Currently 63 streams in the Grampians National Park (GNP), Victoria, Australia, are under increasing pressure from declining rainfall and water extraction (Chester & Robson 2011). Caddisflies are a dominant invertebrate group in perennial and intermittent stream networks in this region and provide important ecosystem services, with larvae performing a key shredding function in aquatic food webs (Holzenthal *et al.* 2007). Three species of caddisfly from the family Leptoceridae are commonly found in these streams: *Lectrides varians* (Mosely); *Triplectides truncatus* (Neboiss); and *Triplectides similis* (Mosely),
with *L. varians* being the most widespread and abundant. Persistence of caddisfly populations under changing environmental conditions depends largely on their ability to disperse to more favourable habitats, and/or ability to adapt to local conditions by genetic or plastic responses (Hoffmann & Sgro 2011; Humphries & Baldwin 2003; Lytle & Poff 2004).

Previous studies indicate that *L. varians* larvae can survive extended periods without surface water by aestivating (Chapter 3; Wickson *et al.* 2012) and by exhibiting specific pupal site selection that may maximize survival (Chapter 4; Wickson, unpublished data). However, additional resilience traits, such as the dispersal potential of GNP caddisflies, remains poorly understood. Direct measures of dispersal in trichopteran adults using trapping have shown great inter-specific variability, with some taxa having limited dispersal ranges from less than 100 m to long-distance dispersal ranging from 8 - 100 km (Sode & Wiberglarsen 1993). Population genetic studies of trichopterans are limited, however recent studies indicate low levels of genetic differentiation over large spatial scales in *Tasiagma ciliata* (Tasimiidae) (Hughes *et al.* 1998) and *Tasimia palpata* (Tasimiidae) (Schultheis *et al.* 2008), indicating high dispersal capacities. Large caddisflies, such as *L. varians*, often are strong flyers, leading to wide lateral dispersion away from streams, but they may also use larval drift for longitudinal in-stream dispersal leading to well-mixed populations over tens of kilometres (e.g. Baker *et al.* 2003; Wilcock *et al.* 2005). So, despite adults being relatively short-lived, strong dispersal appears to be a common trait amongst many large caddisflies. The combination of information concerning dispersal potential and other drought resistance and resilience traits will provide a valuable resource for predicting the likely fate of caddisfly species and ecological consequences under climate change in the GNP and for similar stream ecosystems elsewhere.
Trichoptera are holometabolous insects with aquatic larvae that are found in a broad range of freshwater habitats worldwide. More than 14,000 extant species have been described from 49 families (Trichoptera Checklist Coordinating Committee 2005) and occupy a particularly diverse range of trophic niches and microhabitats (Mackay & Wiggins 1979). Diptera is the only other aquatic insect order with comparable levels of diversity (Holzenthal et al. 2007). Leptoceridae is a cosmopolitan family and is one of the three largest families of Trichoptera, with approximately 1,800 described species within 46 extant genera (Holzenthal et al. 2007). Leptoceridae is also amongst the most common trichopteran families found in temperate Australia, represented by over 70 species from 15 genera (Gooderham & Tsyrlin 2002). The genus Lectrides is endemic to Australia and consists of two named extant species, *L. varians* (Mosely) and *L. parilis* (Neboiss) (Mosely & Kimmins 1953), with another unnamed species (*Lectrides* sp. AV1) from Western Australia (St Clair 2000). *Lectrides varians* has been recorded most often from Victoria and Tasmania, however its distribution also extends through New South Wales to northern Queensland (Fig. 1; Towns 1983; St Clair 1994). The species is known to occupy a wide range of aquatic habitats in warm and cool climates including lakes and streams, alpine and lowland areas, temporary or permanent waters, and, as its name suggests, the species exhibits morphological variation (St Clair 1994). Whether these variants represent plasticity or species-level differences has not been investigated. Consequently, an independent assessment using molecular markers will validate the reliability of current diagnostic morphological characters and clarify the taxonomy of this important invertebrate taxon.

The aim of this study is to investigate the population genetic structure of *L. varians* from the GNP using DNA sequence data from the mitochondrial *cytochrome oxidase subunit I* gene (*COI*) to infer the dispersal potential of this species. These data will complement existing information on drought resistance.
traits and provide a basis for predicting the likely response of *L. varians* to future environmental disturbances, including those associated with climate change. In addition, a comprehensive phylogenetic analysis of *L. varians* from Victoria and Tasmania was conducted to determine the evolutionary significance of the GNP genotypes and address current conjecture around the species taxonomy. This analysis was complemented by assessments of morphological characters to test the reliability of current diagnostic markers and strengthen interpretations of the taxonomic status of *L. varians* in south eastern Australia.

**Hypothesis**
The following hypothesis was tested in this investigation:
1) *L. varians* adults are strong dispersers and will show no genetic structuring between populations within the Grampians NP or across its range in Victoria.

**Methods**

**Larval Sampling**
Approximately 200 *L. varians* larvae were collected from nine GNP sites (4-15 samples per site) between 2006 and 2012. Samples were collected by sweep netting and picking individuals by hand from leaf packs, and were then kept alive for transport and preserved individually in 100% ethanol. An additional 21 specimens from 18 sites across Victoria and Tasmania (mostly from the Glenelg River catchment) were collected by the Environmental Protection Agency, Victoria River Health Unit between 2011 and 2012 and supplied for analysis. Sample sites and collection details are provided in Table 1 and Figure 1.

**DNA Extraction and Sequencing**

Total genomic DNA extractions were obtained using a modified Chelex® extraction protocol (Walsh *et al.* 1991). One leg from each individual was rested
to evaporate the excess ethanol and crushed with a pestle in a 1.5-mL Eppendorf-tube, containing 2 μl of proteinase K from Roche (Penzberg, Germany). Following this, 150 μl of 5% Chelex® 148 resin from Bio-rad laboratories (Hercules, CA) was added and the sample incubated at 60°C for 1 hour and then immediately afterwards at 80°C for an additional 8 min. Tissue extractions were cooled on ice and stored at -20°C until required for analysis. Prior to PCR, extractions were homogenized by inversion and centrifuged at 13,000 rpm for 2 min. Supernatant was subsequently taken for PCR from the bottom half of the supernatant above the Chelex® 153 resin precipitate. A 710 base pair (bp) fragment of the mitochondrial COI gene was successfully amplified and sequenced for 81 individuals representing eight sample locations in the Victoria Range, GNP (Table 1). Polymerase chain reaction (PCR) amplification of mitochondrial COI gene used primers LCO1490 and HCO2195 (Folmer et al. 1994) and was performed in a 20 μl reaction volume containing 2 μl of template DNA (from the extraction above), 0.2 units Taq polymerase from NEB (Ipswich, MA), 1 mM of dNTPs, 0.2 μM of each primer, and 1x reaction buffer. PCR conditions were as follows: 94°C for 2 mins, followed by 35 cycles of 92°C for 30 s, 50°C for 40 s, 72°C for 45 s, and a final extension of 72°C for 3 mins. PCR products were purified and sequenced in both directions by Macrogen Inc. (Seoul, South Korea) on an ABI3730XL DNA sequencer.

**POPULATION DIFFERENTIATION**

Consensus gene sequences were aligned and edited for all individuals using Geneious version 5.6.5 (Biomatters 2012). Shared haplotypes were identified and uncorrected pairwise genetic distance (%) were calculated using Geneious as per standard population genetics analysis. This simple non-parametric distance measure uses matrices of pairwise genetic differences from sequence data to achieve reliable estimates of both intra- and interspecific genetic
variation (Templeton et al. 1992). Genealogical relationships between mitochondrial haplotypes were inferred from a haplotype network (Templeton et al. 1992). Unrooted networks, which do not assume an ancestral grouping a priori, were generated with TCS version 1.21 (Clement et al. 2000), using maximum parsimony to connect haplotypes with a 95% confidence interval. Arlequin version 3.5 (Excoffier & Lischer 2010) was used to estimate global $\Phi_{ST}$ (analogous to Wright’s $F$ statistic - $F_{ST}$) and pair-wise measures of $\Phi_{ST}$ among sample locations from the GNP (Table 1). Significance levels were determined using 10,000 permutations and corrected using the sequential Bonferroni procedure (Rice 1989). An analysis of molecular variation (AMOVA) was also performed in Arlequin with pairwise $\Phi_{ST}$ as the distance measure using 10,000 permutations and a model for analysis that involved partitioning variation among and within sample locations. Regression and Mantel tests of linearized $\Phi_{ST}$ transformation ($\Phi_{ST} / (1 - \Phi_{ST})$; Rousset 1997), with the natural log of geographical distance were calculated using GenAlex (Peakall & Smouse 2006). Significance of Mantel tests was determined by permutation (10,000 randomisations). Population genetic analysis was only performed on selected populations from the GNP, whereas phylogenetic analysis involved all populations (Table 1).

**PHYLOGENETIC ANALYSIS**

Phylogenetic reconstructions were performed via standard methodology using unique haplotypes only and were generated by Bayesian Inference (BI) methods in MrBayes version 3.2 (Ronquist & Huelsenbeck 2003). Prior to BI analyses, MrModeltest (Nylander 2004) was used to identify an optimal nucleotide substitution model for the dataset (GTR+I+G) under the Akaike Information Criterion (AIC) (Akaike 1973). BI analyses consisted of two parallel Metropolis Coupled Markov Chain Monte Carlo (MCMC) runs with four chains each and
a temperature setting of 0.02. The temperature parameter value was optimised from the default setting to improve chain mixing and the efficiency of Metropolis coupling. A total of 10 million generations were run with convergence of parallel runs indicated by average standard deviation of split frequency values being less than 0.01. Burn-in and convergence for each run was determined via assessment of likelihood score stabilisation using the software Tracer (Drummond & Rambaut 2007). Post-burn-in trees were summarised as a 50% majority-rule consensus tree with posterior probabilities as nodal support. All analyses started with a random starting tree and seed with no root specified. The phylogeny was constructed using *Leptorussa darlingtoni* and a variety of *Triplectides* species as outgroup taxa (Genbank accession numbers provided in Fig. 3).

**MORPHOLOGICAL ANALYSIS**

Identification of all larval specimens was verified using a current taxonomic key (St Clair 2000). Samples were examined using 50 x magnification, and drawings made following St Clair (2000). Photographs were taken using a Motic Moticam5 digital camera. Multiple samples from 18 sites representing each ancestral lineage were examined for morphological differences (Table 1). In particular, the head capsule, mid and hind legs, sternum and meso-sternum and the meso-abdomen were compared between the genotypes, as these characters are most commonly used in the morphological delineation of caddisflies (St Clair 1994). Case morphology was also examined.
RESULTS

POPULATION DIFFERENTIATION

Initial assessment of the COI dataset indicated the presence of two genetically divergent *L. varians* lineages, one confined to Deep Creek and the other distributed over the remaining seven GNP sites. Pairwise distance measures indicated an 18.4% difference between the two lineages suggesting possible taxonomic differences. Consequently, the 10 Deep Creek samples were omitted from further analysis in order to avoid biasing population genetic measures.

From the remaining 71 individuals representing seven GNP sites, 26 haplotypes were identified, three of which were found at multiple sample locations. Haplotype 1 was found at five sites accounting for 13% of individuals, haplotype 3 was found at six sites accounting for 15% of individuals and haplotype 5 was found at seven sites accounting for 13% of individuals. Global $\Phi_{ST}$ was not significantly different from zero ($\Phi_{ST} = 0.03, P > 0.05$) indicating gene flow and lack of genetic structuring across sampling locations. Similarly, all pairwise comparisons of $\Phi_{ST}$ among sample locations did not differ significantly from zero indicating panmixia (Table 2). An AMOVA showed that the vast majority of genetic variation was observed within sites ($97\%; P < 0.0001, df = 70, SS = 31$) while 3% of variation was explained by between-site variation ($P < 0.0001, df = 6, SS = 7$), emphasising the lack of genetic structure within the dataset.

Regression analyses and a Mantel test suggest a non-significant association between genetic distance and geographic distance (Regression $R^2 = 0.012$; Mantel $r = -0.110, P = 0.360$) again supporting a high degree of gene flow among sample sites (Fig. 2). The genealogical relationships among haplotypes also revealed minimal genetic differentiation between locations as the distribution of haplotypes provided no apparent geographical pattern (Fig. 3). All
mitochondrial haplotypes have been submitted to Genbank (accession numbers KC348473-KC348498).

**PHYLOGENETIC ANALYSIS**

All specimens used in the current study were identified as *L. varians* using current morphological identification keys, however, phylogenetic analyses indicate *L. varians* is clearly paraphyletic (Fig. 4). Analysis of 245 individuals from 18 sites across Victoria and Tasmania identified two highly-supported monophyletic ancestral clades, each including one of the GNP lineages (depicted as blue and red colour-coded clades in Fig. 4). Clade 1 (coloured blue in Fig. 4) appears to have a broad distribution encompassing Victoria and Tasmania and has two well-supported sister clades. The sister clades represent haplotypes from the Nicholson River (Tambo Catchment) and haplotypes (FN600976 and FN600977) from an unknown location listed on Genbank (Malm & Johanson 2011), respectively. Unfortunately, the authors could not be contacted to confirm the source locality of the FN600976 and FN600977 haplotypes. In comparison, Clade 2 (coloured red in Fig. 4) consists of a stand-alone lineage and appears geographically limited to the GNP. Individuals belonging to the two ancestral clades were found in sympatry at both catchment and stream scales within the GNP. Individuals from both clades were collected from the same site at Mt William Creek and both were found within the Glenelg River catchment (Table 1). Mean uncorrected pairwise genetic distances within *Lectrides* Clades 1 and 2 were 0.06% and 1.0%, respectively, while the mean genetic distance between the clades was 14.0%. Mean genetic distance between Clade 1 and its two sister clades was 13.3% (ranging from 12.4% to 14.4%; Fig. 4). In comparison, mean genetic distance between the two outgroup taxa, *Leptorussa* and *Triplectides*, was 24.8% and these differed from Clades 1 and 2 by 24.1%
and 21.6% (*Leptorussa*), and 18.9% and 17.7% (*Triplectides*), respectively. An analysis of 61 species and all 16 families of Trichoptera known from New Zealand by Hogg et al. (2009) found genetic distances between species within genera averaged 11.5% (range 2.3 to 19.5%), whereas species in different genera showed an average divergence of 21.7% (range 3.2 to 29.4%)

*MORPHOLOGICAL ANALYSIS*

Two distinct *L. varians* morphotypes corresponding with the two ancestral *L. varians* clades were identified using current morphological keys. The first of the two morphotypes was characterised by extremely pale or absent metanotal and metasternal sclerites and the absence of banding on the hind legs, consistent with St Clair (2000) (Fig. 5 and 6). The second morphotype showed distinct sclerotisation and hind leg banding and will henceforth be called ‘dark’. The morphotype where both were absent will be referred to as ‘light’. The dark morphotype is restricted to genotypes from ancestral Clade 1 and is consistent with what has previously been found across Victoria and current keys for *L. varians*. Conversely, the light morphotype was restricted to the ancestral GNP Clade 2. These associations were further confirmed by genotyping a subset of specimens from each site, providing confidence in the association between phenotype and genotype. Although genetic differentiation between Clade 1 and its two sister clades also indicate potential species-level differences, no morphological differences were observed between these three indicating the possibility of cryptic species. Analysis of case morphology revealed no significant difference between genetic lineages.
CURRENT AND REVISED KEYS TO LECTRIDES VARIANS

Key taken from “Preliminary Keys for the Identification of Australian Caddisfly Larvae of the Family Leptoceridae” (St Clair 2000). The second key shows amendment at Step 3 separating L. varians and Lectrides sp. AV2.

Key Prior to Present Study

Key to mature larvae of known Australian species of Lectrides
1 Metasternum with one large and two small sclerites and numerous very short dark setae, case rounded................................................................. Lectrides sp. AV 1
Distribution: WA

- Metasternum with at least eight small sclerites, no large sclerites or very short setae, case very flat................................................................. 2

2 Found in Western Australia......................................................... Lectrides parilis
Distribution: WA

- Found in eastern Australia......................................................... Lectrides varians
Distribution: Northern Qld, NSW, Victoria, Tasmania, South Australia
Revised Key Based on Findings from Present Study

1 Metasternum with one large and two small sclerites and numerous very short dark setae, case rounded.................................................................*Lectrides sp. AV* 1
Distribution: WA

- Metasternum with at least eight small sclerites, no large sclerites or very short setae, case very flat.........................................................................................................2

2 Found in Western Australia.................................................................*Lectrides parilis*
Distribution: WA

- Found in eastern Australia.................................................................................................................................3
Distribution: Northern Qld, NSW, Victoria, Tasmania, South Australia

3 Metasternum with at least eight small well-defined sclerites, distinct striation on hind leg, medial and lateral metanotal sclerites dark and well defined.................................................................................................................................*Lectrides varians*
Distribution: Northern Qld, NSW, Victoria, Tasmania, South Australia

- Metasternum with faint or absent sclerites, striation on hind leg very faint or absent, medial and lateral metanotal sclerites very faint or absent.................................................................................................................................*Lectrides sp. nov.*
Distribution: currently known from the Grampians National Park only
DISCUSSION

LIFE-HISTORY ADAPTATIONS OF LECTRIDES VARIANS

Evidence of gene flow and a lack of genetic structuring across 30 km of the GNP ranges indicates _Lectrides varians_ has a high dispersal capacity. Although estimates of genetic structure were derived from only 346 y a single maternally-inherited genetic marker, this marker has been used successfully to demonstrate high levels of genetic structuring in other invertebrate groups along the same sampling gradient in the GNP (Chester _et al._ unpublished data). These findings are also consistent with previous genetic studies of other large caddisflies, such as _Cheumatopsyche_ (Hydropsychidae) (Baker _et al._ 2003) and _Plectrocnemia conspersa_ (Polycentropodidae) (Wilcock _et al._ 2005), where little genetic structure was observed over distances of up to 80 km. These findings suggest that _L varians_ is potentially capable of dispersing to more favourable habitats as an adult in response to drought conditions and with eventual recolonisation of drought-affected habitats when flows resume. Broad dispersal is also likely to enhance the resilience of this species by maintaining large population sizes and adequate genetic diversity in quantitative traits that enhance its ability to respond to environmental stress by the process of natural selection (Hedrick 2011). Previous research indicates that _L. varians_ exhibits several additional life history traits that should facilitate resistance to drought conditions. Field observations suggest that larvae are able to withstand extended dry periods by occupying refuge pools, or by aestivating (Chester & Robson 2011, Wickson _et al._ 2012). Therefore, the combination of adult dispersal and larval aestivation should facilitate the persistence of GNP _L. varians_ under climate change induced drying (Robson _et al._ 2011). However, aestivation is likely to have fitness costs, with increased larval mortality under extended drought conditions (Masaki 1980). Consequently, _L. varians_ may
become increasingly dependent on adult dispersal to combat severe drought conditions under climate change. Given that the *L. varians* lineage included in the population genetics study appears to be endemic to the GNP (Clade 2; red in Fig. 4), the maintenance of perennial water sources in the GNP are likely to be critical for the persistence of this species. In contrast, the other *L. varians* lineage (Clade 1; blue in Fig. 4) has a much broader geographical distribution potentially indicating more relaxed habitat constraints, and is also likely to be a strong disperser given this appears to be a common trait of large caddisflies. Consequently, this lineage may be comparatively more resilient to changing environmental conditions than the lineage restricted to the GNP.

**EVIDENCE OF A SPECIES COMPLEX**

Phylogenetic analyses indicated that *L. varians* is paraphyletic and consists of two monophyletic and genetically divergent ancestral lineages. These lineages have overlapping, but varied distributions, with one appearing to be limited to the GNP, and the other having a broader distribution encompassing (at least) north central and south western Victoria (including GNP), and Tasmania. We also recorded a single case of sympatric occurrence in a tributary of Mt William Creek (GNP). Multiple sources of evidence suggest that *L. varians* is likely to be a complex of species. Strong evidence for the monophyly of each *L. varians* lineage provides support for the delineation of species based on principles defined by the phylogenetic species concept (Wheeler & Platnick 2000). Also, given that *L. varians* has a large dispersal potential, it seems unlikely that dramatic genetic disjunctions on such fine spatial scales (i.e. within a stream or catchment) would be observed in the absence of reproductive isolation. The fact that these lineages were found in sympatry support a scenario for reproductive isolation and further evidence of a species complex, although this is yet to be formally tested. Genetic distance measures between *L. varians* ancestral clades are
consistent with interspecific estimates reported for other leptocerid genera, providing further weight to our argument towards the recognition of a species complex. However species delineation based on ‘genetic yardstick’ approaches is a very crude taxonomic measure and should be interpreted with caution as nucleotide substitution rates often vary considerably between taxonomic groups (Mayr 1996).

Further support for the recognition of separate species is evident in the morphological analysis. Clear morphological differences between Clades 1 and 2 were revealed, supporting the phylogenetic results and species delineation (Lee 2003). These morphotypes were originally identified by St Clair (1994) following a review of Victorian larval descriptions and we have now incorporated these characters into a revised identification key for Lectides larvae (Table 3). The type specimen of *L. varians* was described from Tasmania in 1953 (Mosely & Kimmins 1953) suggesting new taxonomic nomenclature is likely needed for Clade 2 identified in this study. Further cryptic speciation within *L. varians* is likely, as a high level of genetic differentiation was observed between Clade 1 and its two sister clades (comparable to interspecific divergence estimates in other leptocerids), although obvious morphological differences are not apparent. We suggest a more comprehensive morphometric analysis needs to be conducted to clarify the taxonomy of this ancestral group.

This study highlights the potential limitations in traditional taxonomic classification of Australian caddisflies that are based solely on morphological traits. Current taxonomy of *Lectrides* is based primarily on adult morphological traits, with external male genitalia being the primary diagnostic character (de Moor & Ivanov 2008). However, we have demonstrated that reliable taxonomic classifications can be difficult when characters are variable. It has been estimated that only 20 to 25% of trichopteran species have been described
worldwide (de Moor & Ivanov 2008), therefore this study emphasises the importance of consulting molecular markers for resolving patterns of cryptic speciation and validating taxonomic classifications.

**CONCLUSION**

Caution is now recommended when interpreting previous studies of *L. varians* ecology. Additional work will be needed to determine whether biological and ecological patterns are common to all species and whether previous measures have been influenced by incorrect species assignment. Further research is also required to confirm the geographic range of the new putative species described in this study and to determine whether this group is indeed restricted to the GNP. If so, this species would join a growing list of aquatic invertebrate known to be endemic to this region (Johnston & Robson 2009; Chester & Robson 2011).

The GNP is emerging as an evolutionary hotspot for freshwater biodiversity, however the region is facing significant environmental pressures other than climate change, including agriculture practices (nutrient loading and direct modification of aquatic habitats), habitat disturbance through water extraction for municipal uses and invasive species (Parks Victoria 2003). In addition, the GNP is highly isolated from areas of similar habitat and is particularly fire-prone (Enright & Goldblum 1999). Consequently, future genetic studies will be highly useful for identifying patterns of uniqueness and evolutionary processes shaping biodiversity in this region. This information should then be incorporated into future GNP conservation planning to ensure biodiversity is preserved in the face of future environmental pressures. These findings are directly relevant for other areas worldwide which harbour high levels of species diversity and endemism in environments under increasing levels of threat, indicating that incomplete knowledge of the phylogeny of species under threat can influence the likely success of conservation efforts.
ACKNOWLEDGEMENTS

This project was undertaken as part of the PhD research of S. Wickson at Deakin University and financial support for this study was provided through an Australian Postgraduate Award. Field collections were conducted under Permit No. 10004699 from the Department of Environment and Primary Industries, Victoria. Thanks to Dr Ros St Clair from the Victorian Environmental Protection Agency for provision and identification of samples. Many thanks also go to Associate Professor Belinda Robson for assistance in development of the manuscript.

TABLES

Table 1 Details of sample locations and collection sources for specimens used in this study. Also, details of analysis applied to specimens from each site Phy = phylogenetic analysis (number of individuals analysed provided in parentheses), Pop = population genetic analysis, the diagnosed colour morph, and the haplotypes and frequency (provided as number of individuals in parentheses beside haplotype number) for each site. Haplotype numbers correspond with those presented in Figure 4. GNP = Grampians National Park. Some sites had individuals collected at multiple dates, with both years displayed.
<table>
<thead>
<tr>
<th>Locality</th>
<th>Source</th>
<th>Catchment</th>
<th>Analysis</th>
<th>Date Collected</th>
<th>Morph</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Haplotypes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep Creek, GNP</td>
<td>Author</td>
<td>Glenelg</td>
<td>Phy</td>
<td>2006/12</td>
<td>Dark</td>
<td>-37.439</td>
<td>142.209</td>
<td>34 (4), 41(1), 43(6), 45(1),</td>
</tr>
<tr>
<td>Alex Creek at GNP</td>
<td>Author</td>
<td>Glenelg</td>
<td>Phy/Pop</td>
<td>2006</td>
<td>Light</td>
<td>-37.398</td>
<td>142.210</td>
<td>3(7), 6(2), 7(1), 18(2), 19(1), 20(1), 21(1)</td>
</tr>
<tr>
<td>Wannon River, GNP Tourist Road</td>
<td>Author</td>
<td>Glenelg</td>
<td>Phy</td>
<td>2012</td>
<td>Dark</td>
<td>-37.439</td>
<td>142.461</td>
<td>6(1), 27(1), 32(1), 37(1), 38(1), 42(1)</td>
</tr>
<tr>
<td>Muline Creek at GNP</td>
<td>Author</td>
<td>Glenelg</td>
<td>Phy/Pop</td>
<td>2006</td>
<td>Light</td>
<td>-37.205</td>
<td>142257</td>
<td>3(1), 6(1), 13(1), 29(1)</td>
</tr>
<tr>
<td>Red Rock Creek at GNP</td>
<td>Author</td>
<td>Glenelg</td>
<td>Phy/Pop</td>
<td>2006</td>
<td>Light</td>
<td>-37.223</td>
<td>142.252</td>
<td>1(1), 2(1), 3(1), 4(1), 5(1), 6(1), 7(1), 8(1), 9(1), 10(1)</td>
</tr>
<tr>
<td>Honeysuckle Creek at GNP</td>
<td>Author</td>
<td>Glenelg</td>
<td>Phy/Pop</td>
<td>2006/12</td>
<td>Light</td>
<td>-37.341</td>
<td>142.228</td>
<td>1(2), 3(1), 6(2), 9(3), 21(2), 24(1), 25(1), 27(1), 28(1), 29(1), 30(1), 31(1), 32(1)</td>
</tr>
<tr>
<td>Camp Creek at GNP</td>
<td>Author</td>
<td>Glenelg</td>
<td>Phy/Pop</td>
<td>2006</td>
<td>Light</td>
<td>-37.341</td>
<td>142.228</td>
<td>1(2), 3(1), 6(4), 10(1), 11(1), 12(1), 13(1), 14(1), 15(1), 16(1)</td>
</tr>
<tr>
<td>Number 1 Creek at GNP</td>
<td>Author</td>
<td>Glenelg</td>
<td>Phy/Pop</td>
<td>2006</td>
<td>Light</td>
<td>-37.355</td>
<td>142.213</td>
<td>6(1), 12(1), 21(1), 28(1)</td>
</tr>
<tr>
<td>Mt William Creek tributary at Moyston West Road</td>
<td>EPA</td>
<td>Wimmera</td>
<td>Phy</td>
<td>2012</td>
<td>Dark /Light</td>
<td>-37.331</td>
<td>142.650</td>
<td>48(2), 49(1)50(1), 51(3), 54(3)</td>
</tr>
<tr>
<td>Troopers Creek at GNP</td>
<td>Author</td>
<td>Wimmera</td>
<td>Phy/Pop</td>
<td>2006</td>
<td>Light</td>
<td>-37.016</td>
<td>142.426</td>
<td>1(1), 4(1), 13(1), 14(1), 17(1)</td>
</tr>
<tr>
<td>Locality</td>
<td>Source</td>
<td>Catchment</td>
<td>Analysis</td>
<td>Date Collected</td>
<td>Morph</td>
<td>Latitude</td>
<td>Longitude</td>
<td>Haplotypes*</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------</td>
<td>-----------</td>
<td>----------</td>
<td>----------------</td>
<td>-------</td>
<td>-----------</td>
<td>-----------</td>
<td>-------------</td>
</tr>
<tr>
<td>Kennedys Creek at Kennedys Creek town site</td>
<td>EPA</td>
<td>Otway</td>
<td>Phy</td>
<td>2012</td>
<td>Dark</td>
<td>-38.587</td>
<td>143.258</td>
<td>53(2)</td>
</tr>
<tr>
<td>Asplin Creek</td>
<td>Author</td>
<td>Otway</td>
<td>Phy/Pop</td>
<td>2006</td>
<td>Dark</td>
<td>-38.344</td>
<td>143.376</td>
<td>22(11), 23(1)</td>
</tr>
<tr>
<td>Nicholson River U/S Morgan Creek</td>
<td>EPA</td>
<td>Tambo</td>
<td>Phy</td>
<td>2012</td>
<td>Dark</td>
<td>-37.728</td>
<td>147.694</td>
<td>46(1), 47(1)</td>
</tr>
<tr>
<td>Rodger River at Varney’s Track</td>
<td>EPA</td>
<td>Snowy</td>
<td>Phy</td>
<td>2012</td>
<td>Dark</td>
<td>-37.407</td>
<td>148.362</td>
<td>54(1)</td>
</tr>
<tr>
<td>Fitzroy River, Cobbobonee State Forest</td>
<td>EPA</td>
<td>Portland</td>
<td>Phy</td>
<td>2009</td>
<td>Dark</td>
<td>-38.073</td>
<td>141.429</td>
<td>35(1), 36(1), 44(1)</td>
</tr>
<tr>
<td>Creek with waterfall, Hartz Road, Hartz Mountain National Park (Tasmania)</td>
<td>EPA</td>
<td>Phy</td>
<td>2011</td>
<td>Dark</td>
<td>-43.215</td>
<td>146.769</td>
<td>52 (2)</td>
<td></td>
</tr>
</tbody>
</table>
**Table 2** Pairwise $\Phi ST$ estimates among five *Lectrides varians* sample locations. No significant differences (P<0.001) in $\Phi ST$ values were identified after 16,000 permutations and corrections for multiple comparisons. Number of individuals genotyped per site in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Red Rock</th>
<th>Camp</th>
<th>Alex</th>
<th>Honeysuckle</th>
<th>Number 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Rock</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camp (14)</td>
<td>-0.019</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alex (15)</td>
<td>0.051</td>
<td>0.083</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honeysuckle</td>
<td>0.009</td>
<td>0.025</td>
<td>0.105</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>(23)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number 1 (4)</td>
<td>-0.026</td>
<td>-0.049</td>
<td>0.09</td>
<td>0.019</td>
<td>*</td>
</tr>
<tr>
<td>Muline (6)</td>
<td>-0.053</td>
<td>-0.070</td>
<td>-0.024</td>
<td>0.030</td>
<td>-0.067</td>
</tr>
</tbody>
</table>

**FIGURE CAPTIONS**

**Fig. 1** Map of *Lectrides varians* collection sites from south eastern Australia, including (A) the Grampians National Park, and (B) Victoria and Tasmania (including the Grampians region, outlined in yellow). Colour coding of site locations reflects phylogenetic assignment to ancestral clades (blue = Clade 1, red = Clade 2, purple = sympatric occurrence, yellow = unique lineage sister to Clade 1).

**Fig. 2** Regression analysis for the *Lectrides varians* mitochondrial dataset, linearized $\Phi ST$ against the natural log of the pairwise geographical distance (km). Scores for the accompanying Mantel test is $r = -0.110$, $P = 0.360$.

**Fig. 3** Haplotype network of *Lectrides varians* from the Grampians National Park reconstructed by maximum parsimony. Each circle represents a unique haplotype and sizes are roughly proportional to haplotype frequency within the
dataset. Black lines separated by black shaded circles on connecting branches between haplotypes indicate single base mutations. Colours indicate site where haplotypes were detected (site locations provide as inset).

**Fig. 4** Phylogenetic reconstruction of ancestral relationships among *L. varians* haplotypes (Table 1). Nodal support values provided represent Bayesian posterior probabilities (>0.5). Major ancestral clades present in the GNP are colour differentiated, and an assignment of clade specific morphotypes is provided. Blue clade is the Victoria wide dark morph and the red clade represent the light morph restricted to the GNP.

**Fig. 5** Drawing of key differences between the dark and light morphotypes identified as *L. varians*. (A) leg, dark morph, (B) leg, light morph, (C) meso- and metasternum, dark morph (D) meso- and metasternum, light morph, (E) thorax dorsal, dark morph, (F) thorax dorsal, light morph. Setae on the pronotum are not shown. Arrows highlight significant taxonomical difference between morphotypes.

**Fig. 6** Photographs of the key differences between the dark and light morphotypes identified as *L. varians*. (A) leg, dark morph, (B) leg, light morph, (C) meso- and metasternum, dark morph (D) meso- and metasternum, light morph, (E) thorax dorsal, dark morph, (F) thorax dorsal, light morph. Setae on the pronotum are not shown. Arrows highlight significant taxonomical difference between morphotypes (Table 3).
Figure 1

Figure 2

\[ y = -0.0048x + 0.04 \]

\[ R^2 = 0.012 \]
Figure 5.
Figure 6.
CHAPTER 3  AESTIVATION PROVIDES FLEXIBLE MECHANISMS FOR SURVIVAL OF STREAM DRYING IN A LARVAL TRICHOPTERAN (LEPTOCERIDAE)

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, but over 30% of larvae did not become active until 72 h after re-immersion. L. varians can survive extended periods without surface water (112 days), 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 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 & 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 & 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 & 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 & 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 three months (Pörschmann & Speith 2011), and some mosquito species having adult dormant phases lasting up to eight 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 & 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 approximately 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, a laboratory experiment was used 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 & 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 & 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 & 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(a); Chester & 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. There was no reason to expect that individuals would become active immediately after rewetting, so individuals were observed over a long period to ensure that mortalities were not overestimated. 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 & 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 \( \text{Probit}(\pi(x)) = a + \beta x \), 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 (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 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 four 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(a); Chester & 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 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 (Sterns 1976; Southwood 1977; Winterbourne 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 & 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 & 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 & Robson 2011), although this is not always the case (Chessman 2009). 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

I would like to thank Rebecca Lester and Ty Matthews for their assistance in reviewing this paper and Alecia Bellgrove for her assistance with supervision of SW. Parks Victoria is thanked for permitting the collection of live samples and the Deakin University Warrnambool Aquaculture Group for their kind support of materials for this experiment. Financial support for this study was provided to SW via an Australian Postgraduate Award.
Figure captions

**Figure 1.** Survival estimates from Probit regression (3 levels) over the experiment’s duration (0 to 16 weeks) for each treatment (solid lines), with 95% confidence intervals (dashed lines) based on 3 replicate microcosms per treatment per time ($n = 54$). Mean counts for microcosms are also plotted (open circles).

**Figure 2.** Histogram showing the percentage of surviving *L. varians* larvae that responded to rewetting of sediments from the moist and dry treatment over a period of three days.
Figure 1.
Figure 2.

[Graph showing post-rewet survival over time with bars for Moist and Dry conditions.]
CHAPTER 4 PUPAL SITE SELECTION BY A LEPTOCERID CADDISFLY IN STREAMS WITH VARIABLE FLOW REGIMES

ABSTRACT

Populations are rarely dispersed randomly across a landscape, nor are resources and habitat. These patchy distributions influence population dynamics and are important for ecologists to understand ecosystem function. Patchiness and aggregation can occur over a range of scales and any or all life stages of an organism can show patchiness in their distributions. *Lectrides varians* (Mosely) is a large shredding caddisfly and the most common aquatic macroinvertebrate found in the Grampians National Park, Australia and its resource use may influence population dynamics of aquatic macroinvertebrate communities in the region. Anecdotal evidence indicated that pupae of *L. varians* could be consistently found clustered on large rocks in deep stream sections and other clusters were likely to be found nearby. Data were collected from five streams within the Grampians National Park, Victoria, Australia, to determine whether pupae were actually aggregating. If so, whether this occurred on specific types of resources, such as larger rocks in deeper water and whether these aggregations were spatially clustered was also to be determined. Rocks with *L. varians* pupae were found to be significantly larger than those rocks without pupae. An association between pupal clusters and increasing water depth and rock size was found. However, no consistent spatial pattern was found that described the arrangement of pupal aggregations. Late-stage larvae of *L. varians* appear to aggregate on larger rocks in deeper water prior to pupation, which may be due to environmental pressures or communal behaviour. The cues which drive this aggregation are unclear, but a number of advantages may be associated with this behaviour, including greater protection...
from drying, spates and predators, compared with individuals that pupate on their own, or on smaller rocks in shallower water. This lack of consistent spatial pattern differs from other similar studies that investigated the importance of resources for structuring the spatial arrangement of birds, plant and macroinvertebrates. Thus, resource availability does not appear to be the only factor influencing patterns of resource use, for this aquatic species, with abiotic disturbance (such as drying or flood) affecting distribution of individuals.

INTRODUCTION

The movement and habitat selection of individual organisms on small scales have the potential to influence much larger-scale ecological processes and patterns, including population dispersal and distribution, metapopulation dynamics and community composition (Levin 1992; Lancaster & Downes 2004, Lancaster 2006). Fine-scale structural patchiness of populations can therefore be of importance to landscape-scale ecology in streams (Hoffman et al. 2006). The naturally patchy arrangement of resources, as well historical and ecological pressures over a range of scales from the landscape to micro-habitat scales, often affects population and community structure (Poff 1997; Chesson 2000) with the distribution of those populations changing over different spatial scales (e.g. patterns of regular dispersal at small scales compared with clumping over larger scales) (Strand et al. 2007). What drives this patchiness in the distribution of populations is a question often pondered in population ecology.
In freshwater ecosystems, the classic explanation for the variable distribution of benthic invertebrate taxa is that drift dominates in-stream dispersal and delivers larvae to a random subset of available habitat patches (Downes & Keough 1998). However in reality, the distribution and availability of different habitat patches are important factors that can influence movement, dispersal and post-settlement survival and mortality (Townsend & Hildrew 1994). Factors that affect dispersal can be abiotic or biotic and may operate on populations or individuals (Meyer et al. 2007; Clarke et al. 2008; Wang et al. 2012). The presence or absence of individuals at a particular site can be due to active choice or active avoidance of particular location, post-settlement mortality, or simply ability to disperse to a given site (Downes et al. 2005).

It is uncommon for insects living in flowing waters to live predominantly in the pelagic zone; most are benthic. Life in fast-flowing water requires adaptations of behaviour, body shape or structure to avoid displacement in benthic and pelagic biota (Lancaster & Downes 2013). The movement, behaviour and resource use of benthic insects is strongly influenced by near-bed flow velocities and habitat heterogeneity (Hart & Resh 1980; Kohler 1984). Aquatic macroinvertebrates respond to the direction and force of stream flow by avoiding unfavourable flow conditions, creating asymmetry in the use of patches similar to that caused by wind in flying insect habitats (Hoffman et al. 2006). Other factors, in addition to environmental conditions, may also influence the distribution of biota, particularly at smaller scales. Animals may aggregate by exhibiting similar reactions to ecological conditions or by gregarious aggregation amongst populations (Gotceitas 1985). The location of organisms, such as trichopteran pupae, is unlikely to be random; the suitability of local environmental factors and/or the abundance of predators and parasites during the pupation period (Otto & Svensson 1981) are likely to alter distributions. Some trichopterans
have been known to aggregate in heterogeneous environments during pupal stages or as late larval instars (Svensson 1974; Otto & Svensson 1981; Gotceitas 1985). Two potential explanations can be suggested for pupal aggregation: either the larvae are exhibiting gregarious behaviour or the aggregation is driven by larvae responding similarly to a limiting or influential abiotic factor (Hoffman 1997).

The spatial arrangement of available habitat and post-selection survival can thus produce a variety of patterns in population distribution. Understanding how organisms interact with variable landscapes is key to comprehending population ecology because small-scale movements and processes combine to drive large-scale population dynamics (Lancaster & Downes 2004). The spatial patterns derived from ecological processes can often be described as point processes. A point process can include the result of any natural process which exists as set of geographical points. For example, the distribution of pupae on available pupation sites is an example of a process that could be described in this way. Analyses using point processes often include additional data on the properties of the points (e.g. type of substrate, depth of water, size of rock or velocity of flow) (Lancaster et al. 2003) to identify environmental factors that may be driving patterns. Those additional data can be coded as marks to identify potential external drivers that can then be tested (Dall 1979; Grabarnik, et al. 2011).

Social or biological inter- and intra-specific interactions may also shape spatial distributions. For example, social weavers (colonial nesting birds), have clusters of nests at small spatial scales as a result of communal nesting but, at larger scales, are regularly spaced with competition between communities leading to non-random distribution of nest sites (Giebelmann et al. 2008). Similar
discontinuities across spatial scales are likely to exist for other taxa due to the differential effects of resource distribution and biological interactions. The pattern of small-scale inhibition (i.e. interactions between groups or individuals which drive distribution at a sub-population level) is currently more commonly described in terrestrial landscapes (Olivier & Wotherspoon 2006; Strand et al. 2007; Giebelmann et al. 2008).

Insects exhibit complex life cycles, often with stages that inhabit different environments. These different habitats expose individuals to a range of physical and chemical conditions and pressures that vary as the individual moves through its life cycle as an egg, larva, pupa and finally, as an adult (Lancaster & Downes 2013). As such, different patterns are likely across different life-history stages and under different distributions of available resources. The relationship between resource-specific life-history stages (those which are dependent on a particular abiotic resource) and the spatial distribution of resources has previously been explored for trichopterans (Lancaster et al. 2003; Reich et al. 2011). These studies have showed that some hydrobiosid caddisflies exhibit clumping at small spatial scales when selecting oviposition sites, above the underlying clumping of potentially available oviposition sites (i.e. emergent rocks mostly appear along stream margins). In addition, different patterns of dispersion can exist between similar species, such as the two hydrobiosid species studied by Lancaster et al. (2003) and Reich et al. (2011). These patterns were more pronounced when aspects of patch quality were introduced (e.g. flow velocity), with one taxon (*Ulmerochorema*) being clearly clumped on rocks located within fast-flowing water, while another (*Apsilochorema*) exhibited a distribution that was over-dispersed. Over-dispersal occurs when points are distributed with more variability than would be expected due to chance alone (Lancaster et al. 2003). Similar patterns have not been explored at other life-history stages (e.g. pupae).
Extensive qualitative surveys of benthic macroinvertebrate fauna of the Grampians National Park, Victoria, Australia, suggested that aggregations of leptocerid caddisfly *L. varians* pupae were consistently found on the underside of the largest rock in the deepest section of a reach. Furthermore, where an aggregation of pupae was found on one rock, a number of other rocks with aggregations of pupae would be found nearby, with aggregations generally being found on the largest of these nearby rocks. The number of pupae per rock appeared to increase out of proportion with increasing rock size, so it appeared that factors other than rock size may also be important. To test these anecdotal observations, replicate surveys in different streams were used to investigate whether *L. varians* pupae were aggregating and, if so, which factors were associated with an aggregated distribution.

Two methods were used to test three hypotheses. The first test compared rock size and the presence or absence of pupae and was used to test Hypothesis 1. The second method mapped *L. varians* pupae across the stream bed, noting the number of pupae per rock as well as the surface area and depth of occupied rocks and tested Hypotheses 2 and 3. This provided a test of whether the clumping of sites used by similar caddisflies for oviposition (Lancaster *et al.* 2003) has an analogue in the pupal stages of *L. varians*, or whether patterns of small-scale inhibition better describe the test system. The aim of this study was to determine whether aggregations in *L. varians* pupae actually exist and, if so, at what spatial scale and associated with which environmental factors. This is important because it could provide a greater understanding of how *L. varians* is able to maintain self-sustaining populations in an increasingly dry and patchy environment.
Three alternative hypotheses were tested in this chapter:

1) Rocks occupied with pupae will be larger than rocks not occupied by pupae (thus indicating a relationship between rock size and the presence of pupae).

2) The spatial distribution of pupae on rocks will not be random, but either clumped or over-dispersed depending on the spatial scale (thus illustrating that pupae are exhibiting some differential use of available resources).

3) Rock size and water depth will be correlated with the number of pupae (micro-aggregation) and the number of pupal aggregations found on a given rock, beyond differences expected due to changes in available habitat area.
METHODS

STUDY SPECIES

*Lectrides varians* is a case-forming caddisfly from the family Leptoceridae. It occurs in a wide range of habitats, from alpine lakes and streams to temporary and permanent water bodies in both warm and cool climates throughout eastern Australia, from southern Queensland to Tasmania (Towns 1983; St Clair 1994). Leptocerid caddisflies are among the most abundant stream macroinvertebrates in the Grampians NP in south eastern Australia, with four species recorded (*Lectrides varians*, *Triplectides truncatus* Neboiss, *T. proximus* Neboiss and *T. similus* (Mosely & Kimmins 1953; Chester & Robson 2011). All four species are shredders and are important processors of coarse particulate organic matter (St Clair 1994). The pupae of *L. varians* have been found in clusters on the undersides of small submerged boulders in pool sections of streams throughout the Grampians National Park (personal observation).

*L. varians* eggs are laid in gelatinous masses on hard substrates at heights ranging between 10 and 20 cm above the waterline around permanent pools (Towns 1983). Oviposition occurs in late spring and early summer, with eggs developing rapidly following rain (with no recorded diapause) and hatching four to eight weeks later (Towns 1983). Larvae develop quickly, being able to maximise production during seasonal low-flow periods, and can grow from eggs to 5th instar in 135 days after hatching in captivity (Towns 1991). Larvae show low mobility compared to other trichopterans but consume allochthonous detritus and aggregate on leaf litter accumulations that are closely associated with backwater sections of streams (Towns 1983; St Clair 1994). Larval cases are made from allochthonous leaf litter, with the pupal case formed after modifying the larval case with an oblique silk closure at the anterior and a silk
membrane used to attach the case to the substrate (Jackson 1985). Larvae are able to aestivate for up to 16 weeks under leaf litter on damp sand when held in captivity (Wickson et al. 2012). Pupae appear to require a hard substrate, usually rocks or large woody debris (Jackson 1985), but *L. varians* in the streams studied here are only found on rocks and not on large wood. Very little is known about the ecology of pupae or adult stages of this species. As reported in Chapter 2, population genetics indicate that adults are highly mobile and that all larvae sampled within the Grampians NP form part of a single population (see chapter 2). It should be noted that, as stated in Chapter 2, it is likely that two separate species of *Lectrides* exist in the Grampians NP. This was not known at the time of sampling, so genetic testing was not carried out on pupae used from First and Second Wannon Creeks during this present study, nor could any larvae be found subsequently for morphological identification. Therefore I refer to patterns relating to the *Lectrides varians* species complex, but any as-yet-undescribed species-specific differences may influence the interpretation of these findings.

**STUDY SITES**

Surveys were conducted at five streams within the Grampians National Park Victoria, Australia; Hut (-37.1644, 154.1611), Mosquito (-37.2036, 154.1919) and Honeysuckle (-37.2944, 142.2597) Creeks from the Victoria Range, which is the westernmost of a series of mountain ranges running north-south in the Grampians NP and First (-37.3651, 142.5709) and Second (-37.3140, 142.5473) Wannon Creeks, which are located in the Mt William Range, which is the easternmost range of the series (refer to Robson et al. 2008(a) for a map). The region is classified as a Mediterranean to semi-arid climate (400 – 600 mm average annual rainfall, Chester & Robson 2011). Vegetation is dry sclerophyll woodland and forest, dominated by *Eucalyptus* species (Robson et al. 2005,
Flow is mostly derived from precipitation, but is supplemented by low-pressure springs arising from rock fissures (as opposed to seeps which percolate through permeable soils) (Robson et al. 2005, 2008(a); Chester & Robson 2011).

All study streams are best described as seasonal streams, being generally wet, but drying to disconnected pools over summer and autumn. The flow regimes of a number of streams within the Grampians NP have been affected by regulation and water abstraction and all streams investigated had reduced inflows as a result of drought during the study (Chester & Robson 2011). First and Second Wannon Creeks have diversions upstream of the study sites, but Hut, Mosquito and Honeysuckle Creeks are unregulated (Robson et al. 2008(b)). The study sites within each of the streams feature sandstone boulders and cobbles with areas of bedrock. Streams nearby in the region range from flowing permanently to having seasonal and intermittent flow (Chester & Robson 2011). No flow gauges are present on any of these streams, so detailed hydrological information is not available.

**SAMPLING METHODS**

Data were collected using two methods;

**Method 1**

To determine whether there was any relationship between rock size and the presence and absence of pupae (Hypothesis 1), as well as measuring the dispersion of pupae across the stream bed, a total of 121 rocks were collected, including 42, 33 and 44 from each of Honeysuckle, Mosquito and Hut Creeks, respectively. At each stream, three replicate samples were taken using a haphazardly-placed 1 m² quadrat within a 10 m section (as to include at least one riffle/pool sequence) at each stream (giving a total of nine replicates over
the three streams). Quadrats were placed haphazardly to avoid areas of unsuitable habitat (i.e. bedrock) and every stone (i.e. occupied and unoccupied by pupae) was picked up and measured (range of rock sizes sampled between 5 and 49 cm in length). Anecdotal observations prior to this experiment revealed that pupae did not occur on rocks that were smaller than 5 cm length. The use of three 1-m² quadrats was considered adequate given that the streams sampled were small, ranging from 1 - 2 m wide. The length (longest plane), width (longest plane at right angles to length), height (perpendicular to both length and width) and circumference (along the plane of length and width) of each rock (greater than 5 cm in length) was measured, along with the number of *L. varians* pupal cases. As rocks in these streams are uniformly smooth and ovoid in shape, surface area was estimated using the equation:

\[ SA = \pi/3 (L*W+L*H+H*W) \]

where \( L = \) length, \( W = \) width and \( H = \) height (Dall, 1979).

**Method 2**

The second method aimed firstly to provide data for point pattern analyses (Hypothesis 2) as well as determine whether a correlation existed between the number of pupae per rock and water depth and rock size (Hypothesis 3). Point pattern analyses were used to test for spatial clustering in pupal micro-aggregations (in this study a micro-aggregation is a group of two or more pupae per rock). It involved sampling a single site approximately 10 m long in each of three streams: First Wannon; Second Wannon; and Hut Creeks. Honeysuckle and Mosquito Creeks were not suitable for the second part of the study because pupae could not be found in sufficient densities over the required area. Sites were chosen that were known to have populations of *L. varians* with cobbles substrate and variable depth.
Every rock greater than 5 cm in length at each site was lifted and checked for pupae. Each rock with pupae was measured as described above and mapped using triangulation. Unoccupied rocks were not measured because the hypotheses for this survey related to clusters of pupae on occupied rocks only. The positions of occupied rocks (relative to an arbitrary reference point) were determined using trigonometry. The water depth to the top of each rock was measured relative to the mean strand line at each stream (i.e. the line marking interface between land and water on hard substrate, which was defined by a prominent stain on nearby rocks), and true depth was then calculated by adding the height of the rock. Positions of occupied rocks were converted to an x-y grid format, which were then mapped. This allowed for the spatial arrangement of occupied rocks and pupal clusters to be assessed using 10-m reaches because that typically included at least one riffle/pool sequence.

**DATA ANALYSIS**

**Method 1**

The hypotheses outlined above were tested using a univariate PERMANOVA with a two-way crossed factor design: pupae presence (random factor, 2 levels: pupal presence versus absence) and stream (fixed factor, 3 levels: First Wannon, Second Wannon and Hut Creeks), with the size of occupied and unoccupied rocks used as the units of replication. Rather than using an $F$ distribution, a test statistic of pseudo-$F$ was employed, due to the use of permutations (Anderson et al. 2008). The effect of rock size on the presence or absence of pupae was first tested using all sites, then at each site individually, following a significant interaction between stream and size of occupied versus unoccupied rocks in the initial analysis. Correlation or regression analysis was not suitable in this case due to the complexity of the study design.
Using the same quadrat data, a coefficient of dispersion was calculated to test for the micro-aggregation of \textit{L. varians} pupae. This was done using a coefficient of dispersion test which is a Chi-squared test comparing the observed distribution of pupae with that expected if the distribution of pupae per rock were random. This method provides a simple, but effective way of measuring whether points within a discrete area are dispersed randomly, clustered or over dispersed (more uniformly dispersed than random) but does not describe spatial distribution (Krebs 1989). A more complex method of describing clustering and spatial distribution was employed and is described in below in Method 2.

The Coefficient of Dispersion (CD) is calculated by:

\[
CD = \frac{\sigma^2}{\mu}
\]

where $\mu$ is the mean number of pupae per rock and $\sigma^2$ is the observed variance (Lancaster \textit{et al.} 2003). A CD of 1 indicates a random distribution, while a CD of $< 1$ indicates over-dispersion and a CD $> 1$ indicates clustering. No correction for rock size (e.g. using a measure such as pupae per cm$^2$) was utilized for the quadrat data as analyses were based on the number of pupae per rock as per Lancaster \textit{et al.} (2003).

\textbf{Method 2}

Marked point pattern analysis (MPPA) was used to determine whether pupae were randomly distributed, clustered or over-dispersed. It was also used to identify whether this distribution was consistent across the three study reaches (\textit{sensu} Lancaster 2006). MPPA is an extension of basic point pattern analysis (PPA). PPA is usually used to analyse the position of mapped locations of objects in a plane (Lancaster & Downes 2004). MMPA differs from PPA in that the
points in the MPPA have an associated characteristic or "mark" and allow for the spatial correlation of the marks to be measured against the neutral pattern (Lancaster 2006). The distribution of pupal micro-aggregations (i.e. where there was more than one pupa per rock) were used as marks within this analysis to compare the pattern of distribution of aggregations to the underlying neutral pattern (i.e. the distribution of all L. varians pupae, whether in micro-aggregations or not). This neutral pattern was used to decouple the effects of site selection from the impact of other factors related to resource availability (e.g. differential survival rates), so that the exploited resources could be examined within the context of the pattern of available resources across a landscape (Lancaster & Downes 2004). The effects of water depth and rock surface area on the distributions of pupal aggregations were also explored in the same way (i.e. as marks).

When undertaking a MPPA, Ripley’s $K$ function is often used to compare resource exploitation relative to the neutral landscape (Ripley 1976). Ripley's $K$ is a cumulative function which calculates the expected number of points within concentric circles of increasing radius $r$ for a given point pattern. A higher number of points within $r$ than would be found under Complete Spatial Randomness (CSR), where points are arranged in a fully random pattern, would be found in a clustered point pattern, while fewer points than CSR within radius $r$ would be found in an over-dispersed pattern (Perry 2004; Strand et al. 2007). For example, if more pupae were found in circles of smaller radii than larger radii, then, they would be considered to be clustered.

In a MPPA, the distances between all pairs of points in a dataset form the basis of neighbourhood analysis of point patterns within a particular distance, $t$, of each point. In this analysis, $t$ takes range of values (here $t$ ranges between 0.5 and 28 m). Edge corrections are often made when using Ripley’s $K$-function
(K(t)) (Lancaster & Downes 2004). This is used to give weighting to points near the edge of the study area as the real number of neighbouring points may be underestimated given that some will lie outside the study area boundary. In habitats which have “real” edges (such as aquatic-terrestrial boundaries) there will be no neighbouring points outside the boundary and any weighting may belie their significance (Lancaster & Downes 2004). In this study, the entire streambed was an available resource, but the surrounding terrestrial habitats were not, so an edge correction was not applied (as per the method used by Lancaster & Downes 2004). This constrains the choice of approach to one that compares the observed and null values of Ripley’s K.

It is common for K(t) to be presented in a linearized form as an L-function (Besag 1977). This linearized version is often easier to interpret than K(t) as, under CSR, L(t) = 0 for all t. The results presented for this MPPA represent the difference between the observed L-function and the L-function under CSR (L(t)\text{obs} – L(t)\text{CSR}; i.e. the observed value of L minus what would be expected for L under CSR), as required by the choice not to apply edge corrections. Monte Carlo permutations (n = 999) were used to develop the 95% confidence interval for the difference between L(t)\text{obs} and L(t)\text{CSR} where t = 0.5 m.

Thus, MPPA was used to differentiate between two spatial patterns (i.e. clustered versus non-clustered pupae) and to test whether the pattern of pupal distribution was significantly different between clustered and non-clustered pupae. It was also used to attempt to identify the extent to which the size and depth of rocks influenced those patterns. This analysis was performed on stream-bed mapping data collected at First and Second Wannon Creeks and Hut Creek. Code for MPPA was developed following the method of Lancaster and Downes (2004) using R (The R Foundation for Statistical Computing 2012).
RESULTS

RELATIONSHIP BETWEEN ROCK SIZE AND THE PRESENCE & ABSENCE OF PUPAE

There was a significant interaction between streams and the size of rocks with and without pupae (pseudo-$F_{1,117} = 35.2, p = 0.001$; Figure 1), when data from all streams were analysed together. When streams were analysed separately, rocks with pupae were significantly larger than those with no pupae in Hut (pseudo-$F_{1,42} = 6.4, p = 0.013$) and Honeysuckle Creeks (pseudo-$F_{1, 40} = 37.1, p = 0.001$), but not in Mosquito Creek (pseudo-$F_{1, 31} = 1.0, p = 0.33$, Figure 1). It should be noted that this data was not normally distributed, hence the use of standard error rather than standard deviations. All sites tested for coefficient of dispersion showed a CD greater than 1, indicating small scale clustering of pupae (as opposed to random distribution). This result was supported by differences shown between the observed distribution and a Poisson (expected) distribution of pupae (Figure 2).

CORRELATION BETWEEN ROCK SIZE AND WATER DEPTH AND THE NUMBERS OF PUPAE FOUND ON A GIVEN ROCK

The number of pupae per rock, (excluding rocks with no pupae), was positively correlated with both rock size and depth of rocks at Second Wannon and Hut Creeks, but not at First Wannon Creek (Table 1). The degree of correlation between rock size, water depth and pupae number was very similar for both First Wannon and Hut Creeks. The relationship between pupae number and rock size was stronger than that between pupae number and water depth at Second Wannon Creek. These results confirmed that the number of pupae increased as rock size and water depth increased. There was, however no correlation between rock size and pupae per cm$^2$ (Figure 3).
THE SPATIAL DISTRIBUTION OF PUPAE AND MICRO-AGGREGATIONS

The density of pupae was broadly similar across the three mapped streams however micro-aggregation was less common in Hut Creek than in First and Second Wannon Creeks (Table 2, Figure 3). Each stream surveyed showed similar densities of occupied rocks and micro-aggregations. Rock size was within the same order of magnitude across all streams. The size of the micro-aggregations ranged from 2 to 26, 2 to 28, and 2 to 18 individuals in First Wannon, Second Wannon, and Hut, respectively (Table 2). This suggests that the three streams were broadly comparable in terms of the available rocks and the number of pupae present.

Maps illustrating the position of occupied rocks at each stream were developed to show single pupa and micro-aggregations on rocks smaller and larger than median and on rocks above and below median depth (Appendix 1 First Wannon Creek, Appendix 2 Second Wannon Creek and Appendix 3 Hut Creek). The figures show the spatial relationship between micro-aggregations and rock size and depth at each study site. While some clustering of micro-aggregations can be seen at First Wannon Creek on large rocks and in deeper pools at First Wannon and Hut Creeks, these plots illustrate that no consistent spatial patterns exist across streams and environmental conditions (i.e. rock size and depth) (Table 2).

The dispersion of pupae was markedly different across each mapped stream bed (Figures 4a, 5a and 6a). First Wannon (Figure 4a) and Hut (Figure 6a) Creeks both showed a trend of spatial clustering of pupae (solid line which indicates the mean difference between the observed $L$-function and the $L$-function under CSR, is above the dashed line indicates no difference as stated in
the null hypothesis). However, First Wannon was the only stream to show a significant effect across the majority of the spatial scales investigated. This is shown by the confidence intervals (dotted lines), which did not include $L(t)$ values of 0, indicating marginally significantly clumping between scales of approximately 4 and 8 m (Figure 4a). In contrast, Hut Creek had no significant patterns, but some clustering occurred at larger scales in Hut Creek, while pupae tended to be over-dispersed in Second Wannon Creek (Figure 5a). Larger sample sizes are needed to assess the ecological importance of these trends. The association between rock size and clustering of pupae varied between streams with moderate negative and significant association at First Wannon and Second Creeks but a weak and insignificant negative correlation at Hut Creek (Figure 3, Table 3). As the MPPA undertaken used rock size as a factor, no correction was required for pupae per surface area.

There was no significant clustering of micro-aggregations (at any stream, with micro-aggregations found to be randomly distributed; Figures 4b, 5b & 6b). Also, Hut Creek was the only site to show any significant pattern of dispersion when the two environmental variables were accounted for. Here, significant clustering between 10 and 15 m occurred, when rock surface area (Figure 6c) and water depth (Figure 6d) were included. This suggests that pupae were randomly distributed across the available resources (as described by rock surface area and depth) at First and Second Wannon Creeks.
DISCUSSION

The pupae of *L. varians* were found in clusters of up to 28 individuals, which is comparable to the clustering patterns of other trichopterans (Gotceitas 1985). Pupae of *L. varians* were not distributed randomly across the study sites, but were clustered on larger rocks in deeper water consistently across all mapped sites. However, there was no consistent pattern of spatial distribution across the study streams. The lack of consistency among streams suggests that there were few general patterns in the distribution of *L. varians* pupae in these streams, and that differences in resource availability (e.g. the total number of rocks) may have influenced the patterns observed. Also, it should be noted that, as shown in Chapter 2, two species of *Lectrides* exist in the GNP. While Hut Creek is known to contain the more widespread dark morph, it is unknown which exists in First and Second Wannon Creeks. If the two separate species are found across study sites, this may account for the inconsistent patterns of dispersal.

Table 4 summarizes a number of recent studies using Ripley’s *K* to analyse the spatial patterns of a range of flora and fauna. These studies have shown that spatial distribution can be driven by a range of factors (e.g. inter- and/or intra-specific competition, resource availability) and that clustering is evident only at particular spatial scales. For example, Strand *et al.* (2007) showed that western juniper was regularly distributed at scales less than 15 m due to intra-specific competition for resources, but was clustered at scales greater than 30 m due to the relatively limited dispersal of seed. Unlike other studies which have used Ripley’s *K* (Table 4), no consistent evidence for spatial clumping or over-dispersion was found for pupal aggregations among the streams investigated, beyond the expected patchy distribution which is normally found in rocky streams (Hildrew & Townsend 1980). It is possible that further investigation using larger data sets (e.g. longer reaches) may uncover clustering that was not
found at the current scale measured, but previous research has found consistent patterns of aggregation in the distribution of oviposition sites at similar spatial scales (Lancaster et al. 2003).

It is difficult to draw general conclusions regarding the patterns across studies in Table 4 because of the small number of studies. However, this study adds substantially to our understanding of the generality of spatial patterns across multiple systems, as it is the first of the few studies that have used point pattern analysis for identifying spatial patterns in the distribution of flora and fauna to find highly inconsistent patterns across multiple populations. This may mean that in this case that stochastic events in dynamic systems may override animal behaviour and habitat requirements.

**Micro-aggregation of pupae**

Micro-aggregation (i.e. more than one pupae per rock) was quite common in Mosquito and Honeysuckle Creeks, but not so in Hut Creek (Figure 2). The larger number of rocks available in Hut Creek may have meant that micro-aggregation was unnecessary due to an abundance of suitable rocks, which may suggest that pupae do not aggregate unless rock resources are limiting. This pattern of micro-aggregation was illustrated in the higher values for CD at Mosquito and Honeysuckle Creeks and the large difference between the observed distribution and a Poisson distribution there (Figure 2).

One possible explanation for this is that larger rocks have a greater surface area, which may be the sole reason for the observed increase in the number of pupae on larger rocks. If this were the case, it could be expected that there would be a linear, monotonic relationship between rock size and the number of pupae per unit surface area. Scatter plots showed the relationship between number of pupae per cm² and rock size at each stream (Figure 3), illustrating that the
relationship between pupae number per unit surface area and rock size was not linear and not always monotonic. This suggests that the observed relationship is not solely a function of increasing surface area, and that some factor in addition to surface area was influencing the number of pupae per rock (Figure 3).

Another explanation for the micro-aggregation of *Lectrides* pupae is that larvae actively seek out and settle near conspecifics to pupate. While the most probable drivers for the micro-distribution of pupae are environmental factors (e.g. flow, depth, substrate type), the selection of a final settlement site may be determined by the presence of conspecific pupae (Otto & Svensson 1981, Hoffman 1997). A number of studies have shown that caddisfly larvae are able to recognise conspecific pupae, which results in pupal aggregations in homogenous environments (Otto & Svensson 1981, Hoffman 1997). Tests of gregarious pupation behaviour were attempted for *L. varians* in a separate laboratory study (Chapter 2), but none of the larvae successfully pupated.

**Effect of Depth and Rock Size**

It should also be noted that while there was a strong association between presence and number of pupae with rock size and depth, there was also a strong association between rock size and depth in the streams due to fluvial processes (Twidale 1976). This interaction of the size of rocks found in stream beds being strongly related to depth, occurs due to hydrological process which sort boulders leaving larger rocks in deeper sections (Twidale 1976). Manipulative experiments would be needed to separate the influence of these two factors. However, in practice the question of whether depth or rock size influence the presence of *L. varians* pupae is likely to be moot, due to the strong, consistent association between rock size and depth.
The observed response to depth and rock size may be indirect, with biota responding to other conditions which are directly related to either or both (Lancaster & Hildrew 1993). Therefore, a number of ecological explanations could be made for the strong association of pupae with rock size and depth. As the pupae are sedentary, they are especially vulnerable to predators and are unable to escape abiotic pressures and disturbance such as scour and drying (Lancaster & Downes 2013).

Alternatively, by pupating on the underside of large rocks in deep water, *L. varians* individuals may be much less likely to be exposed to desiccation during periods of drying. They may also be protected from disturbance caused by high flow events. Other authors have shown, that prior to pupation, late instar caddisfly larvae move from exposed, stream-facing rock surfaces (Lamberti & Resh 1983; Vaughn 1986) to form aggregations on the less-exposed undersides or down-stream surfaces of large substrates (Resh *et al.* 1984). Other possible mechanisms driving micro-aggregations include greater protection from predators and parasites (such as nematodes and chironomids) and increased likelihood of finding a mate at emergence (Otto & Svensson 1981; Hoffman 1997; Lancaster *et al.* 2011; Reich *et al.* 2011). Further experiments would be required to determine which of these explanations may be operating in this system.

**Implications of Life History**

While many aquatic insects are able to aestivate, it is unlikely that *L. varians* pupae are able to do so. Larvae of *L. varians* can aestivate (Wickson *et al.* 2012) and insect taxa tend to display dormancy in only one life-history phase (Masaki 1980). The likely absence of dormancy for the pupal life-stage makes behavioural adaptations, such as selecting areas likely to avoid desiccation,
critical to the survival of *L. varians* individuals to adulthood. To remain moist would be crucial for pupae, but as eggs are laid in late spring (Towns 1983), it would appear that pupation period is common during late winter and early spring when the threat of pupation sites drying out is at its lowest (St Clair 1993). It is also possible that larvae select pupation sites at random and that fewer pupae were found on smaller rocks due to disturbance (e.g. it may be that larvae attached to the smaller rocks in shallower pools but these were then removed during a spate).

It is possible that a number of factors in addition to rock size and water depth are important drivers in pupal site selection for *L. varians*. Some pupae do occur outside of deep sections and on small rocks. It is possible that these larvae may be selecting sites based on the availability of food sources to finish larval development prior to pupation, or materials necessary to construct their final cases for pupation (e.g. leaf packs) (St Clair, pers. obs.) or simply settle there by chance. Larvae may be deposited into deeper pools as they fall out of drift in low energy sections of the stream during late winter high-flow events. Other factors such as current velocity and presence of predators restrict available pupation sites, so final site selection may be due to rock size or the presence of conspecific pupa.

**LANDSCAPE EFFECTS**

As stated earlier, small scale movement and habitat selection of individuals have the potential to influence much larger-scale ecological processes and patterns (Levin 1992; Lancaster & Downes 2004; Lancaster 2006). Active pupal site selection at local scales by the late instar larvae of *L. varians* may help explain its wide scale persistence in the Grampians NP, where other stream macroinvertebrates have declined in numbers or become locally extinct (Doeg
The presence of flow refugia, such as those provided by large rocks in deep pools, may prevent high mortality and local extinction of species as a result of spates and floods. These refugia thus provide a source of colonists for rapid recolonisation following high-flow disturbances (Lancaster & Hildrew 1993; Woodward et al. 2002). During periods of high flow, biota that are susceptible to flow are more likely to seek refuges (Lancaster & Hildrew 1993) than rheophilic taxa. It follows, as pupa are immobile for an extended period, that seeking a pupation site that provides refuge from an unpredictable environment would maximise survival and local population persistence.

Extreme flow variability is a key disturbance for stream biota, with high-velocity spates and extended periods of low flow causing severe population losses and changes in community structure. Unravelling how communities recover and persist after such disturbances is important to understanding the fundamental processes occurring within these systems and increases our knowledge of autecology (Resh et al. 1988; Niemi et al. 1990). Furthermore, it can be argued that the distribution of a species over space and time, at a range of scales is the basis for understanding the features of habitat networks (Williams 2011).

ACKNOWLEDGEMENTS

I would like to thank Patrick Russell, Luke Sandri, Peter O’toole, Alistair Becker, Brett McKenzie and Tony Lovell for assistance in field data collection. I also thank Jill Lancaster for assistance in developing Marked Point Analysis Code and reviewing an earlier version of this manuscript as well as guidance and encouragement from Associate Professor Belinda Robson during the development of this manuscript. This project was undertaken using permit No. 10004699 from the Department of Sustainability and Environment - Parks Victoria.
**Tables**

**Table 1.** Spearman’s Rank Correlations describing the relationship between the number of pupae against rock size and water depth at First Wannon Creek \((n = 55)\), Second Wannon Creek \((n = 86)\) and Hut Creek \((n = 71)\). Significant results \((\alpha = 0.05)\) are shown in bold.

<table>
<thead>
<tr>
<th>Stream</th>
<th>Surface Area</th>
<th>Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\rho)</td>
<td>(p)</td>
</tr>
<tr>
<td>First Wannon</td>
<td>0.23</td>
<td>0.093</td>
</tr>
<tr>
<td>Second Wannon</td>
<td>0.50</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Hut</td>
<td>0.35</td>
<td><strong>0.0023</strong></td>
</tr>
</tbody>
</table>
Table 2: Number of rocks occupied by pupae and the number of micro-aggregations per rock for the three streams sampled in an approximately 10-m stream reach. The number of pupae found within a micro-aggregation ranged from 2 – 28 per rock. The total area of stream sampled in Hut Creek was larger than the other two streams because it was much wider and slightly longer.

<table>
<thead>
<tr>
<th></th>
<th>First Wannon</th>
<th>Second Wannon</th>
<th>Hut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area surveyed (m²)</td>
<td>30</td>
<td>33</td>
<td>52</td>
</tr>
<tr>
<td>No. of pupae</td>
<td>141</td>
<td>320</td>
<td>129</td>
</tr>
<tr>
<td>No. of occupied rocks</td>
<td>55</td>
<td>86</td>
<td>71</td>
</tr>
<tr>
<td>Pupae per m²</td>
<td>4.7</td>
<td>9.7</td>
<td>2.5</td>
</tr>
<tr>
<td>No. of micro-aggregations</td>
<td>26</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>Median rock size (surface area cm²)</td>
<td>1926.0</td>
<td>2196.5</td>
<td>947.0</td>
</tr>
<tr>
<td>Mean rock size (surface area cm²)</td>
<td>1980.5</td>
<td>2931.0</td>
<td>1251.8</td>
</tr>
<tr>
<td>Standard error of rock size (cm²)</td>
<td>227.5</td>
<td>289.8</td>
<td>143.7</td>
</tr>
<tr>
<td>No. of rocks occupied below median rock size (cm²)</td>
<td>26</td>
<td>44</td>
<td>35</td>
</tr>
<tr>
<td>Number of occupied rocks above median rock size (cm²)</td>
<td>29</td>
<td>42</td>
<td>36</td>
</tr>
<tr>
<td>Number of occupied rocks below median water depth (cm)</td>
<td>26</td>
<td>42</td>
<td>36</td>
</tr>
<tr>
<td>Number of occupied rocks above median water depth (cm)</td>
<td>29</td>
<td>44</td>
<td>35</td>
</tr>
</tbody>
</table>
Table 3: Spearman’s Rank Correlations describing the relationship between the number of pupae per cm² and rock size (surface area, cm²) First Wannon Creek ($n = 55$), Second Wannon Creek ($n = 86$) and Hut Creek ($n = 71$).

<table>
<thead>
<tr>
<th>Stream</th>
<th>$\rho$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Wannon</td>
<td>-0.46</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Second Wannon</td>
<td>-0.063</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>Hut</td>
<td>-0.37</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Authors</td>
<td>Ecosystem</td>
<td>Taxa</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Strand et al. (2007)</td>
<td>terrestrial</td>
<td><em>Juniperis occidentalis</em> (western juniper)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giebelmann et al. (2008)</td>
<td>terrestrial</td>
<td><em>Philetairus socius</em> (sociable weaver)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olivier &amp; Wotherspoon (2006)</td>
<td>marine</td>
<td><em>Pagodroma nivea</em> (snow petrel)</td>
</tr>
<tr>
<td>Lancaster et al. (2003)</td>
<td>freshwater</td>
<td><em>Ulmerochorema</em> sp. &amp;</td>
</tr>
<tr>
<td>Authors</td>
<td>Ecosystem</td>
<td>Taxa</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Apsilochorema</em> sp. (caddisfly)</td>
</tr>
<tr>
<td>This study</td>
<td><em>freshwater</em></td>
<td><em>Lectrides varians</em> (caddisfly)</td>
</tr>
</tbody>
</table>
FIGURE CAPTIONS

Figure 1. Mean size (surface area cm²) of rocks occupied and unoccupied by pupae at each stream from quadrat survey. Error bars show one standard error,* denotes streams with significant difference in size of occupied and unoccupied rocks (Honeysuckle n = 42, Mosquito n = 33, Hut n = 44).

Figure 2. The proportion of all rocks classified according to the number of pupae per rock based on quadrat data collected at (a) Honeysuckle (n = 42, μ = 1.02), (b) Mosquito (n = 33, μ = 0.88) and (c) Hut (n = 44, μ = 0.16) Creeks. Dots represent observed values, lines indicates the Poisson distribution (i.e. expected distribution of pupa per rock for the given mean if distribution was random).

Figure 3. Scatter plots of relationships between surface area of rocks (cm²), and number of pupae per cm² at (a) First Wannon Creek, (b) Second Wannon Creek and (c) Hut Creek. Trend lines in each figure are logarithmic lines of best fit.

Figure 4: Marked Point Pattern Analysis for First Wannon Creek showing (a) all occupied rocks; (b) rocks with multiple pupae; (c) using surface area of rocks as the mark; and (d) the depth of rock as the mark. n = 55 (based on occupied rocks). The solid line indicates the mean difference between the observed L-function and the L-function under CSR, the dotted lines indicate the 95% confidence interval for this difference and the dashed line indicates no difference as stated in the null hypothesis. Where the mean and confidence intervals are greater than zero, pupae are more clustered than would be expected under CSR, while a mean and confidence interval lower than zero indicates over-dispersion among individuals.
**Figure 5:** Marked Point Pattern Analysis for Second Wannon Creek showing (a) all occupied rocks; (b) rocks with multiple pupae; (c) using surface area of rocks as the mark; and (d) the depth of rock as the mark. Solid line indicates the observed L-Function, the broken line indicates 95% confidence interval for the difference. Sample size $n = 86$ (based on occupied rocks). $L$ of zero indicates random distribution, greater than zero indicates clumping, less than zero indicates over-dispersal. Refer to Figure 4 for additional detail on how to interpret the figure.

**Figure 6:** Marked Point Pattern Analysis for Hut Creek showing (a) all occupied rocks; (b) rocks with multiple pupae; (c) using surface area of rocks as the mark; and (d) the depth of rock as the mark. Solid line indicates the observed $L$-function, the broken line indicates 95% confidence interval for the difference. Sample size $n = 71$ (based on occupied rocks). $L$ of zero indicates random distribution, greater than zero indicates clumping, less than zero indicates over-dispersal. Refer to Figure 4 for additional detail on how to interpret the figure.
Figure 1.
Figure 2.

(a) CD = 8.98

(b) CD = 4.75

(c) CD = 1.15
Figure 3.
Figure 4. 
(a) 
(b) 
(c) 
(d) 
$L(t)_{obs} - L(t)_{CSR}$

Distance of $t$ (m)

Distance of $t$ (m)
Figure 5.

(a) (b)

(c) (d)

Distance of \( t \) (m)
Figure 6.
Appendix 1: Map of surveyed stream bed a First Wannon Creek showing all occupied rocks. (a) Black squares micro aggregations, open diamonds single pupa (b) Black squares large rocks (surface area greater than median), open diamonds small rocks (surface area less than median) (c) Black squares deep rocks (depth greater than median), open diamonds shallow rocks (depth less than median).
Appendix 2: Map of surveyed stream bed at Second Wannon Creek showing all occupied rocks. (a) Black squares: micro aggregations, open diamonds: single pupa (b) Black squares: large rocks (surface area greater than median), open diamonds: small rocks (surface area less than median) (c) Black squares: deep rocks (depth greater than median), open diamonds: shallow rocks (depth less than median).
Appendix 3: Map of surveyed stream bed at Hut Creek showing all occupied rocks. (a) Black squares: micro aggregations, open diamonds: single pupa (b) Black squares: large rocks (surface area greater than median), open diamonds: small rocks (surface area less than median) (c) Black squares: deep rocks (depth greater than median), open diamonds: shallow rocks (depth less than median).
CHAPTER 5 GENERAL DISCUSSION AND CONCLUSIONS

SUMMARY OF FINDINGS

This thesis has examined life-history adaptations of the leptocerid caddisfly *Lectrides varians* that might explain the success of self-sustaining populations during a decade-long drought in an already hydrologically-unpredictable environment. Specifically, the results of this thesis, using a small, aquatic trichopteran as a model organism, have contributed to the field of stream ecology by providing a greater understanding of:

1. The dispersal ability of *L. varians*, based on genetic data that indicate the species has the ability to disperse widely, giving individuals potential access a greater range of suitable habitat patches, such as refuges during dry periods;

2. The morphological and genetic plasticity of *L. varians* in south eastern Australia. This includes the discovery of a new morphologically- and genetically-distinct species that is potentially restricted to the Grampians NP. Another potentially cryptic species was also found in eastern Victoria in the Mitchell River;

3. Aestivation limits with respect to how long and to what degree *L. varians* larvae are able to withstand drying without significant losses. Larvae were able to withstand drying for longer (at least 110 days) and to a greater degree (more than 75% survival) than was expected. Larvae also show a bimodal response to re-wetting post aestivation, allowing for some individuals to respond rapidly to changing environmental conditions while others respond when the hydrology has stabilised. This may be important for the survival of larvae despite potential for there to be ‘false starts’ where surface water is short-lived; and
4. The relationship between the distribution and number of pupae and key habitat variables, including rock size and water depth. As predicted, a strong relationship was found between the presence and number of pupae and the size and depth at which rocks were found. However, no consistent pattern of clustering was found. *Lectrides varians* has developed behavioural and physiological traits at each life history stage. These adaptations enhance individual survival and the maintenance of self-sustaining populations in unpredictable hydrological and climatic conditions. This confirms the hypotheses at the beginning of this study that *L. varians* has a suite of adaptations to cope with variable hydrology. However, as genetic investigations uncovered a species complex within the Grampians NP after the studies of aestivation and pupal site selection were undertaken, these findings may need to be revisited to ensure that all individuals used during the various experiments were the same species. It appears, contrary to initial assumptions, that there are two species from the genus *Lectrides* sharing the same general niche within the Grampians NP.

These species however, may have divergent life histories, exploit different microhabitats or have differing phenologies. The discovery of species complexes, including cryptic species, has been important in the biological control of pest species (Paterson 1991), has provided a clearer understanding of potential disease vectors (Besansky 1999), and been used to clarify the growth and preferred conditions of species used in biomonitoring where species complexes confound results (Geller 1999). Cryptic species complexes have also been shown to drive diversity in ecological communities. Jackson and Resh (1998) found that genotypes of a cryptic species complex of *Gumaga* (Sericostomatidae: Trichoptera) showed distinct behavioural differences between genotypes. Other studies have also shown that species complexes may have broad geographical ranges (Adams *et al.* 2014; Latch *et al.* 2014; Zhang *et
al. 2014), but specific species within the complex may actually have a limited distributions and be much more prone to extinction than previously thought (Stuart et al. 2006). Nonetheless, species revisions are common in ecology, and the findings here are relevant for the species complex as is currently described.

Biological adaptations that enable species to survive adverse conditions are generally placed under three categories: behavioural; life history; and morphological adaptations. Behavioural adaptations, for example, include upstream migration by aquatic beetle larvae and adults to avoid drying (Lytle et al. 2008) or the movement of benthic macroinvertebrates from exposed surfaces (i.e. high flow areas) during spates (Brooks 1998). Life-history adaptations, for example, include diapause synchronized with seasonal drying (Hynes 1970, 1976), metamorphosis (or other life-history stages) timed to avoid average flood season (Lytle 2002; Boulton 2003) and aestivation in response to drying (Boulton 1989; St Clair 1993; Chapter 3). Morphological adaptations, for example, include suction cups on Blephariceridae to attach to substrate in high flows (Gooderham & Tsyrlin 2002) or streamlined body shapes that reduce drag during floods (Statzner & Holm 1989). Morphological adaptations that may facilitate persistence during drying include a highly sclerotised exoskeleton (Masaki 1980) or a shell (e.g. bivalve molluscs) or the ability to burrow into the hyporheic zone (Stubbington et al. 2009).

Life-history adaptations are considered to be more advantageous than behavioural or morphological adaptations for taxa living in changeable environments because such adaptations enable them to react quickly to individual disturbances, rather than seasonally to cues which may vary widely from year to year (Lytle & Poff 2004). Lectrides varians shows a range of behavioural (aestivation, Chapter 2), life-history (pupal site selection, Chapter 3) and morphological (dispersal ability, Chapter 4) adaptations. These are
crucial for population resilience in variable hydrological regimes (Williams 1996). As mentioned earlier, life history studies of Trichopterans are uncommon in Australia (St Clair 1993), but the larvae of other shredding caddisflies (in particular, Limnephilidae) in the Northern Hemisphere have been shown to aestivate under drying conditions (Hickin 1964; MacKay 1977; Colburn & Garretson Clapp 2006). This thesis has explored adaptations to drying of a highly disturbance-resilient taxon at three life history stages. While the results presented here may be used to explain why *L. varians* has continued to persist it may be also be used as a framework for understanding the adaptations of other similar taxa.

**Population genetics and phylogeny**

The investigation into population genetics showed that *L. varians* has a wide dispersal range, with panmixia occurring across much of Victoria and into Tasmania. Its capacity for wide dispersal, as estimated by global $\Phi_{ST}$ (in an analysis of molecular variance between populations), appears similar to other large caddisflies (Baker *et al.* 2003; Wilcock *et al.* 2003). However, this study also uncovered previously unknown speciation, including possible cryptic speciation, which warrants further exploration using molecular and traditional morphological methods. The molecular analysis has uncovered three distinct lineages of *L. varians* across Victoria and Tasmania. Morphological characters such as pigmentation and sclerotisation in late instar larvae were found to be consistently different between the two genotypes found within the Grampians NP. Individuals collected from the Nicholson River in the east of Victoria may be a cryptic species, but more samples will need to be examined to verify this.

Knowledge of trichopteran taxonomy and diversity in Australia is still uncertain, particularly when compared to the Northern Hemisphere (Davies & Stewart
Many species from a range of genera are currently undescribed including; *Ecomina*, *Notalina*, *Notoperata*, *Triplectides* and *Hydrobiosella* (St Clair 2000). Finding two potentially different species that have hitherto been identified as *L. varians* has important implications in terms of understanding the life history adaptations of this species, or species complex. This has been the case for previous authors investigating Trichopteran genera including Harvey *et al.* (2012) who uncovered cryptic diversity within currently recognized *Diplectronia* (Hydropsychidae) species, and Zhou *et al.* (2011) who identified deep genetic differentiation in two caddisfly species, *Limnephilus sansoni* (Limnephilidae) and *Cheumatopsyche campyla* (Hydropsychidae), but could not find consistent morphological traits that would differentiate adults. While no delineation of habitat requirements or behaviour and adaptations are immediately apparent, the two lineages may in fact have different life-history requirements, disturbance thresholds or adaptations which allow them to maintain sympatric populations within sub-catchments (and in-stream sympathy in one case as well) in apparent reproductive isolation. Jackson and Resh (1998) found that genotypes of a cryptic species complex of *Gumaga* (Sericostomatidae) showed distinct behavioural differences between genotypes.

As mitochondrial DNA is only inherited maternally, having this as the only molecular marker potentially limits how the population genetics results from Chapter 2 can be interpreted. The addition of nuclear markers would give greater resolution. Also, to date, I have been unable to collect adult samples of the proposed new species of *Lectrides*, and so have been unable to make a full species description for the new taxon to have the new species formally recognised. Nevertheless, the investigations undertaken have provided a much greater understanding of the life history of *L. varians* and the adaptations which allow it to survive in a particularly harsh and highly disturbed environment.
AESTIVATION LIMITS

Larval *L. varians* can survive at least 16 weeks with no surface water on moist or dry sediment. Mortality of larvae on moist sediments was not significantly different to control specimens which remained submerged for the duration of the experiment, but survival rates declined over time on dry sediments. Importantly, larvae were able to withstand experimental drying conditions that were longer than those that would be normally encountered in the seasonal dry period in Grampians streams (Robson *et al.* 2008(a); Chester & Robson 2011), which would allow some individuals and populations to survive in dry streams over summer. Previous authors have shown that several families of caddisflies inhabit temporary systems in the Northern Hemisphere (Hickin 1964; MacKay 1977; Colburn & Garretson Clapp 2006). Live larvae from the family Leptoceridae have been observed within leaf litter in drying streams and under gravel and under rocks on damp substrate (Boulton 1989; St Clair 1993; Chester & Robson 2011). Aestivation is also 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 are also known to be able to leave temporary pools in summer and aestivate in their unsealed cases under leaf litter (Flint 1958). However, to my knowledge, no-one has ever tested how long they can survive without surface water or the processes of recovery once rewetted. These traits are not common and avoidance of drying rather than withstanding desiccation is the most common strategy of Mediterranean river invertebrate biota (Hershkovitz & Gasith 2013; Robson *et al.* 2011; Mesquita *et al.* 2006; Bonada *et al.* 2007).

Despite this ability to aestivate, indicating that this organism is successfully adapted to periods of drying, its chance of survival decreases with increasing periods of time under completely dry conditions. Predicted outcomes under
current climate change models suggest that south eastern Australian streams are likely to experience extended and more severe drying in seasonal streams than previously (Pittock 2003; Steffen et al. 2009). Models developed for similar systems in south-western Australia have estimated that the frequency of river flow rates may be reduced by up to two months per year and no-flow periods may be extended by up to four months (Barron et al. 2012), potentially reducing the viability of *L. varians* populations by exceeding aestivation capacity. Furthermore, Stewart et al. (2013) expect that predicted climate change will shift water temperatures in smaller streams beyond that which is tolerable for sensitive species. Both points act to strengthen the argument for summer environmental flows in regulated streams (Mackie et al. 2012) which would maintain sediment moisture and enhance the likely survival of the species in the long term, particularly in heavily forested smaller streams.

The aestivation experiment showed a bimodal response in the time to emerge from aestivation, 71% responding to rewetting within 24 hours, whereas 29% remained inactive for three days before showing any response. This bimodal response potentially protects the population from false starts to the resumption of flow (Boulton 2003). Without careful planning, summer environmental flows could provide these ‘false starts’, to the detriment of some species’ ability to survive to pupation. However, *L. varians* appears to have adaptations which allow them to cope with these conditions (i.e. early responders may have high mortality, but the slow starters are more likely to survive). Studies of the bimodal response to environmental cues appear to be uncommon, as no literature describing similar behaviour could be found. Other authors that referred to aestivation in caddisflies reported unimodal responses to rewetting, (Hickin 1964; MacKay 1977; Colburn & Garretson Clapp 2006). These results may be due to aestivation being reported in field observation rather than laboratory experimentation.
It should be noted that the experiments conducted in the present study were only carried out on individuals from Deep Creek, so further investigation is required on other populations within the Victoria Range and elsewhere as individuals in different streams appear to be different species. Attributing unique adaptions and behaviours to each morphotype/genotype will strengthen the argument that they are separate species, such as may occur if Lectrides sp. AV1 individuals do not show a similar bimodal response to drying. Given that the existence of the two species was unknown at the time of this experiment, it is also possible that both were present in the samples taken. Fortunately, this is unlikely as no Lectrides sp. AV1 individuals have been recorded from Deep Creek and stream level sympatry appears to be uncommon (sympatry was detected at only one site on the other side of the Grampians Range). It should be noted that the aestivation study was completed in the laboratory and the limits discovered here may not translate directly to the field. The implications of this are also potentially relevant for the pupal site selection study.

**Pupal site selection**

The pupae of L. varians were found clustered on rocks, as has been observed in other trichopteran species (e.g. Gotceitas 1985) and they were not distributed randomly across the study sites. However, no consistent pattern of spatial distribution was found across the study streams. This lack of consistency suggests that differences in resource availability (e.g. the total number of rocks) may have influenced the patterns observed rather than a general pattern in the distribution of L. varians pupae. The micro-aggregation of Lectrides pupae may be due to late instar larvae actively seeking out and settling near conspecifics to pupate, as this has been shown in other caddisflies (Gotceitas 1985; Martin & Barton 1987; McCabe & Gotelli 2003). However, the most probable drivers for the micro-distribution of pupae are environmental factors (i.e. depth, substrate
type) such as avoidance of high flows or minimisation of the risk of drying
during pupation. Extreme flow variability is a key disturbance for stream biota,
with high-velocity spates (which can occur year-round) causing severe
population losses and changes in community structure. Other factors such as
chance may also be important. The higher concentration of pupae in pools than
riffles (when compared to larvae) may be due to pupal mortality and
disappearance of pupal cases in riffles, rather than active movement of larvae
before pupation. As stated in the previous section, avoidance of drying is far
more common than resistance in aquatic insects. This requires adaptive
behaviour such as rheotaxis, active and passive migration and life-history traits
such as short life cycles and synchronized emergence, as well as morphological
features that allow for active migration (Lytle & Poff 2004).

Unravelling how populations recover and persist after such disturbances is
important to understanding the fundamental processes occurring within these
systems and increases our knowledge of autecology (Resh et al. 1988; Niemi et
al. 1990). It should also be noted that the individuals from Hut Creek were most
likely to be *Lectrides* AV1 as described in Chapter 2. Populations from First and
Second Wannon Creeks had been identified using pupal cases only.

Therefore, it is unknown if they are *Lectrides varians* or *Lectrides* sp AV1 and
whether each species behaves differently at preparation stages, potentially
affecting the outcomes of the study. This study (and other life-history studies of
*L. varians*) may need to be revisited considering that the presence of different
species may be driving variation amongst populations. An obvious example is to
re-run this study comparing known populations of *Lectrides varians* and
*Lectrides* sp AV1.
IMPLICATIONS OF FINDINGS FOR OTHER FRESHWATER TAXA

While permanent streams do experience disturbance, they usually provide a constant source of habitat (i.e. water). This is not the case in intermittent streams, where the size, quality and location of suitable habitat patches vary over time, and disturbances, such as complete drying, tend to be much more frequent (Williams 2006). Recolonisation following disturbance (either flood or drought) occurs via migration from various refuges (Ashcroft 2010; Temunović et al. 2013). These include migration from outside sources, swimming or crawling upstream from downstream, drifting downstream from upstream sources or by emerging from local benthic refuges (Harrison 1966; Gray & Fisher 1981; Delucchi 1989; Morrison 1990). While some taxa with high conservation value, such as freshwater crayfish (Johnston & Robson 2009), caddisflies (St Clair 1993; Chapter 2) and large predatory odonates use aestivation as a refuge, Chester & Robson (2011) found that perennial water was the most important refuge for drought in the Grampians NP and that the faunal composition of the perennial refuge pools was not strongly linked to what was found in intermittent sections downstream.

Perennial waters, such as these refuge pools, have been found by many authors to be the most important refuges in Mediterranean and arid systems (Lake 2003; García-Roger et al. 2011; Chester & Robson 2011; Morrison et al. 2013). This dependence upon seasonal refuges drives a cycle of retreat and recolonisation in these systems. This sets Mediterranean systems apart from more permanent systems where refuges are not central to patterns of river biodiversity (Hershkovitz & Gasith 2013). Accordingly, the integrity of refuges is a key requirement of community persistence, however these processes are poorly understood (Hershkovitz & Gasith 2013). Nevertheless, this importance means that the conservation and management of permanent pools is critically
important in intermittent systems such as the Grampians NP (Sheldon et al. 2010). It is therefore likely that even though a few important taxa (such as *Lectrides*) have adaptations to survive without surface water, perennial refuges are required to maintain biodiversity in this system (Chester & Robson 2011). Also, as these refuges become less common and the distance between them becomes greater (e.g. as is likely with predicted drying under climate change in the region), the ability to disperse between patches of suitable habitat will become increasingly important.

Results from this study have shown that *Lectrides* has traits which allow for resistance to survive disturbance (i.e. aestivation and pupal site selection) as well as resilience traits allowing for rapid recolonisation of suitable patches after disturbance (i.e. wide dispersal capabilities). Chester & Robson (2011) found that the stream networks in the Grampians NP are resilience-driven, where stream biota show high local mortality from disturbance, but recolonise after conditions improve, although most of the fauna relied on permanent surface water to provide refuges from drought. The faunal composition of these pools was unrelated to subsequent assemblage composition of riffles once flows resumed because individuals present in spring arose from oviposition by adults as well as larvae emerging from refuges (Chester & Robson 2011). Therefore, it would appear that permanent surface water is important, not only to the stream in which it is found, but also to the network of streams surrounding it. This is analogous to other source-sink networks such as deciduous/evergreen forests (Dias 1996), agricultural and forested landscapes (Ricci et al. 2005) where colonists from a permanent (or high-quality) habitat supply individuals to intermittent (or low-quality) habitats.

To take full advantage of both near and distant pool refuges across multiple sub-catchments, taxa that use these pools must have dispersal capabilities that
enable them to reach a range of available refuges. Strong dispersal, such as that shown by \textit{L. varians} in Chapter 2, has previously been shown to be linked to long-term persistence of aquatic communities in non-perennial systems, particularly where habitat tends to be ephemeral relative to any particular taxa’s lifespan (Bilton \textit{et al.} 2001). Dispersal is not only important for avoidance of disturbance, but also assists in avoiding inbreeding stress or competition for resources (Bilton \textit{et al.} 2001). Strong dispersal capabilities are likely to provide many taxa with short-term resilience to climate change disturbances. This is likely to be the case for \textit{L. varians}, however as \textit{Lectrides} sp. AV1 appears to have a much more restricted range and therefore lower dispersal capabilities it is likely to suffer greater impacts. However, over longer time scales, even strong dispersal ability is unlikely to protect macroinvertebrate biodiversity from wide-scale refuge loss or degradation due to landscape change, if refuges become scarce, widely scattered or, in some circumstances, lost altogether (Chester & Robson 2011).

Local extinctions of some taxa and the absence of suspension-feeding taxa have been recently observed in the Grampians NP (Robson \textit{et al.} 2005; Chester & Robson 2011) compared with results of a previous study (Doeg 1996). Loss of suspension feeders is often associated with reduced duration of streamflow (Boulton & Lake 2008) which many of these species require for feeding, so the changes are linked to extended periods of drying and/or altered flow regimes in a number of streams. The present study coincided with south-eastern Australia experiencing one of the longest and most severe droughts on record (Lake & Bond 2007). Other unpublished data (Chester \textit{et al.} in review; Chapter 2) suggests that \textit{L. varians} is a stronger disperser than other invertebrate taxa in the Grampians NP, which might be the key to its persistence in this region, where other taxa have become less common. However, it also appears that, of the caddisflies in this system, its ability to aestivate over summer drying periods
is unique (several other species of caddisflies occur within this area and none have been observed aestivating, but laboratory testing would be required to make definitive statements on this). Therefore, both of these traits are probably important for its persistence, particularly given that most aquatic macroinvertebrates appear to rely on a source of permanent surface water (Chester & Robson 2011) and some permanent streams may actually become intermittent given future predictions associated with climate change (Collins et al. 2013).

While *L. varians* exhibits multiple life-history adaptations that allow this species to persist despite severe disturbances (such as prolonged drought) these traits seem to be largely unique to this species. Therefore, the persistence of invertebrate taxa which are unable to escape disturbance within the seasonal or intermittent systems appears to be reliant on the availability of local refuges (or other critical habitat patches) that provide a necessary source of colonists once the disturbance passes (Adler & Nuernberger 1994; Chester & Robson 2011). Individuals must however be able to move through the landscape at a rate relative to that which disturbances occur in order to avoid the disturbance (Crowley 1977) and isolation is accentuated in organisms that incur reduced reproductive fitness due to dispersal (Doak et al. 1992). Mean metapopulation sizes may therefore remain stable, but the size and distribution of local sub-populations will shift through time (Adler & Nuernberger 1994; Chester & Robson 2011). To maintain healthy and diverse communities within systems such as these, refuge pools may need to be maintained via provision of environmental flows in a network which will allow colonisation between them.
FURTHER WORK

Three obvious unresolved areas of enquiry can be seen to follow on from the results presented here. Firstly, additional research is required to complete the process of describing the new species of *Lectrides* discovered from the Grampians NP. This includes the need to collect adults from these populations and compare them to the descriptions of adult *L. varians* from Mosely & Kimmins (1953). As *L. varians* is distributed along most of eastern Australia, further investigation across its entire distribution would be prudent. This should follow the methods used in this study with the addition of nuclear markers, as outlined above. Any variation in the morphology of adults can then be published in line with differences found in the larvae and molecular data.

Secondly, as discussed in Chapter 4, re-visiting stream bed mapping and surveying longer stream sections of pupal sites may reveal greater spatial structuring, at a scale larger than those studied. Manipulations of rocks of varying size both inside and outside of deep pools would also be useful to provide a more powerful test of which abiotic components of stream habitat might influence the spatial clustering of pupae within the stream. Modification of the experimental design so that only living pupa are examined could also be enlightening. The importance of conspecifics could also be tested by replicating the experiment with known populations, which would resolve any interspecies differences.

Finally, after discovering that a potentially-new species of *Lectrides* occurs in the Grampians along with *L. varians*, an investigation to uncover differences between the dispersal characteristics, aestivation potential and habitat use of the two species may yield some understanding of whether resource partitioning
may exist between the two species and if they have comparable adaptations to withstand a variable and unpredictable environment. These findings may be applicable to other resilient taxa in Australia and elsewhere, conversely they maybe unique to Australian seasonal streams. These themes deserve further investigation across Australia and worldwide.

**MANAGEMENT IMPLICATIONS**

Trichopteran larvae are vital components of the food webs in most freshwater habitats (Resh & Rosenberg 1984). These habitats are often under pressure due to the amplification of negative catchment processes (e.g. erosion and sedimentation as a result of land clearing and cultivation). Furthermore, the impact of climate change and associated changes to stream flow are likely to be significant for trichopterans, especially those in alpine, headwater streams where disturbance is expected to be severe, with local extinctions expected in Australia (Steffen et al. 2009) and worldwide (Lawrence et al. 2010). Therefore, monitoring and maintenance of known refuge pools is likely to become important in some Mediterranean and arid regions for trichopterans and other taxa. For example, physicochemical conditions within refuge pools must not exceed the tolerance limits of aquatic invertebrates if they are to persist in intermittent streams (Store & Quinn 2013).

Therefore, to protect the aquatic macroinvertebrate communities within these streams, particularly those in water supply catchments, two main changes to management are recommended:

- Ensure that summer environmental flows are used in regulated streams to maintain refuge pools and water quality therein for reaches downstream of the off-take weirs in all regulated systems. This should ensure that refuge pools are maintained, and this process will also
maintain moist sediments where taxa such as *L. varians* may potentially be aestivating. Maintaining a series of pools is important to provide ‘stepping stones’ for water-dependent taxa to retreat to. This practice is common for larger regulated systems in Australia (Victorian Environmental Water Holder 2014), but less so in headwater systems.

- Maintain vegetation around key permanent refuge pools, which will help to prevent excessively high water temperatures and reduce evaporation during summer and allow for natural nutrient inputs and processing. This will not only assist in maintaining refuge pools, but ensure that they are habitable for other stream fauna.

These activities are realistic and achievable measures that can be incorporated into existing management planning to help protect a unique and threatened ecosystem. River networks can be viewed as a spatial hierarchy of patches (Frissell *et al.* 1986) within which freshwater invertebrates occupy discrete patches bounded by terrestrial landscape that is often inhospitable for aquatic larvae (Bilton *et al.* 2001). Beyond this structure, the spatial configuration of the landscape in which the system sits is important for terrestrial flying adults and must be considered in species conservation programs (Fahrig & Merriam 1994). Networks of streams should be managed as a functional unit rather than single waterways in isolation (Fahrig & Merriam 1994; Gippel 2000). The environmental fluctuations in seasonal systems results in spatial and temporal variability in available habitat and therefore aquatic communities (Boix *et al.* 2010; McManamay *et al.* 2013). When protecting fragmented populations, an understanding of the connections between patches, quality and size of patches, temporal variability of the landscape as well as the dispersal characteristics of the target taxa is vital (Fahrig & Merriam 1994). If not managed, this fragmentation can lead to local extinctions such as those observed for stream macroinvertebrates within the Grampians NP.
Chester & Robson (2011) found that taxa that do not require surface water for refuge are rare in the Grampians NP. It appears that *L. varians* is particularly resilient to drying, but a complete absence of moisture is still likely to be detrimental to populations of this species (Chapter 3). A number of the streams within the Grampians NP are used for town water supplies, and regulation of water in these streams for human use is increasing the duration and severity of drying in this region (GHCMA 2003). Evidence has shown that the impacts of human activity on water quality in regulated streams can push the levels of disturbance during drought beyond the tolerance threshold that most macroinvertebrates can withstand (Lind *et al.* 2006). This thesis indirectly adds to the evidence of the requirement of “summer fresh” flows to maintain moist sediments at the very least, or to provide water in refuge pools for a greater duration and to prevent the declining water quality in some pools. Further to this, all efforts will need to be made to maintain connectivity of appropriate vegetation structure between streams to allow for the dispersal from refuges into new habitat. This will require the maintenance of a matrix of refuges that are structured spatially to allow taxa to move between them. The maintenance of suitable habitat between patches will also be required. This can be achieved through maintaining perennial surface waters where possible, through the provision of environmental flows, and by protection of riparian vegetation. This will be particularly important for taxa that lack resilience adaptations. Negative impacts to biodiversity due to long-term habitat changes can be expected as hydroclimatic extremes are predicted to increase in frequency and magnitude (Beche *et al.* 2009).
CONCLUSION

Alteration of timing and increased drying from reduced runoff and water abstraction has been shown to significantly alter macroinvertebrate assemblages in intermittent streams, with communities becoming less diverse over time (Robson et al. 2005; Sponseller et al. 2010; Mackie et al. 2012). This thesis has shown that Lectrides varians possess what appears to be a unique suite of adaptations to survive the unpredictable hydrological conditions in the Grampians NP and other intermittent waterways within its range. Combined with work from Towns (1983), evidence in this thesis shows that L. varians has adaptations to drying and persisting in dynamic patchy landscapes at every life history stage. No other homobelatous taxa has been uncovered in the literature that shows such distinct adaptations at each part of its life cycle. As water resources are placed under more pressure through increased abstraction and reduced runoff, adaptations to drying will become increasingly important, as will active management of such landscapes.

As two morphologically- and genically-distinct populations have been discovered in sympaty in at least one site within the Grampians NP, caution is suggested when inferring results from previous studies of L. varians ecology. Further research needs to be undertaken to determine the range of the new species described and to find if it is indeed endemic to the Grampians NP, as well as to review the taxonomy of the genus Lectrides through eastern Australia. If the species described in this thesis is endemic to the Grampians, it would join a number of aquatic invertebrate species known to be unique to this region (Johnston & Robson 2009; Chester & Robson 2011). This caution should also extend to all researchers who study the life history and ecology of taxa with potentially uncertain taxonomy.
Extreme variations in stream flow are a primary disturbance for aquatic taxa. Understanding how communities recover and persist is key to understanding the fundamental processes occurring within these systems (Resh et al. 1988; Niemi et al. 1990). Understanding the limits of *Lectrides* provides estimates for the upper tolerance limits that macroinvertebrate assemblages can withstand (at least 16 weeks on moist substrates). Management of waterways within the park to protect these species will also be beneficial to a wide range of other aquatic taxa.

Each chapter has investigated adaptations to drying (including morphological, physical, behavioural and life-history adaptations) exhibited by the larva, pupa and adults of the shredding caddisfly *L. varians*. As stated earlier, aquatic macroinvertebrate biodiversity and distribution in the Grampians NP has declined sharply in the decade following Doeg’s (1997) survey, while Leptocerid caddisflies have maintained stable populations. These results can potentially be integrated with those of previous authors (Towns 1991; St Clair 1994) to understand why *L. varians* has become the dominant aquatic macroinvertebrate throughout the Grampians NP, with implications for the mechanisms of persistence of common taxa in other intermittent stream systems. As a shredder of coarse particulate organic matter, *L. varians* provides an important ecosystem service particularly in intermittent streams which tend to be reliant on allochthonous carbon. This autoecological information for a resilient species such as *L. varians* is important to further our understanding of the drivers of persistence despite disturbance and forms the basis of broader population ecology and management practices.

The work presented within this thesis contains important information for other authors, to be used as the basis for subsequent research. Potential cryptic speciation has implications on past and future work on *L. varians*. Past work
may need to be reassessed in light of the fact that variation amongst populations
may be due to the presence of different species, and will need to be framed by
the possibility that *L. varians* as currently described may actually be a complex
of many species. I have also presented a technique for testing the aestivation
limits for aquatic macroinvertebrates. As aestivation is a common strategy used
by Trichoptera (and other aquatic macroinvertebrates) worldwide, this
approach could also be applied by researchers elsewhere. This work is also the
first time (to my knowledge) that the aestivation capability of an aquatic
macroinvertebrate has been quantified. Finally, I have given greater insight into
resource use and habitat use of late instar larvae, particularly in preparation for
pupation.
REFERENCES


Chester, ET & Robson, BJ 2011, 'Drought refuges, spatial scale and recolonisation by invertebrates in non perennial streams', *Freshwater Biology*, pp. 2094-2104.


Dall, PC 1979, 'A sampling technique for littoral stone dwelling organisms', *Oikos*, vol. 33, pp. 106-112.

Datry, T, Arscott, DB & Sabater, S 2011, 'Recent perspective on temporary river ecology', *Aquatic Sciences*, vol. 73, pp. 453–457.


De Block, M, McPeek, MA & Stoks, R 2008, 'Life history plasticity to combined time and biotic constraints in *Lestes* damselflies from vernal and temporary ponds', *Oikos*, vol. 117, pp. 908-916.


Drummond, A & Rambaut, A 2007, *Tracer: MCMC Trace Analysis Tool*, version 1.5.0, Program Distributed by the Authors.


Hoffman, A 1997, 'To settle or not to settle? The aggregation behaviour of Lasiocephala basilis (Kol.) (Trichoptera: Leptostomatidae) larvae prior to pupation', in Holzenthal, RW & Flint, OS (eds.), *Proceedings of the 8th International Symposium on Trichoptera*, pp. 151-156.


Hughes, JM, Schmidt, DJ & Finn, DS 2009, 'Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat', *Bioscience*, vol. 59, pp. 573-583.


Johnston, K & Robson, BJ 2009, 'Habitat use by five sympatric Australian freshwater crayfish species (Parastacidae)', *Freshwater Biology*, vol. 54, pp. 1629-1641.


McManamay, RA, Orth, DJ & Dolloff, CA 2013, 'Macroinvertebrate community responses to gravel addition in a southeastern regulated river', *Southeastern Naturalist*, vol. 12, pp. 599-618.


Nylander, JAA 2004, *MrModeltest v2*, Distributed by the Author, Evolutionary Biology Centre Uppsala University.

Olivier, F & Wotherspoon, SJ 2006, 'Modelling habitat selection using presence-only data: Case study of a colonial hollow nesting bird, the snow petrel', *Ecological Modelling*, vol. 195, pp. 187-204.


Pörschmann, U & Speith, HR 2011, 'Aestivation in *Pieris brassicae* (L.) affects the parasitoid load caused by *Cotesia glomerata* (L.)', *Entomological Science*, vol. 14, pp. 31-36.


Reid, DJ, Quinn, GP, Lake, PS & Reich, P 2008, 'Terrestrial detritus supports the food webs in lowland intermittent streams of south-eastern Australia: a stable isotope study', *Freshwater Biology*, vol. 53, pp. 2036-2050.


invertebrate communities to hydrological extremes', *Hydrobiologia*, vol. 630, pp. 299-312.


Walsh, PS, Metzger, DA & Higuchi, R 1991, 'Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material', *Biotechniques*, vol. 10, pp. 506-513.


Wilcock, HR, Nichols, RA & Hildrew, AG 2003, 'Genetic population structure and neighbourhood population size estimates of the caddisfly Plectrocnemia conspersa', *Freshwater Biology*, vol. 48, pp. 1813-1824.


