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Title:
Implications of oviposition site selection and habitat limitation for caddisfly populations in sand-bed streams

Date:
2015

Persistent Link:
https://hdl.handle.net/11343/55310

Implications of oviposition site selection and habitat limitation for caddisfly populations in sand-bed streams

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Submitted in total fulfilment of the requirements

of the degree of Doctor of Philosophy

July 2015

School of Geography

University of Melbourne

Produced on archival quality paper

Abstract

Organisms with complex life histories pose a particular challenge to population ecologists seeking to understand why their numbers fluctuate in time and space. Dispersal during one or more life cycle stages can mean that population dynamics may be controlled by factors remote from and over a larger scale than the typical scale of investigation. Marine researchers, among others, demonstrated the importance of considering multiple life stages and showed that the dynamics of sessile adult populations was determined by the number of dispersers arriving in many situations. Populations could thus be supply limited (where habitat was in excess) or habitat limited (where the number of dispersers saturated the available habitat).

Freshwater insects are analogous in some ways to sessile marine larvae because they have mobile adults dispersing that give rise to sessile egg stages in a different niche/habitat. Some freshwater insects require a solid substrate upon which to lay their eggs and if suitable oviposition sites vary in space and time, it is possible that inputs of eggs and thus distribution of larvae are constrained by availability of oviposition sites (i.e. habitat limitation). There is some evidence that oviposition habitat can be limiting in lotic systems, but so far this has been restricted to rocky, typically upland, streams. The broad aim of this thesis is to test whether habitat limitation operates in a different stream type with distinctly different substrate dynamics. The study streams, Hughes, Creighton's and Seven Creeks, have sandy beds and the predominant source of hard substrate is bark and wood. The research program focussed on two caddisflies with contrasting larval habits: *Ecnomus* a retreat-building predator and *Cheumatopsyche* and net-spinning filter-feeder.

A survey of numbers of egg masses and density of oviposition substrate – bark and wood – at 16 sites across three streams and three times showed that *Ecnomus* egg mass numbers were positively associated with the amount of bark, which is consistent with habitat limitation. However, this was not the case for *Cheumatopsyche* whose masses appeared to be associated with substrates in fast flows rather than the total amount of bark present. Subsequent small-scale experiments manipulating the conditions of individual pieces of bark confirmed that *Cheumatopsyche* lay more egg masses on bark placed in fast flows, but no such relationship was found for *Ecnomus*. However, *Ecnomus* was found to lay more eggs on submerged bark than emergent bark, whereas *Cheumatopsyche* showed no response to emergence. A further experiment demonstrated that both taxa used the emergent portion of bark as a landing pad from which to access submerged substrate, however they were not dependent on this pathway because both were also able to lay eggs on partially submerged bark where they had been blocked from walking down from the emerged portion. Thus females must also crawl or swim to access submerged substrate and *Ecnomus* was observed doing the latter. A further round of experiments demonstrated that both species also had strong responses to bark condition, with recently submerged bark and very well-conditioned bark being rejected for oviposition.

A large scale field experiment built upon the preceding work and by (1) manipulating the quantity of bark in 25 m sites with appropriate controls and (2) the characteristics of individual pieces of bark in the resulting sites of high and low bark density, I was able to test whether oviposition was habitat limited (hypothesis 1) and whether female oviposition behaviour was dependent on the overall availability of substrate (hypothesis 2). Consistent with my survey, there was a strong, positive relationship between *Ecnomus* egg masses and the surface area of bark at the site scale, demonstrating that habitat can limit egg input in a non-rocky stream (hypothesis 1). *Cheumatopsyche* was not limited by total bark surface area, which I hypothesise is a result of their preference for fast flows. Taxa with such selective oviposition behaviours may be more likely to be habitat limited, but it is difficult to establish given the difficulty of knowing confidently whether bark is completely unsuitable or simply failed to gain any egg masses, as can happen when species are supply limited. Thus, *Cheumatopsyche* could alternatively be supply limited with a distribution driven by patterns of emergence, dispersal and mortality in adult females. Hypothesis 2 was confirmed with the strength of significant associations with emergence and velocity becoming weaker at high bark availabilities for *Ecnomus* and *Cheumatopsyche*, respectively. It is feasible that large volumes of bark reduced the apparency of the most suitable bark and interfered with females' abilities to express oviposition choices, as has been observed for terrestrial insects searching for particular host plant species surrounded by dense, unsuitable vegetation.

This work has established that oviposition habitat can be limiting in streams, thus adding to a small body of work on this area, but also expanding the generality of this concept in lotic systems to include a different type of oviposition habitat. Bark is a substrate type that is likely to be more variably available than rocks because it degrades, is easily transported by floods, and goes through periods of loss and renewal on a yearly basis. Historically, freshwater researchers have tended to attribute high levels of patchiness observed in the distribution of aquatic insect larvae across multiple scales to factors acting on the larval stage such as supposed habitat requirements related to flow. However, strong variation in the distribution of oviposition habitat combined with selective behaviours of females, where one choice gives rise to hundreds of offspring, make habitat limitation a very feasible alternative explanation for high spatial variation in larval densities. A recognition that larval densities may be best explained by adult behaviours in at least some stream may revolutionize the way that stream populations are viewed, just as it did for marine ecologists over the last 30 years. It is time that the field of stream ecology, especially as represented by studies of invertebrates, caught up to other fields of ecology, which have long since recognized and integrated the importance of multiple life history stages and spatial structure into understandings of population dynamics.

Declaration

This is to certify that:

1. The thesis comprises only my original work towards the PhD except where indicated in the Preface.

2. Due acknowledgement has been made in the text to all other material used.

3. The thesis is fewer than 100 000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signed ________________________

Ashley Macqueen

Preface

Chapter 4 is comprised of a substantially unchanged manuscript that has been accepted for publication in ‘Freshwater Biology’ and is currently in-press. This will be a multi-author paper with the author list:

Macqueen A., Downs B.D.

Ashley Macqueen is the primary author and contributed greater than 50 % of the content.

Acknowledgements

I could not have asked for a more supportive supervisor than Barbara Downs. I will be forever grateful for the unwavering encouragement she offered, especially in returning to the project after an extended break.

The following generous volunteers made my fieldwork program possible: Cammie Kelly, Raku Pitt, Dan Rooke, William Bovill, Alena Glister, Sara Deem, Kate Barnard, Elise King, Keira Mason-Hill, Handoko Wahjudi, Jenny Friedan and Jill Lancaster. In addition to field assistance, many of these friends (and others) have provided companionship and support, particularly Allison Mok, Caterina Mocciola and Kate Barnard.

I relied upon the good will of many landholders to access my field sites and I am particularly thankful for the accommodation provided by the Strong family.

I received generous financial support in the form of an Australian Postgraduate Award (stipend) and two grants from the Holsworth Wildlife Research Endowment, as well as support from the School of Geography.

I also wish to extend my thanks to Bobbi and the Benthettes for hosting me at the Rocky Mountain Biological Laboratory.

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Chapter 1. Research rationale, general aims and thesis structure

1.1 Introduction

Population ecology is fundamentally concerned with understanding spatial and temporal variation in the abundance of populations (Krebs, 2009; Cappuccino & Price, 1995). For instance, what processes regulate populations, steering them away from extinction or unchecked growth (Chesson, 1998)? For organisms with multiple life history stages researchers must also determine at which stage these processes act most strongly. Though seemingly self evident, a review of the ecological literature suggests that the development of a more holistic approach was uneven across the diverse fields of ecology. Marine researchers have understood the influential role of larval supply on the dynamics of benthic marine invertebrates, and the importance of studying multiple life history stages, for some time. In fact, the initial discovery over thirty years ago revolutionised the way populations were viewed. By considering processes occurring during the larval phase, rather than focussing solely on the adult stage, researchers could better explain the distribution of invertebrates over regional scales (Roughgarden, Gaines & Possingham, 1988). Unfortunately, perhaps because of a lack of communication between researchers in different fields, those working in lotic systems (running waters, e.g. streams) have only more recently grasped the potential of this idea, having focussed primarily on the larval phase of freshwater insects (Downes & Reich, 2008). Over the past decade or so, a small group of researchers have begun a concerted investigation of the potentially significant implications of dispersal and subsequent habitat selection through oviposition by females on populations of aquatic insects (e.g. Lancaster, Downes & Arnold, 2010a; Reich & Downes, 2003b; Peckarsky, Taylor & Caudill, 2000; Encalada & Peckarsky, 2012). To date these efforts have been restricted to rocky streams, primarily in upland areas. Advancing this cause through a program of research based in sandy-bed streams in north-eastern Victoria, Australia is the overall purpose of my thesis. This chapter details the theoretical background for the work.

I begin by briefly touching on the relevant marine literature and drawing on the insights of marine researchers to evaluate how dispersal and spatial structure of populations can drive dynamics at small scales, given constraints on habitat availability. I draw parallels between the processes of larval settlement and oviposition in insects and discuss the influence of less tractable life history stages on populations and the progress of ecological thought.

I then focus on the process that marks the transition between adult and egg stage in insects, namely oviposition. Research on terrestrial insects, particularly phytophagus ones, is as bountiful as the freshwater contribution is scant when it comes to describing the evolution of adaptive oviposition behaviour and the implications for particular life-history stages. I briefly summarise the state of knowledge for terrestrial and aquatic insects and highlight some of the weaknesses in the literature.

In the final section, I elaborate how the distribution of habitat and adaptive oviposition behaviours can interact to drive the distribution of the new generation of lotic insects and review the progress made to date. I conclude by outlining my aims and the structure of the thesis.

1.2 The influence of dispersal and supply of recruits versus habitat availability on the structure and dynamics of populations

Population ecologists studying organisms with complex life histories seek to identify the factors that impact on key demographic rates (birth, death, immigration, emigration) and determine upon which life history stage(s) these factors act most strongly to regulate populations (Halpern, Gaines & Warner, 2005). A particular challenge is that these stages often occupy different environments and have different levels of mobility. Plants have a (typically) sessile adult and dispersing pollen and seed stages, as do many benthic marine invertebrates, which commonly release their propagules into the water column (with exceptions e.g. snails, that have crawl away larvae). Phytophagus insects, in contrast, disperse as adults, while their larvae are typically constrained to the plants chosen by their female parent (Floater & Zalucki, 2000). Insects with aquatic larval stages are particularly challenging research subjects as both adult and larvae can disperse to varying degrees within their respective mediums. Sessile stages are typically easier to work with and the differential tractability of life history stages has driven the progress of knowledge in a number of ecological contexts (Hixon, Pacala & Sandin, 2002; Halpern, Gaines & Warner, 2005).

Marine ecologists working on adult stages of benthic invertebrates of the intertidal zone found in early research that for many locales, populations appeared to be regulated by a combination of differential physiological tolerances and competition for a limiting resource, space (Connell, 1961), which could be freed up by predation or disturbance (Connell, 1978). The adult stages of the study taxa (barnacles, mussels, etc.) being more or less sessile allowed a number of elegant experiments (e.g. Menge, 1976; Paine, 1974; Underwood, Denley & Moran, 1983). However, this focus meant that the larval stage was treated as a 'black box' that was assumed to deliver an inexhaustible supply of settlers. However, this did not hold true for all locations and it soon became clear that the strength of biological interactions at the adult stage depended on the density of individuals, which was determined by the supply of larvae (Connell, 1985). Some locations (local populations) received settlers in excess of the available habitat resulting in adults densities sufficiently high to facilitate interactions described as habitat limited. Elsewhere settlers did not saturate the available habitat and adult populations were dictated by the number and identity of immigrants (supply limited, Roughgarden, Gaines & Possingham, 1988). Thus, studying the factors controlling supply – dispersal and the spatial structuring of populations – is critical to understanding dynamics of the adult stage and therefore populations. The idea of habitat versus supply limitation is central to this thesis and will be explored further in the section 1.4. The profound effect of supply (i.e. immigration) in the dynamics of many marine taxa demonstrates the importance of considering multiple life states, particularly dispersive stages, and thus the spatial structuring of populations. I will return to the marine literature and review recent progress but first I will introduce some concepts that are commonly used to describe spatial population structure.

A metapopulation perspective

The spatial structure of populations is often discussed using the language of metapopulation theory as conceived by Levins (1969) and further developed by other authors, notably Hanski (1999). The focus of this theory was on explaining the persistence of a species in a landscape of habitat patches. Although individual local populations inhabiting a single patch may go extinct (extinction risk > 0), dispersal from other patches in the metapopulation allow the population to be re-established (the 'rescue effect'). A classic, well studied example is that of butterfly populations inhabiting a fragmented landscape (Hanski, Kuussaari & Nieminen, 1994; Stevens, Turlure & Baguette, 2010). Metapopulation structure depends on the scale and frequency of dispersal relative to the patchiness in the environment, with smaller, more isolated populations more likely to go extinct. A population that receives immigrants is considered open, while a completely isolated population is considered closed. The degree of openness is defined as "one minus the mean probability that an arriving recruit was born within the population boundaries in question" (Hixon, Pacala & Sandin, 2002, p. 1496). Thus, as the proportion of (potentially) dispersing individuals returning to their natal population increases, the degree of openness declines.

If all of the fragments in a landscape are connected by frequent dispersal then they are likely part of one large patchy population and each of the individual patches or sub-populations would be considered open. Alternatively, if population are connected by infrequent dispersal they comprise a classic metapopulation; dispersal is sufficient for extant populations to 'rescue' unoccupied patches, but low enough for the dynamics of each population to be essentially independent. Finally, at the other end of the continuum, where dispersal distance is less than the distance between populations comprises, exists a landscape comprised of isolated or 'closed' populations. Another way of thinking about this problem is to ask at what scale is the population closed – any population must be closed if we 'zoom out' far enough (Hixon, Pacala & Sandin, 2002). Rates of immigration and emigration from patches are not likely to be balanced and uneven dispersal can give rise to mainland-island (smaller populations supported by a larger population) or source-sink arrangements (Harrison, 1991). A population that exports fewer individuals than it receives is a net sink with the most extreme cases relying solely on immigrants to remain extant. Knowing the scale at which a metapopulation operates allows researchers to identify pertinent hypotheses in the appropriate context.

The data required to distinguish between type and scale of metapopulation structure are dispersal distance and frequency. Unfortunately, the dispersive stages of organisms with complex life histories can be difficult to work with due to factors such as their size, fragility etc. (Levin, 2006; Payne & Dunley, 2002), which limit the application of some methods (e.g. mark-release-recapture) that have been traditionally employed elsewhere. Dispersal distance is affected by a range of factors driven by properties of the organism (e.g. mobility, longevity, provisioning or feeding ability, Marsh *et al.*, 2001) and its environment (e.g. wind/water currents, hospitability and resistance of the inter-patch matrix, Ricketts, 2001). A number of techniques, many quite novel, have been employed to measure or predict dispersal in organisms with complex life histories including direct, indirect and modelling approaches. I'll consider those employed in research of marine organisms and terrestrial insects before moving onto techniques used in the study of freshwater insects, and addressing the current state of knowledge in that field.

Given the minute size of many marine larvae, researchers have utilised passive dispersal models based on ocean currents to estimate dispersal kernels (i.e. the inverse cumulative proportion of individuals reaching certain distances from the source Stevens, Turlure & Baguette, 2010). Models have become more sophisticated and detailed over time (Levin, 2006). Incorporating data on development, behaviour and nutrition was a significant advancement; planktotrophic (feeding) larvae may have longer lives and thus greater potential dispersal distances than lecithotrophic (non-feeding) larvae. Marsh *et al.* (2001) studied the development of tube worm *Riftia pachyptila* and combined this with hydrodynamic this data to estimate the dispersal of this hydrothermal vent resident. They concluded that hydrodynamic factors were responsible for keeping dispersal distances <100 km even though larvae could live for up to a year. Larvae can influence their long distance movements by altering their position vertically thus moving between currents going different directions and may also reduce the probability of being swept from their natal habitat (Witman *et al.*, 2003). Analysis of microelemental signatures was instrumental in challenging the assumption that all marine populations were demographically open. The concentrations of elements in seawater vary with proximity to shore and between different coastal regions and the incorporation of these elements into persistent structures (e.g. shells, carapace etc.) reflects the location(s) in which an individual as developed. Swearer *et al.* (1999) found that elemental signature of otaliths (ear bones) of reef fish indicated that a high proportion of individuals on the leeward side of islands were retained i.e. the populations were somewhat closed. The persistence of populations that are 'up-current' from all sources of immigrants provides further evidence of retention (Gaylord & Gaines, 2000). However, Levin (2006) cautions that the recent focus on retained larvae may have gone too far and points out that even in relatively low numbers dispersing larvae may be significant to a population. Studies of genetic markers have been widely used to infer the connectivity of populations of organisms (see Palumbi, 2003) and revealed some surprising findings. For instance, high levels of differentiation were found between populations of corals separated by 6.5-155 km on the Ningaloo Reef, Western Australia. Whitaker (2004) ascribed the finding to restricted gene flow, i.e. low connectivity, at the gametic and larval stages, despite the latter being teleplanic (long lived). The early and now widespread recognition of the importance of the spatial structure of populations among marine researchers is demonstrated by the well-developed literature on dispersal across an array of taxa, relative to that of other fields.

Butterflies are certainly the most studied insect and may be more researched than any other organism with regards to dispersal (Stevens, Turlure & Baguette, 2010). The importance of dispersal for the conservation of butterflies was highlighted by early work on metapopulations (e.g. Gall, 1984). Since that time a significant proportion of research has focussed on rare and threatened species, particularly European ones, whose habitats has been fragmented due to human impacts (Stevens, Turlure & Baguette, 2010). Often data on dispersal is combined with life history information to assess the viability of populations and inform management decisions (Schtickzelle *et al.*, 2005). Traditionally, dispersal was elucidated using mark-release-recapture techniques, which suffer from low catch rates. The addition of pheromones to passive trapping methods can increase the capture rate (Svensson *et al.*, 2011). Miniature transmitters have been successfully used in concert with harmonic radar to track the movements of individual insects (Svensson *et al.*, 2011; Wang *et al.*, 2011) and gives measurements of actual dispersal paths rather than a minimum distance. As such, it offers a promising tool for investigating behaviour more broadly. Genetic methods have been used on terrestrial insects too, often suggesting that individuals disperse further than previously thought. For example, Ugelvig *et al.* (2012) found that the butterfly *Maculinea arion* must travel at least twice as far than expected (~10 km) to maintain the well mixed populations present on the Swedish island of Öland. It is worth noting, however, that genetic connectivity is not equal to demographic connectivity and works over much longer timescales (Bohonak, 1999). A few infrequent dispersal events may be sufficient to stave off genetic differentiation without having a strong impact on local density dependent processes. A meta-analysis of butterfly dispersal by Stevens, Turlure and Baguette (2010) found that the results from the variety of methods employed were broadly congruent.

Freshwater insects often have terrestrial adult stages and aquatic larval stages, which adds complexity to the task of understanding dispersal and hence population structure and boundaries. Some techniques for measuring dispersal in adults are common to fully terrestrial insects. Passive interception trapping methods, such as sticky traps and malaise traps, have been widely deployed in studies attempting to assess the distance and direction of adult flight, although there is some evidence that the latter methods may provide spurious results with regard to direction (Macneale, Peckarsky & Likens, 2004). Nonetheless they have consistently shown that adults predominantly stay close to the stream. However, while much research has focussed on movement along stream, even relatively rare lateral movements may have significant implications for populations (Macneale, Peckarsky & Likens, 2005). Passive methods are more powerful when combined with mass marking methods which include the addition of isotopes (e.g. nitrogen, ^{15}N) into a water body and which are incorporated into the insect tissues (Macneale, Peckarsky & Likens, 2005; Caudill, 2003; Briers *et al.*, 2004; Hershey *et al.*, 1993). Macneale, Peckarsky and Likens (2005) found that females of the stonefly *Leuctra ferruginea* travelled an average of 211 m upstream of the addition site, with a maximum of 663m. Importantly, some individuals were caught over 500 m away in an adjacent stream demonstrating dispersal between catchments is possible even for taxa considered to be poor fliers. Like the seeds of trees, adults insects can be dispersed by wind and models developed to predict seed dispersal have been adapted to model that of insects too, for instance mosquitoes (Estep *et al.*, 2010).

Larvae of lotic insects are also transported advectively by the downstream flow of water, collectively referred to as 'the drift'. Unlike transport by wind, drift is unidirectional and confined to the (typically) dendritic branches of stream drainage catchments. The drift and its role in the rapid colonisation of disturbed or introduced substrates has been the subject of much research (see Mackay, 1992), though crawling and swimming have been shown to deliver more colonists in some studies (e.g. Doeg, Lake & Marchant, 1989). Historically there has been an implicit assumption that in-stream connectivity is high, likely a result of apparent ubiquity of drift (Downes & Reich, 2008). However, presence in the drift does not indicate long distance dispersal (Downes & Lancaster, 2010); differentiating between movement for dispersal and movement over short distances for other activities, e.g. foraging, is an impediment in lotic systems as it is elsewhere (Stevens, Turlure & Baguette, 2010). Furthermore, there is some evidence that the assumption of high larval connectivity is misplaced. Lancaster, Downes and Arnold (2011) found that *Baetis rhodani*, which is a strong swimmer and thus assumed to disperse long distances, rarely dispersed between adjacent riffles during early instars and less than expected in late instars. Support for this finding can also be found in analysis of chromosomal and mitochondrial DNA. Reviewing their work in tropical Queensland, Bunn and Hughes (1997) concluded that adult movement was the primary mechanism of dispersal with low dispersal at the larval stage. High levels of differentiation were found at small scales across a range of taxa, and were attributed to the larvae arising from a few matings and thus not being representative of the wider population. Surprisingly, genetic distance was not correlated with geographic distance i.e. individuals in locations in proximity are not necessarily more closely related than those that are further removed. More recently this finding was supported by work elsewhere on mitochondrial DNA, but not chromosomal DNA, suggesting that females a stay close the channel while males disperse (Hughes *et al.*, 2003). Despite a growing interest in dispersal and population structure, it is safe to say that the freshwater literature is significantly behind that of many marine organisms and select terrestrial insects in pinning down the scale at which metapopulations occur.

As the body of research on dispersal develops, researchers will be better able to identify and explore the implications of different structures on populations dynamics through the testing of hypotheses. Possible structures can be brought into focus using the metapopulation concepts described above superimposed on the distinctive branching structure of a stream. Figure 1.1 illustrates three possible scenarios for the spatial structuring of populations given different motilities during larval and adults phases. The first (a) shows isolated populations where both larval and adult movements are very limited and thus the populations are effectively closed; with no immigration or emigration it is likely that numbers of individuals in subsequent life history stages would be highly correlated. In the second scenario (b) the adult stage is restricted (closed), whereas larvae disperse widely (open) and we could expect numbers of adults to be set initially by local emergence of adults. The final scenario (c) is the reverse with adults dispersing widely (open, closed at larval stage). A scenario where both stages disperse widely at the given scale is not shown but would be effectively a 'zoomed-in' view of scenario (a). The frequency of dispersal at the larval stage and adult stage in (b) and (c) respectively will determine the type of metapopulation structure at a given scale. Frequent dispersal (i.e. highly open) would make for a patchy population, low dispersal would make for metapopulation with populations linked demographically, while rare dispersal may be sufficient to connect the sub-populations such that landscape consists of one well-mixed (panmictic) population *genetically*. The latter scenarios differ from a patchy population in that sub-population sizes vary independently of each another. Distinguishing which scenario most closely represents the population structure of a taxa is very difficult (Bohonak & Jenkins, 2003), but worthwhile as it has profound effects on the influence of other processes. For instance, habitat limitation would be most likely to have the greatest effect under scenario (c) as females can disperse and respond to the distribution of oviposition sites, and lack of dispersal at the larval phase would mean that the patterns established during oviposition would persist.

1.3 Adaptive behaviours facilitate transitions between life history stages

In this section I look more closely at the processes occurring at the end point of dispersal (i.e. habitat selection) as these may ultimately determine the balance between supply or habitat limitation. I explore the factors that drive the evolution of post-dispersal behaviours and how they may benefit an organisms fitness, or that of subsequent life history stages. I review what is known about the process of oviposition in terrestrial and aquatic insects, including the senses employed, as well as some examples of apparent oviposition site preferences observed. I also note the challenge of establishing apparent preferences for certain types of oviposition sites.

The end point of dispersal marks a critical transition to a new habitat through which the success of the next life-history stage may be determined. Organisms differ in the level of influence they have over the location which they or their offspring ultimately inhabit. Plant seeds, relying predominantly on passive dispersal, have little control over where they end up. Marine larvae returning to the shore have a range of motilities and may also reject sites deemed unsuitable on contact (Zimmer, Fingerut & Zimmer, 2009). Adult insects can have relatively sophisticated sensing and motility (i.e. flight) that allows more agency in dispersal and habitat choice for many taxa (though some are poor flyers, dispersed by wind and some have less specific oviposition behaviours). This has obvious advantages as the growth and survival (i.e. the fitness) of the following stage (seedling, sessile adult, or egg stage and beyond) is reliant on habitat quality in terms of food resources, the presence of predators etc.

Indeed, for insects the selection of habitat (in this case, oviposition site) is equally important in determining fitness as so called 'hard' reproductive characteristics (egg number, size etc.) with which they co-evolve (Resetarits, 1996). Equally, the specificity of oviposition behaviours, i.e. the number of types of site accepted, is likely to be dependent on the strategy used to distribute eggs. For example, it is more important to make the most adaptive decision if eggs are laid in a batch rather than singly, as the entire genetic legacy of an individual is in the balance (Díaz-Fleischer & Aluja, 2003). Furthermore, the selective pressure towards specific oviposition behaviour may be stronger for organisms where the following life-history stages have little mobility and thus limited ability to select their own habitat (Gripenberg *et al.*, 2010). For example, larvae of some herbivorous insects (such as caterpillars, Floater & Zalucki, 2000) are constrained to the natal habitat, whereas larvae of freshwater insects are often assumed (perhaps incorrectly, Downes & Reich, 2008) to be able to disperse to new habitat. Thus, females of the former would be expected to have more specific requirements and adhere to them more closely.

The search for habitat can be modelled as a decision tree with the individual following a particular decision pathway, depending on the cues that it receives at different distances from a potential site. Certain cue(s) will allow an individual to locate a patch of sites (reef, stand of plants, riffle of rocks), a site (free rock surface, individual plant, suitable rock) and finally a specific location within the site, i.e. 'micro-site', that is most suitable, at each point accepting or rejecting locations based on the cues received. For example, herbivorous insects use olfactory structures to sense volatile compounds ('smell') released by plants, the ratio of which is thought to be species specific and allow females to locate preferred hosts from a distance (pre-alighting, Bruce, Wadhams & Woodcock, 2005). Some freshwater insects use visual cues (specifically, light polarisation) to locate suitable water bodies and, potentially, areas of suitable velocity (i.e. a patch, Encalada & Peckarsky, 2006; Reich & Downes, 2003b). Bacterially rich 'containers' (e.g. water-filled tree hollows, Albeny-Simoes *et al.*, 2014) and conspecific egg masses (McCall, 1995) emit plumes of volatile compounds that act as an olfactory beacon for ovipositing culicids. Chemosensory organs may be used once a suitable plant has been located to sense for excretions of conspecific larvae (Almohamad *et al.*, 2010) or predators ('taste'). Mechano-receptive hairs may be used to select a suitable micro-site too, allowing aquatic insects to sense water velocity directly when choosing where to oviposit. Given the sophisticated sensing structures and behaviour that have evolved to facilitate habitat selection by ovipositing females it seems likely that there would be substantial adaptive benefits.

If we assume that females are acting in an adaptive way, i.e. that behaviours are the product of a selection gradient, the question arises: how is behaviour increasing fitness and for what life history stage? There is a long history of interest in this question in the insect herbivore literature (Gripenberg *et al.*, 2010). Early work focussed on the quality of plant host as nutrition for larvae and gave rise to the Preference Performance Hypothesis (PPH, Jaenike, 1978). However, in some cases less nutritious plants may be preferred if they provide another benefit, such as protection of eggs or larvae from predators or competitors (Gripenberg *et al.*, 2010; Awmack & Leather, 2002). In addition, females may respond positively or negatively to the presence of conspecific larvae that can physically affect the host quality by modifying roots (Clark, Hartley & Johnson, 2011) or foliage (Awmack & Leather, 2002). Larvae of the same species, or indeed from different species with overlapping resource requirements, may be a source of competition and be avoided by females (Atkinson & Shorrocks, 1984). Aside from effects on larvae the site selected by females can affect the successful development of egg themselves – laying in proximity to other conspecific egg masses may lower the mortality due to reduced predation by the 'dilution' effect (Bovill, Downes & Lancaster, 2014; Williams, Smith & Stephen, 1993). In a rare test of the PPH on lotic insects, Bovill, Downes and Lancaster (2013) found that *Apsilochorema* egg masses transplanted from preferred slow flowing locations to faster flowing locations were scoured by the elevated shear stress, indicating that site choice directly impacted on egg survival. Alternatively, in some cases it appears that selection gradients have generated behaviours that benefit adult females rather than their offspring, e.g. selecting hosts that are optimum for adults rather than larvae nutrition (Clark, Hartley & Johnson, 2011). While this seems counterintuitive, assumedly this is the most adaptive strategy and benefits the next generation is a different way (e.g. hosts that provide quality food resources may allow for more or better provisioned eggs Awmack & Leather, 2002). In some cases the distribution of egg masses does not appear to reflect an adaptive choice, for example where something interferes with a female's ability to successfully select the preferred oviposition site. Floater and Zalucki (2000) found that reduced apparency of preferred tree species in mixed species forest led females of *Ochrogaster lunifer* to aggregate masses on a small number of individuals of the preferred hosts while other individuals were spared. High densities of egg masses lead to increased larval mortality compared to populations in low diversity forest, resulting in a reduced likelihood of 'boom' and 'crash' cycles.

In light of the potential influence that oviposition behaviours can have on population dynamics it is important that preferences are accurately described. To that end, I briefly review some of the pitfalls that can lead to misidentification of preference and supposed fitness benefits. There are a number of challenges in establishing preferences in the first instance and a fitness benefit in the second. The word "preference" is often used loosely to describe an association of high organism density with a particular habitat type, but such correlations do not necessarily capture actual preference and certainly do not provide good evidence for it. Carefully planned experiments are required to establish true preferences when organisms have choices; preferences are often context dependant (e.g. density of prey, presence of predators etc.) and therefore experiments require multiple controls to reach valid conclusions (Underwood, Chapman & Crowe, 2004). Such experiments may be logistically feasible in a laboratory or greenhouse, however this has its own limitations. For example, in a series of neat laboratory experiments Albeny-Simoes *et al.* (2014) made the puzzling discovery that female *Aides aegypti* (Culicidae) would lay eggs preferentially in containers with the predator *Toxorhynchites* spp. (Culicidae), which would seem mal-adaptive. The reason was that partially consumed larvae created substrate for bacterial growth and increased volatile metabolite cues that attract females above control levels. However, in field conditions where there is a baseline abundance of bacteria, the contrast in bacterial activity between containers (e.g. tree hollows) with and without predators may not be meaningful i.e. sufficient to elicit a response from females. It is also worth noting that a non-significant result for a cue does not mean the cue is unimportant under other conditions or that females are not making adaptive decisions based on some other cue not considered, of which there is a long list. Even where experiments are conducted in the field care must be taken when applying the findings to other conditions. Furthermore, though it is often assumed that behaviours are adaptive, behaviours without any fitness benefit may arise as a product of genetic drift in the absence of strong selective gradients (Raine *et al.*, 2006).

The literature on oviposition by freshwater insects is much less developed relative to phytophagus insects, with the notable exception of disease vectors such as mosquitoes and blackflies (Lancaster & Downes, 2013). In addition, eggs of lotic insects have been quantified by several authors (Elliott, 2013; Elliott, 1982; Willis & Hendricks, 1992) for the production of life tables, which provide important insights into the rates of mortality throughout the lifecycle, but are not intended to elucidate the processes underlying oviposition. Like terrestrial insects, aquatic insects exhibit a range of oviposition strategies, which typically involve depositing eggs into the water, though some taxa lay above the water on overhanging vegetation or emergent wood (Hoffmann & Resh, 2003). The scale of selectivity varies between taxa and methods; some species simply choose a water body and broadcast their eggs onto the surface, while others will choose a specific location and lay their eggs in a mass. However, the former may still exhibit selective behaviours, for instance a two step process (e.g. water body, channel unit) rather than a three step process (e.g. water body, rock, position on rock). Females adopting the latter strategy are therefore likely to evolve more specific adaptive behaviours. Furthermore, egg masses are able to be located, identified and counted readily in the field and offer the possibility of experimental manipulation (Bovill, Downes & Lancaster, 2013; Hoffmann & Resh, 2003). Females from a number of taxonomic groups have been shown to have specific oviposition preferences that relate to physical and biological characteristics of the prospective site and surroundings. Members of Hydrobiosidae (Bovill, Downes & Lancaster, 2013; Reich *et al.*, 2011) Rhyacophilidae (Lancaster, Downes & Arnold, 2010b), Uenoidae (Hoffmann & Resh, 2003) and Baetidae (Encalada & Peckarsky, 2006; Lancaster, Downes & Arnold, 2010a; Peckarsky, Taylor & Caudill, 2000) have been shown to lay egg masses exclusively on emergent rocks with some species showing a preference for those with large 'landing pads' (area of emergent portion)(Reich & Downes, 2003b), and for specific locations upon the rock (Reich & Downes, 2003a). Emergent wood is also utilised by some taxa (e.g. *Onocosmoecus unicolor,* Limnephilidae; Hoffmann, 2003). Preferences are also exhibited for particular locations in the stream such as particular channel units (i.e. riffle vs. pool) and proximity to the bank, which may be related to flow conditions (Bovill, Downes & Lancaster, 2013), or other factors such as shading (Hoffmann & Resh, 2003). Velocity has been demonstrated experimentally to be an important cue, with some taxa showing preference for fast flows (e.g. *Ulmerochorema*), others slow flows (e.g. *Apsilochorema,* Reich, 2003).

There is little definitive information available on the adaptive benefit for these choices by lotic taxa, with some notable exceptions (e.g. Bovill, Downes & Lancaster, 2013). Decisions are often assumed to benefit the egg stage, as larvae are generally considered to be mobile and able to select appropriate habitat (Peckarsky, Taylor & Caudill, 2000), though there is some evidence to suggest this assumption is misplaced (Lancaster, Downes & Arnold, 2011). Positive associations between conditions selected by ovipositing females and conditions of habitat occupied by larvae in sites without oviposition substrate provide some evidence that females may make decisions based on larval requirements for some taxa (Reich & Downes, 2004). It is also worth considering that survival of the adult stage may drive oviposition preferences, as drowning or predation are very real risks (Encalada & Peckarsky, 2007). Adult behaviours, such as swarming, have been advanced as a possible explanations for the high aggregation of egg masses, often on particular substrates over successive generations (Reich *et al.*, 2011), in spite of an excess of unused, apparently suitable oviposition substrates (Peckarsky, Taylor & Caudill, 2000). Attraction to conspecific egg masses could also explain this pattern, though it has been ruled out for some taxa (Reich *et al.*, 2011; Encalada & Peckarsky, 2006). Of course, there may be countless other cues that influence suitability and many of the oviposition sites that appear suitable according to our present knowledge may be rejected by females.

1.4 Oviposition substrate as a limiting resource for lotic insects

Having reviewed the present knowledge of female oviposition behaviours in aquatic insects, I will now discuss how these small scale decisions can have population scale implications. I will review the evidence for habitat limitation in streams and touch on the factors that influence the persistence of patterns established during oviposition, before identifying some promising avenues for further progress in this area.

As discussed in section 1.2 with reference to marine invertebrates, the organisms with complex life histories that include mobile/dispersive stages transitioning to a sessile phase requiring particular habitat may be supply or habitat limited. Section 1.3 detailed the many selective oviposition behaviours exhibited by females requiring hard substrates upon which to lay. Thus oviposition by females is analogous to settlement by marine larvae and may have similarly large effects on population dynamics.

Taxa with more specific oviposition requirements are hypothetically more likely to be habitat limited. For example, species requiring emergent rocks (e.g. *Baetis*) will have fewer sites available than a species that lay on fully submerged rocks (e.g. *Cheumatopsyche*), barring other requirements. Similarly, Bovill, Downes and Lancaster (2013) concluded that *Apsilochorema*, which avoid laying in proximity to conspecific masses would be more likely to be habitat limited that *Ulmerochorema* that lays in aggregations.

The distribution of potential oviposition substrate varies in both space and time. Emergent boulders, for example, are typically more abundant in the upper reaches of streams.

Lancaster, Downes and Arnold (2010a) found a three-fold difference between comparable stretches of different streams having different channel morphologies and rock sizes. Additionally, seasonal hydrological factors, such as snowmelt and spates can restrict the availability of suitable substrate at critical times (Peckarsky, Taylor & Caudill, 2000). A number of studies have shown that egg masses of some taxa co-vary with substrate availability (Encalada & Peckarsky, 2011; Lancaster, Downes & Arnold, 2010a) suggesting that egg inputs may be substrate limited. Encalada and Peckarsky (2012) manipulated natural substrates and found that a 50% increase in emergent rocks gave rise to a 400% increase in *Baetis bicaudatus* egg masses, thus establishing a causal constraint for this species. Alp, Indermaur and Robinson (2013) added bricks to stream reaches and found that substrate was a significant predictor of egg mass densities of both *Baetis* and *Hydropsyche* species. In contrast, an unreplicated study by Blakely *et al.* (2006) in an urban stream found that egg mass distribution remained unchanged despite the addition of rock substrates. Built structures (e.g. culverts), however, may have inhibited the dispersal of females to the new sites (Smith, Alexander & Lamp, 2009).

The implications of substrate limitation for populations hinges on how long the effects persist throughout the lifecycle and research on the relevant drivers is limited. Nonetheless, elevated *Baetis* spp. densities have been shown to persist through to late instar larvae after experimental manipulation in North America (Encalada & Peckarsky, 2012), and in Europe, 80% of variability in distribution of late instars could be attributed to oviposition substrate (Lancaster & Downes, 2014a). In contrast, a transplant experiment that doubled egg inputs did not lead to a persistent change in densities of the predatory alderfly, *Sialis fuliginosa*, over two generations (Hildrew *et al.*, 2004). However it is worth noting that, due to logistic constraints, the experiment was unreplicated and the outcome may be peculiar to the individual study site. In an observational study, Reich and Downes (2004) found no difference in larval densities between sites with and without oviposition substrate and they, like Hildrew *et al.* (2004), attributed this to high levels of density dependent mortality and dispersal among early instar larvae. High mortality among early instar larvae has been found for some taxa (e.g. *Baetis rhodani,* Elliott, 2013; *Odontocerum albicorne*, Elliott, 1982) and has been attributed to cannibalism in some cases (e.g. *Hydropsyche slossonae*, Willis & Hendricks, 1992). However, there is little evidence to suggest that drift is density dependent, i.e. higher in locations with more substrate and thus more eggs and first-instar larvae

(Lancaster, Downes & Arnold, 2011). Furthermore, recent evidence suggests that *Baetis*, considered to be very agile, rarely moves between riffles (Lancaster, Downes & Arnold, 2011), which suggests that assumptions of frequent dispersal be scrutinised for other taxa as well. It is worth noting too that high abundance in the drift does not necessarily reflect high rate of, or indeed, successful dispersal (Downes & Lancaster, 2010).

1.5 Conclusions

I have summarised the compelling evidence that availability of oviposition substrate can limit egg inputs and that these maternal effects persists through to late instar larvae for select taxa. However, research to date has been focused on a limited number of species predominantly in cobbled, upland streams. In order to test the generality of substrate limitation, research is needed in different stream types and with different taxa. *Baetis* species have been central to progress in this area and researchers have been able to develop detailed knowledge of its lifecycle, dispersal propensity etc. I suggest that investing that level of focus an alternative taxa, or better two contrasting taxa, would be a fruitful next step. Useful attributes of focal species include 1) laying a single egg mass, 2) well resolved taxonomy. Furthermore, the evidence of habitat limitation on oviposition uncovered by research so far suggests that other relatively neglected stages of insect lifecycles (e.g. egg and pupal mortality), may yield new insights into the dynamics of populations.

The idea that populations might be limited by the supply of new individuals via a dispersive life history stage was primarily developed by marine researchers over 30 years ago. The fact that this concept, so significant in progressing ecological understanding elsewhere, has been investigated by a handful of researchers for half the time underlines the importance of communication between colleagues addressing the same questions in different systems. This observation is not new and aquatic systems, both saline and fresh, have been identified as natural partners for such dialogue (e.g. Downes & Keough, 1998).

1.6 Thesis structure and aims

The overall aim of this thesis is to investigate the possibility of habitat limitation on rates of oviposition by two taxa not previously studied in this context – caddisflies of genera *Ecnomus* and *Cheumatopsyche* – in streams very different to the cobbled, upland streams that have typified research to date. My research was conducted in Creighton's, Hughes and Seven Creeks, three sandy-bed streams arising in the Strathbogie Ranges, Victoria, Australia. Given the paucity of rock in these streams, females oviposited on submerged bark and wood. I exploited the manipulable nature of bark to test a range of hypotheses. The following summaries of Chapters 2-4 briefly outline my approach and detail the hypotheses tested in the summers of 2007/08, 2008/09 and 2009/10 respectively. In Chapter 5, I summarise my findings, discuss the implications for our understanding of population dynamics and suggest some fruitful avenues for future research.

Chapter 2

I surveyed the abundance of egg masses of two caddisflies *Ecnomus* and *Cheumatopsyche* and surface area of bark and wood substrates in 16 sites across three streams (Figure 2.1), Creighton's, Hughes and Seven Creeks, on three occasions during the summer of 2007/08. This provided baseline data in a hitherto unstudied egg stage in this system with which I could test the hypothesis that egg supply was limited the availability of oviposition sites i.e. habitat limited, rather than the supply of gravid females i.e. supply limited. A positive relationship would support the former, while no relationship would support the latter, though not conclusively, it being an observational study.

Chapter 3

I conducted three experiments investigating whether *Ecnomus* and *Cheumatopsyche* females lay on substrates with particular characteristics i.e. 'preferences' and the ways in which females accessed substrates (i.e. directly from the air or swimming or crawling underwater). In experiment 1, I manipulated emergence of individual pieces of bark (emergent vs. submerged) and the velocities (< 0.15 ms-1 vs. > 0.2) in which they were located. The aim was to test whether factors that had been shown to be important in cobbled streams for a number of taxa were also applicable in different substrate and stream types. Observations from the survey (chapter 2) suggested that *Cheumatopsyche* tended to lay masses predominantly in fast flows so it was expected that the pattern would be repeated and thus established formally in the experiment. Experiment 2 and 3 tested whether the suitability of bark as an oviposition substrate changed the longer it spent submerged underwater (i.e. level of 'conditioning': Fresh vs. Partially Conditioned vs. Fully Conditioned). Unlike rock, bark degrades appreciably over time so I hypothesised that fewer masses would be laid on more highly conditioned bark. In experiment 3, in addition to bark condition, I manipulated the pathways via which females could access the submerged portion of an emergent piece of bark, by placing a sticky barrier (Tangle trap) at the waterline (Figure 3.1B, D). Some taxa are able to dive directly into the water (including hydropsychids, (Deutsch, 1984)), while others utilise emergent landing pads (Peckarsky, Taylor & Caudill, 2000). I tested whether a significant number of females used this latter pathway.

Chapter 4

The large-scale, manipulative experiment detailed in Chapter 4 draws together two threads from previous chapters; (1) habitat limitation and (2) oviposition preferences, and tests multiple hypotheses in concert at two scales: whole sites and individual pieces of bark, respectively. Bark was manipulated in fourteen 25 m long sites to create four site-level treatments: High density (4 sites), Low density (4), control (3) and procedural control (3). Effectively, I tested the same hypothesis as in Chapter 1 – that the number of egg masses laid would be habitat limited – but with the inferential power of an experiment. Within High and Low sites, individual pieces of bark were manipulated with respect to emergence and velocity as in experiment 1, Chapter 3. However, this experimental design tested whether the oviposition behaviours varied with the amount of substrate available. Parasitic wasps are able to alter their feeding and oviposition behaviour in response to different densities of host (Yang *et al.*, 2012), but there have been no equivalent experimental studies of this in lotic insects.

1.7 Figures

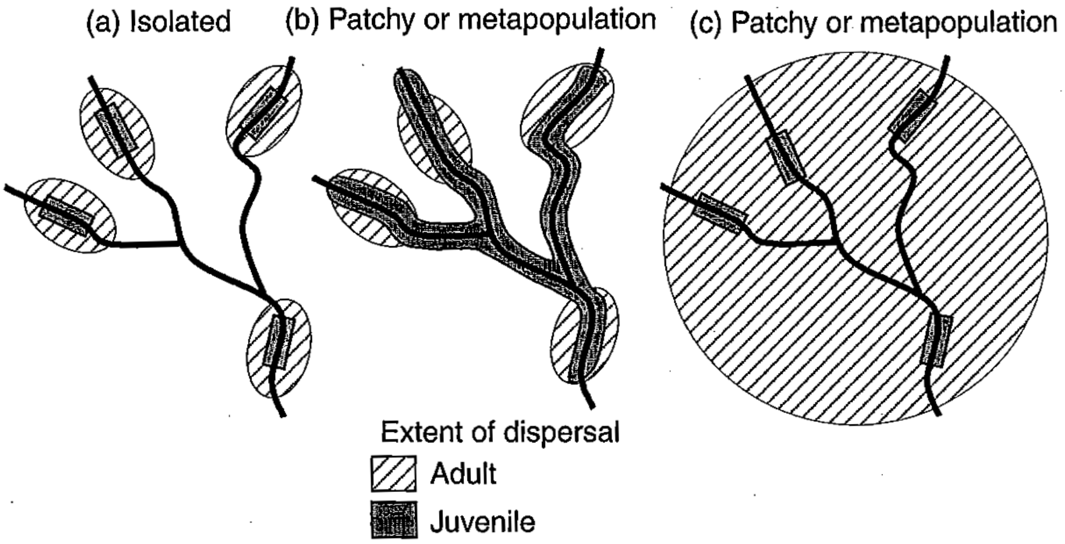

Figure 1.1: Possible metapopulation structures for aquatic insects in streams (Downes & Reich, 2008)

Chapter 2. Distribution of caddisfly egg masses and oviposition substrate in the Strathbogie Ranges.

2.1 Introduction

Complex life cycles have at least two distinct phases and two phase transitions that can be marked by dramatic changes in form and habitat, especially where the stages occupy different mediums (e.g. aquatic vs. terrestrial). For instance, aquatic insects have a typically long lived, free living aquatic larval stage, sometimes a pupal phase, followed by a terrestrial winged adult phase that completes the cycle by returning to water to lay their eggs in (or near) the water. Barnacles begin life as a mobile pelagic nauplius larva that moults to a cipris larva in preparation for settlement onto the shoreline and metamorphosis into a sessile adult. Plants too have complex life histories, and like barnacles, have a sessile adult phase. However, the dispersing seed phase, has no active role in determining where they end up. The number (supply) of individuals transitioning (recruiting) into a new phase can have profound consequences for the dynamics of populations i.e. by determining the strength of density dependent processes (e.g. competition between *Chthamalus* and *Balanus* barnacles, Connell, 1961). If we focus on dispersive (or at least mobile) phases (e.g. marine larvae, adult insects) transitioning into new habitat, the starting distribution of the new phase (sessile invertebrate adult, adult fish, insect eggs i.e. 'recruits') can be driven by processes occurring during, 1) the mobile phase (e.g. mortality, dispersal i.e. connectivity with source populations; supply limited) or 2) by the availability of suitable sites for the new phase (habitat limited) (Schmitt & Holbrook, 2000).

When arriving into a new habitat, behavioural requirements for territory (e.g. some reef fish, Swearer *et al.*, 1999) or requirements for particular conditions (e.g. emergent rocks, Encalada & Peckarsky, 2006) mean that the number of sites available is less that the absolute area (i.e. m^2 or m^3) of habitat available. The selectivity or choosiness of a species (i.e. breadth of acceptable conditions), will determine the number of suitable sites in a location. In a terrestrial example, ovipositing herbivorous insects vary in the number of plant hosts they will accept with those accepting multiple species likely to have more sites in a given habitat (Thompson & Pellmyr, 1991). Where all suitable sites are occupied, the remaining portion of the settling cohort must disperse elsewhere (and suffer associated fitness costs) or expire (Peckarsky, Taylor & Caudill, 2000).

Adult females of freshwater insects effect the transition to the next life history stage by placing their eggs into or near water via a variety of strategies. Here we will focus on insects that inhabit flowing waters (i.e. lotic insects). Many taxa lay their eggs together in a mass and require a solid substrate upon which to lay and can display apparent preferences for particular substrate characteristics. *Baetis* females, for example, alight upon large, emergent, un-embedded boulders, crawl into the water and lay their eggs on the underside (Peckarsky, Taylor & Caudill, 2000). The availability of suitable substrates has been shown to constrain the distribution of *Baetis* egg masses through correlative and experimental studies in both North America (see Encalada & Peckarsky, 2012) and Europe (see Lancaster, Downes & Arnold, 2010a). However, females do not use every apparently suitable rock, or indeed the entire submerged surface of individual rocks (Reich & Downes, 2003b). Of the small body of literature testing for habitat limitation – that is, whether females encounter a shortage of suitable oviposition sites – the majority has been conducted in cobbled and/or upland streams (but see Blakely *et al.*, 2006), on a small range of taxa, with the strongest evidence being for *Baetis* species. However, many streams do not have abundant rocks, instead having beds of sand or mud. In these streams, hard surfaces, like those required by ovipositing females, are predominantly in the form of wood or bark that falls into the stream from riparian vegetation (Bond *et al.*, 2006). Hughes, Creighton's and Sevens Creeks in central Victoria, south-eastern Australia, are three such streams and are the subject of the study described in this chapter.

In order to test the generality of habitat limitation, it is vital to see if it applies in different types of stream and with different taxa, which is the overall aim of this thesis. The first step is to acquire baseline information about the distribution and identity of common egg masses and substrates in the study streams, as well as gaining preliminary evidence of habitat limitation if it occurs. I hypothesise that there will be a positive relationship between the number of egg masses in a length of stream and the amount as measured by surface area (SA) of substrate (bark and wood) present (hypothesis 1). As this is an observational study I will be unable to establish causality – a positive relationship could indicate that substrate is limiting, or that the distribution of eggs is driven by a factor that co-varies with substrate area. A lack of relationship could indicate that egg inputs are limited by supply of ovipositing females rather than by shortages of oviposition substrate or that the absolute measure of habitat (i.e. total SA of all bark and wood regardless of type, condition, location, size, etc.) does not accurately reflect the availability of suitable site as determined by female behaviour. The effect of female behaviour on availability of suitable habitat and causality are addressed by experiments detail in chapters 3 and 4 respectively.

2.2 Methods

Study area

Hughes, Creighton's and Seven Creeks arise in the Strathbogie Ranges approximately 100 km NNE of Melbourne, Victoria, Australia and drain north-westerly, before joining the Goulburn River (Figure 2.1). Aside from occasional granite outcrops their beds are naturally sandy, though vegetation clearance for grazing is thought to have increased sediment loads in some areas (Davis & Finlayson, 2000). Remnant vegetation is concentrated in the riparian zone and is most intact in the upper reaches (Downes *et al.*, 2011). River red (*Eucalyptus camaldulensis*) and mana gums (*E. viminalis*) dominate the vegetation, which lacks a mid-storey along much of the study streams. The eucalypts drop large amounts of bark during summer (Reid *et al.*, 2008), along with the occasional limb, providing the predominant source of hard substrate in the streams. However, detrital loads are still relatively low: <6 % areal cover and <400 g dry weight m^{-2} (Lancaster & Downes, 2014b). The characteristics of the streams (particularly their sandy beds), proximity to Melbourne, and prior research in the area made them ideal for testing the concept of habitat limitation. Predominance of bark and wood substrate sets the study streams apart from rocky upland streams and offered an opportunity to test habitat limitation in a new type of system. The light and tractable nature of bark facilitated small and catchment scale experimental manipulations.

The climate in the region is temperate having hot dry summer and cool wet winters. Temperatures during the study exceeded 45 °C, though average maximum is 21 °C. The average minimum temperature is 9 °C, but the area does receive frosts in the winter (i.e. < 0 °C) (R. Strong, personal communication). On average the area receives 800-1000 ml rainfall per year concentrated in winter and spring (Australian Bureau of Meteorology). However, the study was carried out in the final years of the ‘Big Dry’, a drought that affected the region for over a decade (Ummenhofer *et al.*, 2009) (Figure 2.4). Consequently, stream discharge typically declined significantly over summer and lower reaches became disconnected in Seven and Creighton's Creeks. Velocities ranged between 0 and 0.55 ms^{-1} (Section 3.2, Lancaster & Downes, 2014b).

Study taxa

Two caddisfly taxa are the focus of this study: *Cheumatopsyche* spp. and *Ecnomus* spp. As will be explained below (Results), it is easy to distinguish between the egg masses of different caddisfly families in the field (Figure 2.3), but it is not possible to differentiate between genera within families. However, previous work on other life history stages in the study area shows that *Cheumatopsyche* dominate the hydropsychids in the study system, especially *Cheumatopsyche* AV4 and *C. deani* Neiboss (Downes *et al.*, 2011). Ecnomids are exclusively from the genus *Ecnomus* with *E. continentalis* being an order of magnitude more common than its congeners at both adult and larval stages (Downes *et al.*, 2011; Lancaster, Downes & Glaister, 2009).

Ecnomus larvae are predatory, constructing nets to catch prey (Wiberg-Larsen, 1993), while *Cheumatopsyche* uses nets to filter-feed (Dean & Bunn, 1989). Both taxa inhabit sand and leaf packs in similar numbers, though *Cheumatopsyche* is found in fast flowing and *Ecnomus* is more common in areas of slow flows where fine sediments accumulate (Lancaster, Downes & Glaister, 2009). *En masse, Cheumatopsyche* nets can stabilise sandy areas in concert with development of biofilm (O'Connor, 1993). Larval densities for both taxa reach 500 to >1000 individuals m^{-2} in benthic samples and are also common in the drift (Downes *et al.*, 2011; Lancaster, Downes & Glaister, 2009; Lancaster and Downes, unpublished observations). Adults are abundant along the stretches of Seven and Hughes Creeks used in this study (Downes *et al.* unpublished observations). *Ecnomus* spp. populations in the La Trobe River (~150 km SSE of study location) are thought to be bi-voltine with a slow growing winter generation and a fast growing summer generation (Marchant *et al.*, 1984). While there is some data on the voltinism of *Cheumatopsyche* in other locations, the closest geographically being Hong Kong (Dudgeon, 1997), it is unlikely that they are applicable to our study taxa given the plasticity observed in such traits (Sanchez & Hendricks, 1997). *Hydropsyche orris* , a member of Hydropsychidae like *Cheumatopsyche*, has been reported to have an adult span of 10-13 days, with females succumbing post oviposition (Badcock, 1953). A thorough search of the literature did not yield equivalent information for members of Ecnomidae.

A survey in nearby (< 100 km away) upland streams found egg masses of two hydropsychid taxa, *Asmicridea* and *Smicrophylax*, on submerged rocks. They were described as flat masses comprised of concentric rows of closely packed, pale to dark pink eggs and were observed to hatch within 14 days (Reich, 2004). I am unaware of any descriptions of *Ecnomus* egg masses in the area. A search for egg masses in two 40 m sites in areas with bedrock was conducted to assess the contribution of rocky outcrop to egg inputs. Very few masses were found especially given the large surface area available (see Section 4.2[1]). Egg masses have not been observed on sand despite the large number of benthic samples taken and processed from these streams (Downes & Lancaster, 2006; Downes *et al.*, 2011; Lancaster & Downes, 2014b)

Egg mass identification and hatching times

Approximately 100 egg masses were collected on bark from multiple locations in Hughes (HCA, HTA, HDU, HBB) and Creighton's Creeks (CGF, CBL) and hatched in the laboratory in Petri dishes following procedure of Reich (2004). Hatchlings were identified to the lowest possible taxonomic level to ensure accurate identifications in the field. To my knowledge there is no published data on hatching times of *Ecnomus* or *Cheumatopsyche* in the study area. However, two hydropsychid taxa were found to hatch within two weeks in upland streams nearby (< 100 km)(Reich, 2004). A short pilot study, conducted in tandem with the survey detailed in this chapter, followed the fate of 85 *Ecnomus* and 118 *Cheumatopsyche* egg masses and found that 97% and 98% of masses, respectively, hatched within 19 days (for further detail see Chapter 4[2]). Similarly high rates of hatching success have been observed for *Hydropsyche slossonae,* in the Appalachian Mountains (Willis & Hendricks, 1992).

Survey design

The number of egg masses on bark and wood was counted at sixteen, 40 m long sites on Hughes (6 sites), Seven (6) and Creighton's Creeks (4) at three times (11-19 December 2007, 6-21 February and 17-25 March 2008). Study sites (Figure 2.1) were selected haphazardly from accessible locations that 1) had some native overstorey, 2) no rocky outcrops, 3) had

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[1] Some information that would normally be reported more fully in this chapter has been reported in chapter 4 as it was required as supporting context for the publication.

[2] Some information that would normally be reported more fully in this chapter has been reported in chapter 4 as it was required as supporting context for the publication.

both pool and run channel units and 4) were at least 500 m from any other site. The lower portion of the study streams are prone to drying over summer and are more degraded, so the upper portion of the creeks were used. The suitable section of Creighton's creek was shorter than the other streams and had fewer access point allowing only four sites. Each piece of bark or wood with a minimum dimension greater than about 2 cm was inspected and the number and identity of any eggs recorded along with the substrate's dimensions. Large pieces of wood i.e. logs were included. Bark and wood without egg masses were tallied and the dimensions of up to 20 randomly selected substrates of each type were recorded. In sites with extraordinary amounts of bark a minimum of 20 m of site length were randomly sampled in 10 m lengths . The percentage of the site area occupied by pool channel type (i.e. typically deeper areas with slow flows and unbroken water surface) was assessed by eye and the total wetted area calculated from average wet widths

Statistical Analyses

Egg mass totals were calculated for each site, scaling up to 40 m where 20 m lengths had been sampled. Total bark and wood surface areas at sites were calculated by summing the areas of measured bark. The surface area of unmeasured bark and wood (i.e. most pieces without egg masses) was estimated by multiplying the number of pieces by the median SA of bark and wood for each site.

My primary interest in this survey was to test whether there was a relationship between the amount of substrate and number of egg masses that were laid (hypothesis 1). I approached the analysis as a multiple linear regression treating all possible explanatory variables (Creek, Trip, Bark SA, Wood SA, Percentage pool) equally, to ascertain which was the most predictive. Specifically, different trips were considered to be independent samplings, rather than repeated measures of the same sites, as sites are likely to be quite different from one sampling period to the next. New eggs and bark would have replaced those that had hatched or washed away respectively and the sandy bed changes quite markedly over time (Pers. obs.). In addition, graphical exploration of egg mass data showed no correlation between times.

An analysis comprising two steps was performed in R (R-Core-Team, 2014) used for each genus: 1) a model selection procedure ('leaps' package in R) was used to identify highly predictive terms i.e. those likely to be of most interest, and 2) a model containing these terms was analysed using a hierarchical partitioning procedure ('hier.part' package in R). The 'leaps' procedure uses an efficient branch-and-bound algorithm to select the best predictive terms for a given size of model, i.e. 1 predictor, 2 predictors... etc. (Lumley, 2009). Unlike other discredited methods, such stepwise model selection (Quinn & Keough, 2002), the 'leaps' package fits all possible combinations of terms. Interaction terms that were consistently found in models with low BIC (Bayesian Information Criterion) values (i.e. most parsimonious) were identified and included, along with the main effects, in a model of 'best fit' for each taxon. The independent explanatory power of terms in the best fit models were calculated using the hierarchical partitioning package in R (MacNally & Walsh, 2013). Hierarchical partitioning calculates the independent R^2 for each term averaging across all possible combinations of the terms i.e. the proportion of variation in the dependent variable that can be attributed to each term (MacNally, 1996). Three sites in Seven Creeks dried to a chain of pools in March 2007 and the analyses where conducted with and without these data to assess the effect on the results.

Variation in bark SA and wood SA over time (Trip) and space (Creek) was analysed using a two-way ANOVA. Creighton’s Creek was not sampled during Trip 1, so it was not possible to obtain a test of interaction between Trip and Creek for this creek. Pearson correlation coefficients were used to asses the relations between bark and wood SAs. Transformations were applied to egg mass counts ($\log_e$) and bark ($\log_e$) and wood surface areas (square root) to meet model assumptions and reduce the influence of outlying individual data points.

2.3 Results

Egg mass identification

Ecnomus egg masses are white or light grey and are typically laid in small, tightly arranged, loosely ovaloid masses with ragged edges (Figure 2.32D), but can also be found in more linear arrangements (Figure 2.3C1). *Cheumatopsyche* masses vary in colour: cream or white with various tints of pale blue, green or yellow being observed (Figure 2.3C2). The masses are often thumb-nail shaped, consisting of orderly rows of ovaloid eggs (Figure 2.3E) and can found in aggregations (Figure 2.3B). *Cheumatopsyche* eggs are larger and more elongate than *Ecnomus* eggs and may become deformed where they meet neighbouring eggs in tightly packed masses.

Analysis of egg masses

Bark SA explained 20% of the variation in *Ecnomus* egg masses while Creek explained over 50% (Table 2.2). The relationship between egg mass number and bark SA appears to be strongest in Hughes Creek where Bark SA varied the most widely (Figure 2.5). Preliminary analyses showed Bark SA to be the dominant predictor explaining over 60% of variation. However, transformation reduced the influence of the most extreme sites such as HCA3, which had close to 5000 egg masses and about 60 m^2 of bark SA, reducing the variability in egg masses predicted by bark to 20%. Hughes Creek had consistently higher numbers of egg masses than Creighton's and Seven Creeks; the latter had the lowest on average (Table 2.1, Figure 2.6). The models of 'best fit' explained 80% of variation for *Ecnomus* and 50% for *Cheumatopsyche*, while the 'main effects' models, included for comparison and having less terms, explained a lower proportion of the overall variability. It appears that substrate SA at the site level is not an important predictor for *Cheumatopsyche*, with both bark and wood SAs being relatively poor predictors (Table 2.1). Numbers of *Cheumatopsyche* masses were quite similar at creeks during trips 1 and 2, but declined in Creighton’s and especially Seven Creeks by trip 3 (Table 2.1, Figure 2.6). Percentage pool was not present in any models of best fit for either taxa.

Bark SA varied significantly between Creeks and Trips (Table 2.3) with SA being highest in Hughes Creek, while Creighton’s Creek had the lowest. Bark SA increased over the study and was higher at Trips 2 and 3 (Figure 2.7). Wood showed the opposite pattern with SA declining from the start of the experiment (Figure 2.7). Analysis of variance indicates that Trip is a significant term, but this test may be generous since the variation could be underestimated by the model because sites were measured at multiple times. Bark SA was higher than wood SA as a proportion of total SA in January and March (76%, 72%), but not December (29%) and reached a maximum of 86% at some sites (Table 2.1). Furthermore, bark contributes more variability in substrate SA at sites over time and space (SD = 11.58 m2) relative to wood (SD = 2.42 m2). There is little correlation between bark and wood SAs (r = 0.06, t = 0.3678, df = 37, p-value = 0.7152).

2.4 Discussion

The amount of bark surface area found in a site predicted a substantial amount of variation in the number of *Ecnomus* egg masses present. This correlation is consistent with habitat limitation, i.e. that sites where the is little bark receive fewer egg masses than sites where bark is abundant. If substrate were limiting, it would set the initial distribution of the new generation and could have important flow on effects for subsequent life history stages such as the strength of inter- and intra-specific interactions as has been observed in other systems (e.g. Connell, 1961). The persistence of any effect will depend on the post hatching mortality and dispersal (Reich & Downes, 2004), which are often assumed to be high (Downes & Reich, 2008). While these assumptions have been challenged by recent work on other taxa (Lancaster & Downes, 2014a), *Ecnomus* and *Cheumatopsyche* do travel via the drift in the study streams (Downes *et al.*, 2011), which may or may not indicate dispersal (i.e. rather than local movements, Downes & Lancaster, 2010).

In contrast to *Ecnomus*, substrate was a relatively poor predictor of the number of *Cheumatopsyche* egg masses in a site. This indicates that (1) *Cheumatopsyche* is supply limited or (2) that my measure of habitat i.e. total surface area does not accurately reflect the number of suitable oviposition sites for *Cheumatopsyche* females. In the first case, the distribution of egg masses would be determined primarily by that of *Cheumatopsyche* females, which is driven by patterns of emergence and rates of mortality and dispersal. Unfortunately, there is a paucity of data on these factors in the literature generally and only baseline information on the distribution of females: both adult and larval *Cheumatopsyche* have been observed throughout the study area (Downes *et al.*, 2011). Without more detailed information on the factors affecting the abundance and distribution of females it is impossible to assess the likelihood of supply limitation.

Cheumatopsyche egg masses where consistently observed in higher numbers on substrate in 'fast' flows (personal observation) and I hypothesise that this was the result of adaptive behaviours exhibited by females and an explanation of why substrate was not a strong predictor of *Cheumatopsyche* egg mass distribution. If females were focussing their masses on substrate in fast flows, a measure of substrate that includes bark in slow flow (i.e. total bark SA) is unlikely to accurately represent the amount of suitable oviposition substrate. In upland streams, the total number egg masses laid in a riffle has been related to the number of emergent of emergent rocks for some taxa, but not others which prefer emergent rock in very particular flow environments (Bovill, Downes & Lancaster, 2013; Bovill, unpublished data). Since bark can be transported in the flow I did not measure velocity during the survey as I could not be certain that point measurements would reflect the velocity when the egg masses where laid. My estimates of percentage pool were an attempt to account for this, but were perhaps too coarse a measure to be a useful predictor of egg mass numbers. Flow velocity has been shown to be an important cue for ovipositing females in upland streams (Reich & Downes, 2003a) and subsequent chapters will look more closely at this topic.

The difference in number of *Ecnomus* masses found in the different streams suggests a substantial difference in the number of adults and different population sizes. This is consistent with the findings of Downes *et al.* (2011), who found *Ecnomus* spp. larvae to be over 7 times more abundant in Hughes creek, relative to Seven Creeks. Although we know little about the dispersal capabilities of *Ecnomus* adults, it seems unlikely that females would disperse such distances (>10 km) in search of oviposition substrate. Adults typically stay close to the stream with small fraction dispersing 100s of metres (Macneale, Peckarsky & Likens, 2005) or up to several kilometres (Briers *et al.*, 2004). Numbers of *Cheumatopsyche* egg masses were initially similar in all streams, but numbers declined in both Creighton's and Seven creeks by trip three. The study took place during a drought and discharge had fallen significantly by this time, so that some of the study reaches in these creeks had become a chain of ponds (SKU, SKD), or had dried entirely (SNR). Given that *Cheumatopsyche* filter feed as late instar larvae, it is likely that females would avoid laying in still water, though they do oviposit in pools elsewhere, albeit in fast flowing, mountainous streams (Lancaster, Downes & Arnold, 2010b).

Bark was more abundant and more variable than wood in both space and time, a likely explanation for its predominance in predicting variation in *Ecnomus* egg masses. Bark accumulates over summer as bark drops from riparian trees. In general, riparian vegetation is more intact in Hughes Creek (Pers. obs.) making it no surprise that bark is more abundant. However, bark is vulnerable to transport by spates, which may also bury bark under the shifting sands common to streams in the area. While the same is true of small woody debris, larger snags are more likely to stay in place and may alter the bed form (Brooks *et al.*, 2004). Additionally, they may serve to anchor smaller debris and bark (O'Connor, 1992). Thus while bark is likely to be the most significant source of oviposition substrate in summer months, larger pieces of wood are may provide a more stable baseline of substrate during periods of frequent flooding, i.e. winter and spring.

Being correlative, this study cannot establish a causal link and it is possible that egg masses and substrate could co-vary with a third variable. For instance, trees that produce a lot of bark might also provide a habitat for mating adults, resulting in a high number of egg masses not because of the available bark substrate, but because it attracts a large number of gravid females. Swarming is a common mating behaviour for aquatic insects such as chironomids (Oliver, 1971) and is typically associated with a erect physical marker (e.g. a tree branch). It has been observed in hydropsychids elsewhere (Sullivan, 1981), but the mating behaviours of ecnomids are unknown. However, analogous correlations have been established elsewhere between baetid egg masses and emergent rocks (Encalada & Peckarsky, 2011; Lancaster, Downes & Arnold, 2010a), and subsequent studies have shown experimentally that substrate can be a limiting factor in upland streams (Encalada & Peckarsky, 2012). My own large-scale, manipulative experiment is detailed in chapter 4.

It would have been desirable to conduct this study over several years so as to better understand the generality of the observed patterns, however, this was beyond the scope of this PhD. It is possible, for example, that *Cheumatopsyche* oviposition, may be limited by the supply of adults. However, if recruitment to the adult stage was particularly low during the survey, as a result of the drought say, subsequent more favourable years may tip the population towards habitat (versus supply) limitation. In addition, more intensive study of the study organisms, particularly of their egg taxonomy, would be desirable. It is presently unclear whether minor variations in egg colour represent genetic variability between (or within) species, or are due to phenotypic variability. An egg's appearance is likely to vary with its developmental stage. Species level egg mass data would be ideal. The disadvantage of genus level classification is that some of the observed variation between streams and between trips could be due to a the mix of species varying in space and time. The high numbers of egg masses in Hughes Creek relative to the other creeks during trip 3 (Figure 2.6) could be due to the presence of a species that lays later in the summer, or that the species present were able to complete an additional life cycle.

This observational study provided baseline information on the distribution of egg masses and oviposition substrate in three creeks in the Strathbogie Ranges, which are quite different to those represented in the literature to date. Bark has been shown to be an important, possibly limiting, resource for ovipositing females, particularly ecnomids. Any relationship between egg masses and substrate will likely be mediated by a taxon's oviposition preferences, as I hypothesise is the case for *Cheumatopsyche*. I take advantage of the tractable nature of bark to address this experimentally in chapter 4.

2.5 Tables and Figures

Table 2.1: Summary statistics for substrate surface area (mean, m^2) and number of egg masses (sum) by creek and trip

	Bark			Wood		
	Trip 1	Trip 2	Trip 3	Trip 1	Trip 2	Trip 3
Creighton’s		3	1.59		1.19	1.57
Hughes	3.25	12.8	21.17	4.08	2.1	3.51
Seven	0.94	7.68	3.49	5.62	2.89	0.77
	Ecnomidae					*Cheumatopsychidae*
Creighton's		140	115		617	181
Hughes`	1505	1867	10421	427	1653	1493
Seven	25	169	1	397	1516	0

Table 2.2: Summary of hierarchical partitioning results. Cells contain percentage of variation in egg masses explained by each term. The total explanatory power is higher for 'best fit' models than 'main effect' models as the former contain more terms.

	Cheumatopsyche		*Ecnomus*	
	Main Effect	Best Fit	Main Effect	Best Fit
Creek	10.1	10.3	54.8	56.3
Trip	7.3	6.7	1.9	2.1
Bark SA	9.1	8.4	20.9	19.2
Wood SA	2.8	4.8	1.3	1.1
Creek:Trip		17		1.6
Creek:Bark SA		6.7		
Creek:Wood SA				2
Trip:Bark SA				1.6
Model Total	29.2	54	79	82.4

Table 2.3: Summary of two-way ANOVA of bark and wood SA

Source	df	SS	MS	F	p
Bark SA					
Creek	2	2.461	1.231	14.783	<0.001
Trip	2	2.554	1.277	15.341	<0.001
Residual	34	2.83	0.083		
Wood SA					
Creek	2	0.459	0.23	0.572	0.57
Trip	2	3.621	1.811	4.511	0.018
Residual	34	13.643	0.401		

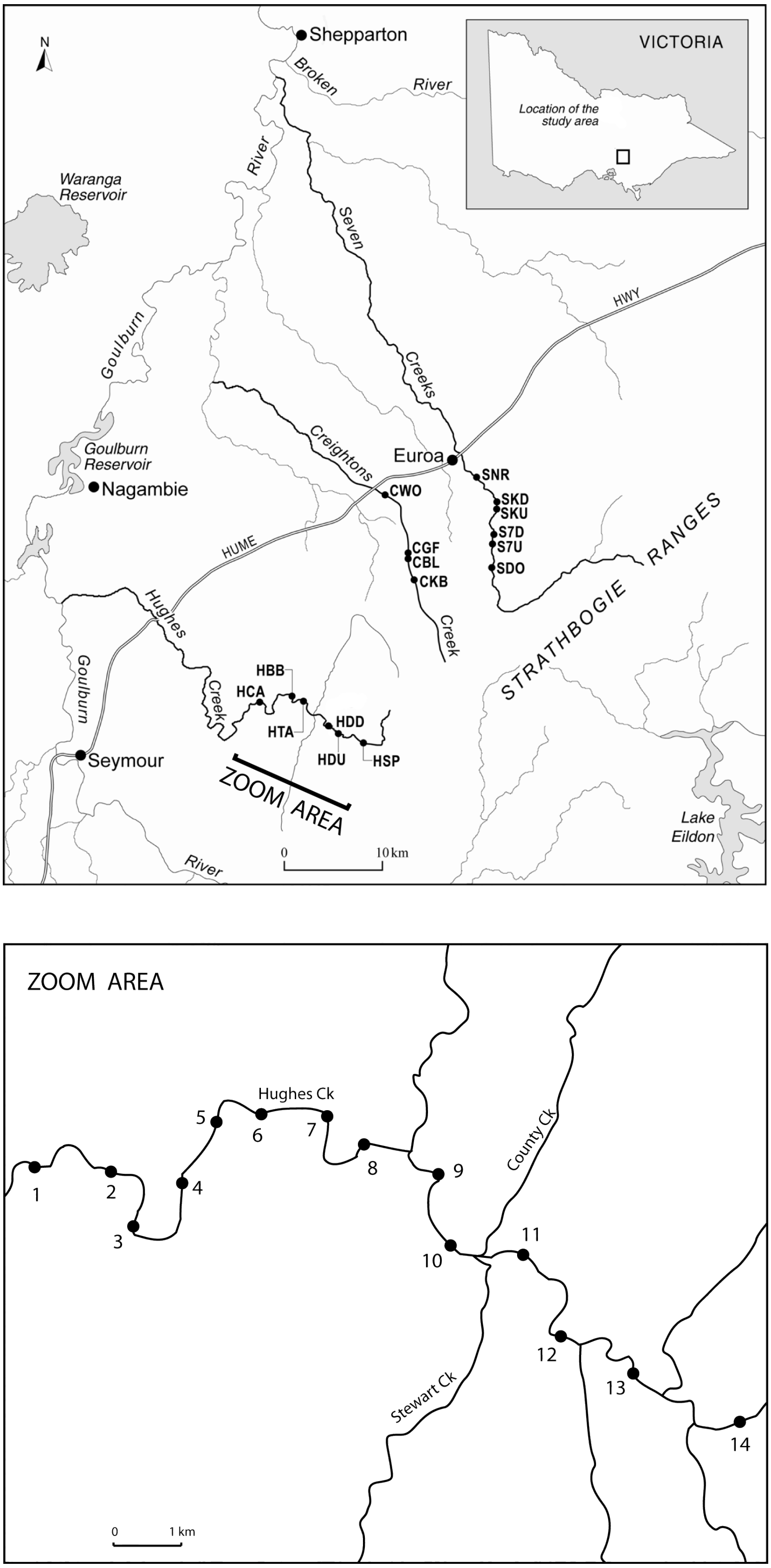

Figure 2.1: Study area. Zoomed area pertains to experiment detailed in Chapter 4.

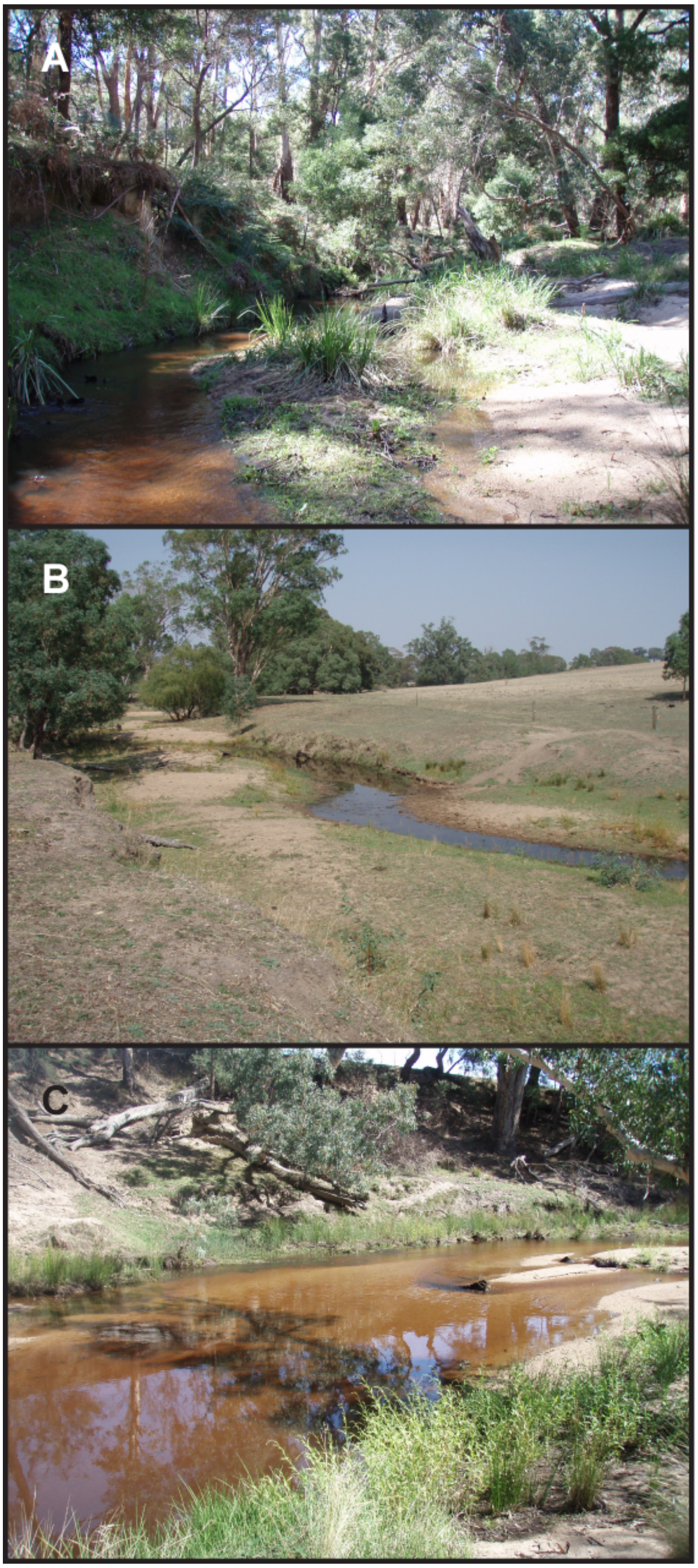

Figure 2.2: Representative photographs of the study streams: (A) uppermost site in Hughes Creek (site 14), (B) a 'middle' site in Seven Creeks (S7D), and (C) lower site in Hughes Creek (near site 1). Note the more intact riparian vegetation upstream (A) and the 'sand slug' in (C).

Figure 2.3: (A) *Ecnomus* egg masses including one hatched egg mass. (B) *Cheumatopsyche* egg masses are often aggregated. (C) *Ecnomus* (1), *Cheumatopsyche* (2) and gyrinid (3) egg masses are occasionally laid together. (D) An *Ecnomus* egg mass. (E) A *Cheumatopsyche* egg mass. Ruler gradations are at 0.5 mm intervals.

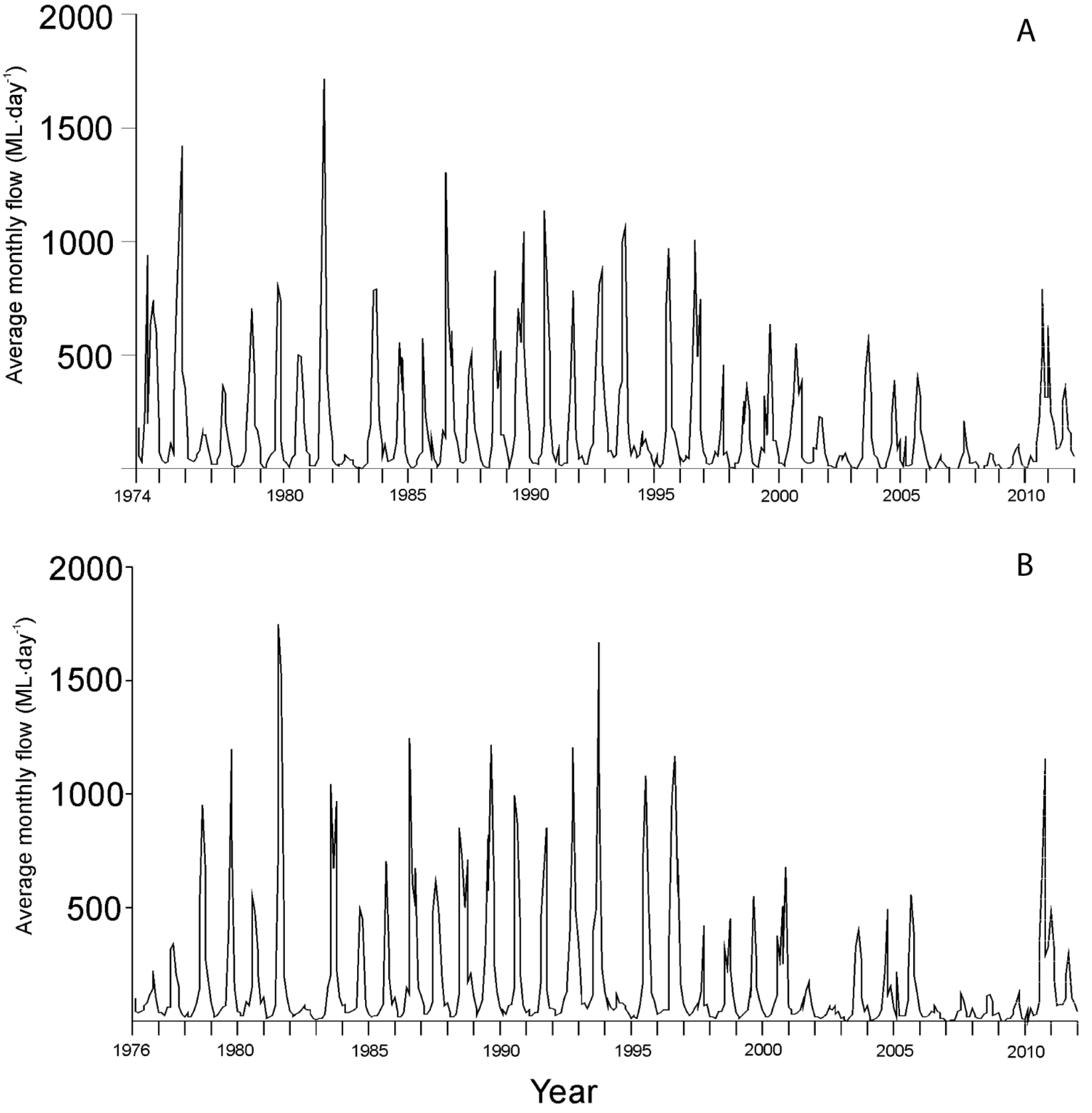

Figure 2.4: Discharge of (A) Hughes Creek 1976 to 2012 from gauge at Tarcombe Rd site 405228, Victorian Water Resource Data Warehouse, www.vicwaterdata.net) and (B) Seven Creeks from near Euroa (site 405237).

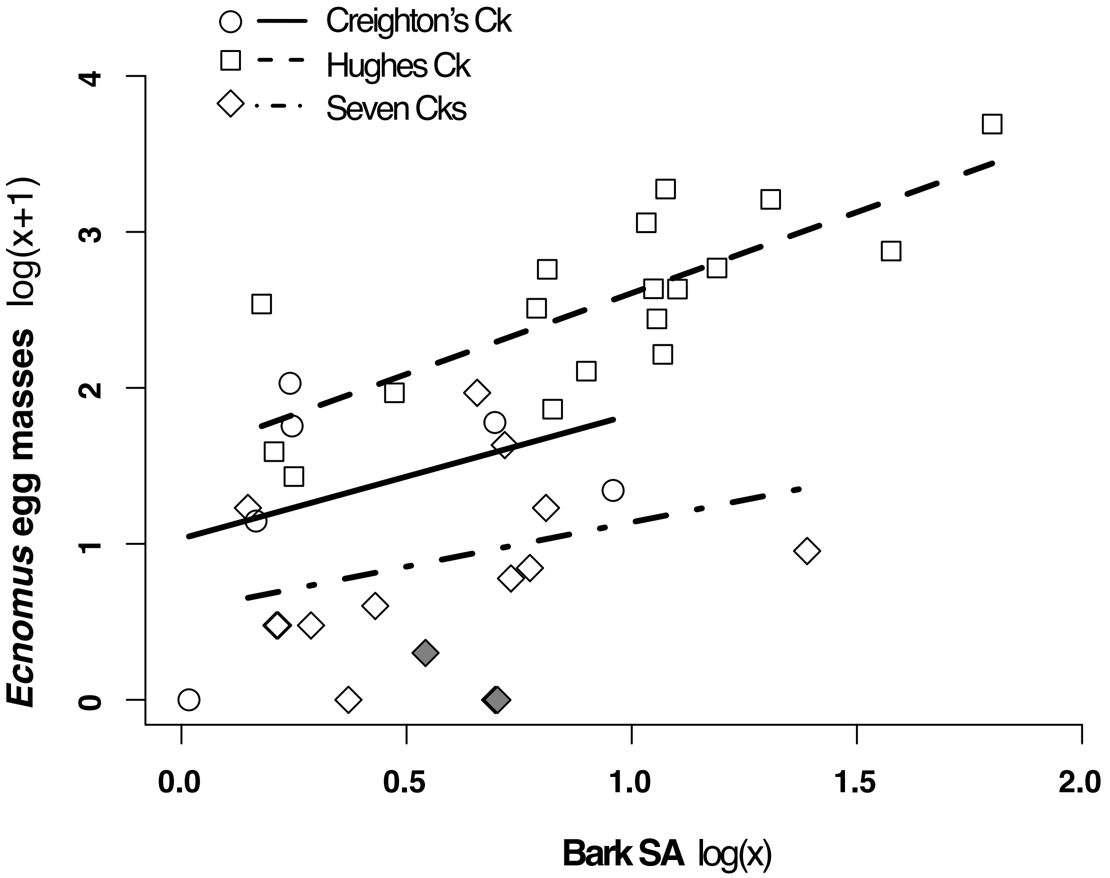

Figure 2.5: The number of *Ecnomus* egg masses in a site varies with creek and bark SA. Filled symbols represent sites which were not flowing at the time of sampling.

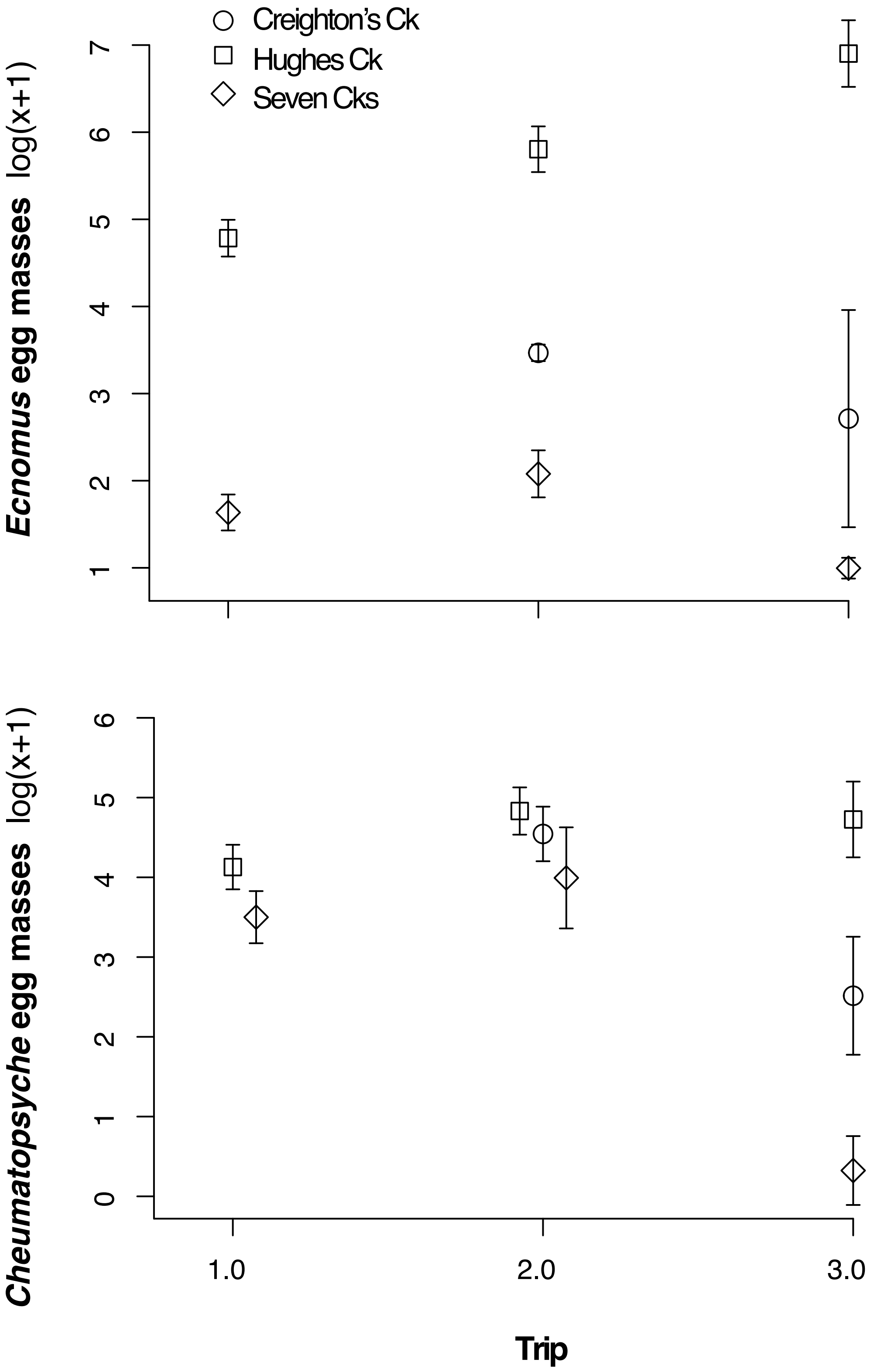

Figure 2.6: Variation in egg masses of *Ecnomus* and *Cheumatopsyche* between creeks and times. Error bars represent ± one standard error

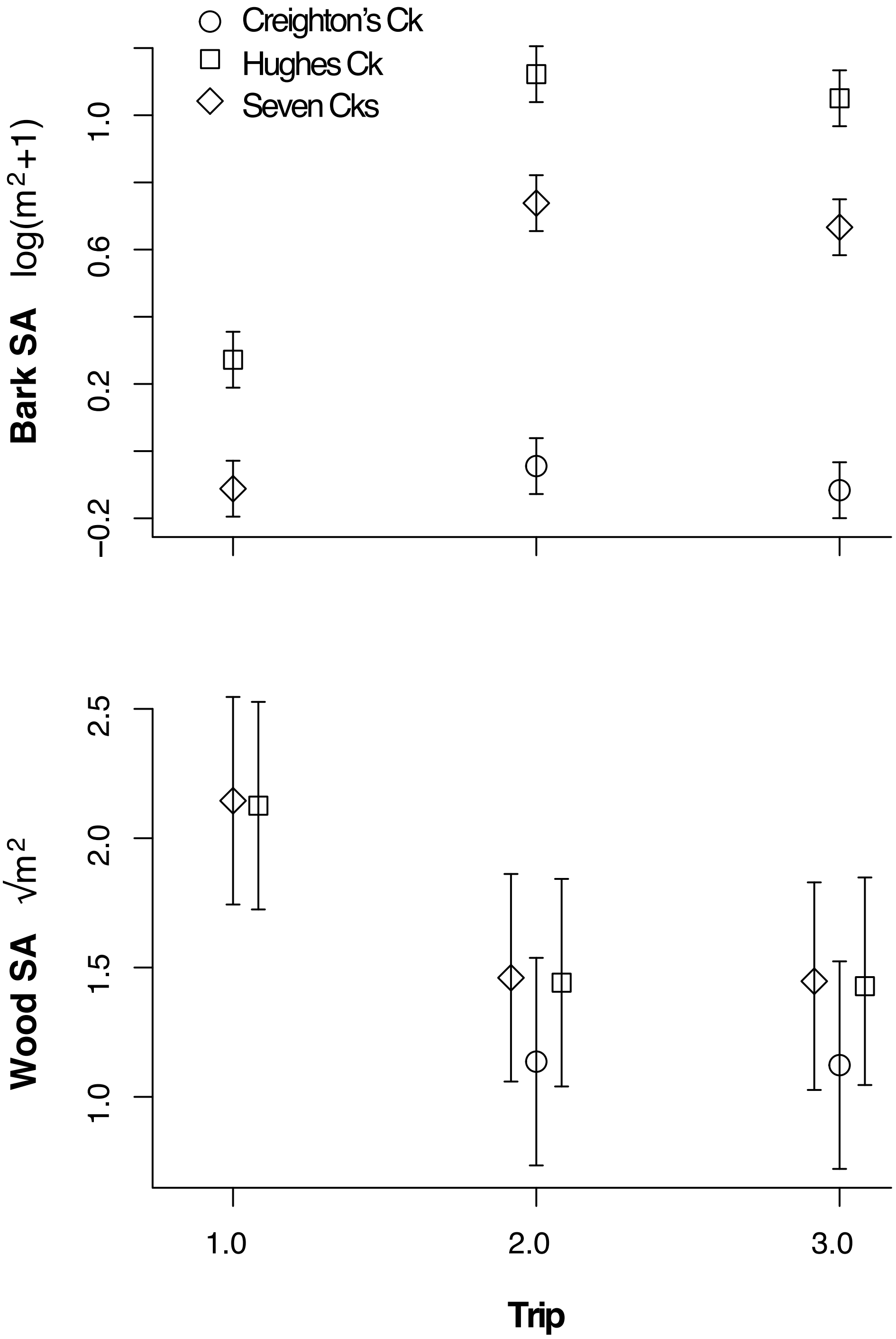

Figure 2.7: Variation in bark and wood SA between creeks and trips (fitted values). Error bars represent ± one standard error

Chapter 3. Selective behaviour of ovipositing females

3.1 Introduction

Preference for habitat has historically been used to describe associations of organisms with particular habitats. However, just because an organism is found in a location does not infer that it maximises fitness with respect to a single variable of interest to the researcher e.g. food resources. Other factors such as biological interactions (competition and predation) and ability to disperse to and locate habitat can constrain an organism's distribution. Thus organisms may to forced to utilise sub-optimal habitat while some optimal habitats remain undiscovered i.e. vacant. Manipulative experiments, rather than correlative studies, are crucial in establishing causal links and those which address multiple factors simultaneously are preferable. To establish 'true' preference, carefully planned experiments with multiple controls are required (Underwood, Chapman & Crowe, 2004). I do not aim to do this here and use 'preference' as a shorthand only.

Determining where, how and why females insects lay their eggs can be important in understanding the dynamics of populations as it allows the accurate identification of suitable oviposition sites, which ultimately drive the distribution of the next generation. Additionally, this information is critical in determining if a taxon is supply or habitat limited. Many females of terrestrial insects show specific habits for laying on certain species of plant (Wiklund, 1977) or animal host (Yang *et al.*, 2012) and are able to discriminate between hosts of high and low quality. However, successful oviposition not only depends on their ability to sense a host, but also locate and access the hosts, which may be impaired by the complexity of the environment (Floater & Zalucki, 2000). In many cases, these preferences are associated with maximising the performance (fitness) of their offspring. In the insect herbivore literature there is a focus on the nutritional quality of hosts (Gripenberg *et al.*, 2010). For example, oviposition on longer willow shoots by *Euura lasiolepis,* a galling sawfly, increased survivorship in larvae (Craig, Itami & Price, 1989). However, some hosts may provide other benefits, such as protection from predation. Furthermore, adult behaviours appear to benefit the egg or adult stages in some cases (Clark, Hartley & Johnson, 2011).

There are a number of different oviposition strategies exhibited by females ovipositing in streams, including laying their eggs as a mass upon hard substrates (Encalada & Peckarsky, 2007). Research in upland streams shows that females lay in quite specific conditions with respect to velocity, rock size and emergence (Reich & Downes, 2003b; Peckarsky, Taylor & Caudill, 2000). These characteristics may promote female survival, e.g. by allowing safe access to an oviposition site, or performance of their offspring either at the egg stage (e.g. amenable physiological environments) or larval stages (e.g. proximity to food resources). Emergent rocks facilitate oviposition by providing a dry landing pad for females, which may be important for taxa that lay in riffles where flows are fast and turbulent. High flow velocities are selected by some taxa and may speed egg development (Sivajothy, Gibbons & Pain, 1995) or suit offspring that filter feed. In contrast, *Apsilochorema* lays in slower flows to prevent damage due to shear stresses on their high profile, bulbous masses (Bovill, Downes & Lancaster, 2013). Peckarsky, Taylor and Caudill (2000) hypothesised that laying on large rocks may reduce the risk of movement during floods and the likelihood of desiccation as they were found more often in deeper water.

While the oviposition preferences of *Baetis* have been well studied in rocky upland streams, to date there had been little research on other taxa or in other stream types. My study streams have sandy beds and the most common hard substrate is bark, followed by wood (Chapter 2), offering a unique opportunity to assess whether the behaviours observed in upland streams are found on different substrates and a new taxon. Notably, unlike rocks, bark degrades over time, making the level of conditioning in the stream a variable of interest and the subject of one of three experiments, which investigated where and how *Ecnomus* and *Cheumatopsyche* lay their egg masses in Hughes and Sevens Creeks. Firstly, I assessed the influence of emergence (Hypothesis 1) and flow velocity (Hypothesis 2) on the number of egg masses laid by females, thereby testing the generality of these cues outside upland streams where they have been shown to be important. Personal observations made during my survey (Chapter 2) suggested that there may be a positive relationship between *Cheumatopsyche* egg masses and flow velocity. Secondly, I tested whether the suitability of bark as oviposition changes as it changes condition with the length of time it is in the water (Hypothesis 3). Finally, to address how females access oviposition sites, I tested whether females that alight on emergent bark and crawl into the water contribute a significant number of egg masses (Hypothesis 4), relative to those that access bark by other means, i.e. crawling or swimming.

3.2 Methods

Study taxa and Study area

The experiments detailed in this chapter focussed on two taxa with the highest number of egg masses as identified in my initial survey in 2007/08, *Ecnomus* spp. and *Cheumatopsyche* spp., and were conducted in the same study area (see Chapter 2 for details). Experimental sites in this chapter were randomly selected from a pool of potential sites in Hughes and Seven Creeks that had the following properties: 1) having some native overstorey (no exotics), 2) containing pool and run channel units, 3) readily accessible, 4) separated by at least 1 km, 5) no rock outcrops. Efforts were made to select sites that minimised the chance of livestock interference. The start of each site was chosen haphazardly.

Experimental designs

Experiment 1: flow velocity and emergence

Experiment 1 was conducted at eight 40 m sites, four each in Hughes (HCA, HBB, HTA, HDU, Figure 2.1) and Seven Creeks (SNR, SKU, S7D, S7U) from 17 December 2008 to 6 January 2009. Bark emergence (emergent vs. submerged) and flow ('Fast' > 0.25 ms^{-1}, 'Slow' < 0.15 ms^{-1}) were selected to test oviposition preferences (Hypotheses 1 & 2). Flow velocities were taken at 40% depth. Relatively thick bark (> 2 mm) common in the study area was collected from riparian zones in order to ensure reliability of treatments. Pieces of bark of equal surface area (approximately 0.06 m^2) were secured with screw-in hooks to stakes that had been hammered into the stream bed; the hooks allowed easy removal (Figure 3.1A, C). Within each site, four replicates each of four treatments (Fast flow, Emergent; Fast flow Submerged; Slow flow, Emergent; Slow flow, Submerged) were installed in randomly selected locations that met the appropriate velocity criteria, which were derived from previously collected pilot data (0.176 ± 0.146 ms^{-1}, range: 0 - 0.542 ms^{-1}). Emergent treatments were positioned so that a portion of bark projected above the water's surface. After 18 days, egg masses were identified and counted.

Experiments 2 & 3: Bark Age and Access

Experiments 2 and 3 were conducted in four 50 m sites in Hughes creek (HCA, HBB, HTA, HDU, Figure 2.1). Experiment 2 (25 February – 11 March 2009) focussed on the effects on ovipositing females of bark condition (Hypothesis 3), contrasting 'Fresh' bark (Fresh) with

Partially Conditioned (PC) bark. Partially Conditioned bark was stored in the stream for 30 days prior to the experiment using mesh sacks to exclude gravid females. Bark of each condition was attached to stakes as described above (Figure 3.1A,C) and replicated 10 times in each site. All bark was fully submerged. Egg masses were identified and counted after 14 days.

Experiment 3 two considered bark condition (Partially vs. Well Conditioned) and a new factor Access, with three treatment levels: Control, Procedural Control (Part Access) and No Access. In combination there were six treatments, which were replicated three times at each site. Bark from Experiment 2 was fitted with small polystyrene floats at one end such that a constant portion projected out of the water regardless of discharge, while the other end was secured to a stake (Figure 3.1B, D). No Access treatments had a complete Tangle trap barrier (The Tanglefoot Company, Grand Rapids, USA) where the bark emerged from water surface, preventing adults that landed on the dry portion from crawling into the water. A partial Tangle trap barrier (i.e. halfway across each piece of bark) was added to Procedural Controls to capture any additional, unwanted effects of the Tangle trap other than its role as a barrier. Egg masses were identified and counted at days 2, 3, 6, and 10. Multiple census dates allowed to the novel experimental units to be adjusted where necessary. Egg masses were removed after each visit to avoid double counting and to avoid any influence on subsequent censuses.

Statistical Analyses

Experiment 1: flow velocity and emergence

Ecnomus egg masses were analysed using a 3-way ANCOVA on Emergence (Submerged vs. emergent, fixed factor) crossed with Creek (2 levels, fixed factor), Site nested in Creek (4 levels, random factor), and Velocity (covariate). Due to the random factor, Site, the main effects were to be tested over the appropriate 2-way interaction (Sokal & Rohlf, 1981). However, following Quinn and Keough (2002), interactions with p-values greater than 0.25 were combined with the residual, making this the appropriate denominator for significance tests. Initially the same approach was used for hydropsychid egg masses; however, the pattern of significance among terms was sensitive to points of high Cook's distance, i.e. a few points had disproportionate influence over the outcome of tests. Analysing Hughes and

Seven Creeks data in separate 3-way ANCOVAs mitigated this problem and simplified the model.

Experiments 2 & 3: Bark Condition and Access

Experiment 2 was analysed with a simple 2-way crossed ANOVA on Site (4 levels, random factor) and Bark Condition (2 levels: Fresh vs. Partially Conditioned, fixed factor), again using appropriate denominators for significance tests. Egg masses from Experiment 3 were summed across census days and analysed with a 3-way ANOVA on Site (4 levels, random factor), Bark Age (2 levels: Partially vs. Well Conditioned, fixed factor) and Access (3 levels: Control, Procedural Control, No Access; fixed factor). Interactions with $\alpha > 0.25$ were combined with the residual as described above. Planned contrasts were included to elucidate the relationship between Access treatment levels. In an initial ANOVA I tested the contrast No Access = Procedural Control and since this was not significant I combined the two in a stronger contrast against the Control term (i.e. No Access + Procedural Control = Control). I thus tested whether the Tangle trap barriers trapped a significant number of insects and therefore Hypothesis 4 Where interactions were significant, unplanned pair wise comparisons were used to compare treatment levels. Units that did not function correctly (e.g. overturned, destroyed by cattle) were excluded from analyses. Two and 3-way ANOVA models were used to test whether velocity varied systematically across treatments in Experiments 2 and 3, respectively to test whether water velocity played a role in experimental outcomes. All data were $\log_{10}$ transformed to improve fit with model assumptions and analyses were performed in R v.3.10 (R-Core-Team, 2014).

3.3 Results

Experiment 1: flow velocity and emergence (Hypotheses 1 and 2)

The velocity of water flowing over bark units declined over the course of the experiment leaving only 13% of units in fast flows, thus the spread of velocities was not as wide as anticipated. Nonetheless, the distribution of velocities as was similar for emergent and submerged bark permitting its use as a covariate. Furthermore, 8 submerged units became emergent, so that only 43% of bark was submerged. However, ANCOVA is relatively robust to modest imbalance, especially where there is good replication (Quinn & Keough, 2002). Given that the assumptions of ANCOVA were met (independence, normality, homoscedasticity of dependent variable and covariate) I was confident that the significance tests were reliable, rejecting possible remedies (discarding data or generating data for missing cells to create balance) that introduce problems of their own (Quinn & Keough, 2002).

Ecnomus laid significantly more egg masses on submerged bark than emergent bark in both Hughes and Seven Creeks, and significantly more in Hughes overall (554 egg masses vs. 142 in Seven Creeks: Table 3.1, Figure 3.2). In contrast, females of *Cheumatopsyche* were not significantly influenced by emergence, but did lay many more egg masses in Hughes creek also (833 vs. 90 in Sevens Ck), though numbers of eggs were also significantly affected by flow velocity and site in both streams (Table 3.2). There were positive relationships between the number of egg masses and the flow velocity in which a unit was located for all sites in Hughes Creek, though there was some variation in slopes (Figure 3.3). At sites SKD and S7U there were sufficient masses to show a relationship with velocity, though not in the remaining sites, which had fewer masses and in the case of SNR, very low velocities.

Experiments 2 & 3: Bark Age and Access

Tangle trap barriers were effective and trapped a significant number of adults: 54 *Cheumatopsyche* on No Access (complete barrier) and 6 on procedural controls (partial barrier) and 125 and 6 *Ecnomus* adults on No Access and Procedural Controls, respectively. Though analyses showed that water velocity did not vary significantly between treatments in Experiments 2 and 3 (Table 3.4), in the former velocity at 30 days old bark was a higher than at 0 day old bark (0.164 ± 0.037 vs. 0.117 ± 0.037). Females of *Ecnomus* and *Cheumatopsyche* did not appear to differentiate between bark that was 14 or 44 days old (Experiment 2; Table 3.3, Figure 3.5). However, females of *Ecnomus* did lay more egg masses on 38 than 68 day old bark (Experiment 3; Table 3.5, Figure 3.6), while *Cheumatopsyche* did not show any preference (Table 3.5). Interestingly, almost half of all 68 day old bark curled in on itself to resemble a cylinder. There were 12 times more *Cheumatopsyche* masses in the most upstream site than the remaining sites combined, accounting for the significant site effect during Experiment 2. The number of masses laid appears to have been constrained by Tangle trap barriers for both taxa (Table 3.5). However, post hoc pair wise tests on *Ecnomus* masses showed that this effect was only significant at one site, where the No Access treatments had significantly fewer egg masses than Controls (Figure 3.7). Although Access was not a significant factor in the ANOVA for

Cheumatopsyche, a planned contrast of Controls against treatments with Tangle trap barriers (i.e. No Access and Procedural Controls) was marginally significant (Table 2.1) indicating that fewer egg masses were laid on the latter treatments (Figure 3.8)

3.4 Discussion

Contrary to expectations and the literature from upland streams (Peckarsky, Taylor & Caudill, 2000; Reich & Downes, 2003b), emergence was either not important (*Cheumatopsyche*) or less desirable (*Ecnomus*) than fully submerged bark. Discharge declined significantly over the summer, so it is possible that females of *Ecnomus* preferred submerged bark because their egg masses were less likely to become exposed and dry out. However, if desiccation was a strong selective factor one might also expect fewer *Cheumatopsyche* egg masses on emergent bark, which was not the case. I hypothesise that this is because other factors (e.g. velocity) had more influence on females' choice. It is also worth noting that up to three spiders were observed on individual pieces of emergent bark. It is possible then that the lower numbers of egg masses on emergent bark was due to females becoming prey or that females avoided landing on bark to mitigate that possibility.

Females of *Cheumatopsyche* showed a strong preference for bark in fast flows: the velocity effect was still significant when tested over the much reduced degrees of freedom associated with the site-velocity interaction (Table 3.2). Furthermore, in sites where there were very low velocities, e.g. SNR, there were few egg masses at all. Variation in the strength of relationship between egg masses and velocity at different sites may be due a different mix of species at the sites. For instance, the most downstream site in Hughes Creek, Campsite, may be home to species that are less sensitive to velocity than their upstream relatives. Though there is some noise in the relationship, there does not appear to be any levelling off at high velocities suggesting that velocities higher than those recorded could attract even higher number of egg masses. Although analyses on velocity for Experiments 2 and 3 did not show any statistically significant differences between treatments, the differences could be large enough to influence the behaviour of *Cheumatopsyche* females. For instance, a 0.5 ms^{-1} difference between average velocities at controls and procedural controls in Experiment 3 could have contributed to the lower numbers of egg masses observed on the latter. The strong dependency of *Cheumatopsyche* masses on velocity established experimentally here is consistent with observations from my survey (Chapter 2), and adds weight to the hypothesis that the reason that *Cheumatopsyche* were not constrained by the overall amount of bark is because only a subset, i.e. bark in fast flows, was suitable for oviposition.

My results suggest that the suitability of bark as an oviposition substrate declines over time submerged in water. However, after several days in the stream, fresh bark was covered with a gelatinous exudates, which is thought to have arisen from the bark, making it unsuitable for oviposition. The exudate was more pronounced in slow flowing areas, presumably being sloughed off in fast flows, and was mostly absent after about a week. Thus, bark suitability likely increases quickly from a low value, then declines more gradually. The approximate four week difference between our Bark Condition treatments in Experiments 2 and 3 significantly impacted on egg mass numbers, but only once the more conditioned bark was about two months old. Presumably bark becomes less desirable as the surface degrades over time and becomes more likely to disintegrate. Egg masses of both species where most often found on relatively smooth, clear surfaces, which included tyres and beer bottles. Furthermore, the tendency for older bark to curl may reduce its apparency to searching females (Floater & Zalucki, 2000), thus reducing the probability of receiving eggs though the surface area remains the same. Taxa in other contexts are also faced with the challenge of ovipositing on substrates that degrade over time, e.g. fruit flies requiring rotting fruit (Atkinson & Shorrocks, 1984).

Experiment 1 established that females of both taxa were able to access bark without an emergent landing pad. However, Experiment 3 showed that where bark was emergent, adult females did use it as a way of accessing the submerged portion and that this pathway contributed a significant number of egg masses. The fact that the reduction in egg masses on bark with Tangle trap occurred for both taxa (albeit only one site for *Ecnomus*), and the large number of adults trapped on Tangle trap, suggests that the treatments were effective and induced a real effect. The marginal statistical significance is consistent with females accessing the bark via other means (e.g. swimming, crawling) in addition to via an emergent landing pad. I hypothesise that females use emergent objects such as bark as a way of entering the water, but may move on to assess other potential oviposition sites in the vicinity. In fact, I personally observed a female of *Ecnomus* in captivity crawl down a piece of bark into the water before swimming between submerged objects with apparent ease and, finally, laying her eggs. This may explain the different patterns observed for *Ecnomus* at different sites; at a site with many emergent objects, females can access a particular piece of bark after entering the water via a number of different objects, i.e. the importance of any one particular object as a means of entering the water is reduced.

These findings and observations are a significant contribution to our scant knowledge about the oviposition behaviours of aquatic insects, especially *Ecnomus*, but there are many unanswered questions about how females locate and access oviposition sites, the sequence of decisions involved and whether they are made aerially or aquatically. Considering what we know about the behaviour of *Cheumatopsyche* we can construct a conceivable chain of behaviours leading to oviposition. Some insects are able to sense polarized light (Lancaster & Downes, 2013), which would allow *Cheumatopsyche* to locate areas of fast flow as the broken water surface scatters light. Experiment 3 showed that *Cheumatopsyche* used landing pads at least some of the time, although hydropsychids are known to dive elsewhere (Deutsch, 1984; Deutsch, 1985). Hydropsychids have also been observed swimming in other places (Deutsch, 1985), so it is possible that once in the water, *Cheumatopsyche* may swim to locate a suitable piece of bark like *Ecnomus*. A oviposition event might unfold thus: *Cheumatopsyche* fly along the stream channel searching for fast flow locations from the air, then enter the water via emergent object or diving, then swim to choose a suitable site. There are other factors that could influence this sequence which we still know little about. Hydropsychids have been observed to swarm (Sullivan, 1981), which might lead to aggregation of egg masses if females exit the swarm and oviposit in the immediate vicinity. Another prospective cause of aggregation would be attraction to conspecific egg masses (Reich *et al.*, 2011).

In addition to knowing where *Ecnomus* and *Cheumatopsyche* lay and how they access these sites, it is interesting to speculate on why masses are laid in those places. Larvae of *Cheumatopsyche* filter feed as late instar larvae so it is possible that females’ tendency for laying in fast flows is an effort to give their offspring ready access to the conditions they require. It is worth noting, however, that earlier instars may obtain nutrition via other means. Furthermore, hydropsychids lay in pools elsewhere (Lancaster, Downes & Arnold, 2010b), which is not the preferred habitat of later instar larvae, but may improve female survival. It is possible that laying in pools is a way of avoiding the high turbulence associated with fast flows in upland streams (riffles), whereas fast flowing areas of Sevens and Hughes Creeks ("runs") are typified by relatively laminar flows. Indeed, it is possible that emergent ‘landing pads’ are less important in the calmer flows of my study creeks than the upland streams where multiple taxa are dependent on them (Peckarsky, Taylor & Caudill, 2000; Reich & Downes, 2003b). *Ecnomus* are retreat-based predators (Wiberg-Larsen, 1993), which is not a velocity dependent feeding mode. Rather, it is likely that the preferences for submerged bark and younger bark are driven by survival of the female (spider avoidance), successful oviposition (clean surface) and egg survival (desiccation avoidance) as described above.

While there is evidence that a few species are numerically dominant in the study area within families Ecnomidae (*Ecnomus continentalis*) and Hydropsychidae (*Cheumatopsyche* AV4 and *C. deani* Neiboss) (Downes *et al.*, 2011), it is possible that the mix of species varies in space and time. Given that closely related species can have different behaviours, but not always, this could impact on my results in several ways. Consider a scenario where one species (A) has a strong apparent preference for submerged bark, while another (B) prefers emergent bark and a third (C) is ambivalent. If the proportion of A:B is 90:10 then a clear pattern favouring submerged substrate would be expected, a 50:50 would result to no pattern being detected, while a 10:90 ratio would result in a clear pattern in favour of emergent substrate. Any presence of species (C) would simply add noise to the observed effect. Following this logic, any observed effect, such as the pattern of oviposition seen here for *Ecnomus*, is a real effect despite the presence of additional species.

To my knowledge, all studies of oviposition behaviour in lotic insects have found females express some level of preference for particular habitat conditions. It is likely then, that there is an adaptive benefit that outweighs the fitness costs of employing the behaviours (e.g. increased risk of predation during search, Encalada & Peckarsky, 2007). Specific oviposition behaviours also increase the likelihood of being habitat limited, as the proportion of potential sites is reduced. However, the paucity of research makes it impossible to make accurate generalisations about which life history phase accrues the benefit. Nonetheless, it has been suggested that it is more likely that adult or egg stages benefit since aquatic larvae are considered to be mobile and thus able to disperse to suitable habitat (Peckarsky, Taylor & Caudill, 2000). In contrast, the larvae of many herbivorous insects are limited to the plant selected by their mother, suggesting female oviposition behaviour would be more critical (Floater & Zalucki, 2000). Indeed, a meta-analysis by Gripenberg *et al.* (2010) found strong support in the literature for larval performance as a driver of female behaviour. Many studies focus on commercially important species and are conducted in controlled laboratory or greenhouse conditions (Gripenberg *et al.*, 2010), which may limit the applicability to more complex, natural systems. While my experiments do not suffer this problem, they potentially average across other factors that influence the apparent strength of selectivity shown by females. For instance, does the density of oviposition sites influence the behaviour of females in lotic insects as has been found for parasitic wasps in response to variation in prey densities (Yang *et al.*, 2012)?

Many factors might conceivably influence the choosiness of females including both biological and environmental (see Section 1.3). Species with long lived and mobile adult phases might be expected to spend longer searching and in more locations, increasing the probability of finding a substrate that closely meets its oviposition requirements. Also, The number of masses laid by a female could influence their choosiness (Resetarits, 1996). Theoretically, a species laying multiple masses would be less picky than one laid a single mass, as each decision would have less weight with respect to their genetic legacy. However, this difference would be much less pronounced than comparing a broadcast spawner with a mass laying taxa. Of course, spending longer searching involves a trade-off against the increased probability of mortality (e.g. due to predation) or drowning during oviposition (Encalada & Peckarsky, 2007).

I have established a baseline of new information on oviposition behaviour in a new type of stream with substrate that is functionally different to that found in upland streams. While this work represents significant progress it also highlights the gaps in present knowledge and the need for further research. Indeed such basic biological knowledge is critical in understanding the distribution of populations as the events in one stage, such as oviposition behaviours, can strongly influence later life history stages, strong evidence of which will be presented in the following chapter.

3.5 Tables and Figures

Table 3.1: Experiment 1. Summary of ANOVA of *Ecnomus* egg masses on emergent and submerged bark at sites in Hughes and Sevens Creeks with velocity as a covariate. Interaction terms with α > 0.25 in initial analysis were excluded (see text).

Source	df	SS	MS	F	p
Creek	1	2.552	2.552	12.297	<0.001
Emergence	1	3.262	3.262	15.717	<0.001
Velocity	1	0.273	0.273	1.316	0.254
Site:Creek	6	0.21	0.035	0.168	0.985
Creek:Emergence:Velocity	3	0.498	0.166	0.801	0.496
Residuals	105	21.792	0.208		

Table 3.2: Experiment 1. Summary of ANOVA on *Cheumatopsyche* egg masses on emergent and submerged bark with velocity as a covariate. Hughes and Sevens Creeks were analysed separately to reduce the impact of highly influential data on the analysis. The non-significant, three-way interaction (α > 0.25) was removed so that remaining interactions were tested over the residual.

Source	df	SS	MS	F	p
Hughes Creek					
Site	3	1.274	0.425	3.001	0.041
Emergence	1	0.412	0.412	1.756	0.277
Velocity	1	11.621	11.621	14.56	0.032
Site:Emergence	3	0.703	0.234	1.656	0.19
Site:Velocity	3	2.395	0.798	5.64	0.002
Emergence:Velocity	1	0.368	0.368	2.602	0.114
Residuals	44	6.227	0.142		
Seven Creeks					
Site	3	0.454	0.151	4.744	0.006
Emergence	1	0.019	0.019	1.725	0.281
Velocity	1	3.304	3.304	27.292	0.014
Site:Emergence	3	0.033	0.011	0.349	0.79
Site:Velocity	3	0.363	0.121	3.798	0.016
Emergence:Velocity	1	0.03	0.03	0.954	0.334
Residuals	48	1.53	0.032		

Table 3.3: Experiment 2. Summary of ANOVA on *Ecnomus* and *Cheumatopsyche* egg masses on Fresh and Partially Conditioned bark. Interaction terms with α > 0.25 were excluded (see text)

Source	df	SS	MS	F	p
Ecnomus					
Age	1	0.024	0.024	0.14	0.71
Site	3	1.123	0.374	2.221	0.093
Residuals	75	12.641	0.169		
Cheumatopsyche					
Age	1	0.209	0.209	0.681	0.47
Site	3	5.279	1.76	11.938	<0.001
Age:Site	3	0.92	0.307	2.081	0.11
Residuals	72	10.613	0.147		

Table 3.4: Summary of ANOVA on velocity at experimental units for experiments 2 and 3, showing that velocity did not vary systematically between treatments.

Source	df	SS	MS	F	p
Experiment 2					
Age	1	0.005	0.005	1	0.391
Site	3	0.011	0.004	0.444	0.722
Age:Site	3	0.014	0.005	0.556	0.65
Residuals	72	0.632	0.009		
Experiment 3					
Age	1	<0.001	<0.001	0.289	0.628
Access	2	0.034	0.017	1.528	0.291
Site	3	0.008	0.003	0.309	0.819
Age:Access	2	0.021	0.01	1.795	0.245
Age:Site	3	0.004	0.001	0.146	0.932
Access:Site	6	0.067	0.011	1.306	0.273
Age: Access:Site	6	0.035	0.006	0.683	0.664
Residuals	47	0.402	0.009		

Table 3.5: Experiment 3. Summary of ANOVA on *Ecnomus* and *Cheumatopsyche* egg masses on Partially Conditioned and Well Conditioned bark, with free access (i.e. Control), partial access (Procedural Control) or No Access via the emergent portion. Contrast compares Control (C) against Procedural Control (P) and No Access (N) treatments. Interaction terms with α > 0.25 are excluded (see text).

Source	df	SS	MS	F	p
Ecnomus					
Age	1	0.146	0.146	5.414	0.024
Access	2	0.014	0.007	0.437	0.649
Site	3	0.669	0.223	14.056	<0.001
Access:Site	6	0.224	0.037	2.347	0.045
Age:Access:Site	6	0.161	0.027	1.694	0.141
Residuals	51	0.81	0.016		
Cheumatopsyche					
Age	1	0.031	0.031	1.931	0.17
Access	2	0.065	0.033	2.021	0.142
C vs P + N	1	0.065	0.065	4.033	0.049
Site	3	0.424	0.141	8.789	<0.001
Age:Site	3	0.094	0.031	1.942	0.133
Residuals	60	0.965	0.016		

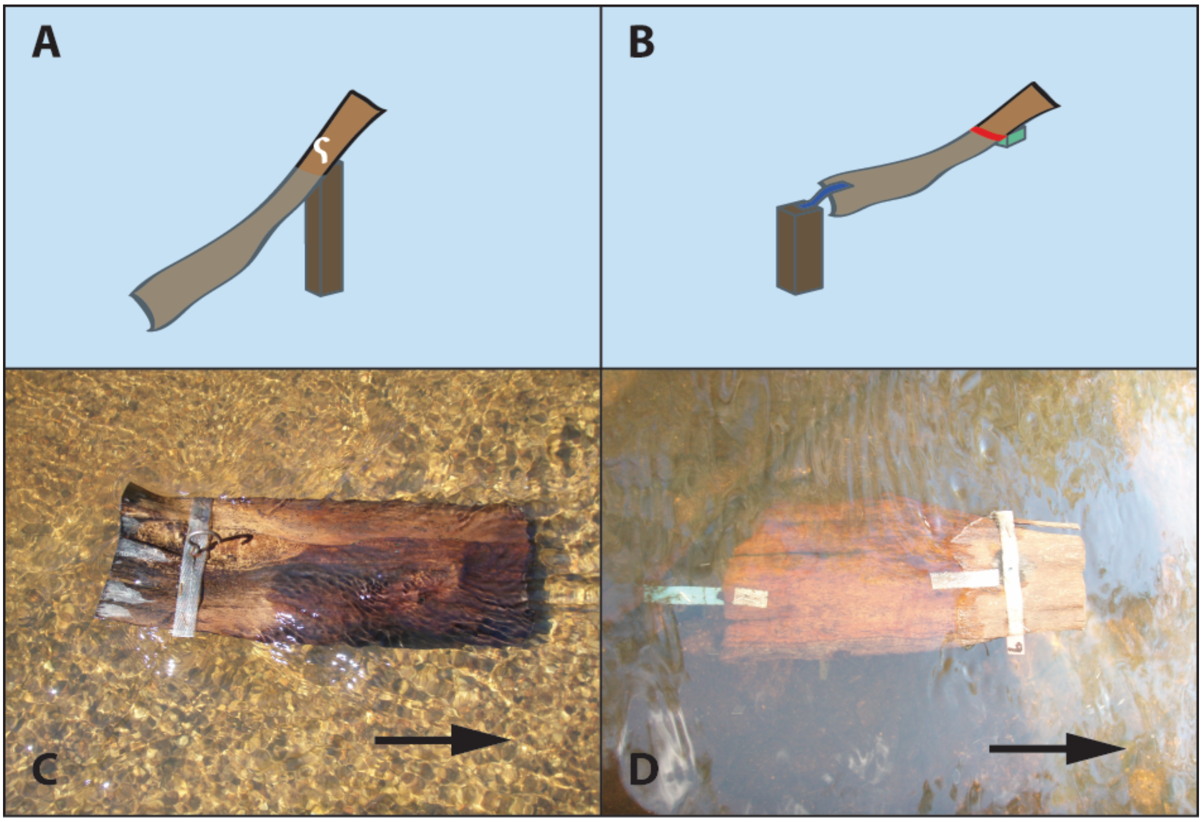

Figure 3.1: Experimental units for Experiment 1 and 2 (A, C) and experiment 3 (B, D). B shows bark (light brown) attached to stake (dark brown) with industrial strapping tape. The float (green) and Tangle trap barrier (red) are also shown.

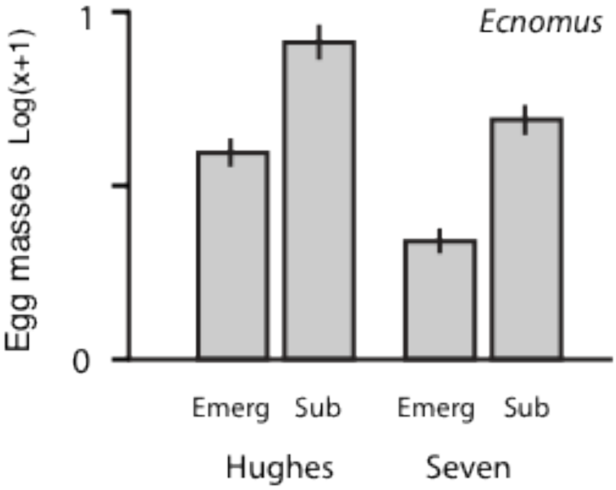

Figure 3.2: *Ecnomus* masses were more abundant on submerged bark in both streams. Error bars represent one standard error.

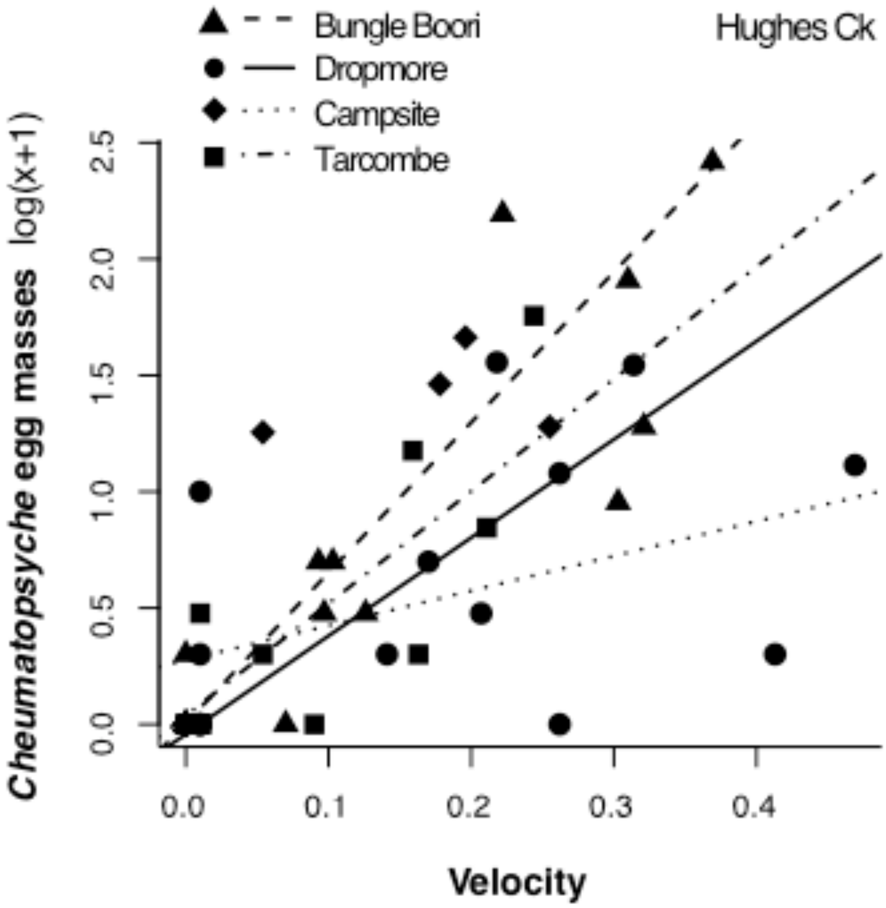

Figure 3.3: Relationship between *Cheumatopsyche* egg masses and velocity shows some variation between sites in Hughes Creek.

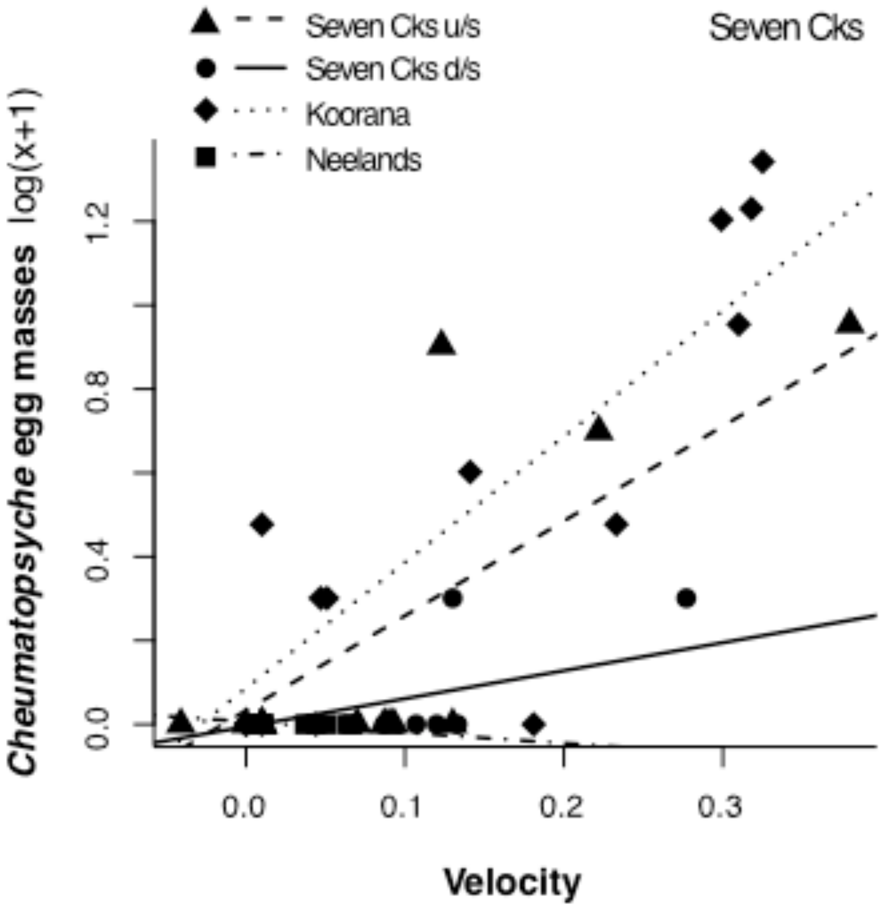

Figure 3.4: Relationship between *Cheumatopsyche* egg masses and velocity varies strongly between sites in Seven Creeks

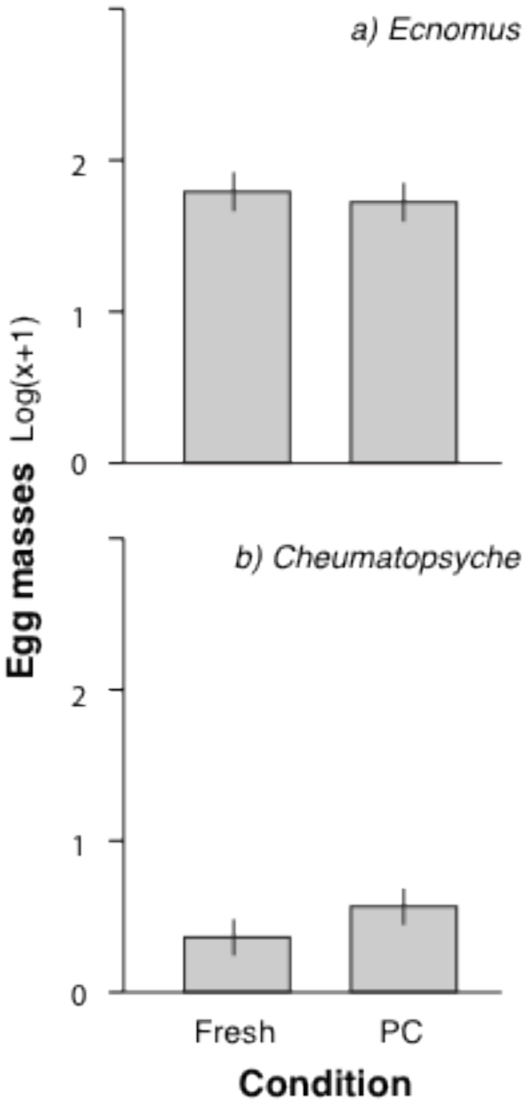

Figure 3.5: No difference between number of *Ecnomus* or *Cheumatopsyche* masses on Fresh and Partially Conditioned (PC) bark. Error bars represent one standard error

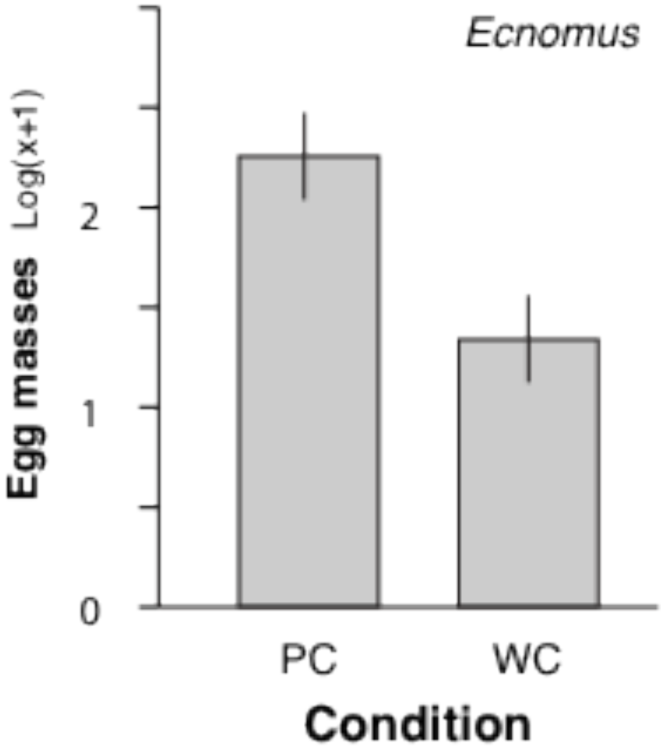

Figure 3.6: *Ecnomus* laid more masses on Partially Conditioned (PC) than Well Conditioned (WC) bark. Error bars represent one standard error

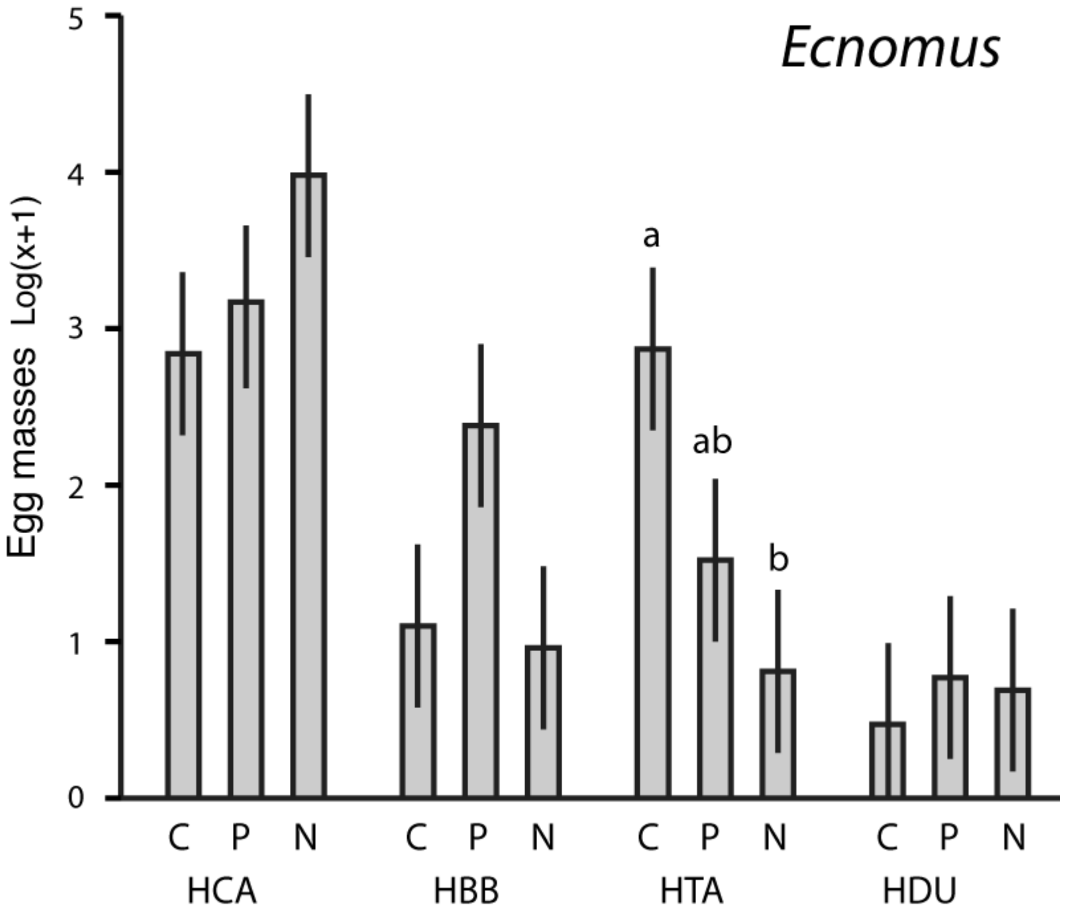

Figure 3.7: *Ecnomus* lay significantly fewer eggs on No Access (N) treatments than Controls (C), but only at one site. Capital letters denote Control (C), Procedural Control (P) and No Access (N) treatments. Means (bars) labelled with different lower case letters are significantly different from each other. Site codes (e.g. HCA) correspond to sites shown in Figure 2.1. Error bars represent one standard error

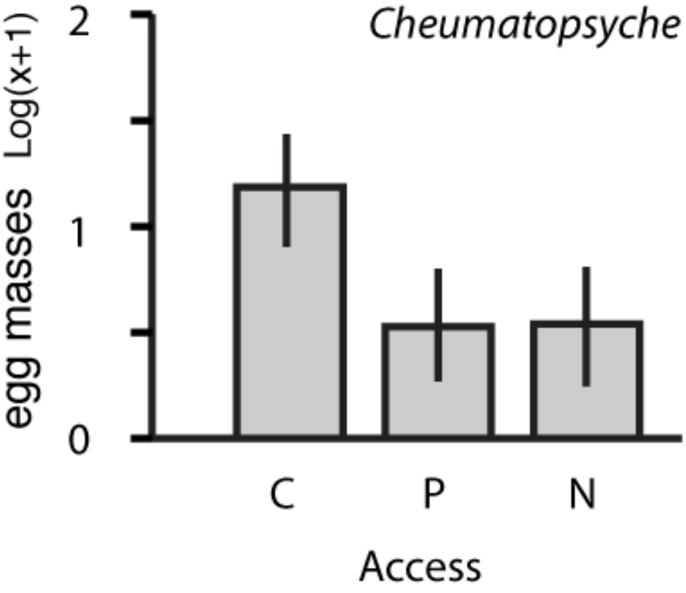

Figure 3.8: *Cheumatopsyche* laid fewer masses on bark with Tangle trap barriers. Capital letters denote Control (C), Procedural Control (P) and No Access (N) treatments. Error bars represent one standard error

Chapter 4. Large-scale manipulation of oviposition substrata affects egg supply to populations of some stream-dwelling caddisflies

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4.1 Introduction

To understand the population dynamics of organisms with complex life histories requires an understanding of variability in vital rates (births, deaths, immigration, emigration), and also at what life history stages these are most influential (Halpern, Gaines & Warner, 2005). For instance, what are the points in the lifecycle where the numbers of individuals transitioning to the next stage is most constrained by mortality? Are there critical resources that are in sufficiently scarce supply as to limit this process? For example, on the coast of Oregon, North America, the supply of planktonic barnacle larvae exceeds the availability of settlement space in the rocky intertidal zone, which constrains the number of individuals recruiting to the adult phase (Roughgarden, Gaines & Possingham, 1988). The answers to such questions are vital for advancing our understanding of populations.

There is mounting evidence that events during the egg stage of some riverine insects can have lasting effects on their population dynamics (Encalada & Peckarsky, 2012; Peckarsky, Taylor & Caudill, 2000). Females of these taxa require a hard, submerged surface, such as rocks, upon which to lay their egg masses (Reich, 2004), and there is evidence that relative scarcity of oviposition substrata can limit the input of eggs into the stream with consequences for larval densities. For instance, in studies by Lancaster *et al.* (2010a), Peckarsky *et al.* (2000) and Encalada *et al.* (2012), fewer egg masses of baetid mayflies were laid at times and locations where fewer emergent rocks were available, which thus determined the densities of larvae. While it has been suggested that high levels of larval dispersal could erode patterns established during oviposition (Reich & Downes, 2004), recent evidence suggests that *Baetis*, considered one of the most mobile and agile taxa, disperses less often and over shorter distances than previously thought (Lancaster, Downes & Arnold, 2011). Furthermore, in a test of competing drivers of larval distribution, Lancaster and Downes (2014) found that the distribution of oviposition substrata accounted for a significant proportion of the variation in densities of larvae of the mayfly *Baetis rhodan*i and caddisfly *Rhyacophila dorsalis*. Thus, the constraint of egg inputs by the availability of substrata, demonstrated for a few taxa in upland streams, can have a lasting impact on population dynamics.

Adult females of many aquatic insects require a hard substratum upon which to lay their eggs and can display taxon-specific preferences for particular types of substrata, their location and surrounding flow conditions (Reich & Downes, 2003; Lancaster, Downes & Arnold, 2010b). However, to our knowledge no studies have tested whether female oviposition preferences vary depending upon the types and amounts of substrata available to them. Indeed, quantifying preference for any organism is difficult, especially as individuals can change their preference according to the context of the experiment (Underwood, Chapman & Crowe, 2004). For example, the ability to express a preference may be compromised if the apparency of the optimal resource is reduced. Floater and Zaluki (2000) found that adults of the processionary caterpillar *Ochrogaster lunifer* were less able to locate the favoured food tree of their offspring in complex multi-species, multi-age stands, resulting in aggregated oviposition on lower quality trees and higher larval mortality

Previous studies on oviposition have been conducted primarily in rocky, upland streams, but many streams in Australia and elsewhere have a dearth of rocks. Instead, many streams have soft sediments such as sand; this includes our study system, Hughes Creek. A prior observational study in Hughes Creek found hydropsychid and ecnomid masses in large numbers on bark and, to a lesser extent wood, the availability of which varies widely according to season and flooding as well the presence of donor trees, many of which have been cleared from extensive stretches of the creek (Downes *et al.*, 2011). Furthermore, the quantity of bark and egg masses co-varied, suggesting that availability of substrata might limit the input of eggs in Hughes Creek (A Macqueen, unpublished data).

In a landscape scale, manipulative experiment we looked for a causal relationship between abundance of egg masses and abundance of bark, thus testing the hypothesis that the distribution of oviposition substrata limits egg supply into the stream (Hypothesis 1). This is the first such study not performed in upland, cobbled streams, and a positive result would provide strong evidence that the egg stage can play an important role in population dynamics, irrespective of substrata type and location. Alternatively, if there is no relationship between availability of substrata and egg masses it might suggest that not all bark was equally suitable to females, a possibility we address in our second hypothesis. Thus, we also tested whether females chose bark that was emergent versus submerged, or situated in particular water velocities, and whether these preferences varied with the overall abundance of bark at sites (Hypothesis 2). Any apparent change in preference with bark availability could be due to a change in behaviour or in females' ability to express their preferences. For instance, a positive relationship between preference strength and bark availability might indicate that females were more selective when choosing among abundant bark substrata. Alternatively, should preference be more weakly expressed when bark was abundant, this could suggest that the most suitable bark was less apparent. Any significant variation in preference strength would have important ramifications for the study of preference: those studies ignoring the density of resources may lack generality or even fail to find an effect.

4.2 Methods

Study area

This study was conducted at 14 sites, each 25 m long and distributed along a 20 km length of Hughes Creek (37° 00' 43" S, 145° 26' 20" E; 36° 59' 1', 145° 20' 39"), which arises on the Strathbogie Ranges, approximately 100 km NNE of Melbourne, Victoria, Australia and flows in a north-westerly direction before emptying into the Goulburn River. The land use in the area is predominantly grazing. Land clearance for pasture has greatly reduced the cover and extent of vegetation, including that within the riparian zones, which are dominated by a few remaining river red gums (*Eucalyptus camaldulensis*) and manna gums (*E. viminalis*). The stream is probably naturally sandy, but land clearing has led to the erosion and deposition of large quantities of granitic sand into other streams in this region (Davis & Finlayson, 2000). This process is likely to have contributed to the long stretches of sand bed, punctuated by occasional granite outcrops, which typify much of Hughes Creek. The vast majority of large, hard substrata are pieces of wood and bark supplied by riparian eucalypts, which shed significant quantities of bark, especially during summer (Reid *et al.*, 2008), and are typically more abundant in the less impacted upper reaches.

The region has a temperate climate with hot dry summers and cool wet winters. The average maximum and minimum temperatures are 21 °C and 9 °C, respectively, but the maximum ranges up to 40 °C or more and the minimum to less than 0 °C. Yearly average rainfall is 800-1000 ml, which falls mostly in winter and spring (Australian Bureau of Meteorology). Discharge varies substantially over the year with average monthly maxima of 1000 - 1500 $ML.day^{-1}$ during winter to spring, which contrasts with summer flows of less than 100 $ML.day^{-1}$ on average; however, summer storms may occasionally generate high, bed-moving flows (Downes *et al.*, 2011). Our study was conducted at the end of a drought in the region, dubbed the “Big Dry” (Ummenhofer *et al.*, 2009). All sites had relatively stable flows throughout the study period, with the exception of a spate on day 32 (Fig. S1). When this study was conducted, average wet stream widths ranged from 3 – 5 m and dry widths from 10 – 25 m with average stream depths of 0.1 – 0.5 m in summers.

Study taxa

This study focussed on species in two families of Trichoptera where the eggs could be clearly distinguished: Hydropsychidae (*Cheumatopsyche* spp.) and Ecnomidae (*Ecnomus* spp.). Though it was not possible to identify egg masses to genera in the field, previous work in Hughes Creek indicates that larval and adult hydropsychids are almost all from the genus *Cheumatopsyche,* and predominantly *Cheumatopsyche* sp. AV2 and *C. deani* (Downes *et al.*, 2011; B. J. Downes, unpublished data). Additionally, ecnomids are exclusively from the genus *Ecnomus* and dominated by *E. continentalis*, which is an order of magnitude more abundant than any other species at both larval and adult stages (Downes *et al.*, 2011; Lancaster, Downes & Glaister, 2009). *Cheumatopsyche* larvae are net-building filter feeders, while *Ecnomus* larvae construct nets to catch prey. In Hughes Creek, both taxa are equally abundant on leaf packs and sand (Downes *et al.*, 2011), with densities reaching 500 to 1000 or more individuals m^{-2} (Downes *et al.*, 2011; Lancaster, Downes & Glaister, 2009; J. Lancaster , B. J. Downes, unpublished observations). Nets built directly onto sand are often near the stream margins. *Cheumatopsyche* occur in relatively fast-flowing areas where sand grains are bound together by biofilms (O'Connor, 1993) while *Ecnomus* is associated with slow flows and fine sediments (Lancaster, Downes & Glaister, 2009). Larvae of both taxa are often found in the drift, and adults are abundant during summer months (B. J. Downes, unpublished data).

Ecnomids lay their tiny white, ovaloid eggs in a single mass with ragged edges. In contrast, hydropsychids lay an orderly thumbnail shaped mass comprised of neatly arranged rows of ovaloid eggs. Their masses vary in colour from cream to white with a bluish tinge (A. Macqueen, unpublished observations; Reich, 2004). Egg masses from multiple locations were hatched in the laboratory and the larvae identified following the procedure of Reich (2004) to ensure accurate identifications in the field. Reich (2004) established a maximum incubation period of two weeks for two hydropsychid genera in nearby upland streams (<100 km away). Estimates of local hatching times were obtained by installing 14 bark substrata (wooden stakes with 3 pieces of bark attached) in a single location. Masses laid on substrata were marked (using a hole punch) and monitored weekly for 4 weeks in summer 2008.

Both taxa require a solid substratum underwater upon which to lay their eggs. In Hughes Creek, eggs occur almost exclusively on bark and wood, even in areas where some rock is available. A thorough search of two 40 m bedrock stretches (surface area: 190 m^{-2}) yielded only 47 egg masses of *Ecnomus* spp. and 3 of *Cheumatopsyche* spp. These densities are much lower than on wood and bark, which can reach over 70 and 300 masses m^{-2} of substrata, respectively. Egg masses have never been observed on sand despite many field observations and collection of hundreds of sand samples (references given above). There is no published information on the preferences for type of oviposition substrata of these taxa in sandy bed streams. Research in cobbled, upland streams found that partial emergence of substrata from the water and flow velocity were important aspects of selection for various aquatic insect species (Reich & Downes, 2003; Bovill, Downes & Lancaster, 2013; Encalada & Peckarsky, 2006).

Experimental Design

The two distinct hypotheses tested are reflected in the two scales or levels in our design: site level, where each site is a replicate, and bark level, where individual pieces of bark are replicates. At the site-level (Hypothesis 1) densities of bark were manipulated to create four treatments, which were randomly allocated to sites: ‘High’ density (4 sites), ‘Low’ density (4 sites), ‘Control’ (3 sites) and ‘Procedural Control’ (3 sites) (Table 1). Each site met the following criteria: (1) some native overstorey, (2) both pool and run channel units, (3) no outcropping rock, (4) separated from other sites by approximately 1 km or more. Within a suitable location, the starting point of each site was randomly selected. For hypothesis 1, bark was removed or added to sites (as 'density units') to create consistent Low and High treatments (Table 1). Controls were not manipulated in any way and provided information on natural, background rates of bark loss or accumulation. Procedural Controls controlled for the effect of walking through the stream when sampling and for the effects of stakes, which trap naturally drifting bark.

At the start of the experiment, initial densities of natural bark were estimated for each site by measuring the length and width of each piece of bark, calculating and summing the areas and dividing this total surface area by the wetted area of the site (average width x site length) to produce an estimate of total bark density in m^2m^{-2}. Sites were randomly allocated to treatments to de-couple any pre-existing effects of bark densities on experimental outcomes. The bark density of sites randomly allocated to Low and High treatments were then standardised by removing and adding bark, as follows. Initially, the density of bark was reduced in Low sites by haphazardly removing a known quantity of bark. Density units were then added to both High sites and Low sites (Table 1). The number of units added to Low (4-12 units) and High sites (14-27 units) resulted in bark densities of approximately 0.025 m^2m^{-2} and 0.1 m^2m^{-2}, respectively, which reflect the minimum and maximum densities observed during the previous two years (A. Macqueen, unpublished observations). The amount of bark in 'fast' flows and amount of fully submerged bark was expected to decline over the experiment as discharge dropped. To offset these changes, approximately 60% of density units were placed in flows greater than 0.2 ms^{-1}, while density units were installed at (slightly emergent) or below the water's surface (fully submerged) to maximise availability of submerged bark during the experiment. Fourteen stakes without bark attached were installed in Procedural Control sites, which were also subject to a similar amount of disturbance by walking.

Estimates of the number of egg masses and bark density were obtained for each site 22 and 55 days after the experiment commenced on 4 February 2010. All natural bark and all density units in three, randomly selected 5 m sections (up to 5 sections if densities were low) were searched for egg masses. The walking disturbance associated with these activities was simulated in each of the procedural control sites. Water velocities were measured in 8 random locations in each site prior (12-14 January), during (2-3 March: day 28-29) and after the experiment (29-30 March).

Our second hypothesis (Hypothesis 2) was tested by manipulating bark emergence (emergent vs submerged) and water velocity ('Fast' > 0.2 ms^{-1}, 'Slow' < 0.15 ms^{-1}) over individual pieces of bark (preference units) within High and Low sites (Table 1). (Note that we use the term "preference" for convenience only given the aforementioned difficulties of demonstrating strict preferences in the field). The four treatments, namely Fast, Emergent; Fast, Submerged; Slow, Emergent; Slow, Submerged, were each replicated twice at random locations within High and Low sites. Egg masses on preference units were identified, counted and removed on days 9, 15, 22, 38 and 50. Egg masses were removed to ensure that overall densities of eggs remained low on these units to avoid any competition for space. If necessary, preference units were raised or lowered to ensure that the emergence treatments were maintained.

Data Analysis

To evaluate the efficacy of the site-scale manipulation, a repeated measures ANOVA was used to test whether the total surface area of bark differed between treatments (Low, High, Control, Procedural Control) and over time (Day 22 & 50). Although manipulations were effective, as explained below, high and spatially variable inputs of natural bark blurred the degree of difference. As a result, site-level bark surface area (SA) was treated as a continuous variable in analyses of egg masses, rather than a discrete factor as planned. Final analyses were conducted on total bark SA and number of masses per site rather than densities, as initial analyses showed that standardising by site area (70 ± 19 m^2) obscured the effects present because of a lack of relation between egg mass numbers and area. For each taxon, an ANCOVA was used to test the explanatory power of two variables, Bark SA (continuous factor) and Time (discrete, fixed factor; days 22 & 50) on egg mass numbers. The total numbers of *Ecnomus* and *Cheumatopsyche* egg masses in the 5m sections surveyed at each site were summed and scaled up to estimate the total number of egg masses per site. As stakes at Procedural Controls were found to collect bark and increase bark SA (see Results), sites without stakes (i.e. Controls) were excluded from the analysis. Bark SA and egg mass estimates were log transformed to satisfy model assumptions.

Originally we intended to employ a split-plot ANOVA with planned contrasts to test hypothesis 2, which became impossible once it was apparent that the site-level variable (Bark SA) should be treated as continuous. Instead, we developed an alternative approach using a series of tests. The numbers of egg masses in each bark-level treatment (Fast flow, Emergent; Fast flow, Submerged; Slow flow, Emergent; Slow flow, Submerged) were summed across replicates (preference units) for each site at each sampling time, and then subject to a three-step analysis. First, a chi-square test of independence determined whether numbers of egg masses were affected by water velocity and emergence independently (i.e. a 2-way contingency table). In all cases these tests were non-significant, i.e. velocity and emergence had independent effects upon numbers of egg masses. Second, to test for a significant departure of numbers of egg masses from a 50:50 ratio for each factor (velocity, emergence) a chi-square goodness-of-fit test was applied. The residuals were used to test whether any preference by females for particular types of bark varied with background bark densities. The standardised residuals are a measure of how different the observed ratio is from the expected ratio (Sokal & Rohlf, 1981). Third, using regression analysis, we compared the residuals with bark SA to test whether female oviposition choices were dependent on the total amount of bark at the site (hypothesis 2). A significant relationship would indicate that the preference strength varied with Bark SA. Due to elevated flows prior to sampling on days 15 and 38, which affected the integrity of treatments, analyses were performed with and without the data from these times to determine whether they influenced statistical outcomes. This approach assumes that egg masses laid on the same piece of bark represent independent decisions by females; this could be problematic if females' choices are affected by egg masses laid previously. If this was the case, spurious preferences would not be observed where none were present, but real effects could be amplified or dampened in several ways. An attractive effect of previously laid egg masses, would accentuate females' preferences, but dampen or reduce preferences if females avoid other masses.

Analyses were conducted using SPSS v.18 (Chicago, USA), R v.2.10 (Vienna, Austria) and GenStat v.13 (Hemel Hempstead, UK).

4.3 Results

Hatching time and success

Of the 118 egg masses of *Cheumatopsyche* monitored, 33% hatched within 12 days and 97% within 19 days. Egg masses of *Ecnomus* hatched more quickly, with 51% and 98% of the initial 85 masses hatching within 12 and 19 days, respectively. The fates of the remaining masses are unknown, but there was no observed mortality of egg masses during the study period. We are confident that all masses observed on day 22 had hatched by day 50, allowing the two times to be treated as independent in our site level analyses.

Efficacy of experimental manipulation

Bark SA was significantly greater at High sites than at all other treatments (Table 2, Fig. 2). On average, bark SA at sites declined over time, but not evenly across sites, so that the range of surface areas at sites in the High treatment group overlapped those of the other treatments as the experiment proceeded (Fig. 2). Although bark SA was statistically greater at High sites than Control sites on most occasions, the control site with the most bark was within the range of High treatments, indicating that such high bark SAs occur naturally. Removing this site produced a significant difference in the amount of bark found in Control sites and Procedural Control sites ($F_{1,9}$= 17.08, *p* = <0.01). This very high bark SA is attributed to a single overhanging tree dropping an extraordinary amount of bark. It is, however, a real data point and we only remove it in this case to illustrate the important effect woody debris can play in trapping and holding bark in place.

Variability in discharge over the study period is likely to have altered the flow velocities and emergence of bark units on some occasions. However, removing the affected data did not change the outcome of the analyses (outcomes of goodness-of-fit tests for individual sites are available as Supporting Information: *Ecnomus*, Table S1; *Cheumatopsyche*, Table S2). Variability in velocity changed the proportion of density units in Fast flow. Prior to the commencement of the study 65% of randomly measured velocities in High treatment sites were over 0.2 ms^{-1} (0.238 ± 0.141 ms^{-1}), falling to 32% by day 28 (0.170 ± 0.117 ms^{-1}). The creek was in spate for the final measurements (post experiment) and 94% of locations had flows exceeding 0.2 ms^{-1} (0.416 ± 0.129 ms^{-1}).

Test of egg mass limitation by substratum availability

The highest numbers of *Ecnomus* egg masses were found at sites where bark was most abundant, a trend that that was consistent through time and that did not show any sign of levelling off over the range of bark SAs recorded (Table 3, Fig. 3). The distribution of *Cheumatopsyche* masses was independent of the total amount of bark at a site. The number of egg masses appears to have a common upper bound across the range of bark SAs (Table 3, Fig. 3).

Test of female preferences

The proportion of egg masses laid by *Ecnomus* on emergent bark was sometimes significantly greater than on submerged bark units (Fig. 4a), but this relatively weak effect declined significantly with increasing total bark SA ($F_{1,19} = 7.25$, $p = 0.011$). *Ecnomus* did not respond to velocity (statistics not reported for brevity). These outcomes were maintained when the spate-affected data (Day 38, details below) were excluded (Fig. 4a). In contrast, in the overwhelming majority of sites, a greater proportion of *Cheumatopsyche* masses were laid in Fast than Slow flows than was expected by chance (Fig. 4b), but *Cheumatopsyche* showed no significant response to whether bark was emergent. The strength of the response to velocity also declined with increasing bark SA ($F_{1,19}=8.48$, $p< 0.01$), but the sizes of the residuals show that the effect remains strong even at high bark SA.

4.4 Discussion

We have demonstrated experimentally that *Ecnomus* egg supply is limited by the availability of oviposition substrata in a sandy bed stream. We successfully manipulated bark surface areas over an order of magnitude, which is within the natural range observed in Hughes Creek. As a consequence, numbers of *Ecnomus* egg masses also varied by an order of magnitude, providing strong evidence that local availability of bark limited local input of eggs. This strong, positive relationship did not level off (i.e. no evidence of saturation), which suggests that there were no shortages of female *Ecnomus,* and that further increases in bark densities could result in even more egg masses. This outcome also suggests that where little bark was available some females may either have failed to oviposit or dispersed elsewhere in search of suitable substrata. In stark contrast to the results for *Ecnomus*, there was no relationship between the total amount of bark and the number of *Cheumatopsyche* egg masses in a site.

This difference in response by the two taxa may be explained by different oviposition preferences for types of bark and how the strength of these preferences varied with bark availability at the site scale (Hypothesis 2). *Ecnomus* laid proportionally more eggs on emergent than submerged bark, but the effect was small; they did not respond to water velocity. The apparent preference of *Ecnomus* for emergent bark varied with bark density and was not significant at high bark densities. Thus *Ecnomus* were less choosy when a lot of bark was available. In contrast, female *Cheumatopsyche* had a strong propensity to lay on bark in fast-flowing water across all bark densities and, although the effect also became weaker at high bark densities, it was still significant. The amount of bark in fast water flows is relatively small because bark typically settles in areas of slow flow unless caught on woody debris (O'Connor, 1992). Additionally, bark in fast flows is further limited by the proportion of stream in which such flows occur, which declined during the experiment (from 65% to 32% of locations) with falling discharge. Thus, *Cheumatopsyche* preference for bark in fast flows meant that the amount of *suitable* bark did not increase at High sites and may have actually declined at some sites during the experiment. This strong preference for bark in fast-flowing water explains why *Cheumatopsyche* did not respond to increased bark densities.

It is interesting to consider biological differences between these taxa that may explain their preferences for different types of bark. Females of species in both Hydropsychidae and Ecnomidae can swim (Badcock, 1953; Deutsch, 1984; A. Macqueen, pers. obs.), which is facilitated by modified (flattened) limbs. However, while hydropsychids are capable of diving (Deutsch, 1985) there is no record of this ability in ecnomids and it is feasible that they use substrata to enter the water, which would explain their preference for emergent bark. Interestingly, hydropsychids oviposit in pools (i.e. slow flows) elsewhere in cobbled upland streams (Lancaster, Downes & Arnold, 2010b), which may be because high turbulence and flow velocities in riffles makes swimming difficult. In contrast, the flow in Hughes Creek was much less turbulent and relatively slow compared to upland riffles, potentially allowing females to oviposit outside of pools. We hypothesise that the decline in the strength of preferences in both taxa with increasing background densities of bark was either because females actively adjust their decisions to suit the conditions they encounter (as do parasitoids searching for appropriate hosts: Yang *et al.*, 2012) or because the most preferred kind of bark is less apparent when bark is abundant (i.e. females are swamped with potential substrata and may not always locate the best sites efficiently). If the latter, this outcome would be analogous to the effects of host plant apparency, which can influence the oviposition habits of terrestrial insects (Floater & Zalucki, 2000). These results have important implications for the study of oviposition preference in aquatic systems because failing to consider the density of the resource in question may lead to conclusions that lack generality (i.e. preferences at one density may not apply at others, or no preferences may be detected at all). Thus it will not always be safe to assume that correlations represent evidence of preference and experiments seeking to establish preferences must be very carefully thought out (Underwood et al. 2004).

The strong response of *Ecnomus* to increased bark densities would deliver profound increases in local densities of 1st instar larvae. Our observations demonstrated that the majority of *Ecnomus* eggs (98%) hatched successfully under field conditions. Hence, the order of magnitude increase in egg masses we observed would translate into a difference of several hundred thousand 1st instar larvae, given that *Ecnomus* egg masses contain approximately 250-350 eggs (A. Macqueen, unpublished observations). It remains to be determined whether these increases result in high densities of later instars or whether the majority of first instars die quickly so that increased numbers have no lasting effects. We have no information on mortality rates of *Ecnomus* larvae, and very high mortality of early instars is possible (e.g. Elliott, 1982). For instance, Hildrew *et al.* (2004) manipulated egg inputs by transporting egg masses of the megalopteran *Sialis fuliginosa* (oviposited on streamside vegetation) between sites and found no lasting effects on aquatic larval densities. However, studies on *Baetis rhodani* and *Rhyachophila dorsalis* in a Scottish stream found that high densities of larvae produced by high densities of oviposition substrata persisted through the life cycle (Lancaster & Downes, 2014): between-site differences in density of oviposition substrata explained up to 80% of variation in densities of late instar larvae. Such results suggest that ecnomid densities may also be strongly affected by the provision of oviposition substrata. Certainly, this hypothesis is a viable explanation for otherwise puzzling high between-site variation in larval densities observed in other sand-bed streams nearby (Downes *et al.*, 2006).

The research that established oviposition substrata can act as a constraint on aquatic insect densities is typically correlative (Encalada & Peckarsky, 2011; Lancaster, Downes &

Arnold, 2010a) but also includes some experiments (Encalada & Peckarsky, 2012; Alp, Indermaur & Robinson, 2013), which, to our knowledge, have been conducted exclusively on species that oviposit on emergent rocks in cobbled streams (particularly *Baetis*). Thus, our results expand knowledge of the role of oviposition constraints to new taxa and a new stream type, soft sediment streams, which are more common than cobbled streams in Australia. Moreover, bark and wood are inherently different to rocks and have different dynamics, although they share some common drivers. For instance, flow variability in upland streams can determine the availability of suitable substrata. However, whereas a large rock substratum will merely be submerged by all but the largest floods, bark (and any attached egg masses) is vulnerable to being transported downstream and stranded by receding flood waters or buried beneath shifting sand. A particularly large flood may leave some sections of stream all but devoid of oviposition substrata. Indeed, Downes *et al.* (2011) reported very low densities of detritus in Hughes Creek in spring, when adults of many species become active. Furthermore, bark fall is seasonal and, unlike stone, bark degrades. Given all this, it is feasible that the taxa of Hughes Creek may suffer more unpredictable and extreme substrata shortages than their upland analogues, and this may have interesting implications for population dynamics.

Human impacts also have a role in driving oviposition substrata dynamics in sand bed streams. Widespread historic clearing of vegetation has led to some stretches of channel having few local sources of bark and wood substrata. Additionally, clearing is likely to be at least partially responsible for large volumes of sand that can bury bark and wood and make it inaccessible, which is significant as woody debris can strongly influence the availability of substrata by trapping drifting bark (often in fast flows) (O'Connor, 1992). While riparian vegetation is widely recognised as a source of material that provides habitat and food to larval stages of aquatic insects, we have demonstrated that riparian inputs are also a critical resource for another life history stage, the egg stage, and hence recruitment. This is important because the losses of species associated with a decline in woody debris in many streams (Lester & Boulton, 2008) may hypothetically be due to constraints on female oviposition opportunities for some species, not a shortage of food or living space for larvae. Increasing the provision of oviposition substrata may involve different amounts and distributions of detritus than that provided for larvae. Our results illustrate the importance of considering essential resources at multiple life history stages, not just one, and to examine the cause-and-effect mechanisms that link woody debris and other detritus to species diversity in streams.

4.5 Acknowledgements

This research was supported by an Australian Postgraduate Award (to AM) and grants to BJD from the Australian Research Council (Discovery Grants DP0772854 and DP120103145) and grants from the Holsworth Wildlife Trust. We wish to thank local landholders, especially the Strong family, for their hospitality and creek access. We are most grateful for the generous field assistance of Dan Rooke, William Bovill, Alena Glaister, Sara Dehm, Kate Barnard, Elise King, Keira Mason-Hill, Handoko Wahjudi, Jenny Frieden and Jill Lancaster. Jill also provided welcome feedback on the manuscript along with two anonymous referees.

4.6 Tables and Figures

Table 1: The experimental design, showing the four treatments and each experimental component. A tick indicates that the component was present, while a plus (for Density units) indicates presence, with number of pluses indicate relative number of units. Density units comprised multiple pieces of bark of known surface area (collectively 0.12 m^2 in Low sites and 0.3 m^2 in High sites) tied together and anchored to a stake (Fig. 1). At High sites, density units were used to increase the amount of bark present, while, at Low sites, density units ensured bark was distributed in clumps similar to those at High sites while still keeping overall bark surface area low (this controlled for the addition of bark density units in High sites). Preference units each comprised single pieces of bark, approximately 0.042 m^2 in area, secured to a wooden stake (Fig. 1) and placed in either slow or fast flows and as either emergent (part of the bark was above the water's surface) or fully submerged. Preferences units tested the responses of females to bark placed into different conditions (water speeds, emergence). The presence of preference units at both High and Low sites enabled us to test whether females' responses varied with background surface area of bark.

Treatment	Natural bark	Stakes	Density units	Preference units
Control	✓			
Procedural Control	✓	✓		
High	✓	✓	+++	✓
Low	✓	✓	+	✓

Table 2: Efficacy of site-level manipulation. Repeated measures ANOVA comparing total bark surface area (m^2) between treatments (C: Control, P: Procedural Control, L: Low, H: High) and times (day 22 and 50). Planned contrasts indicate that bark surface area is significantly greater in High treatments than other treatments.

Source	**df**	**MS**	**F**	**p**
Between Subjects				
Treatment	3	3.453	10.117	0.002
C vs P	1		4.216	0.067
L vs H	1		17.628	0.002
H vs P	1		8.511	0.015
Error	10	0.064		
Within Subjects				
Time	1	1.359	12.31	0.006
Time x Treatment	3	0.273	2.474	0.121
Error(Time)	10	0.11		

Table 3: Summary of ANCOVA testing for effects of bark surface area (SA) and time on the number of egg masses of *Ecnomus* and *Cheumatopsyche*. Control sites are excluded (see text for explanation).

Source	**df**	**MS**	**F**	**p**
Ecnomus spp.				
Bark SA	1	5.570	20.235	<0.001
Time	1	0.207	0.075	0.787
Bark SA x Time	1	0.183	0.665	0.425
Error	18	0.276		
Cheumatopsyche spp.				
Bark SA	1	1.363	0.366	0.553
Time	1	0.729	0.192	0.667
Bark SA x Time	1	0.025	0.007	0.937
Error	18	3.803		

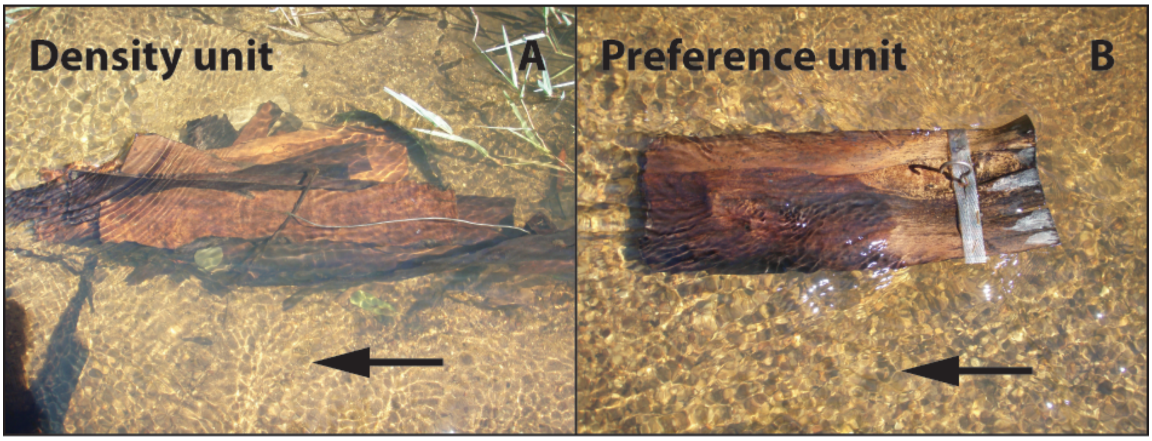

Fig. 1. (A) A density unit and (B) a preference unit *in situ*. Arrows indicate direction of flow. Density units comprised multiple pieces of bark tied together into a bundle, which was anchored to the bottom by tying it to a wooden stake hammered into the streambed. Preference units comprised a single piece of bark situated in either slow or fast flow and either completely submerged or partially emerged. The bark was kept in place with a screw hook, which was screwed into the top of a stake hammered into the streambed. The preference unit illustrated is emergent, with one end out of the water and the rest submerged.

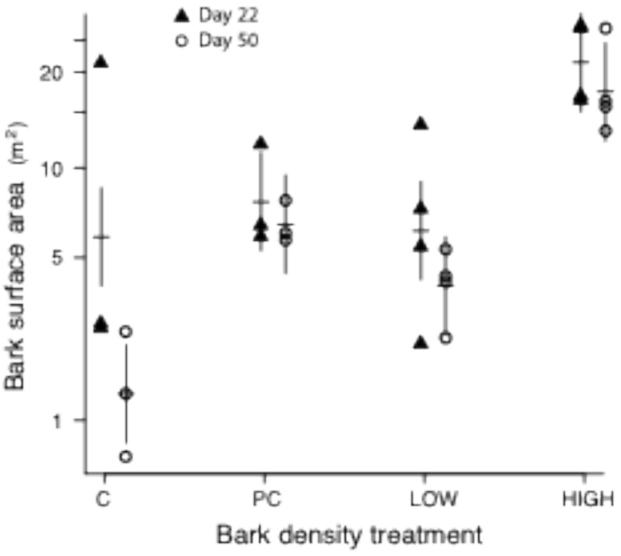

Fig. 2: Bark surface area for individual sites (symbols) and means (±1SE) for each site-level treatment (Control, Procedural control, Low and High density) at day 22 (triangles) and day 50 (circles). Note y-axis is on a log scale. See Table 2 for statistical analyses.

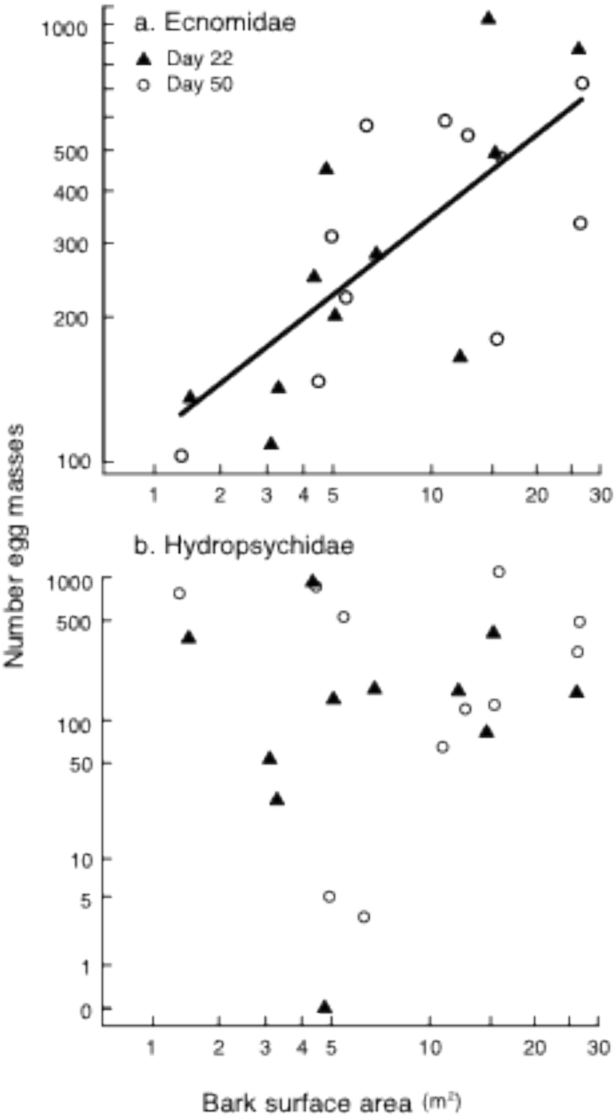

Fig. 3: Relationship between the number of egg masses and bark surface area for each site at day 22 (triangles) and day 50 (circles) for (a) *Ecnomus* and (b) *Cheumatopsyche*. Line in (a) is for data pooled across time. Note both axes are on a log scale. See Table 3 for statistical analyses.

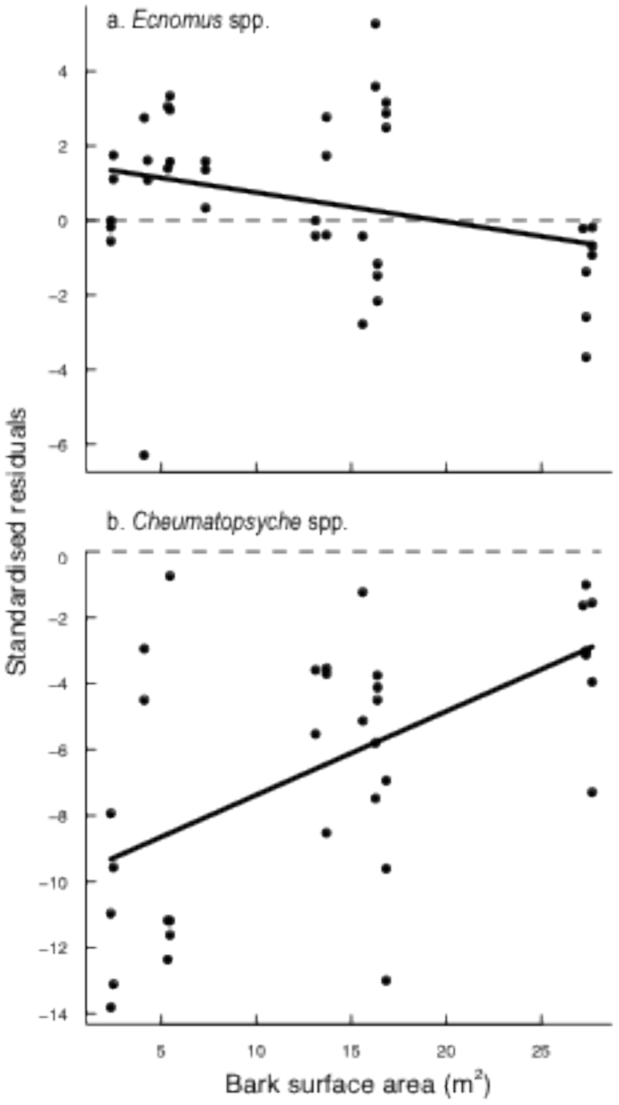

Fig. 4: Relationship between standardised residuals from chi-square tests and bark surface area for (a) *Ecnomus* on emergent bark and (b) *Cheumatopsyche* on bark in Slow flow. Each residual is the product of a chi-squared, goodness-of-fit test on the number of egg masses on replicate preference units (n~4) with an expectation of 50% on each type. A positive residual indicates a propensity to lay on bark in a certain micro-scale condition (e.g. emergent bark and slow flows, respectively) with residuals above about 2 representing a significant effect. For instance a large negative residual in (b) reflects a strong preference for Fast flow.

4.7 Electronic Supplementary Material

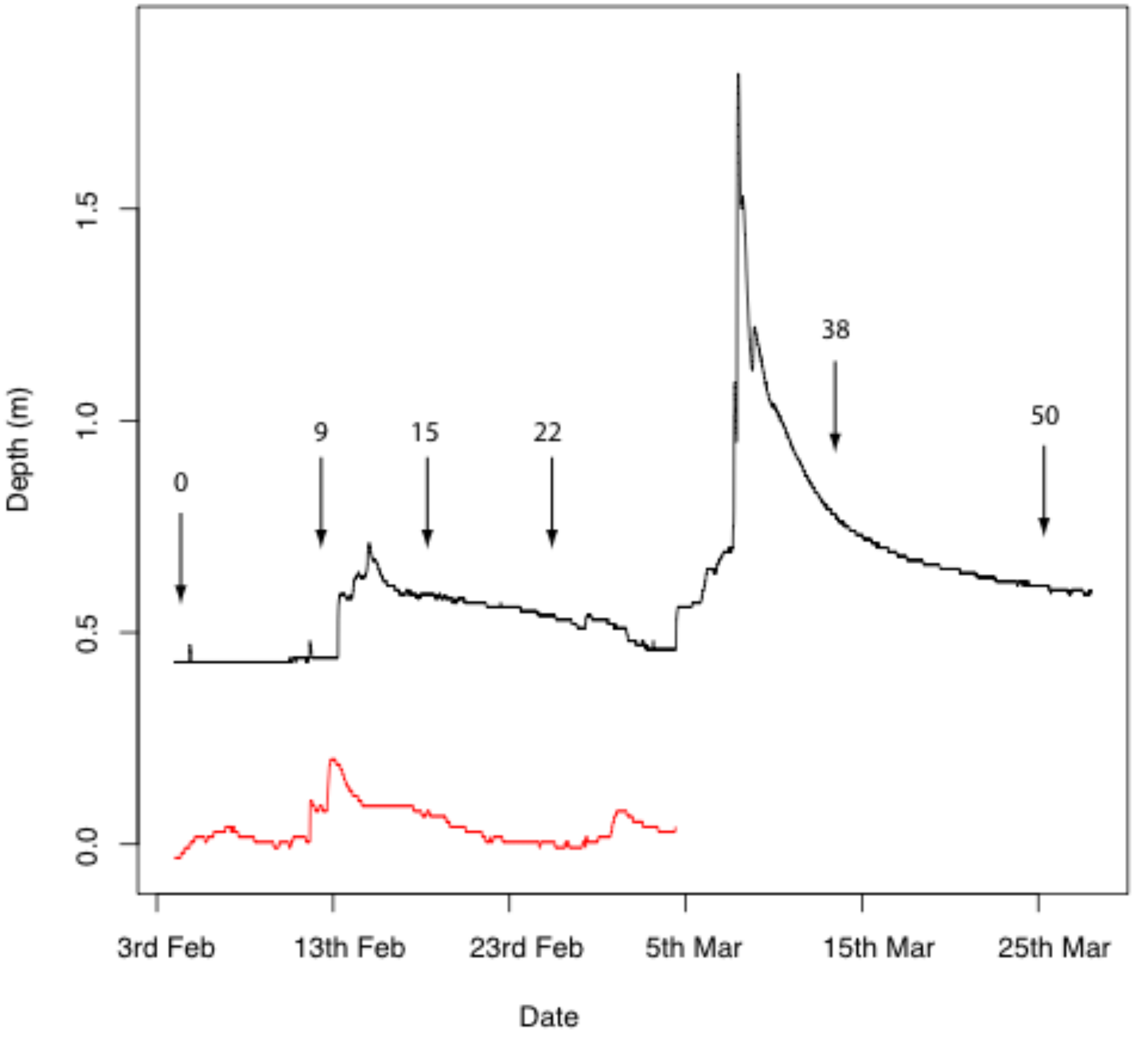

Fig. S1: Variation in water depth during the study period at site 4 (red; Odyssey depth/temperature logger, Christchurch, NZ) and gauging station approximately 20 km downstream of the study area (black; Hughes Creek at Tarcombe Road, www.vicwaterdata.net, site 405228). Arrows indicate sampling days.

Table S1: Chi-square goodness-of-fit tests for *Ecnomus* masses on emergent and submerged bark

		Observed		Residual			
Time	**Site**	**Emergent**	**Submerged**	**Emergent**	**Submerged**	**Chi-sq**	**p-value**
1	1	40	29	2.78	-2.15	12.34	<0.001
	3	23	3	1.36	-1.92	5.56	0.018
	6	19	28	-0.93	0.93	1.72	0.189
	7	3	10	-1.37	1.37	3.77	0.052
	11	1	12	-2.16	2.16	9.31	0.002
	12	23	2	2.97	-2.97	17.64	<0.001
	13	1	2	-0.55	0.63	0.69	0.405
	14	23	1	3.18	-3.18	20.17	<0.001
2	1	42	33	1.74	-1.51	5.29	0.021
	3	21	18	0.34	-0.34	0.23	0.631
	6	11	16	-0.68	0.68	0.93	0.336
	7	4	23	-2.59	2.59	13.37	<0.001
	11	3	9	-1.47	1.7	5.06	0.024
	12	15	5	1.58	-1.58	5	0.025
	13	8	9	-0.17	0.17	0.06	0.808
	14	22	4	2.5	-2.5	12.46	<0.001
3	1	25	29	-0.38	0.38	0.3	0.586
	3	37	20	1.59	-1.59	5.07	0.024
	6	7	8	-0.18	0.18	0.07	0.796
	7	7	44	-3.66	3.66	26.84	<0.001
	11	1	5	-1.15	1.15	2.67	0.102
	12	37	6	3.34	-3.34	22.35	<0.001
	13	10	10	0	0	0	1
	14	22	2	2.89	-2.89	16.67	<0.001
4	1	22	15	2.75	-1.95	11.36	0.001
	3	35	5	1.61	-2.28	7.81	0.005
	7	17	30	-0.42	0.34	0.29	0.592
	11	5	7	-0.41	0.41	0.33	0.564
	12	37	8	3.06	-3.06	18.69	<0.001
	13	23	9	1.75	-1.75	6.13	0.013
	14	39	1	5.28	-4.57	48.77	<0.001
5	1	32	153	-6.29	6.29	79.14	<0.001
	3	42	29	1.09	-1.09	2.38	0.123
	6	20	29	-0.22	0.19	0.08	0.773
	7	41	85	-2.77	2.77	15.37	<0.001
	11	5	5	0	0	0	1
	12	21	10	1.4	-1.4	3.9	0.048
	13	21	12	1.11	-1.11	2.45	0.117
	14	34	3	3.6	-3.6	25.97	<0.001

Table S2: Chi-square goodness-of-fit tests for *Cheumatopsyche* masses on bark in fast and slow flows

		Observed		**Residual**			
Time	**Site**	**Fast**	**Slow**	**Fast**	**Slow**	**Chi-sq**	**p-value**
1	1	39	3	3.06	-3.54	21.88	<0.001
	6	34	1	3.94	-3.94	31.11	<0.001
	7	54	1	1.51	-3.02	11.36	0.001
	11	46	2	4.49	-4.49	40.33	<0.001
	12	185	275	0.95	-0.74	1.45	0.229
	13	381	0	13.8	-13.8	381	<0.001
	14	98	6	8	-6.93	112.08	<0.001
2	1	32	0	3.21	-3.7	24	<0.001
	6	15	9	2	-1.55	6.4	0.011
	7	13	9	1.16	-1.01	2.37	0.124
	11	27	1	4.33	-3.75	32.81	<0.001
	12	259	3	11.18	-11.18	250.14	<0.001
	13	110	0	9.15	-7.93	146.67	<0.001
	14	168	10	12.39	-9.6	245.73	<0.001
3	1	100	13	20.87	-8.52	508.21	<0.001
	6	85	0	9.41	-7.29	141.67	<0.001
	7	17	0	3.6	-3.12	22.67	<0.001
	11	32	1	4.75	-4.11	39.46	<0.001
	12	359	0	8.99	-11.6	215.4	<0.001
	13	210	0	12.65	-10.95	280	<0.001
	14	295	0	14.99	-12.98	393.33	<0.001
4	1	13	0	4.16	-2.94	26	<0.001
	7	3	1	2.46	-1.23	7.56	0.006
	11	30	0	3.11	-3.59	22.5	<0.001
	12	624	0	5.59	-11.17	156	<0.001
	13	358	5	13.1	-13.1	343.28	<0.001
	14	212	1	2.59	-5.79	40.23	<0.001
5	1	65	10	4.49	-4.49	40.33	<0.001
	6	32	16	1.63	-1.63	5.33	0.021
	7	46	0	5.92	-5.13	61.33	<0.001
	11	61	0	5.52	-5.52	61	<0.001
	12	320	5	12.36	-12.36	305.31	<0.001
	13	186	1	9.57	-9.57	183.02	<0.001
	14	126	5	7.48	-7.48	111.76	<0.001

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Chapter 5. Summary and conclusions

This thesis details a thorough investigation into the possibility of habitat limitation on ovipositing females in lotic systems. This chapter brings together the findings from each step of the project from observational baseline data collection (Chapter 2), specific experiments testing female behaviours (Chapter 3) and a large, catchment scale experiment (Chapter 4) to present a compelling array of evidence for habitat limitation and its potentially profound influence on populations of freshwater insects. Firstly, I summarise the results from each chapter and how they build upon the preceding work to create a cohesive progression of ideas. I then discuss the significant implications of my findings for understanding of populations in lotic systems and, finally, situate my work in a broader ecological context.

5.1 Chapter summaries

Correlative evidence for habitat limitation

The observational study detailed in Chapter 2 sought to provide a preliminary test of the hypothesis that egg inputs were constrained by the availability of oviposition sites i.e. habitat rather than supply limited. Bark and wood surface areas were quantified across three streams and found that the former was more variable in both time and space (over an order of magnitude) than the latter. Bark accumulated over summer, quickly becoming more abundant than wood. As almost all of the literature on oviposition comes from studies in cobbled streams, this information is quite novel. Importantly, bark and wood are different to rock in a number of ways, including breaking down over relevant timescales (bark more quickly). Similarly, the natural processes generating wood and bark (tree growth and mortality, seasonal bark drop) operate over shorter scales than those generating distribution of rocks, i.e. geomorphology. Variation in substrate has potential population-level implications for recruitment in taxa that depend on hard surfaces for oviposition.

Hydrological variations, i.e. spates, are a common driver of substrate variability. Setting behavioural requirements aside, the absolute amount of hard substrate (i.e. considering all rocks, rather than just emergent rocks) in cobbled streams is likely to be higher and less variable than in other stream types. Detritus loads are lowest in the study streams after the flood seasons (winter and spring) (Downes *et al.*, 2011) and some sections of the study streams can have virtually no suitable substrate at all, submerged or emergent. Spates can transport bark and wood and any attached egg masses downstream (Reich, 2004), strand it on the bank, or bury it beneath sand. Significantly, this means that eggs laid on bark or wood are more likely to change location than those laid on rock.

Ecnomus and *Cheumatopsyche* egg masses were most abundant in this system. Interestingly, a substantial amount of variation in the distribution of *Ecnomus* egg masses was explained by variation in the surface area of bark present, indicating that bark could potentially limit the input of eggs. However, a third factor that co-varied with both egg mass and bark distributions could, theoretically, be driving the pattern. A possible candidate would be an interaction between trees (i.e. source of bark) and adult behaviour that could influence the distribution of eggs. Adults of many aquatic taxa exhibit swarming behaviour that centres on visually distinct "swarm markers" (Sullivan, 1981), such as erect stems or branches. Swarming behaviour is prevalent among chironomids (Oliver, 1971), and has been observed among caddisflies including hydropsychids (pers. comm., Leon Barmuta; Sullivan, 1981). It is possible that mated females exiting swarms lay nearby, providing a mechanism by which the distribution of riparian vegetation could influence that of egg masses, which is correlated with, but not caused by, the distribution of bark. Wood explained only a small proportion of variation in egg masses, perhaps due to the lower variability in surface area of wood relative to bark.

In contrast, neither bark nor wood were substantial predictors of *Cheumatopsyche* egg masses, which I hypothesised was due to a preference for bark in fast flows, based on personal observations. If correct, only a subset of available bark was suitable for oviposition. As I could not ascertain the velocity in which the eggs were laid (i.e. bark observed in survey could have shifted between time of laying and census), it was not possible to accurately quantify the available substrate that met the perceived flow criteria of *Cheumatopsyche* females. An alternative explanation for a lack of relationship is that the number of females (supply) rather than substrate (habitat) was limiting. An excess of bark would mean the number of eggs would be determined by factors driving the distribution of females, such as rates of emergence, dispersal and mortality, about which little is known. For instance, spatial patchiness in rates of successful emergence coupled with low dispersal in females, could create strong variability in supply of females.

Experimental evidence of selective oviposition and substrate access pathways

Following the findings of my first field season and after observing a consistent association of *Cheumatopsyche* with faster flows, I undertook a series of experiments (Chapter 3) to ascertain whether *Ecnomus* and *Cheumatopsyche* showed a propensity for particular substrate characteristics, such as emergence and flow velocity, which are important for some taxa in rocky upland streams (Reich & Downes, 2003b). Additionally, I tested whether the suitability of bark for oviposition changed over time as is the case for fruit flies utilising fallen and decomposing fruit (Atkinson & Shorrocks, 1984). Finally, I tested if a significant number of females utilised the emergent portion of bark substrates as landing pads to access the submerged portion, relative to other pathways such as crawling or swimming.

As hypothesised, there was a strong relationship between the velocity in which a bark substrate was located and the number of *Cheumatopsyche* egg masses that were laid. This indicates that, as suspected, *Cheumatopsyche* were associated with higher velocities and thus would have fewer suitable substrates available than a taxa with less specific oviposition behaviours. That is, if females of two taxa, one with strong oviposition preferences and one without, were equally abundant the former would be more susceptible to habitat limitation as fewer substrates would meet their criteria. It is possible that *Cheumatopsyche* lay in fast flow for the benefit of their offspring, which filter-feed using silk nets.

Ecnomus females did not appear to respond to flow velocity, but did lay more egg masses on submerged bark than emergent bark. Submerged bark is relatively common so it is less likely to strongly limit the proportion of suitable bark available to females. Therefore, this finding is compatible with the relationship between total bark surface area and *Ecnomus* egg masses established in Chapter 2. Female *Ecnomus* may have avoided emergent bark as it is often frequented by spiders that likely prey upon them. *Cheumatopsyche* did not exhibit a preference for emergent or submerged bark.

In rocky streams a number of taxa rely on emergent landing pads to enter the water (Reich & Downes, 2003b; Peckarsky, Taylor & Caudill, 2000). However, I showed that although females of both *Cheumatopsyche* and *Ecnomus* could access submerged bark by other means, i.e. swimming or crawling, females still alighted on emergent bark as there were significantly fewer masses on the submerged portion of bark with partial or full

Tangle trap barriers. This finding was further supported by the substantial number of adults trapped on Tangle trap barriers over the course of the study. *Cheumatopsyche* may dive directly into the water and swim as has been observed elsewhere (Deutsch, 1984), but there is no information on whether or not *Ecnomus* can do the same. However, I observed an *Ecnomus* female swimming underwater and ovipositing in captivity – a first for this genus. Swimming was likely facilitated by flattened limbs which are substantially broader in the female than the male (J. Lancaster, personal communication). It is feasible that *Ecnomus* females use emergent objects to enter the water then swim and oviposit on a submerged substrate that is most suitable. Alighting on emergent substrates may have fitness benefits where females are a risk of being entrained in the drift and eaten by predators (Encalada & Peckarsky, 2007).

As expected, the suitability of bark appeared to vary according to how long it was in the stream (i.e. with increasing level of 'conditioning'). During the first days to a week bark suitability was likely to be low as a slimy substance, apparently arising from the bark, covered the surface and precluded oviposition. Once the exudate cleared the bark was presumably at its peak suitability, which then declined over time. A significant difference in the number of egg masses was detected between partially conditioned bark (~ 4 weeks old) and fully conditioned bark (~8 weeks old). Females may avoid older, more conditioned bark, because the surface becomes uneven or covered in biofilm. Additionally, older bark is more likely to disintegrate and separate eggs within a mass resulting in the loss of any fitness benefits to eggs or hatchlings (e.g. predator swamping; Williams, Smith & Stephen, 1993) gained by this strategy. Bark shedding by Eucalypts appears to peak in summer, so the combination of disintegration and the action of spates (transporting, burying) suggests that the total surface area of substrate would be low in winter and spring. In contrast, in cobbled streams rocks may be submerged (i.e. unavailable to some taxa) but rarely removed from the channel and certainly not degraded over relevant timescales.

In summary, both *Ecnomus* and *Cheumatopsyche* make specific choices during oviposition, indicating that not all bark is equally suitable. Both taxa can access submerged bark by swimming or crawling. It is likely that these behaviours have fitness benefits for the adult, egg or larval stages as has been demonstrated elsewhere (Bovill, Downes & Lancaster, 2013).

Manipulative experiment demonstrates habitat limitation and density dependent selective oviposition

Having found patterns consistent with habitat limitation in my survey and established some selective oviposition behaviours used by my target taxa, I was ready to test further hypotheses in a large scale experiment that would give stronger inferential power and control over the context in which females choose where to lay (Chapter 4). I tested two major hypotheses: (1) that egg inputs were limited by the availability of oviposition substrate, and (2) that the selectivity of females when choosing substrate would vary with the abundance of bark at a site. The first was tested by comparing the number of eggs laid in sites manipulated to create high and low surface areas of bark. The second was tested by comparing numbers of egg masses on individual pieces of bark, which were manipulated with regard to emergence and velocity, and were situated in sites with high and low bark availabilities.

I successfully manipulated bark densities over an order of magnitude eliciting concomitant variation in the number of *Ecnomus* egg masses. Thus, I established a causal link between surface area of bark and densities of eggs masses, whereas my survey was only able to show association, and conclusively demonstrated that habitat limitation occurs outside of rocky streams. Oviposition sites were not saturated even at maximum total bark surface areas recorded in the experiment, which were among the highest observed in any of the study streams, suggesting that further increases in substrate would lead to even higher numbers of masses. Furthermore, since bark densities were as high or higher than those measured in 2007/2008 across all three streams it is likely that *Ecnomus* are routinely limited by access to bark. Despite efforts to ensure that a proportion of added bark was in fast flows, *Cheumatopsyche* didn't respond to increased amounts of bark. This suggests that the proportion of suitable bark, i.e. in fast flows, did not vary in proportion to the total amount of bark in a site and thus *Cheumatopsyche* egg inputs were independent of total bark surface area. This is not surprising as bark typically accumulated in areas of slow flow unless trapped on a piece of wood. I hypothesise that *Cheumatopsyche* is limited by the availability of suitable habitat, but this is hard to determine as it would require measuring velocity at every substrate, which can change if bark moves around or flow varies. Alternatively, *Cheumatopsyche* could instead be limited by supply factors.

Both *Ecnomus* and *Cheumatopsyche* responded to the manipulation of bark characteristics (preference units) within sites of varying overall bark surface area. In contrast to my findings in Chapter 3, a higher proportion of *Ecnomus* egg masses were found on emergent bark than expected. However, the strength (i.e. effect size) of this apparent preference declined with increasing total surface area of bark, approaching the expected 50:50 ratio at high surface area sites. It is possible that conditions in December 2008 were different to those in February and March 2010 in a way that altered *Ecnomus* behaviour. Certainly bark is likely to have been more scarce during the initial experiment in spring, i.e. before bark loads accrued. Indeed, this may taken as a warning against assuming that behaviours are fixed and independent of context. Alternatively, it is possible that the species mix within the genus changed between the two times altering the average response at the genus level from favouring submerged bark to favouring emergent bark. Though studies to date have shown *E. continentalis* to be dominant at the adult and larval stages (Downes *et al.*, 2011; Lancaster, Downes & Glaister, 2009), it is conceivable that another taxa, say *E. pansus,* dominated during one experiment and had contrasting oviposition preferences. The findings for *Cheumatopsyche* were consistent across experiments: a strong apparent preference for bark in fast flow. Like *Ecnomus* the effect was strongest at low bark surface areas with very large effects sizes and declining, but still strongly significant effects, at low surface areas. One possible explanation is that the preferred bark is less apparent when there is an abundance of other non-preferred bark. Reduced apparency has been established as a process that causes deviations from the expected (i.e. optimal) distribution of egg masses in terrestrial insects (Floater, 2001). Alternatively, if locations with large amounts of bark attract and create high densities of females, and females only assess the quality of the bark once in the water, the resulting demand for oviposition sites may force some females to settle for lower quality sites.

In summary, I have demonstrated that *Ecnomus* are habitat limited in this system while *Cheumatopsyche* could be supply limited, though the latter finding is less definitive. Furthermore, the behaviour of females is dependent on the amount of potential oviposition sites available.

5.2 Implications for populations and future research

I have demonstrated that oviposition habitat can be a limiting resource for aquatic insects in streams with few rocks and sandy beds, thus extending the generality of this idea in lotic systems (Encalada & Peckarsky, 2012; Lancaster, Downes & Arnold, 2011). Streams without rock are common in many places around the world and are likely more common in Australia than cobbled streams, which have been the focus of previous research. At this point, however, it is difficult to say how long the patterns established during oviposition will persist into the next stage of the life cycle. The logical progression in my study system would be to test for an association between the abundance of suitable oviposition sites and larvae of various instars (Reich & Downes, 2004). Benthic samples were collected during the experiment in Chapter 4, so this can be done in the future. There has been some significant work on this question in recent years, but overall there is a paucity of information on post hatching behaviours such as dispersal. The persistence of patterns established at oviposition also depend on rates of mortality, which is also understudied. One potential source of mortality for which there is specific data for the study taxa in Hughes Creek is the prevalence of nematode parasites. *Ecnomus* adults have been observed with abdomens bursting with nematodes, which likely impact heavily on mortality and fertility rates. While 20% of *Ecnomus* adults were infested, no nematode parasites have been observed in *Cheumatopsyche* (Bovill and Lancaster, unpublished data). Though somewhat out of vogue, life table analyses, such as those conducted by Elliott (1982; 2013), would be highly instructive in determining where post hatching mortality is highest. Interestingly, Willis and Hendricks (1992) found high levels of mortality during first instar and pupal stages of *Hydropsyche slossonae,* using this approach. The latter stage is virtually unstudied and further research on both would be very worthwhile.

Interpretation of my results would have been aided by a lower taxonomic level of egg mass identification. Further detailed work may yield advances in the identification of hydropsychid taxa, but ecnomid eggs were very consistent in their appearance, leaving little prospect of improved identification by eye. Genetic analysis of egg masses may be a fruitful way of determining the mix of species ovipositing and, combined with additional work on adults, could shed some light on the spatial structuring of the study populations (e.g. Hughes *et al.*, 2003). Repeating this type of work on another Ecnomid species and other related taxa would be helpful in determining the phylogenetic consistency of my findings. The genus has a world wide distribution with the greatest diversity in Africa where there are about 50 species (Barnard & Clark, 1986), so there is no shortage of possible subjects and locations.

A greater understanding of adult dispersal and population structure for the study taxa would be of great benefit, because of the influence on the supply of females. The small size of ecnomid and hydropsychid adults likely precludes the use of harmonic radar even with the miniature transmitters now available (but see Briers, Cariss & Gee, 2002). Stable isotope analysis would likely be a better option for studying dispersal (and thus spatial structure) for these populations (Macneale, Peckarsky & Likens, 2005). If females lay their eggs back into the section of stream from which they emerged (i.e. closed at the adult stage) it would be possible to predict the abundance of females from the distribution of late instar larvae (Downes & Reich, 2008). If females disperse, however, understanding supply is much more difficult, requiring a larger scale approach. Of course, female supply has a direct influence on the balance between supply and habitat limitation. For instance, if larvae and thus emergent females are more abundant in upstream sections and females dispersal ability is limited, downstream sections could be supply limited, while upstream sections are habitat limited (i.e. upstream source for adults, downstream sink). This is analogous to marine systems where some locations receive fewer settlers due to prevailing currents (supply-limited) and others receive more settlers than can be accommodated on the shore (habitat-limited) (Roughgarden, Gaines & Possingham, 1988).

Testing for habitat limitation relies upon accurate quantification of suitable substrate. For some oviposition habitat requirements this may be relatively straight forward, e.g. emergent rocks are simple enough to count. I was fortunate that suitable substrate for *Ecnomus* varied in proportion to the total amount of bark in a site, which was simple, if laborious, to quantify. Whether a lack of relationship between numbers of egg masses and a given measure of oviposition site abundance (as observed for *Cheumatopsyche*) is due to habitat being in excess, i.e. egg inputs supply limited, or due to a mismatch between the measure and actual female requirements is harder to determine. Indeed, it may be near impossible to consider all of the factors that drive where females choose to lay. *Ecnomus* showed a strong relationship with bark surface area and yet not every piece of bark, or indeed every m^2 of bark, was utilised. Whether bark without eggs was a result of chance or because it is unsuitable may be impossible to determine, making it difficult to demonstrate habitat limitation definitively. It also suggests that females will not lay on just any piece of bark, even if bark is in short supply (Lancaster, Downes & Arnold, 2010b). Furthermore, the accuracy of a measure of oviposition substrate may also vary over space and time as females respond to the context, such as density of resources, and change in the suitability individual site (e.g. experimental bark substrates). For instance the suitability of substrates can vary over time as was demonstrated by the conditioning of bark and changing velocity observed in chapters 3 and 4. Observations need to be made at an appropriate frequency otherwise the accuracy of treatments with respect to suitability will be reduced and the ability to identify preferences compromised.

While freshwater insects present particular challenges to population ecologists, they also offer opportunities to study processes that are much more difficult to approach in other systems. For instance, egg masses represent an active decision by females, allowing closer study of recruitment in aquatic insects, compared to analogous processes such as settlement in benthic marine invertebrates where observing settlement choice is more difficult due to their size. Furthermore, it is possible to actively manipulate the supply of new individuals to a particular location and investigate the ramifications on subsequent stages (Hildrew *et al.*, 2004), which would be a much more difficult undertaking in marine systems (i.e. controlling supply of settling larvae). Indeed, while the transition in marine invertebrates (i.e. settlement) is of a single individual, that of insects is a transition of one to many individuals (eggs), so the effect of individual oviposition decision is magnified in the next life history stage.

This has important ramifications for the potential impact of habitat limitation on population dynamics. Indeed, the order of magnitude variation in the number of egg masses at sites with high and low bark abundances observed in my large-scale experiment (Chapter 4) could potentially lead to a difference between sites of several hundred thousand first instar larvae. Whether such substantial differences are quickly eroded by high rates of mortality (Elliott, 1982) or dispersal as suggested by some authors (e.g. Reich & Downes, 2004) is unclear. However, some recent work suggests that dispersal via drift is not as common or extensive as commonly assumed (Lancaster, Downes & Arnold, 2011) and that patterns established during oviposition can be observed in late instar larvae (Lancaster, Downes & Arnold, 2010a). In any case, it is feasible that a combination of a highly variable distribution of substrate and selective female behaviours could account for the surprisingly high variability in larval densities observed in similar streams nearby (Downes *et al.*, 2006).

If the most recent literature is any guide then the interactive effect of adult supply and habitat availability could revolutionise the way we think about populations of lotic insects. For example, a recent study on *Baetis rhodani* – considered to be highly mobile and thus unlikely to show persistent effects of oviposition patterns – found that 80% of variation in late instar larvae could be attributed to the distribution of oviposition habitat (Lancaster & Downes, 2014a). My work has shown that habitat limitation can operate beyond cobbled, upland streams. In light of such evidence, freshwater ecologists must set aside the long held implicit assumption that factors acting on the larval stage have the most influence over population dynamics and capitalise on the progress made in other fields of ecology. The discovery of the importance of supply in determining dynamics of benthic marine invertebrates represented a leap forward in the understanding of those populations. The consideration of processes occurring during other life stages, like oviposition, may represent an equivalent turning point in freshwater ecology.

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