The ecological role of tadpoles in streams of the Australian Wet Tropics

Thesis submitted by

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for the degree of Doctor of Philosophy

in the College of Marine and Environmental Sciences

James Cook University

October 2015

Statement on the contribution of others

Intellectual input and editorial assistance were provided by my supervisors, Prof. Richard Pearson, Prof. Ross Alford and Dr Robert Puschendorf. Others who helped with editing of the thesis chapters include Faye Christidis, Ng Ee Ling, Betsy Roznik, Fiona McDui and Yui Sato. Field assistance during my project was provided by a large number of volunteers, who are listed in the acknowledgements section of this thesis. Other specific input for the thesis chapters was provided as follows:

Chapter 2: The tadpole survey data during 1989 to 1994 was generously provided by Stephen Richards. It was used in combination with my more recent survey data for this chapter. Water nutrient analyses were carried out by the Tropical Water and Aquatic Ecosystem Research Centre (TropWATER) laboratory at James Cook University.

Chapters 4 and 5: Miranda Starr helped to process samples in the laboratory. Nutrient analyses were carried out by Waite Analytical Services at the University of Adelaide and the CSIRO Adelaide.

Chapter 6: The stable isotope chapter was carried out in collaboration with Melanie Blanchette, who helped with field and lab work and provided intellectual input. Electrofishing was carried out by Aaron Davis, who also provided advice and support. The samples were analysed by the Stable Isotope Laboratory at the University of Hong Kong.

During my PhD I was supported by a Postgraduate Research Scholarship and a Research Tuition Scholarship, both from James Cook University. The study was funded by the Margaret Middleton Fund Award for endangered Australian native vertebrate animals from the Australian Academy of Science, a Marine and Tropical Science Research Facility grant (to Richard Pearson), an Internal Research Allocation (IRA) from the College of Marine and Environmental Sciences at James Cook University and a grant from the Graduate Research School at James Cook University.

Ethics approval and permits

The research in this thesis was conducted under animal ethics guidelines in compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th edition, 2007, and the Queensland Animal Care and Protection Act 2001. The research was approved by the Animal Ethics Committee at James Cook University under the approval number A1689. Scientific permits WITK09928211 and WISP09948311 for this research were issued by the Queensland Department of Environment and Resource Management.

Acknowledgements

First of all, I'd like to thank my supervisors, Richard Pearson, Ross Alford and Robert Puschendorf. They have been supportive and encouraging from the start and have enabled me to see this project through. Richard, thank you for your endless patience and guidance, and for your tireless input into every aspect of this project. I really appreciate your understanding for when things got tough and for allowing me to progress at my own pace. Ross, your input has been very valuable and I thank you for all your advice, especially towards the end of the project. Rob, thank you for always believing in me and for giving me support when I needed it. Also, thank you for going bush-bashing with me to find the artificial streams!

I want to thank the many volunteers who have helped me with field work under often difficult conditions. Being wet all day, fighting off marchflies, mosquitoes and leeches, and spending long hours at the stream sorting animals, regardless of extreme hot or cold weather and endless rain, was challenging to say the least. I loved all the creative names you gave the animals – names like "sticky butts" (Simuliidae), "platypus mayfly" (Ameletopsidae), "wiggly flies" (Plecoptera), "camel neck" (Neurorthidae) and "pretty bum" (Diphlebiidae) made sorting that much more enjoyable. A big thank you to all of you, especially those who came out with me regularly: Victoria Graham, Richard Duffy, Kiyomi Yasumiba, Alexander Foster, Julia Koburg, Elisabeth Kernan, Larry Webster, Anita Heim, Nadiah Roslan, Miranda Starr, Jana Köhne, Anna Kluibenschedl, Helios Martinez, Rosa Arribas, Kelsey Miller, Carly Lawson, Michael Lee, Karen Chong-Seng, Margaret Murray, Sasha Greenspan, Dominik Schmid, Sefano Katz, Craig Kelly, Julie Jourdan, Betsy Roznik, David Pike, Annie Portal, Mateusz Czarnota, Cody Wolfe, Christopher Heckathorn, Maeghan Gade, Rachel Heckathorn, Alexandra Ainscough, Manuela Giammusso, Madeleine Emms, Oda Almland, Scott Harte, Jamie Canepa, Yui Sato, Takuma Karaoka, Julie Goldsbury, Kristina McOmber, Allen Wootton, Alexandra Grand, Sebastian Hoepker, Yannick Hartmann, Tory Chase, Jaime Bosch, Swati Banerjee, Kahleana Stannard, Jacinta Tonkin, Jessica Gautherot and Katherine Schatz.

When I started this project I was completely new to stable isotope techniques and I am very grateful to Melanie Blanchette for assisting me with this part of the study. Thank you for explaining the basic concepts of the technique, for helping with sample collection and lab work, and for providing valuable input to the chapter. Also, thank you

to Aaron Davis for advice and for conducting electrofishing for the stable isotope work. I would also like to acknowledge Thomas Mannering and Megan Kent for giving me access to a muffle furnace when I needed it. Thank you for going out of your way to help me process my samples. Betsy Jakes helped with collection and identification of plants and enabled me to find the species I was looking for. Thank you!

Several people have assisted me with the statistical analyses. Thank you to Karen Chong-Seng, Richard Rowe, Scott Parsons, Deborah Bower and April Reside for your help and advice. Thank you to my editors, who often read several chapters and improved them tremendously. Faye Christidis, Ng Ee Ling, Betsy Roznik, Fiona McDui and Yui Sato – thank you! This project would not have been possible without the help of the technical and research staff at JCU, especially Janice Cran, Savita Francis and Sue Reilly. Also, thank you to Ross Barrett from the engineering workshop for preparing some of my field work equipment. Debbie Ford, thank you for massively reducing my anxiety towards the end of the PhD! Julie Fedorniak, you were always very helpful and incredibly efficient – you made organising field work so much easier. Also, thank you to you and Tony for rescuing me from Tully when I lost the car keys!

I received a lot of advice and support throughout this study and I am grateful for all the help I got during this long journey. Thank you to Scott Cashins, Andi Cairns, Melanie Blanchette, Betsy Roznik, Sara Bell, Megan Higgie, Conrad Hoskin, Sarah Sapsford and David Pike for sharing your knowledge and experience with me and for just having a chat when I needed company. It's been great to have someone to turn to. Also, thank you to Lin Schwarzkopf for encouraging me to do a PhD and for allowing me to be part of the lizard lunch group. Carmen Panayi, thank you for your company during my many trips to Paluma. I always looked forward to spending time with you up there, especially after long days of field work. You made the house so much more "home".

A special thank you to Karen Chong-Seng and Thomas Mannering for being such great friends and for all the fun and wonderful times we spent together in Townsville. Kiyomi Yasumiba, it has been great to share the ups and downs of the PhD with you, especially towards the end when we were both writing up. And Fiona McDui, I enjoyed spending time together during our writing retreat – thank you for the much-needed laughs, movie nights and hikes! Thank you also to Noriko Iwai, who has supported me from afar throughout this time. Your friendship means so much to me. I would also like to thank Rebecca Shanks and Dr. Evelyn Lamari for all the times you

listened to me and for helping me when I was down. Thank you to Margit Cianelli, who opened her home to me during my frequent trips to the Tablelands. It's been great to relax and work at your beautiful place with so many animals around, especially my favourite tree kangaroo, Kimberley. Thank you for all the cuddles, Kimberley, and for keeping me company while I was sitting in front of the computer for hours on end.

Lastly, I want to thank my family. Yui, you have been there for me every step of the way, providing me with love and support throughout this journey. Thank you for everything! And thank you to my parents Elisabeth and Raju, who have supported my dreams and ambitions from the very beginning and who have always had faith in me. You are the best! Also, thank you to my brothers Matthias and Christian, for just being you.

Abstract

Stream-breeding amphibians are an important link between terrestrial and aquatic habitats, and frogs and tadpoles may be important contributors to ecosystem functioning in their habitats. Amphibian declines due to fungal disease have affected many frog assemblages, especially in upland rainforest sites, leading to reduced abundance and diversities of stream-dwelling tadpoles. This raises the question of what role tadpoles play in streams and what the impact of their declines might be. In the Australian Wet Tropics bioregion, some frog species have declined or disappeared, but others have persisted, and tadpoles can still be seasonally abundant, possibly having important effects on stream ecosystems.

The aim of this study was to investigate the role of tadpoles in the ecology of Wet Tropics streams using inferences from natural populations and manipulative experiments. Monthly surveys of streams near Paluma and Tully showed that tadpole and invertebrate assemblages fluctuated seasonally, with abundances highest in spring and summer, reflecting the main reproductive period. Variability of flow was the most important environmental influence on the animals in both locations, explaining up to 25% of the variation in the tadpole assemblages and up to 40% in the invertebrate assemblages. There were no indications of major interactions between tadpoles and invertebrates.

The role of tadpoles in stream processes, including leaf litter breakdown and sediment removal, was tested using field experiments in artificial stream channels. *Litoria serrata* tadpoles and invertebrates interacted during leaf processing, most likely through direct physical activity by invertebrates that facilitated tadpole feeding, but there was no evidence of effects of nutrient regeneration by tadpoles. *Mixophyes coggeri* tadpoles did not contribute to leaf processing, but they were important in sediment removal, which benefits smaller consumers. Stable isotope analysis was used to determine the trophic position of tadpoles and their place in the food web in Paluma and Tully streams. Tadpoles assimilated mainly biofilm and algae, but they were generalist feeders that used different food sources depending on nutrient quality and availability. The food web structure was simpler in the Paluma riffles, where tadpoles of two species disappeared in the early 1990s, suggesting that tadpoles increase food web complexity.

The study showed that the loss of tadpoles from a stream, or from a particular habitat within a stream, affects overall benthic assemblage composition, food web structure and stream ecosystem functioning. Tadpoles of different species can have different functional roles and it is therefore important to consider the identity of species lost, as well as the effects of reduced diversity, during amphibian declines resulting from disease, climate change or land-use changes. Although tadpoles are only abundant during part of the year, their peak occurs in the spring-summer period when invertebrates are also most abundant, so their influence on the ecosystem is likely to be substantial and may well carry over to the cooler season.

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1. General Introduction

1.1 Overview

Frogs and tadpoles are important in the transfer of energy within and between terrestrial and aquatic habitats (Whiles et al. 2006). Amphibians can efficiently use the nutrients derived from food for growth and reproduction; they require only minimal energy for maintenance and convert the remaining energy into new tissue, which then becomes available to predators (Dodd 2010). The role of tadpoles in community processes and ecosystem functioning in aquatic systems is not yet well understood, although there have been recent advances identifying the importance of tadpoles in Central American streams (e.g., Whiles et al. 2006; Barnum et al. 2013; Connelly et al. 2014; Rantala et al. 2014). However, it is known that tadpoles are important consumers in aquatic ecosystems, and they may influence the structure and function of these systems through their feeding activities and interactions with other organisms (Whiles et al. 2006). This influence may differ among species, depending on their functional roles and abundances in specific habitats.

1.1.1 Tadpole ecology

Tadpoles occur in many freshwater systems, including ephemeral pools, permanent ponds, lakes, rivers and streams (Alford 1999). These systems differ in a range of biophysical properties, such as plant growth, organic matter accumulation, turbidity, substratum composition and flow regime; these properties can also differ among habitats within each system (Boulton and Brock 1999). Therefore, community composition may change from one site to the next, depending on the requirements of individual species. The tadpole assemblage can also vary with season, depending on the temporal breeding patterns of the various frog species. Many species breed primarily during one season, and the influx of tadpoles occurs during that time (Cashins 2009). However, in areas with a constant climate throughout the year, reproduction and tadpole abundances may not change seasonally (Inger 1969). Some species have more than one breeding period within a season, so various tadpole size classes may co-exist (Alford 1999).

Tadpoles are mainly focussed on feeding to fuel growth and development (Altig and Johnston 1989). The feeding modes of tadpoles differ depending on their morphological characteristics and habitat (Hoff et al. 1999). They typically obtain food by filtering fine particles and algae from the water column, collecting accumulated organic matter on the substratum, or scraping biofilm and other material from submerged surfaces (Hoff et al. 1999). However, tadpoles are opportunistic feeders and they may also ingest animals, including tadpoles and eggs of conspecifics or other species (Alford 1999).

Tadpoles of different species have specific morphological characteristics that represent adaptations to their habitat. Some species are adapted to living in fast-flowing waters and have suctorial mouthparts with which they attach to rocks, as well as large tail muscles and low fins to withstand high current velocities (Altig and Johnston 1989). Other species live and forage in still water and usually have small mouthparts and less muscular tails (Altig and Johnston 1989). Riffle specialists use their sucker-like mouthparts to graze on epilithic biofilm, whereas pool tadpoles spend more time in the water column and consume suspended or accumulated organic material (Hoskin and Hero 2008). The variability in mouthpart structures also influences the size of the food particles tadpoles can consume (Hoff et al. 1999).

Predation and competition affect the survival and growth of tadpoles, and therefore the structure of amphibian populations (Gonzalez et al. 2011). Larval amphibians, as well as adults, are important prey in aquatic systems (Tyler 1976; Ranvestel et al. 2004), and predators such as fishes and dragonfly larvae have a major influence on the presence of tadpoles, either through direct predation or as a result of their influence on breeding site choice by adults (Heyer et al. 1975; Eterovick and Barata 2006). The behaviour and appearance of tadpoles can influence the success of predators in detecting and catching them; for example, large tadpoles are more likely to escape predation than smaller ones (Richards and Bull 1990). Tadpoles can prey on individuals of their own or other species, depending on species and relative sizes of individuals (Heyer et al. 1975; Alford 1999). Such predation may be common in temporary waterbodies due to limited and declining space, and competition for food (Hoff et al. 1999).

Competition among tadpoles and with other organisms for space and food is probably greatest when densities are high. Interactions of tadpoles with invertebrates may depend on particular species' traits and their abundances, as well as resource use

and availability. It is likely that in some situations tadpoles compete with freshwater invertebrates, many of which rely on the same resources as tadpoles (Alford 1999; Kiffney and Richardson 2001), especially grazers that feed on epilithic biofilm, and fine-particle gatherers feeding on accumulated detritus (Cummins and Klug 1979). Species interactions between tadpoles and invertebrates may also be positive: for example, facilitation occurs when one or both species benefit from an interaction and there are no negative consequences for either (Stachowicz 2001). In freshwater systems, facilitation may occur between different invertebrate feeding groups, or between invertebrates and other organisms, such as tadpoles (Iwai et al. 2009; Rugenski et al. 2012).

1.1.2 Amphibian declines

Amphibians worldwide have recently been experiencing population declines, and approximately one third of the world's anurans are threatened or extinct (IUCN Red List 2015). Habitat loss, alteration and fragmentation as a result of human activities are very concerning (Gallant et al. 2007). The fungal disease chytridiomycosis, caused by the pathogen *Batrachochytrium dendrobatidis* (*Bd*), has been identified as another major cause of declines (Berger et al. 1998; Skerratt et al. 2007; Crawford et al. 2010). Some regions have been more affected by chytridiomycosis than others: for example, disease-driven declines are prevalent in Mesoamerica and Australia, whereas habitat-loss related declines are more common in South-East Asia (Stuart et al. 2004). Stream-associated amphibians from high-elevation rainforest sites have been most affected by chytridiomycosis (Stuart et al. 2004). In the Australian Wet Tropics biogeographic region (hereafter, the "Wet Tropics"), many endemic rainforest frogs declined or disappeared in the late 1980s to early 1990s, particularly stream-breeding frogs from upland areas, with chitridiomycosis as the putative cause (Richards et al. 1993; McDonald and Alford 1999). Of the sixteen frog species that breed in rainforest streams in the Queensland Wet Tropics (QWT), half have been listed as endangered or critically endangered (Hoskin and Hero 2008) and two species have disappeared from Paluma, where this study was conducted (Richards et al. 1993).

Amphibian populations respond differently to the disease, and while some have been eliminated, others have survived unchanged, or have recovered and persist despite the continuing presence of the fungus (Woodhams and Alford 2005). Cool and moist

conditions appear to favour fungal growth (Johnson et al. 2003; Stevenson et al. 2013), and species distributed along elevational gradients can be more resistant to or tolerant of infection at lower elevations (McDonald and Alford 1999). Others seem to coexist at high densities in high-elevation dry forests that are peripheral to rainforests, where they are commonly infected by *Bd* but do not seem to develop the disease, due to exposure to warmer and drier microclimates (Puschendorf et al. 2011). The behaviour of frogs also affects their vulnerability to the disease and transmission rates (Rowley and Alford 2007). Physical contact between individuals and contact with infected water or substrata increases the likelihood of transmission, and solitary species that spend substantial time away from the water appear to be less affected by *Bd* (Rowley and Alford 2007). Individuals of several species of Wet Tropics frogs that maintain higher body temperatures are less likely to carry *Bd* infections (Rowley and Alford 2013), and some species may increase their body temperature through thermoregulation, making them less vulnerable to *Bd* infections (Richards-Zawacki 2009).

Tadpoles can be infected by *Bd*, in some cases leading to deterioration of their mouthparts, which reduces foraging efficiency and limits growth rates (Blaustein et al. 2005). Tadpoles do not usually develop the disease or seem to suffer significant mortality due to *Bd*, but they act as a reservoir for the pathogen (Woodhams and Alford 2005). Infected tadpoles of several species seem to have some tolerance to the infection, and can regrow their mouthparts, feed and metamorphose successfully (Cashins 2009). However, the impact of chytridiomycosis on the abundance of tadpoles in a system depends on how well the adult population copes with it, and is species-dependent. In the Wet Tropics, for example, *Litoria nannotis* are more vulnerable than *L. serrata*, possibly because of behavioural differences (Rowley and Alford 2007). This affects rates of recruitment into tadpole assemblages because it influences rates of breeding by adults. *Bd* can affect male calling effort, which could alter rates of frog reproduction and therefore population dynamics and tadpole abundance (Roznik et al. 2015). In streams where frogs have recovered or continue to persist, tadpoles can still be very abundant, but elsewhere there have been significant losses of frog species and their tadpoles.

1.1.3 Stream-dwelling tadpoles

Tadpoles in streams are exposed to a wide range of habitat types with different flow conditions, substratum compositions and food sources. Many species have specific adaptations and are therefore confined to certain sections of a stream (Allan and Castillo 2007; Dudgeon 2008). They can use isolated pools, connected pools, runs, riffles or fast-flowing torrents, depending on their ability to withstand high current velocities (Richards 2002). The distribution and abundance of tadpoles in streams may also depend on the availability of suitable breeding conditions for the adults (Gillespie et al. 2004), presence of predators such as fishes (Eterovick and Barata 2006) and competition between species (Alford 1999). Stream ecosystems in different areas may have unique assemblage compositions, determined by the species pool, flow patterns, in-stream habitats, available resources, climate and environmental disturbances (Allan and Castillo 2007; Pearson et al. 2015).

While seasonality is most obvious in high latitudes, and may be absent in the equatorial tropics (Yule and Pearson 1996), seasonal tropical ecosystems have distinct wet and dry seasons that influence the abundance, growth and reproduction of organisms in streams (Flecker and Feifarek 1994). Seasonal tropical streams are influenced by rainfall patterns and by natural disturbances, which can lead to changes in flow rate and substratum composition over short periods of time (Flecker and Feifarek 1994; Pohlman et al. 2008). In the Wet Tropics, for example, adults of *Litoria serrata* at Birthday Creek, Paluma, were most abundant during spring and summer, and were often absent along the stream during the winter months (Richards and Alford 2005). This temporal distribution of frogs influences the timing of reproduction and therefore the presence and development stages of tadpoles present in streams (Flecker and Feifarek 1994).

The amount of time that tadpoles of stream-breeding frogs spend in the stream ranges from a few months to more than a year, depending on the adult breeding period and climate (Cashins 2009). Therefore, the cohorts from different breeding peaks may overlap, resulting in tadpoles of various size-classes co-existing in the stream (Alford 1999). These tadpole stages may have different food and habitat requirements and may therefore occupy different microhabitats within the stream (Werner and Gilliam 1984). Size can also affect the interaction between tadpoles and other aquatic organisms, as predation risk and resource use change (Werner and Gilliam 1984).

The community structure of a system is determined by the species that make up an assemblage, their functional roles and trophic interactions (Allan and Castillo 2007). Different species have different traits and behaviours, and therefore different functional roles in a stream, which collectively and interactively contribute to ecosystem functioning (Allan and Castillo 2007). Community structure differs between pools and riffles in a stream (Brown and Brussock 1991; Cheshire et al. 2005), and therefore interactions between tadpoles and invertebrates involve a suit of different species depending on the habitat. With the recent worldwide decline in stream-breeding frogs and their tadpoles, organisms sharing the same habitats may be affected, which may greatly impact community structure in these systems (Colon-Gaud et al. 2010a).

1.2 The role of tadpoles in streams

Studies in Neotropical streams have found variable effects of tadpoles on community composition, food web structure and stream functioning before and after amphibian declines (Ranvestel et al. 2004; Whiles et al. 2006; Colon-Gaud et al. 2009). However, in most systems the effect of tadpole loss cannot be directly measured using direct comparisons of pre- and post-decline data because pre-decline data on tadpole assemblages and ecosystem variables are not available. Experimental approaches have therefore been useful in studying the role of tadpoles in ecosystems (e.g., Lamberti et al. 1992; Kiffney and Richardson 2001; Connelly et al. 2008). Such experiments are important in determining the contribution of tadpoles to various stream processes, their interactions with other organisms, and the potential effects of tadpole loss on particular systems. They also provide insight into the mechanisms causing the ecosystem shifts that have been observed in streams after amphibian declines.

1.2.1 Stream food webs and nutrient cycling

Food webs combine the links between basal sources, producers and consumers within an ecosystem (Allan and Castillo 2007). Each stream has a unique food web structure, which is influenced by external factors that affect in-stream habitat and flow patterns, as well as by the sources of nutrients (Allan and Castillo 2007). Species richness, interactions between organisms, and feeding mechanisms at different life stages also influence energy pathways (Pimm and Rice 1987; Polis and Strong 1996).

Although the structure and complexity of food webs vary depending on the resources and organisms in the system, the flow of energy is often through a few specific pathways and is largely controlled by a few key species (Allan and Castillo 2007).

In forest streams, allochthonous leaf litter from the surrounding riparian vegetation is a significant source of organic matter (Cummins 1973; Pearson et al. 1989; Wallace and Webster 1996). These streams are typically shaded and there is little autochthonous energy input from phytoplankton or macrophytes (Anderson and Sedell 1979; Yule and Yong 2004). Nutrient input therefore comes mainly from heterotrophic sources (Boulton and Brock 1999; Graça 2001), and nutrients are cycled within the system through the transformation, consumption and egestion of leaf litter by stream organisms (Boulton and Brock 1999). Freshwater invertebrates play an important role in the cycling of nutrients and energy transfer from allochthonous leaf litter in stream systems (Cummins 1973). Leaves are first colonised by microorganisms, which partially degrade the plant material, making it more nutritious for invertebrates (Graça 2001). Shredding invertebrates, which process coarse particulate organic matter, further break down the conditioned leaves into fine material that then becomes available for other organisms (Cummins 1973; Cummins et al. 1973). Microbial and invertebrate activities, as well as physical factors such as abrasion, are therefore responsible for the breakdown of leaves and the release of nutrients into the stream (Graça 2001). Tadpoles may not be able to directly feed on the coarse organic material due to their jaw structure and fine teeth, but may indirectly contribute to leaf processing through their interactions with other stream organisms (Iwai et al. 2009).

Tadpoles are important primary consumers in stream systems due to their high abundances and broad resource use (Alford 1999). They feed on algae, sediments, detritus or other animals (Flecker et al. 1999; Ranvestel et al. 2004; Whiles et al. 2006), but their feeding ecology and trophic status are still poorly understood (Altig et al. 2007). Little is known about assimilation and nutritional requirements in tadpole feeding, including their main sources of energy and nutrients (Altig et al. 2007). For example, tadpoles thought to feed on detritus may not actually be obtaining their required nutrients from the plant material itself, but from the microbes attached to it (Hunte-Brown 2006; Altig et al. 2007). To understand the role of tadpoles in stream systems, it is essential to determine their position in the stream food web and their contribution to nutrient cycling within the system.

1.2.2 Tadpole-invertebrate interactions

Tadpoles are omnivores or detritivores with broad diets, which probably overlap with those of other aquatic consumers (Alford 1999). Tadpoles may compete with each other (Flecker et al. 1999; Kim and Richardson 2000) or with other organisms, especially with macroinvertebrates, which are abundant consumers in streams (Colon-Gaud et al. 2009). Freshwater invertebrates may also interact with tadpoles through predation or facilitation (Richards and Bull 1990; Kiffney and Richardson 2001; Ranvestel et al. 2004; Iwai et al. 2009). The nature of interactions between tadpoles and invertebrates depends on the species involved and their traits, behaviours and abundances, as well as on resource availability. In seasonal environments, both tadpole and invertebrate abundances fluctuate over time, and interactions may only be important when animal abundances are high. Space and resources become limited when habitat availability decreases and animal densities increase, leading to greater competition within and among species.

Facilitation between tadpoles and invertebrates is a positive interaction for one or both participants and may enhance stream processes. Bioturbation and nutrient regeneration by tadpoles are examples of tadpole activities that may benefit invertebrates. Bioturbation occurs when tadpoles stir up the substratum during feeding, exposing underlying food material (Ranvestel et al. 2004). Small grazers are able to access these resources, thereby benefiting from the tadpole activity (Ranvestel et al. 2004). In Panamanian headwater streams, some insect grazers declined following tadpole loss, indicating that tadpoles probably facilitated their feeding (Colon-Gaud et al. 2010a).

Nutrient regeneration by tadpoles may benefit microorganisms directly by providing them with extra nutrients, and this may promote shredder activity (Iwai et al. 2012; Rugenski et al. 2012). Nutrient regeneration occurs when tadpoles convert organic material to inorganic nutrients through their feeding activities and release these nutrients into the system in their excreta. The extra nutrients lead to increased microbial activity, which stimulates greater nutrient release from leaves during conditioning (Iwai and Kagaya 2007; Iwai et al. 2012). This results in a lower C: N ratio of the conditioned leaves, making them more nutritious for shredders (Iwai and Kagaya 2007). Therefore, tadpoles may indirectly enhance shredder activity during leaf breakdown.

Nutrient regeneration by tadpoles also encourages biofilm or algal growth, and results in increased biomass of these resources (Iwai and Kagaya 2007). Stream organisms may benefit from such an increase in food availability, especially grazing invertebrates. However, in a pond experiment, Iwai et al. (2012) found that the tadpoles themselves consumed the extra biofilm, thereby benefiting from their own nutrient regeneration and not providing other organisms with a surplus resource. This may be a common occurrence, so nutrient regeneration by tadpoles may not always lead to facilitation of invertebrates. However, this has not been tested in a stream system.

Invertebrates may also facilitate tadpoles. This can happen as a result of direct physical activities during leaf processing. Shredders break down coarse particulate organic matter during their feeding activities, making smaller particles available for other consumers (Cummins and Klug 1979). Tadpoles may then be able to feed on these particles, thereby benefiting from the shredder activity (Iwai et al. 2009). Facilitation between tadpoles and invertebrates, in one or both directions, leads to faster leaf litter breakdown than would be expected from the combined effects of the animals' individual activities (Iwai et al. 2009).

1.2.3 Functional redundancy

Ecosystem functioning includes the transport of materials such as water and nutrients, and the flow of energy within the system (Naeem 1998). It is directly influenced by the specific functional roles of species in a community (Giller et al. 2004). Functional redundancy occurs when species have overlapping roles, in which case one species may fulfil the role of another and compensate for its loss (Allan and Castillo 2007). However, when one species is lost, processes to which it contributes may become less efficient (Allan and Castillo 2007). Species are often grouped into functional groups, but individual species within a functional group may not necessarily play exactly the same role and therefore may not be functionally redundant (Vaughn 2010). For example, different invertebrate species within a particular feeding group may not have the same ecological role in a stream system (Boyero et al. 2006; Vaughn 2010).

Amphibian diversity in the Neotropics is high and prior to amphibian declines streams usually contained tadpoles of a suite of species (Whiles et al. 2013). These species have distinct feeding modes (Rugenski et al. 2012), and are therefore likely to

have specific functional roles. High species richness may safeguard the system if species are lost, thereby maintaining ecosystem function, as it is more likely for such a system to contain other species which are similar to those lost (Allan and Castillo 2007). However, a species may be invaluable to a system if it is extremely abundant, plays a key role in the transfer of energy, or strongly affects the activities of other species, in which case its function cannot be compensated for by high biodiversity (Allan and Castillo 2007). Whiles et al. (2013) found that despite high amphibian diversity in Neotropical streams, there were no signs of functional redundancy in the amphibian assemblage in a stream where 98% of the total tadpole biomass of more than 18 species was lost.

Stream ecosystem function may shift as a result of tadpole declines, depending on the functional roles of the remaining organisms. Invertebrates may or may not take over the role of tadpoles in streams. If invertebrate grazers are able to compensate for tadpole activity in the stream, the shift could simplify the food web structure. Barnum et al. (2013) measured functional redundancies between invertebrates and tadpoles in streams by comparing the isotopic niches of invertebrates after amphibian declines to those of tadpoles before the declines. They found that invertebrates had not taken over the isotopic niche of tadpoles two years after tadpoles disappeared, indicating that the ecological roles of tadpoles had not been compensated for. In another study, however, grazers in a Neotropical stream maintained resource availability two years after tadpole populations declined (Colon-Gaud et al. 2010a). Functional redundancy in a stream may thus depend on the roles of individual species, and while invertebrate grazers, for example, may be able to compensate for the functional role of tadpoles of one species, they may not take over the function of another species. The role of a species may also depend on environmental factors, and some species may perform a particular function only under certain environmental conditions (Wellnitz and Poff 2001). Therefore, even if functionally redundant species are present, they may not be able to perform under certain conditions.

1.3 Study aim and objectives

This study aimed to assess the role of tadpoles in stream functioning by investigating tadpole population and assemblage dynamics and tadpole-mediated processes, including tadpole-invertebrate interactions and the contributions of tadpoles to trophic processes. This was achieved using stream surveys, manipulative experiments and stable isotope analyses of tadpoles and other stream organisms in the Wet Tropics. Tadpole assemblages were investigated in four streams, two of which were in the uplands at Paluma Range National Park (~800m ASL) and two in the lowlands at Tully Gorge National Park (~150m ASL). These two locations contained different suites of species. The experiments were conducted in streamside artificial channels at Birthday Creek near the village of Paluma. The combination of these methods was used to answer specific questions about tadpole ecology and their contribution to stream functioning, as follows.

- **Chapter 2. What is the composition of tadpole assemblages in QWT streams and how do they vary in abundance spatially and temporally?**

In this chapter I determine tadpole habitat preferences, frog breeding patterns and the influence of environmental variables on tadpole populations. Data from sampling undertaken during amphibian declines in combination with new data in two of the streams allowed for analysis of long-term patterns of assemblage composition and abundance. The results, along with results from other chapters, allowed for estimation of the magnitude and duration of tadpole influence on stream functioning.

- **Chapter 3. Do tadpole and invertebrate assemblages in Wet Tropics streams show similar abundance patterns?**

I determined whether invertebrates were influenced by the same environmental variables as tadpoles, and whether abundance patterns were likely to be the result of tadpole-invertebrate interactions or of similar responses to the environment. This chapter also aimed to provide information on possible functional redundancy between tadpoles and invertebrate feeding groups.

- **Chapter 4. How do tadpoles influence basic stream processes?**

I investigate the contribution of tadpoles to leaf litter breakdown, sediment removal and biofilm growth, with or without invertebrates, and the importance of tadpole-invertebrate relationships for the maintenance of stream functioning. I also determine whether facilitation readily occurs among the species.

- **Chapter 5. Is nutrient regeneration by tadpoles important in stream systems?**

I determine whether tadpole nutrient regeneration facilitates invertebrate leaf litter processing, and whether nutrient regeneration by tadpoles leads to increased biofilm growth and provides tadpoles or invertebrates with an extra food resource.

- **Chapter 6. What is the trophic status of tadpoles in the stream food web?**

I identify the main food sources for tadpoles and determine whether they are generalist or specialist feeders in the stream. I also investigate how tadpoles and invertebrates are linked in the stream ecosystem and whether there is potential for functional redundancy.

- **Chapter 7. Synthesis – what is the role of tadpoles in Wet Tropics streams?**

I summarise the results of the data chapters and address the question of the role of tadpoles in streams of the Wet Tropics.

2. Tadpole population dynamics

2.1 Introduction

The contribution of organisms to ecosystem functioning depends on their temporal and spatial distribution, abundance, and functional role within the system. Understanding these factors in rainforest stream tadpoles was therefore important in the present study.

Tadpole distributions are influenced by the physical environment, habitat and resource availability, the presence of other aquatic organisms such as predators, and by suitable breeding sites for adult anurans (Inger et al. 1986; Eterovick and Sazima 2000; Gillespie et al. 2004; de Oliveira and Eterovick 2010). In streams, flow and substratum composition are important in determining tadpole spatial distributions (Richards 2002; Allan and Castillo 2007). Stream-dwelling tadpoles may use isolated or connected pools, runs, riffles or fast-flowing torrents, depending on their ability to withstand high current velocities and on resource availability at the various sites (Inger et al. 1986; Richards 2002). This may lead to habitat partitioning, which occurs when different species occupy different microhabitats within a stream (Inger et al. 1986).

The morphology of tadpoles varies among species and reflects the physical characteristics of the environment and the feeding mechanism of the tadpoles (Candioti 2007; Cashins 2009). Tadpole morphology largely depends on the animals' adaptations to flow conditions. For example, species adapted to fast currents often have large tail muscles, low fins and suctorial mouthparts with which they cling to rocks (Altig and Johnston 1989; Hoskin and Hero 2008), enabling them to resist and minimise exposure to high current velocities (Richards 2002). Species without these characteristics live and forage in slow-flowing water, where finer particles and organic matter can accumulate (Boulton and Brock 1999). These tadpoles rely on the interstitial spaces of the streambed for cover and foraging (Welsh and Ollivier 1998).

Habitat selection within a stream may also vary with the developmental stage and size of tadpoles. Frogs often have several breeding periods within a season, and several cohorts of different size classes may therefore co-exist in a system (Alford and Crump 1982). Food and habitat of animals change during growth, as body size affects the ability to use resources (Werner and Gilliam 1984). For example, larger, more developed larvae are capable of inhabiting faster sections of a stream (Wahbe and

Bunnell 2003). Size also influences an animal's interaction, such as predation, with other species sharing the same habitat (Werner and Gilliam 1984). Tadpoles of different sizes within a species may also compete with each other, causing larger individuals to displace smaller ones from suitable habitat (Alford and Crump 1982).

The roles tadpoles play in stream functioning and their interactions with other organisms may only be important for certain parts of the year. This is because tadpole abundances vary according to the seasonal breeding patterns of adult frogs and the length of time the larvae spend in the stream during development. Tadpoles may contribute to stream functioning through their feeding activities and their interactions with other organisms. For example, tadpoles can remove sediment from the substratum through bioturbation, which leads to physical changes in their environment (Ranvestel et al. 2004). Tadpoles also influence the activities of other stream organisms, and they interact with invertebrates during leaf processing (Iwai et al. 2009). These effects are likely to be greater when tadpole densities are high.

The temporal distribution of adults, which influences the timing of reproduction and the presence and developmental stages of tadpoles in streams, is determined by climate and weather (Flecker and Feifarek 1994; Gillespie et al. 2004; Richards and Alford 2005). Streams are highly variable systems, influenced by seasonal or episodic rainfall, which can change the flow conditions and substratum composition over short periods of time (Flecker and Feifarek 1994; Pohlman et al. 2008). The Australian tropics are seasonal in temperature and rainfall, with distinct wet and dry seasons, which influence the breeding patterns of adult frogs. In the Wet Tropics, breeding occurs mainly in the warm, wet spring and summer months, although adult frogs and tadpoles of various developmental stages may be present throughout the year (Richards and Alford 2005; Cashins 2009; Sapsford et al. 2013).

Many stream-breeding rainforest frogs in the Wet Tropics declined or disappeared in the late 1980s to early 1990s (Richards et al. 1993; McDonald and Alford 1999). These declines have been linked to the fungal disease chytridiomycosis, caused by the pathogen *Batrachochytrium dendrobatidis* (Berger et al. 1998; Skerratt et al. 2007; Crawford et al. 2010). The resulting loss of tadpoles may cause changes to the stream system by simplifying the food web structure, changing autochthonous production and affecting nutrient and energy transport and cycling in the stream (Colon-Gaud et al. 2010a). However, where frog populations have persisted or recovered, tadpoles may still be abundant and therefore contribute to stream functioning.

This study surveyed tadpole populations in a number of Wet Tropics streams, using new data from surveys carried out in the Paluma Range and Tully Gorge National Parks between 2011 and 2013, and data from surveys at Paluma between 1989 and 1994 by S. Richards (pers. comm.), when large-scale amphibian declines occurred in the Wet Tropics region. No other pre-decline data on tadpole assemblages are available for the streams surveyed or for any other streams in the region. The aim of this study was to determine temporal and spatial patterns of tadpole abundance, tadpole habitat use and frog breeding patterns in these streams. This information was used to generate hypotheses regarding the extent and timing of tadpole influence in the stream ecosystem. The combined records allowed analysis of long-term patterns of assemblage composition and abundance, including periods during and after the decline in frog populations. The results were interpreted to highlight which species at Paluma were most affected by the amphibian declines and to show whether there were signs of recovery.

2.2 Methods

2.2.1 Sampling sites

For the first survey period (1989-94), sampling was undertaken in Birthday Creek (-18.98°, 146.17°, 795 m elevation) in the Paluma Range National Park (Figure 2.1); for the second survey period (2011-13), samples were collected at the same site in Birthday Creek and in Camp Creek (also referred to as Little Birthday Creek, -18.97°, 146.17°, 766 m) at Paluma, and in two unnamed streams flowing into the Tully River (Stream 1, -17.77°, 145.65°, 102 m, and Stream 2, - 17.75°, 145.61°, 237 m) in the Tully Gorge National Park (Figure 2.1).

The climate of the Queensland Wet Tropics is seasonal, with a distinct wet season during the warm summer months (November-March). Heavy monsoon rains, often associated with cyclonic activity, cause spates in the streams during this time. When flow was normal, three major habitats could be recognised in the streams: (1) pools, usually up to 1.0 m deep (within the sampling transect) with negligible water movement at the surface (< 0.05 ms^{-1}); (2) runs, less than 0.8 m deep with non-turbulent water movement (0.05 - 0.2 ms^{-1}); and (3) riffles, usually shallow with swift turbulent flow over a rocky substratum (> 0.2 ms^{-1}). The distinctions between these categories

became less evident following heavy rain, when flow throughout the stream became fast and turbulent.

Figure 2.1. Location of streams in the Australian Wet Tropics (Google Earth image 2015).

The Paluma and Tully streams differed in terms of gradient, substratum composition, flow, depth, and the surrounding topography and vegetation (Table 2.1). Between 2011 and 2013, the streams at Paluma were generally shaded and slow-flowing (except when there was heavy rainfall), with shallow riffles and runs, and some deep pools (Figure 2.2). The streams at Tully Gorge were more open due to damage caused by Tropical Cyclone Yasi in February 2011. They were steeper than the Paluma streams, and were generally fast-flowing with riffles, cascades, waterfalls, runs and deep pools, and had a high proportion of large boulders (Figure 2.3). All streams flowed through simple notophyll vine forest, characteristic of Wet Tropics upland rainforests (Tracey 1982). Maximum litter fall occurs in spring, but leaves continue to fall throughout the year in both locations and accumulate, together with other organic material, in pools and slow-flowing stream sections (Benson and Pearson 1993). There was greater leaf accumulation in the Paluma streams than in the Tully streams as a result of the higher flows at Tully. Thick algal mats were found in some stream pools at

Tully, particularly during periods of low flow and water level at sites with low canopy cover.

Table 2.1. Stream characteristics within sampling reaches at Paluma and Tully, 2011-2013. Three pools and three riffles were sampled in each of the Paluma and Tully streams. Canopy cover, leaf cover, algal cover and substratum composition were visually estimated at each sampling site. The substratum size distribution is presented as proportions (%) of sand/gravel, cobbles and boulders, with percentages averaged across riffles or pools.

Stream characteristics	Measurement	Paluma 1		Paluma 2		Tully 1		Tully 2	
		Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle
Canopy cover (%)	Minimum	0-25	0-25	0-25	0-25	0-25	0-25	0-25	0-25
	Median	25-50	25-50	75-100	25-50	0-25	0-25	25-50	0-25
	Maximum	75-100	75-100	75-100	75-100	75-100	75-100	75-100	75-100
Leaf cover (%)	Minimum	0-25	0-25	0-25	0-25	0-25	0-25	0-25	0-25
	Median	50-75	0-25	50-75	0-25	0-25	0-25	0-25	0-25
	Maximum	75-100	0-25	75-100	75-100	50-75	0-25	25-50	0-25
Algal cover (%)	Minimum	0-25	0-25	0-25	0-25	0-25	0-25	0-25	0-25
	Median	0-25	0-25	0-25	0-25	0-25	0-25	0-25	0-25
	Maximum	0-25	50-75	0-25	0-25	75-100	0-25	75-100	0-25
Substratum composition Sand/gravel: cobbles: boulders (%)	Mean	76:14:10	28:54:18	52:38:10	24:68:8	16:22:62	10:28:62	16:34:50	10:20:70
Stream width (m)	Mean	7.2		4.1		6.6		4.2	
	Min-maximum	3.9-10.6		2.6-7.3		3.2-9.1		1.8-7.2	
Stream depth (cm)	Mean	20		24		28		23	
	Min-maximum	5-85		5-70		5-60		5-45	

a b

Figure 2.2. Paluma Range National Park: (a) Birthday Creek and (b) Camp Creek.

a. b. c.

Figure 2.3. Tully Gorge National Park: (a) Stream 1 – dry season, (b) Stream 1 – wet season, and (c) Stream 2.

2.2.2 Survey methods

During 1989-94, two pools, two runs and three riffles were sampled along a 310-m transect in Birthday Creek. Each adjacent pair of sites was separated by a stretch of non-sampled stream of a different habitat type. In 2011-13, three riffles and three pools were sampled along a 150-m transect in each of the four streams, except that during the first two months of sampling at Tully Gorge, two pools and two riffles were sampled in each stream. In September 2013, Tully Stream 2 was not sampled and for this month, total tadpole abundance and biomass for both streams were estimated from Stream 1 data. Runs were not sampled in 2011-2013 as they were difficult to categorise, especially after heavy rain, and Trenerry (1988) reported that tadpoles were not commonly found in runs in Birthday Creek. Where possible, deep pools were sampled, but where these were not available, shallow pools were sampled instead. The 2011-13 Birthday Creek transect was a 150-m subsection of the 1989-94 transect.

The 1989-94 surveys at Paluma were carried out at approximately fortnightly intervals between March 1989 and May 1991, monthly intervals between May 1991 and August 1992, and every two to three months between August 1992 and February 1994. During 2011-2013, samples were collected every four to six weeks at Tully and Paluma; sampling commenced in October 2011 at Tully, but at Paluma it was delayed until February 2012 because cyclone damage prevented access to the sites. In pools, three main microhabitats were recognised: leaf packs, sand patches and rocky substratum. At Tully, pool substratum consisted mainly of boulders and cobbles, with small patches of gravel and sand; leaf packs were sparse and accumulated only in the drier months when the water level was shallower. At Paluma, a large proportion of the pool substratum consisted of cobbles, gravel and sand, with few boulders, and there was substantial leaf litter accumulation. Runs and riffles had predominantly rocky substrata in all the streams.

Sampling techniques were standardised within each habitat, but differed among habitats because of differences in current velocity, substratum composition and tadpole behaviour. All sampling measured relative rather than absolute abundance. Relative abundance sampling is a well-established technique, useful for comparing both across time and across sites as long as the comparisons are among sampling locations with comparable habitats; it was recommended for stream tadpoles by Heyer et al. (1994). Pools and runs were sampled with rapid sweeps through the water column of a

triangular-framed dip net (0.9 x 0.3 mm mesh size), and the net was also "bounced" along the substratum to force tadpoles into the water column where they could be captured. Loose rocks were dislodged to expose sheltering tadpoles. Riffles were sampled by placing the net downstream from rocks, which were turned to dislodge suctorial tadpoles; these were then swept into the net by the current. During the 1989-94 surveys, five one-minute dip net sampling episodes were used to capture tadpoles in runs and riffles, and three 30-second sampling episodes in each microhabitat in pools. In the 2011-13 surveys, five one-minute dip net samples were taken in each pool and riffle.

In the 1989-94 surveys, each subsample of tadpoles was sorted and counted in white trays; in the 2011-13 surveys, the five subsamples per site were combined for sorting and counting of tadpoles. Tadpoles were identified and staged when possible (Gosner 1960); in *Litoria nannotis*, *L. rheocola* and *L. dayi*, the hind limbs of tadpoles develop under a sheath and Gosner stages cannot be determined without dissection (Davies and Richards 1990; Cashins 2009). Tadpole body length was measured to the nearest 1 mm using a ruler (or 0.1 mm using callipers in early samples). Tadpoles were released back into the stream before moving on to the next sampling site. Vinyl gloves (Greer et al. 2009) were worn at all times during sampling to avoid touching the animals directly.

Water temperatures were recorded using data-loggers or maximum-minimum thermometers. Two data-loggers (Thermochron® iButtons) were sealed in zip-lock bags within a metal container that was placed into a pool in each stream and attached by wire to a nearby tree. Current velocity was measured using a flow meter (Owen's River Hydroprop). The average velocity for each sampling site was calculated from three readings, which were taken at locations likely to have near minimum, near maximum and intermediate velocities. A Hydrolab Quanta was used to measure pH, conductivity and dissolved oxygen in the streams at the time of sampling. Monthly rainfall data were obtained from the Australian Bureau of Meteorology website. For the Paluma streams, rainfall data from the Paluma township, 6 km south-east of the study sites, were used (Congdon and Herbohn 1993); for the streams at Tully, rainfall records were obtained from Kareeya station, 5 km from Stream 1 and 10 km from Stream 2. In some instances, rainfall per day had to be estimated because data were only available for a number of days combined.

Stream width and depth measurements as well as substratum cover estimates were taken along the transects in September or October 2012, before the start of the wet

season. Width was measured every 25 m and the values were averaged to obtain mean stream width (Table 2.1). All other measurements were taken in the riffles and pools sampled during the surveys (Table 2.1). Leaf litter cover and canopy cover were visually estimated as one of four categories for each sampling site. Minimum, maximum and mean depths for each stream were estimated from measurements at five points at each sampling site. Water samples for nutrient analysis were taken in summer 2011/2012 and were analysed for nitrate, phosphate, sodium, calcium, magnesium and potassium concentrations by the Tropical Water and Aquatic Ecosystem Research Centre (TropWATER) laboratory at James Cook University.

2.2.3 Data Analyses

Seasonal abundance patterns of tadpoles were similar between the two streams in each location (see Appendix 1.1), and therefore tadpole abundances for the two streams were combined in subsequent analyses. Tadpole abundance and biomass data were collected for (a) Paluma, 1989-94 and 2012-13, from Birthday Creek only, and (b) from the two Tully streams combined, 2011-13. If there was more than one sampling date per month, mean monthly abundances were calculated. Biomass for each tadpole was calculated using wet weight - length relationships from measurements taken during experiments (Chapters 4 and 5) and from Tully survey data (Cashins 2009). Tadpole size structure was examined in each monthly sample by counting the numbers of individuals of each species that fell into one of three size classes, as determined by body length.

Water temperature, current velocity and algal data, and tadpole abundance, biomass and size-class data, were plotted using SigmaPlot Version 12.5. Current velocity comparisons were made with Kruskal-Wallis one-way nonparametric analysis of variance (ANOVA) followed by Dunn's test for multiple comparison procedures. The same method was used for the analysis of tadpole distributions over a range of current velocities, including size-class comparisons for each species at different current velocities. Tadpole abundances among the various stream habitats were analysed using one-way ANOVA on rank-transformed data and Tukey's post-hoc tests where appropriate. Tadpole abundance in relation to algal cover was plotted only for Tully pools, where algal growth was most prevalent. The data were separated for pool and riffle species.

Ordination and permutational MANOVA/ANOVA (PERMANOVA) were carried out using PRIMER 6 Version 6.1.15 and PERMANOVA+ Version 1.0.5. Bray-Curtis similarity, using square-root-transformed abundances, was applied to generate the resemblance matrix, as is appropriate for biological assemblage data (Clarke and Gorley 2006). Non-metric multi-dimensional scaling (NMDS) was used to visualise the differences among the tadpole assemblages at Paluma and Tully. Tadpole abundances were compared between locations and habitats with PERMANOVA, using a nested design, with habitat (riffle or pool) nested within location (Paluma or Tully). Permutations of residuals were applied under a reduced model, which is the best model for multi-factorial designs with regard to type 1 error (Anderson et al. 2008). No corrections for multiple comparisons were carried out as the permutational P-values reported provide an exact test for each individual null hypothesis and corrections may be inaccurate and conservative (Anderson et al. 2008).

The relationships between the tadpole assemblages and environmental variables were analysed with distance-based linear models (DistLM in PRIMER 6). This method allows for predictor variables to be fit to the resemblance matrix of a multivariate dataset, with probability values obtained using permutations, avoiding the assumptions of parametric statistics (Anderson et al. 2008). The DistLM model partitions the variation in the data according to a regression (or multiple regression) model (Anderson et al. 2008). Location, stream, habitat and sampling date were selected as factors for the tadpole assemblage data. Draftsman plots indicated whether the environmental data were normally distributed (Anderson et al. 2008), and right-skewed variables were corrected with square-root transformations. Draftsman plots were also examined for multi-collinearity, but none of the environmental variables were sufficiently correlated to eliminate one from the analysis. The selection procedure *Best* in PRIMER was applied to model the environmental variables; this examines all possible combinations of the predictor variables (Anderson et al. 2008). AIC_c, suitable for small sample sizes, was the selection criterion to determine which model best described the tadpole assemblage in the streams (Johnson and Omland 2004). Delta AIC_c was calculated as the change in AIC_c between the model being examined and the best model, and was used to compare the models. A lower delta AIC_c indicated more evidence for the particular model.

2.3 Results

2.3.1 Stream characteristics

Data for the stream characteristics are presented for the 2011-13 surveys. Current velocities differed between habitat types in each stream, being lower in pools than in riffles across the two locations (one-way ANOVA with Dunn's pairwise comparison test, $H = 279.4$, $df = 7$, $P < 0.001$; Figure 2.4). The riffles at Paluma and Tully did not differ significantly, whereas the pools at Camp Creek had a significantly lower velocity than those at Tully Stream 1. In general, current velocity was lower at Paluma than at Tully across streams and habitats.

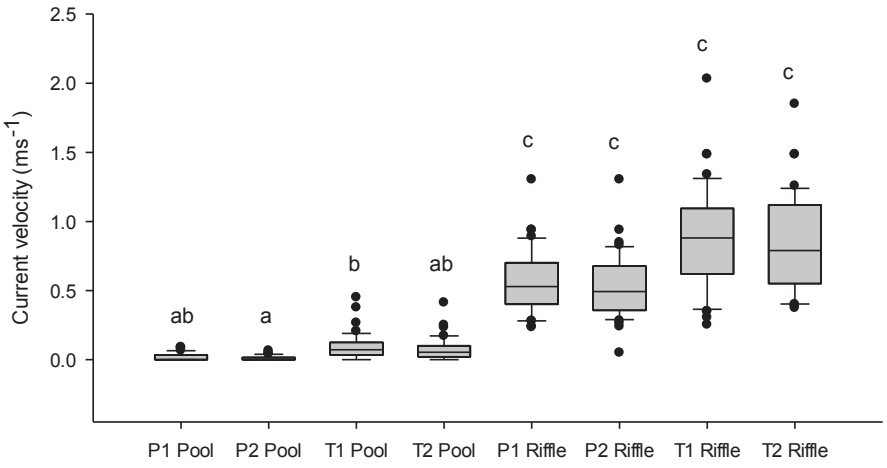

Figure 2.4. Current velocities in riffles and pools of four streams at Paluma and Tully, summarising flow data for 2011 – 2013. The lower, middle and upper boundaries of the box represent the 25th, median and 75th percentiles respectively. The whiskers represent the 10th (lower) and 90th (upper) percentiles. Significant differences between habitat types for each stream (indicated by Dunn's test with $\alpha = 0.05$) are shown by letters a – c. Stream abbreviations: P1 = Birthday Creek, P2 = Camp Creek, T1 = Tully Stream 1, and T2 = Tully Stream 2.

Water temperatures were more similar between the two streams at Tully than between the two streams at Paluma, and temperatures at Camp Creek fluctuated the most (Figure 2.5). Temperatures in winter were about four degrees lower in the Paluma streams than in the Tully streams, in keeping with their greater altitude. The temperature data loggers in Camp Creek were washed on to the stream bank several times by flash floods. The time of the flood was estimated and data taken during these periods were omitted.

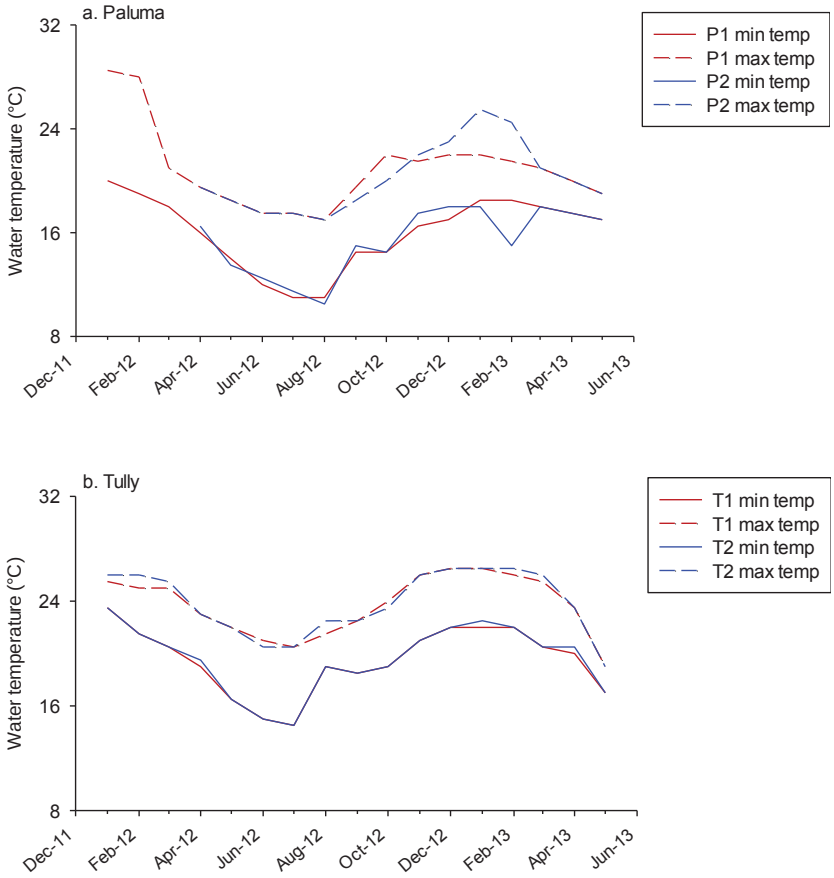

Figure 2.5. Minimum and maximum water temperatures (°C) for streams at Paluma (P1 = Birthday Creek, P2 = Camp Creek) and Tully (T1 = Tully Stream 1, T2 = Tully Stream 2) during the 2011-2013 surveys.

Overall, the water quality was similar in the two locations (Table 2.2). Dissolved oxygen and pH tended to be higher in the streams at Tully compared to Paluma, corresponding with the greater flow. Conductivity was consistently low at all the sites. Trace amounts of calcium, magnesium and potassium, and higher concentrations of sodium were present in all the streams, a characteristic of Australian coastal streams (Williams and Wan 1972). Total phosphorus was lower than total nitrogen, and the Paluma streams had higher concentrations of nitrogen than the Tully streams.

Table 2.2. Water quality of streams at Paluma and Tully, with minimum, maximum and mean values where appropriate.

Stream characteristics		Birthday Creek	Camp Creek	Tully Stream 1	Tully Stream 2
Conductivity (μScm^{-1})	Mean	0.032	0.026	0.028	0.034
	Min-maximum	0.030-0.032	0.025-0.029	0.026-0.031	0.032-0.036
Dissolved oxygen (mgL^{-1})	Mean	8.25	7.86	8.39	8.23
	Min-maximum	6.81-9.55	6.47-8.55	6.96-9.30	7.10-9.10
Dissolved oxygen (% saturation)	Mean	85.18	82.05	94.39	92.08
	Min-maximum	75.30-92.20	68.40-89.90	83.40-99.20	83.50-97.70
pH	Mean	6.44	7.08	7.74	6.90
	Min-maximum	5.61-7.40	6.78-7.33	7.37-8.40	6.40-8.10
Filterable Reactive Phosphorus (µg P/L)		4	4	3	3
Nitrate and nitrite (µg N/L)		46	17	136	226
Calcium (mg/L)		<1	<1	<1	<1
Magnesium (mg/L)		<1	<1	<1	<1
Sodium (mg/L)		4	4	3	4
Potassium (mg/L)		<1	<1	<1	<1

2.3.2 Tadpole abundance

In the 2011-2013 samples, seasonal patterns of tadpole abundance were similar in the two streams in each location but differed between the locations and habitats within each location (Table 2.3). The Paluma streams clustered separately from the Tully streams (Figure 2.6). Only one species, *Litoria serrata,* was present at Paluma during this period, in varying abundances, causing the Paluma points to cluster in a line along axis 1. Species and environmental variables in the streams differed between Paluma and Tully, and the data for the two locations were analysed separately.

In Birthday Creek at Paluma, between 1989 and 1994, *Litoria serrata* and *Mixophyes coggeri* were more abundant in pools or runs, whereas *L. nannotis* and *L. dayi* were more abundant in riffles (Figure 2.7a). In the 2011-13 surveys, only *L. serrata* and *M. coggeri* were found in the Paluma streams (Figure 2.7b). The distributions in pools and runs were similar, so in later surveys runs were not sampled as a separate habitat. Three of the four species in the Tully streams showed a strong preference for one of the habitat types (Figure 2.7c): *L. nannotis* and *L. dayi* were most abundant in riffles, *L. serrata* in pools, but *L. rheocola* tadpoles were found at similar abundances in both habitat types.

Table 2.3. PERMANOVA results for location (Paluma and Tully) and habitat (riffle and pool) similarity based on the tadpole assemblage. A nested design was applied, with (1) location and (2) habitat nested in location as factors. Square root transformations and Bray-Curtis similarities were used.

Source	Df	SS	MS	Pseudo-F	P(perm)	Unique perms
Location	1	95091	95091	70.744	0.0001	9963
Habitat (Location)	2	87952	43976	32.716	0.0001	9934
Res	222	2.98E5	1344.2			
Total	225	6.77E5				

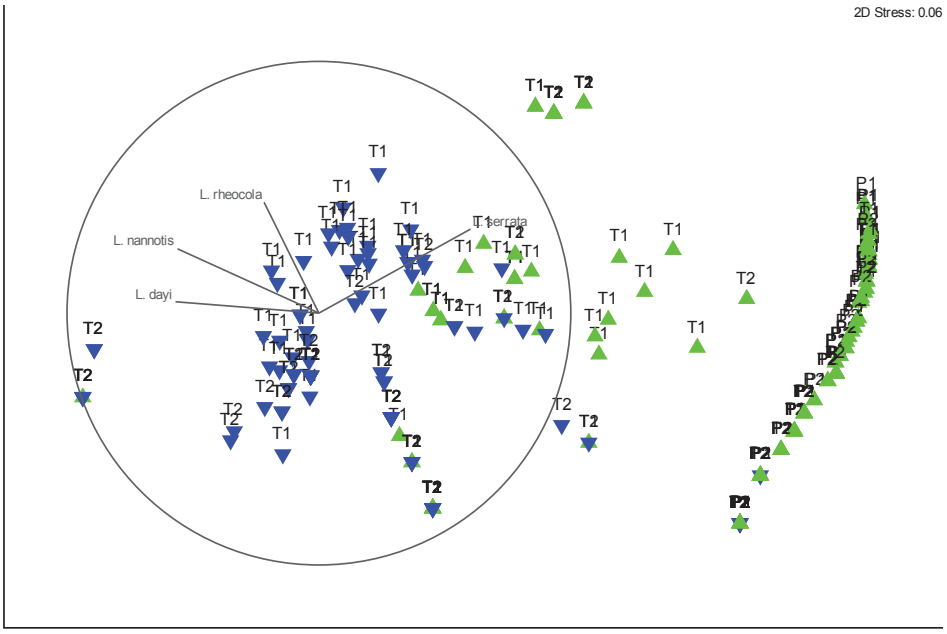

Figure 2.6. NMDS of location and habitat based on the similarities among the tadpole assemblages, with vectors representing anuran species. Four species were present at Tully: *Litoria serrata, L. nannotis, L. rheocola* and *L. dayi;* only one species (*L. serrata*) is included for Paluma (*Mixophyes coggeri* tadpoles are not included because only a few individuals were found over the survey period). Abbreviations: P1 = Birthday Creek, P2 = Camp Creek, T1 = Tully Stream 1, T2 = Tully Stream 2; symbols: green triangle = pool, blue triangle = riffle.

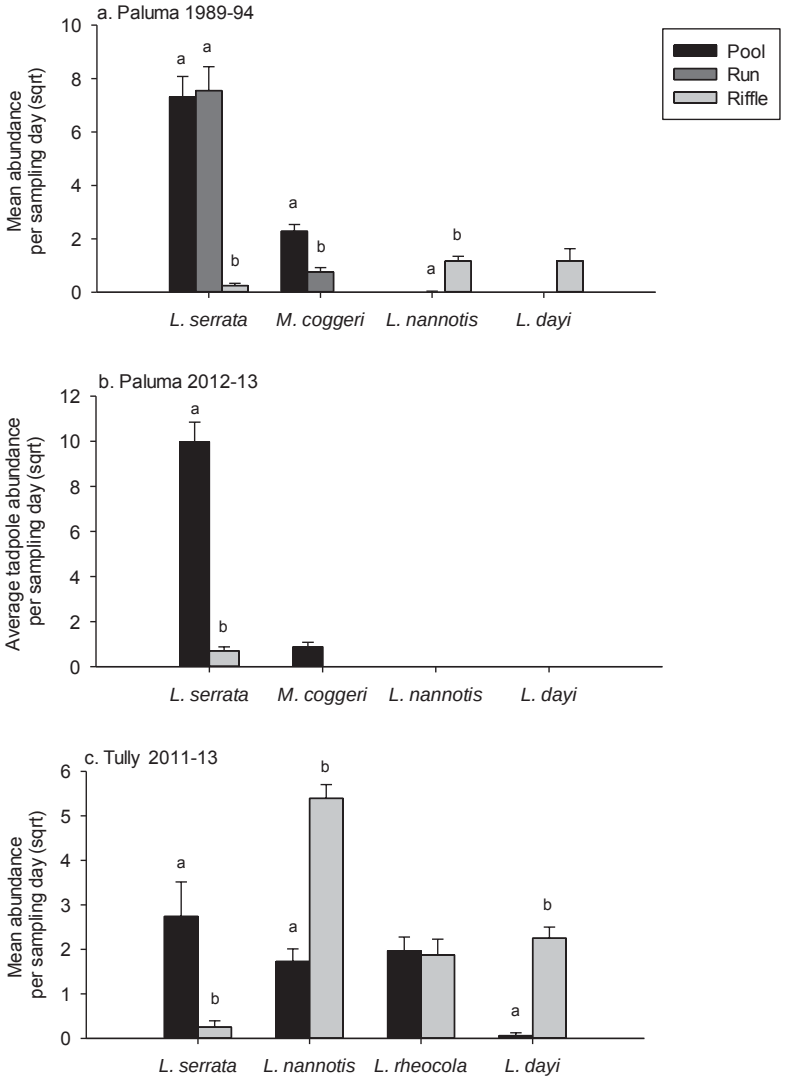

Figure 2.7. Mean tadpole abundance (square root) per day for different habitats in streams at Paluma and Tully. Abundances at (a) Paluma (Birthday Creek only) from pools, runs and riffles during 1989-94, (b) Paluma (Birthday Creek and Camp Creek) from pools and riffles during 2011-13, and (c) Tully (Tully Stream 1 and Stream 2) from pools and riffles during 2011-13. The letters a and b indicate significant differences between habitats (within locations as shown by one-way ANOVAs and Tukey's post-hoc tests ($\alpha = 0.05$).

At Paluma, all four species (when present) were most abundant in the summer months. There were distinct peaks of *L. serrata* tadpole abundances during summer in 1989-94, and in 2012-13 (Figure 2.8a). *Mixophyes coggeri* tadpoles were most abundant in late 1990 and beginning of 1991, but numbers dropped over the second half of the survey period (Figure 2.8b). *Litoria dayi* and *L. nannotis* tadpoles disappeared after 1990 and 1991 respectively, and remained absent over subsequent years (Figure 2.8c-d).

There were two abundance peaks for *L. nannotis,* in early and late summer. The other species each had a single peak, in months that differed among the species.

In the Tully streams, tadpole abundances were also highest in the warmer months; *L. serrata* tadpoles were abundant in early summer but their abundance decreased sharply over winter, whereas the other three species remained present at more constant numbers in the stream throughout the year (Figure 2.9). *Litoria nannotis* was also abundant in winter in 2012. The four species peaked at different times: *L. nannotis* abundance peaked between late winter and spring (Figure 2.9b), followed by *L. serrata* and *L. rheocola* in spring and early summer (Figure 2.9a and c respectively). *Litoria dayi* were most abundant in spring and late summer depending on the year, but they were never present in large numbers and therefore did not have a distinct peak (Figure 2.9d).

Overall, tadpole abundance and biomass followed the same trends at both sites, with an increase in biomass when tadpole numbers increased (Figures 2.8 and 2.9). In some instances, however, the decrease in tadpole numbers following peak abundances was compensated for by an increase in individual biomass (e.g., *M. coggeri,* Figure 2.8b). At Paluma, *L. serrata* and *M. coggeri* tadpoles had higher biomasses than *L. nannotis* and *L. dayi* (Figure 2.8). *Litoria dayi* abundance was high in comparison with that of *L. nannotis* at the start of the survey period, but the low biomass indicated that this consisted of small tadpoles. At Tully, the highest biomass came from *L. nannotis* tadpoles (Figure 2.9b). *Litoria serrata* tadpoles contributed to total biomass only for two to three months a year when abundances were high (Figure 2.9a).

Total tadpole biomass also changed over time among habitat types in the streams at Paluma and Tully (Figure 2.10). From 1989 to 1994, pools and runs at Paluma had the highest biomass, which fluctuated according to tadpole abundances (Figure 2.10a). The biomass in riffles decreased to zero over the course of the survey period. Therefore, during the second half of these surveys, tadpole biomass included only tadpoles in the still or slow-flowing sections of the stream. From 2012 to 2013, biomass in riffles was low and tadpoles living in pools made up almost the entire tadpole biomass in the Paluma streams (Figure 2.10b). At Tully, tadpole biomass in riffles and pools fluctuated during the year, with riffles representing more than half of the total biomass for most of the months (Figure 2.10c).

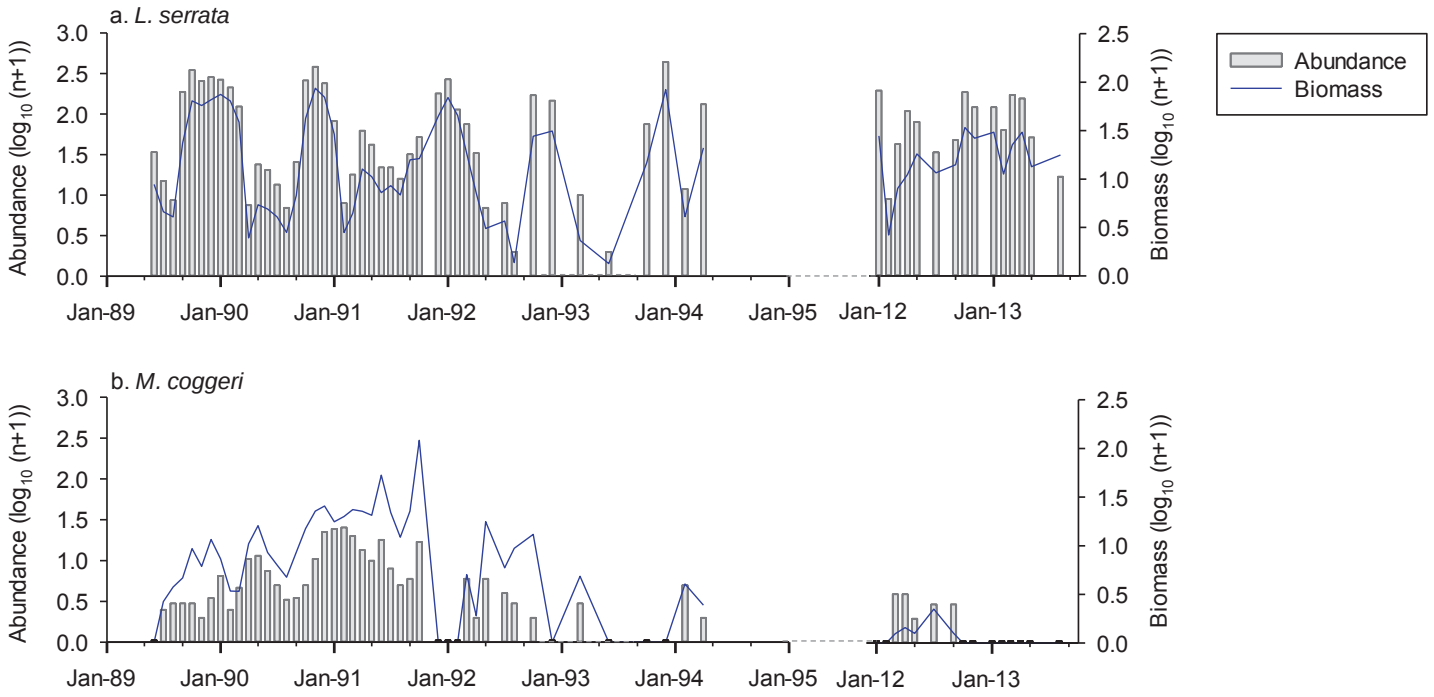

Figure 2.8. (Continued on next page)

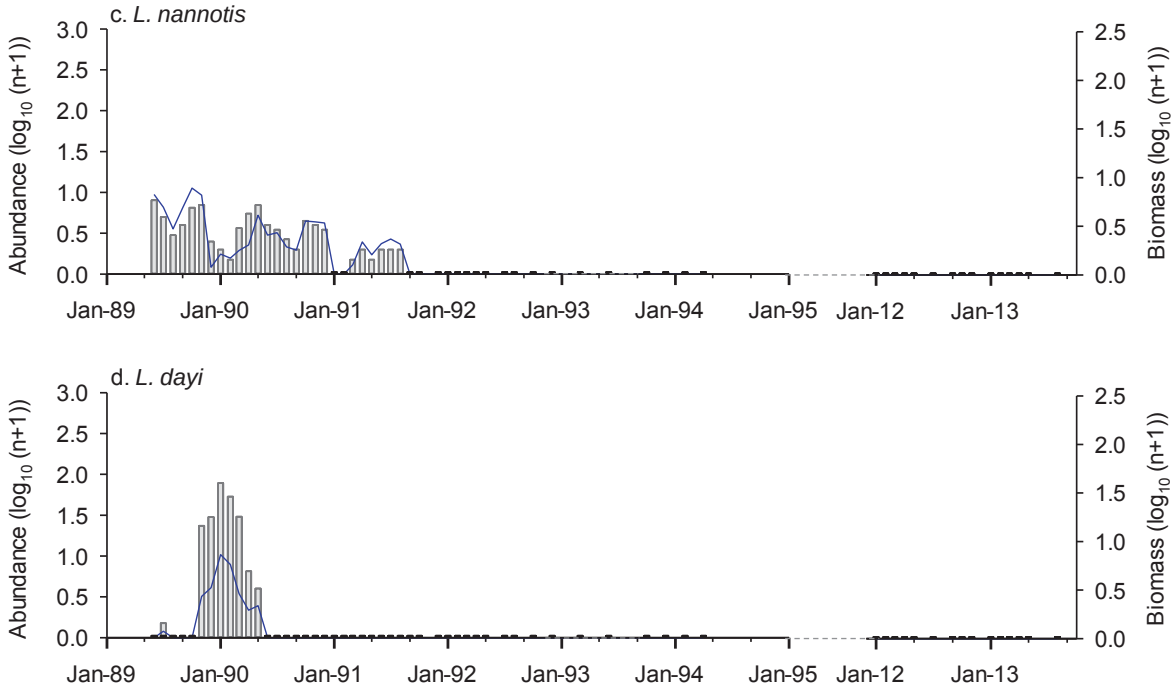

Figure 2.8. Tadpole abundance (bars) and biomass in grams (line) in Birthday Creek at Paluma between June 1989 and May 2013 for (a) *Litoria serrata,* (b) *Mixophyes coggeri*, (c) *L. nannotis* and (d) *L. dayi*, with a gap in sampling between April 1994 and October 2011. Biomass refers to the wet weight of the animals. The short black bars represent sampling periods where no animals were caught.

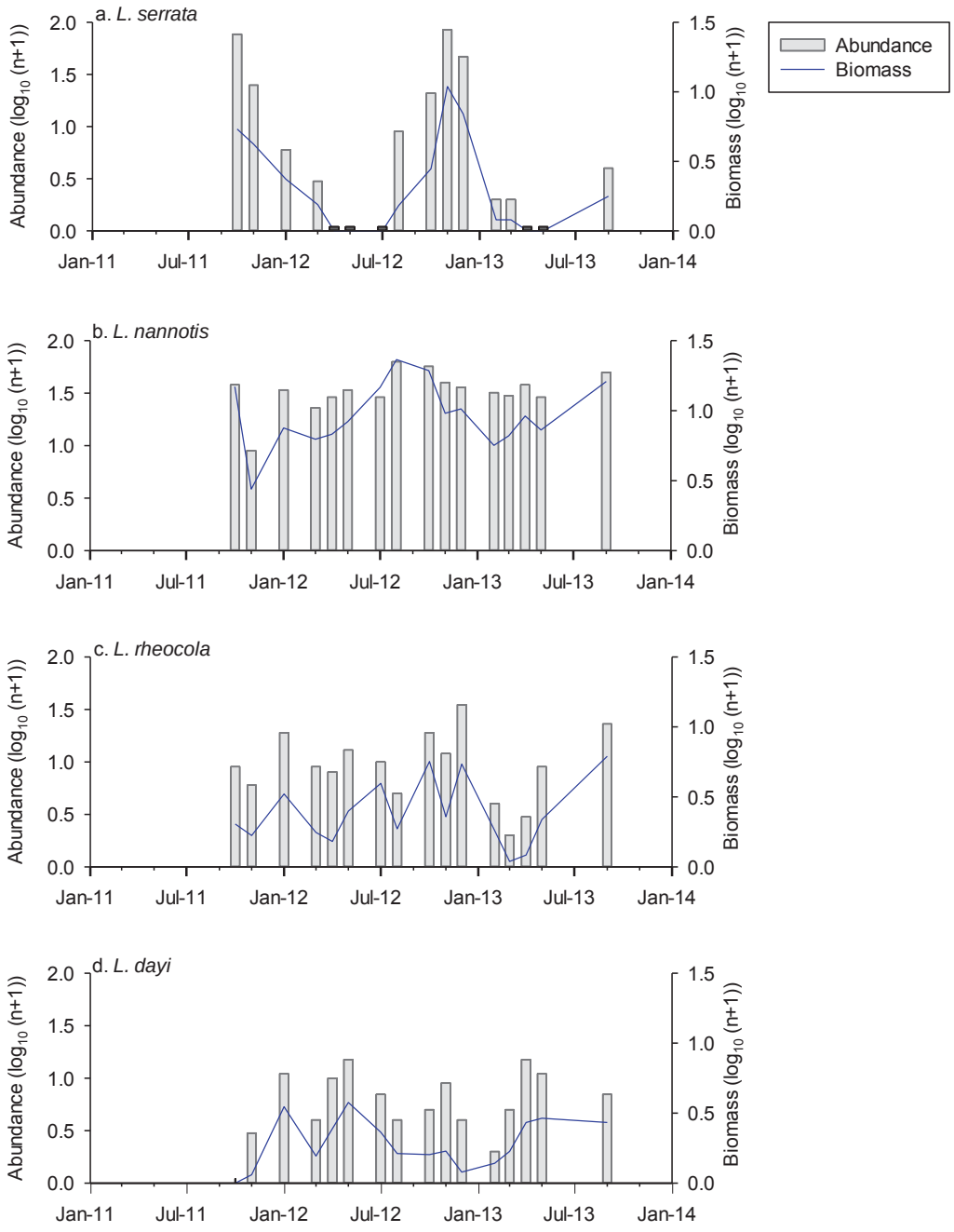

Figure 2.9. Tadpole abundance (bars) and biomass in grams (line) in two streams at Tully between October 2011 and May 2013 for (a) *Litoria serrata*, (b) *L. nannotis*, (c) *L. rheocola* and (d) *L. dayi*. Biomass refers to the wet weight of the animals. The short black bars represent sampling periods where no animals were caught. In October and December 2011, only four out of six sites were sampled per stream (two riffles and two pools), and in September 2013, only one of the two streams was sampled.

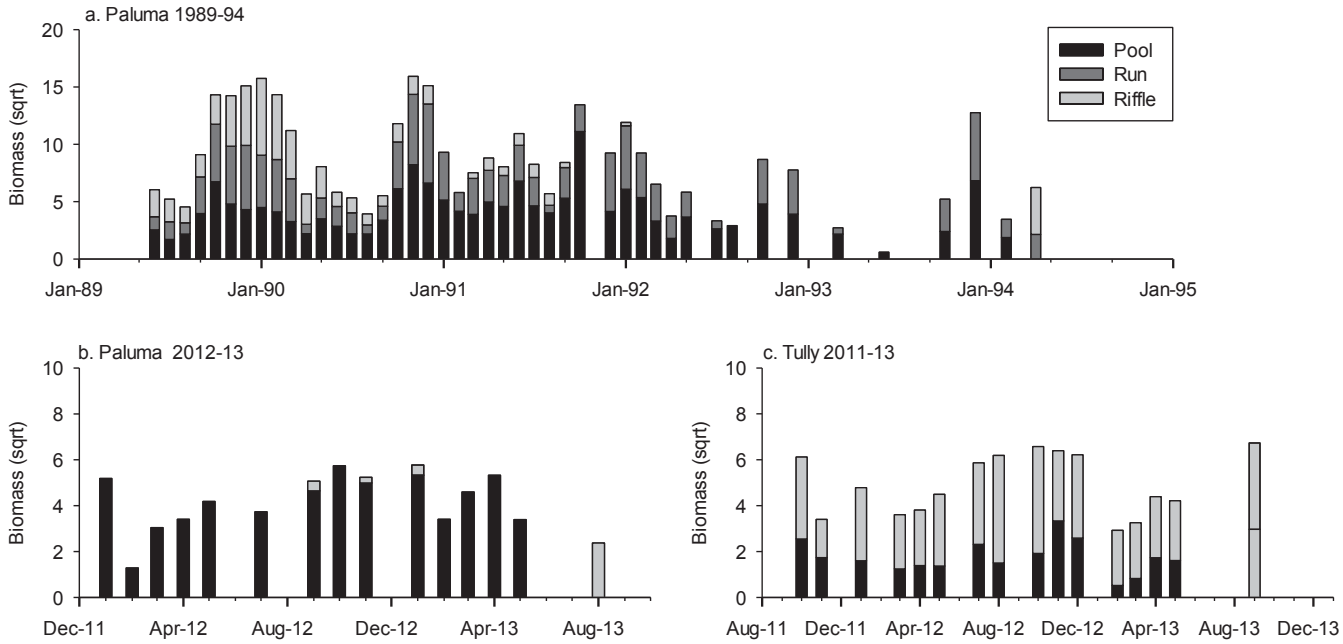

Figure 2.10. Total tadpole biomass (wet weight in grams) in pools, runs and riffles at (a) Paluma from June 1989 to April 1994, (b) Paluma from January 2012 to August 2013, and (c) Tully from October 2011 to September 2013.

2.3.3 Tadpole size structure

Three tadpole size classes were assigned based on body lengths (Table 2.4). *Litoria serrata* tadpoles were generally smaller than *L. nannotis, L. rheocola* or *L. dayi* tadpoles, and *M. coggeri* tadpoles were the largest. From 1989 to 1994, when there was more than one sample per month, the mean abundance of each species in each size class per month was calculated.

Table 2.4. Size classes for *Litoria serrata*, *L. nannotis, L. rheocola, L. dayi* and *Mixophyes coggeri* tadpoles according to body length measurements.

Size class	Body length		
	Litoria serrata	*Mixophyes coggeri*	*Litoria nannotis, L. rheocola, L. dayi*
1	≤ 5.5	≤ 10	≤ 7.5
2	> 5.5 to < 10	> 10 to < 20	> 7.5 to < 12
3	≥ 10	≥ 20	≥ 12

Litoria serrata tadpoles of the smallest size class were not abundant throughout the first survey period at Paluma, but there were annual peaks of the two larger size classes (Figure 2.11a). Size-3 tadpoles typically peaked just after size-2 tadpoles were most abundant each year. In most years at Paluma, *L. serrata* tadpoles of size class 1 increased in abundance in September, and again in March or April, indicating two breeding periods; this trend was clear in 2012-13 (Figure 2.11a). Tadpoles of the three size classes peaked in succession during both survey periods.

Mixophyes coggeri tadpoles of the two larger size classes were present throughout 1989-94, whereas size-1 tadpoles were present intermittently (Figure 2.11b). There was a peak in size-1 tadpoles in November 1990, after which size-2 tadpoles peaked, and larger tadpoles were present in the stream over the second half of 1991. After this period, all three size classes declined. In 2012-13 there were too few *M. coggeri* sampled to allow for size comparisons.

The two riffle species were only present at the start of the early surveys. *Litoria nannotis* tadpoles of size classes 2 and 3 were abundant in 1989-90 (Figure 2.11c), but there appeared to be no recruitment after 1989. The number of larger tadpoles remained high for the first half of 1990, after which tadpoles of all sizes decreased in abundance

and disappeared from the stream by the end of 1991. Size-1 *L. dayi* tadpoles peaked in abundance in January 1990 with some larger tadpoles present during this time, but the numbers of all the size classes dropped to zero by mid-1990 (Figure 2.11d).

At Tully, size-1 *L. serrata* tadpoles increased in numbers from August in 2012 and peaked in October, followed by size-2 tadpoles (Figure 2.12a). Size-3 tadpoles were present in spring and early summer, but not during winter. There was only one peak breeding period for *L. serrata* at Tully and the abundances of tadpoles in the three size classes overlapped during this time. Breeding periods for *L. nannotis* were in August and February, with another possible breeding occasion in April (Figure 2.12b), leading to an increase in, firstly, size-1 tadpoles, followed by size-2 tadpoles. Small tadpoles were present throughout spring and most of summer, whereas large ones were present throughout the year, with peaks in summer between August and October.

The breeding period of *L. rheocola* started a few months later than that of *L. nannotis*, with size-1 tadpoles peaking in December (Figure 2.12c). Size-2 tadpoles were present most of the year, with peaks in early and late summer. Larger tadpoles also overwintered in the streams and their abundances declined in spring. *Litoria dayi* started breeding between the other two riffle species and the size-1 numbers peaked in November and in April (Figure 2.12d). The larger tadpoles were most abundant in early or late summer, depending on the year, and declined throughout winter.

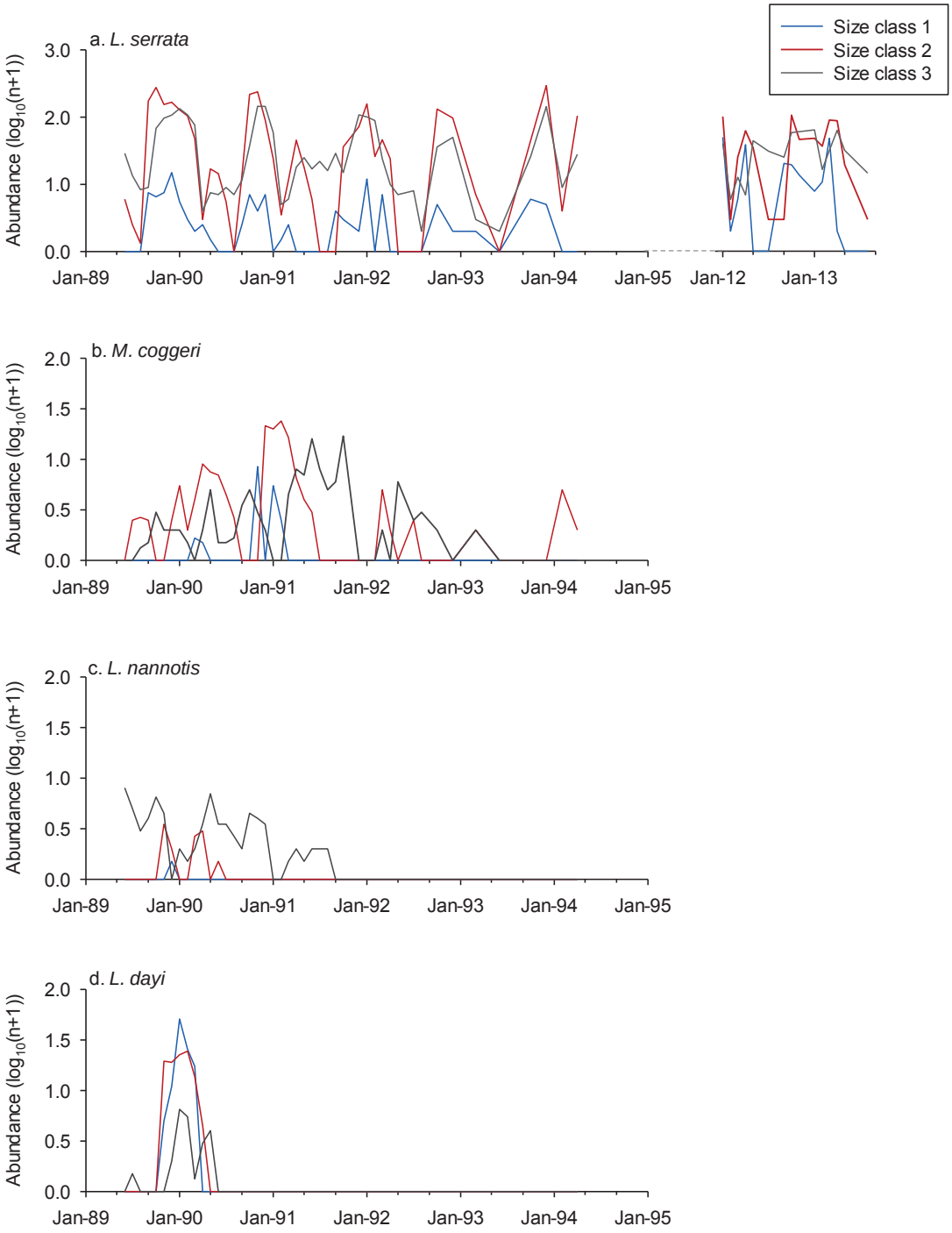

Figure 2.11. Size class distributions of tadpoles at Paluma: (a) *Litoria serrata* from 1989 to 1994 and 2012 to 2013, and (b) *Mixophyes coggeri*, (c) *L. nannotis* and (d) *L. dayi* from 1989 to 1994. The size-class ranges for each species are presented in Table 2.4.

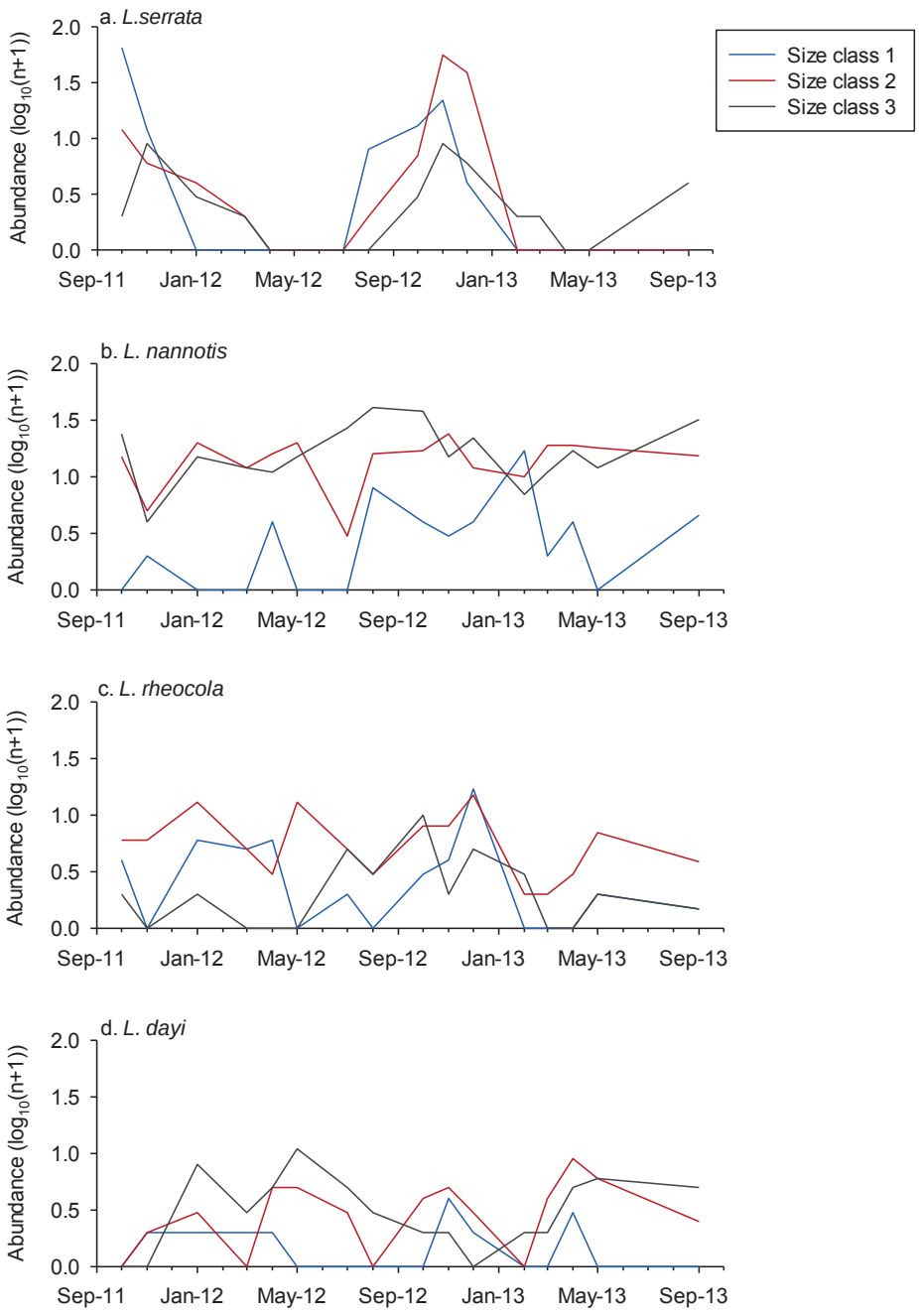

Figure 2.12. Size class distributions of tadpoles at Tully from 2011 to 2013: (a) *Litoria serrata,* (b) *L. nannotis*, (c) *L. rheocola* and (d) *L. dayi*. The size-class ranges for each species are presented in Table 2.4.

2.3.4 Tadpole abundance in relation to environmental variables

The DistLM model indicated the combination of environmental variables that most influenced the tadpole assemblage (species richness and relative abundances), based on lowest AIC_c values (Table 2.5). Rainfall data were tested separately for total rainfall that occurred 3 days, 7 days, and 14 days before the day of sampling to represent the effect of cumulative rainfall over several days on the assemblage. Of the eight variables at Paluma, current velocity was the single most important influence on the presence and abundance of tadpoles, and it explained 11% of the variation (Table 2.5a). The best model included current velocity, water temperature and canopy cover, and the three variables accounted for 23% of the variation in the tadpole assemblage. Water temperature effects were probably indicative of seasonal effects on the tadpole assemblage. Canopy cover on its own did not show a relationship with tadpole abundances in the Paluma pools (Figure 2.13).

At Tully, current velocity was also the single most important environmental variable, explaining 25% of the variation (Table 2.5b). The best model with the lowest AIC_c included 7-day and 14-day antecedent rainfall, current velocity and algal cover. However, the AIC_c values were similar between the models with three and four variables (0.3 units difference) and therefore the model with the lower number of environmental variables was selected as the most appropriate model: 14-day antecedent rainfall, current velocity and algal cover. If AIC_c values are within 1 or 2 units of each other this indicates that there is some redundancy between the predictor variables and these can be used interchangeably (Anderson et al. 2008). The selected model explained 29% of the variation in the tadpole assemblage. The influence of rainfall was most likely related to flow. Algal cover was either negatively or positively associated with tadpole abundances, depending on species. The abundance of pool species (*L. serrata)* was more likely to increase with algal cover, whereas riffle species (*L. nannotis, L. rheocola* and *L. dayi)* were less abundant when algal cover was higher (Figure 2.14).

Table 2.5. DistLM with model selection for increasing number of environmental variables at (a) Paluma and (b) Tully. *Best* selection procedure with AIC_c as the selection criterion was used to determine which environmental variables most influenced the tadpole assemblage. Delta AIC_c was calculated as the change in AIC_c between the model being examined and the best (lowest AIC_c) model. RSS is the residual sum of squared deviations. The best ten models are shown. Environmental variables: 1 = water temperature, 2 = 3-day rainfall, 3 = 7-day rainfall, 4 = 14-day rainfall, 5 = current velocity, 6 = canopy cover, 7 = leaf litter cover, and 8 = algal cover.

a

AIC_c	delta AIC_c	r^2	RSS	No. of variables	Variables selected
547.18	7.3	0.11479	53931	1	5
542.19	2.4	0.18695	49535	2	1,5
539.83	0.0	0.22993	46916	3	1,5,6
540.92	1.1	0.24062	46265	4	1,2,5,6
542.54	2.7	0.24694	45880	5	1,2,5,6,7
544.29	4.5	0.25261	45534	6	1,2,4,5,6,7
546.64	6.8	0.25343	45484	7	1-7
549.12	9.3	0.25375	45465	8	1-8

b

AIC_c	delta AIC_c	r^2	RSS	No. of variables	Variables selected
1061.7	3.7	0.2509	0.00002438	1	5
1059.5	1.5	0.2734	0.00002365	2	5,8
1058.3	0.3	0.2902	0.00002310	3	4,5,8
1058.0	0.0	0.3021	0.00002271	4	3,4,5,8
1058.7	0.7	0.3095	0.00002247	5	1,3,4,5,8
1059.6	1.6	0.3158	0.00002227	6	1,3,4,5,6,8
1060.5	2.5	0.3223	0.00002205	7	1,2,3,4,5,6,8
1062.6	4.6	0.3234	0.00002202	8	1-8

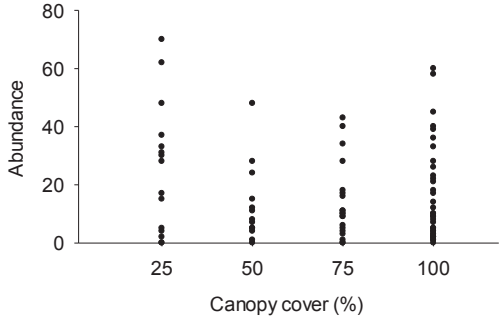

Figure 2.13. The abundance of tadpoles in Paluma pools over a range of canopy covers. The species consisted of *Litoria serrata* and *Mixophyes coggeri.* Canopy cover was measured as a percentage range: 0-25%, 25-50%, 50-75% and 75-100%.

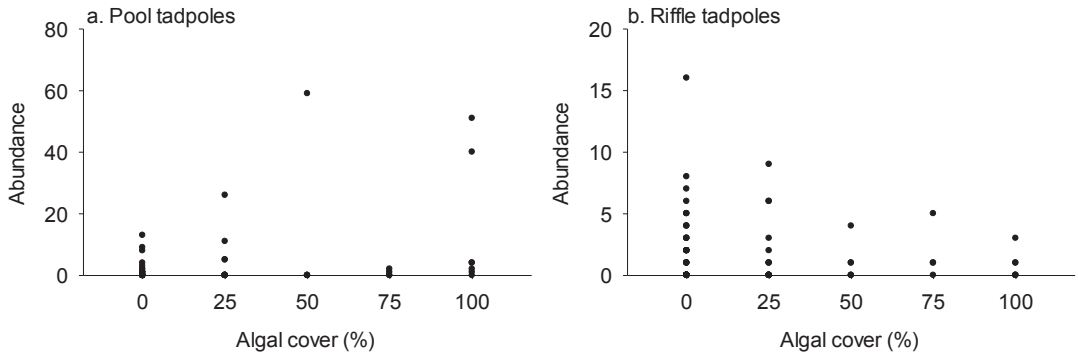

Figure 2.14. The abundance of (a) pool tadpoles and (b) riffle tadpoles in Tully pools over a range of algal covers. The pool species consisted of *Litoria serrata* and the riffle species consisted of *L. nannotis, L. rheocola* and *L. dayi.* Algal cover was measured as a percentage range: 0%, 0-25%, 25-50%, 50-75% and 75-100%.

Litoria serrata tadpoles occurred at velocities significantly closer to 0 ms^{-1} than did riffle species (one-way ANOVA with Dunn's pairwise comparison tests, $H = 1663.5$, $df = 4$, $P < 0.001$; Figure 2.15). Most locations at which *L. serrata* tadpoles were found at Paluma and Tully had current velocities below 0.1 ms^{-1}. *Litoria nannotis* and *L. rheocola* were present over a wide range of flow conditions, but *L. nannotis* was found at significantly higher velocities than *L. rheocola*. *Litoria dayi* were also found at these higher current velocities. There was no difference in distributions across current velocities among the three size classes for the riffle species: *L. nannotis* ($H = 0.78$, $df = 2$, $P = 0.676$), *L. rheocola* ($H = 3.16$, $df = 2$, $P = 0.207$) and *L. dayi* ($H = 0.30$, $df = 2$, $P = 0.222$) at Tully. Large (size 3) *L. serrata* tadpoles at Tully were tolerant of a broader range of flow conditions and were found at locations with a higher median velocity compared to smaller tadpoles ($H = 29.08$, $df = 2$, $P < 0.001$). However, *L. serrata* tadpoles of size 3 at Paluma showed the opposite pattern, being found at locations with lower flows than were the two smaller size classes ($H = 44.57$, $df = 2$, $P < 0.001$).

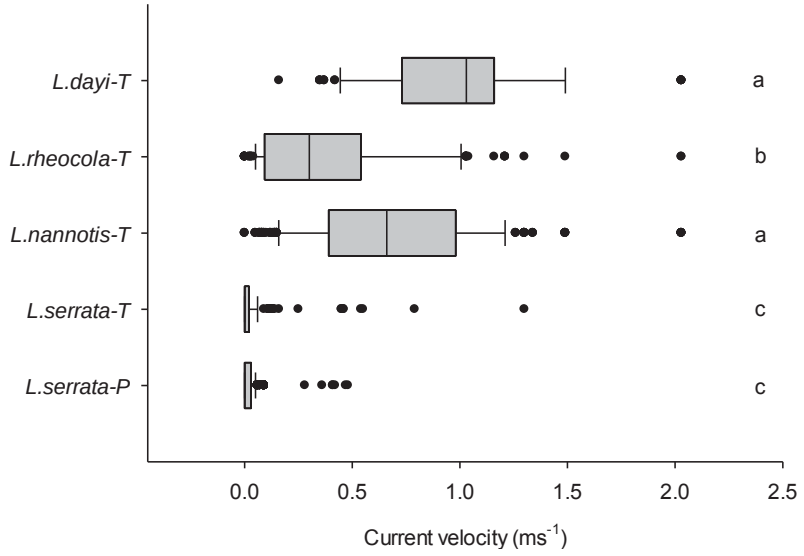

Figure 2.15. Tadpole distribution of four species over a range of current velocities during the 2011-13 surveys at Paluma and Tully. The lower, middle and upper boundaries of the box represent the 25th, median and 75th percentiles, respectively, of velocities at which at least one tadpole was found in a sample. The whiskers represent the 10th (lower) and 90th (upper) percentiles. Significant differences between habitat types for each stream (indicated by one-way ANOVA followed by Dunn's pairwise comparison test with $\alpha = 0.05$) are shown by letters a – c. Abbreviations included with the species names: P = Paluma, T = Tully.

2.4 Discussion

Stream tadpole occurrence and abundance depend on the available species pool and on environmental and biological factors that influence behaviour and survival of adult and larval populations (Flecker and Feifarek 1994; Wahbe and Bunnell 2003; Gonzalez et al. 2011). The frog species and their tadpole distributions differed between the streams at Paluma and Tully, and in 2011-13 only one species was present in both locations. Tadpoles were generally more abundant during spring and summer, coinciding with adult breeding periods, but the timing of peak abundance for the various size classes depended on the species.

The streams at Paluma and Tully differed in their physical characteristics, and therefore in the habitats available for tadpoles. At Paluma, the streams were generally shaded, slow flowing, with a sand and cobble substratum, whereas the Tully streams were more open, had higher current velocities and rocky streambeds with large boulders. Leaf litter accumulation was greater in the Paluma streams because of denser canopy cover and lower flows. Although leaf packs were removed by spates, they were

quickly replaced by new litter fall. At Tully, most pools had some flow for much of the year and frequent spates prevented leaf litter from accumulating. Nevertheless, stream water quality was similar in the two locations, with low nutrient and mineral content, as is typical of these small forest streams (Connolly and Pearson 2013).

Tadpole spatial distributions at Paluma and Tully depended on feeding mode and tolerance of high current velocities. Four species initially occurred at Paluma: two pool species, *L. serrata* and *M. coggeri*, and two riffle species, *L. nannotis* and *L. dayi*. At Tully there was one pool species, *L. serrata*, two riffle species, *L. nannotis* and *L. dayi*, and one species found in both habitats, *L. rheocola*. The pool-dwellers were also found in runs with generally slow current velocities, but they could not withstand the stronger flows typical of riffles (Richards 2002). They have small mouthparts and are not able to firmly attach themselves to the substratum using their oral discs (Richards 2002; Cashins 2009). The riffle specialists at Tully could tolerate strong current velocities, but were found over a wide range of flow conditions. *Litoria rheocola* apparently had a lower tolerance of high flow than the other riffles species, as its distribution was centred on lower current velocities in pools and riffles. *Litoria nannotis* was more abundant in riffles and *L. dayi* was never found in still water. The findings are similar to those of Cashins (2009) who found that *L. dayi* could withstand the highest current velocities, followed by *L. nannotis* and *L. rheocola*. Although the species' habitats overlapped, their different tolerances of high current velocities probably led to partitioning of microhabitats within the stream, reducing the likelihood of competition.

Habitat choice of tadpoles also differed between the two locations over the two survey periods. During 1989-94 at Paluma, *L. nannotis* tadpoles were never found in pools, whereas at Tully, they occurred in both pools and riffles. Current velocities were generally higher at Tully, and the higher velocities in pools may have been more within the range tolerated by riffle tadpoles. Also, riffle specialists were more abundant in the Tully streams, and competition may have caused some of the tadpoles to use less suitable habitats. Although young *L. serrata* tadpoles occurred almost exclusively in pools or runs, they were occasionally found in riffles at Paluma. Breeding sites selected by adults do not necessarily correlate with microhabitat selection by tadpoles (Alford 1999; Strauß et al. 2010); for example, the eggs of *L. serrata* may be laid in flowing water (Hoskin and Hero 2008), and hatchlings may therefore spend some time in riffles before being washed into pools.

In 1989, tadpoles of four species were found in Birthday Creek, but their abundances decreased through 1994. The declines of the riffle-dwelling tadpoles coincided with the period in the late 1980s and early 1990s during which amphibian losses were recorded throughout the Wet Tropics (Richards et al. 1993; McDonald and Alford 1999) as a result of the disease chytridiomycosis (McDonald and Alford 1999). Tadpoles can be infected by *Bd*, in some cases leading to deterioration of their mouthparts (Blaustein et al. 2005), but they do not usually develop the disease or suffer significant mortality from it (Woodhams and Alford 2005). Terrestrial populations of some species, such as *L. nannotis* and *L. dayi* at Paluma, were eliminated by the disease, whereas others, such as *L. serrata,* have recovered and survive despite the fungus being present (Woodhams and Alford 2005).

The tadpole abundances in the streams were directly affected by the collapse in the adult breeding populations, as indicated by surveys of adults (Richards et al. 1993) and the tadpole abundance data. The most likely cause for the decline of *L. nannotis* and *L. dayi* tadpoles at Paluma was the lack of recruitment as there appeared to be no breeding after 1990. However, whereas some medium and large *L. nannotis* tadpoles continued to be present in the stream until the end of 1991, the tadpole population of *L. dayi* crashed by mid-1990, indicating that most of the recruits that had entered the stream a few months earlier did not survive. Both species remained absent from the streams in 2012-13 because the adults did not re-establish themselves in the area. There were fewer fluctuations in size-1 tadpole abundances of *L. serrata*, which probably meant fewer breeding peaks as a result of adult frog declines (Richards and Alford 2005). Nevertheless, the overall tadpole abundance of *L. serrata* appeared stable over both survey periods.

Mixophyes coggeri continued to breed between 1989 and 1994, but abundances of all size classes of tadpoles decreased over this period, indicating that the adult population had declined. No small tadpoles were detected from 1992 onwards, and lower abundances of larger tadpoles suggest that there were probably few recruits. There has been no concrete evidence for the decline of this species (formerly named *M. schevilli*) in the literature and populations have been recorded as stable (Richards et al. 1993; Williams and Hero 1998; McDonald and Alford 1999). However, the extremely low abundances of *M. coggeri* tadpoles recorded during the 2012-13 surveys strongly suggest that the species had declined in these streams. In eastern Australia, populations of *Mixophyes fleayi* recovered following amphibian declines (Newell et al. 2013). The

stability of the populations depended on adult frog survival, whereas juvenile recruitment was low (Newell et al. 2013). This suggests that *M. coggeri* at Paluma may recover to pre-decline abundances if survival of adults is high.

Overall, tadpole numbers were highest in spring and summer, which roughly coincided with the annual influx of small tadpoles. Abundances of the various species peaked at different times, reducing densities and probably competition if they occupied the same habitat type (Altig and Johnston 1989; Bertoluci and Rodrigues 2002). The timing of the breeding peaks of *L. serrata, L. nannotis* and *L. dayi* differed between Paluma and Tully, possibly due to temperature differences (Afonso and Eterovick 2007). The adults at Tully may have been less restricted in their breeding periods because winter temperatures were not as low as at Paluma, given that the two locations differ in altitude by about 600 m. They also co-occurred with, and may thus have competed with, different species, which may have led them to adjust their breeding periods.

For some species, the timing of breeding and the influx of small tadpoles varied from year to year; this was also reported for earlier surveys at Tully by Cashins (2009). This may be due to inter-annual differences in rainfall, stream flow and temperature. The size-1 abundance peaks were usually followed by an increase in size-2 tadpoles. Size-3 tadpoles without limbs were found throughout most of the winters, presumably maturing in the warmer months. During previous surveys, tadpoles at Tully typically hatched in spring or summer, underwent most growth and developed over winter, and then metamorphosed the following spring or summer (Cashins 2009). The high abundance of large *M. coggeri* tadpoles throughout 1991 at Paluma also indicated that most of them remained in the stream over winter and metamorphosed in spring or summer.

Tadpole biomass reflected tadpole numerical abundance in the streams across seasons, but varied widely among species. Although *M. coggeri* tadpoles were not as common as *L. serrata* at Paluma, the biomass of a single tadpole was typically four times that of one *L. serrata* tadpole. Tadpole biomass at Tully was represented largely by *L. nannotis*, which was the most abundant species throughout the year. Although biomass was usually higher in riffles than in pools at Tully, some riffle species were also found in pools, especially when the pools had discernible flow. Therefore, tadpole activity took place in both habitat types throughout the year. Even though *L. nannotis* and *L. dayi* tadpoles were present at the start of the first survey period at Paluma, riffle

species contributed little biomass, and after 1992, their biomass contribution was zero. Riffle tadpoles at Paluma may have contributed to stream functioning through activities such as bioturbation, but their low biomass before the declines indicates that riffle specialists at Birthday Creek may have only had minor effects on the stream ecosystem.

Current velocity was an important influence on the tadpole assemblages at both Paluma and Tully. Pool-dwelling species are probably washed downstream when current velocity increases above the threshold level, and riffle-specialists may also be displaced when velocities exceed their capacity to hold on to the substratum. Although tadpoles may not have the tendency to return to a particular area following spates, their abundance at a site can remain relatively stable, as they tend to be replaced by other individuals (Cashins 2009). Higher current velocities may set an upper limit to tadpole density, as places for tadpoles to attach and protect themselves from the current become more limiting. Although tadpoles of different size classes may act like ecologically distinct species (Alford 1999), the tolerance to strong flows was the same between small and large riffle tadpoles at Tully. Large *L. serrata* tadpoles at Tully could withstand higher current velocities than small tadpoles. At Paluma, however, small *L. serrata* tadpoles were found at higher velocities, possibly because they hatched from eggs laid in high-velocity sites.

Daily rainfall influenced flow patterns in the streams, and there was probably a cumulative effect of antecedent rainfall on flow and current velocity over a longer period of time. At Paluma, current velocity, water temperature and canopy cover were the most important environmental factors influencing the tadpole assemblage, with 3-day antecedent rainfall included in the second-best model. At Tully, the best model included current velocity, 14-day antecedent rainfall and algal cover. The animals at Tully were generally more adapted to faster flows and were more affected by the cumulative effect of rainfall. Nevertheless, *L. serrata* abundances in both locations typically fell dramatically following heavy rains as water levels rose and animals were exposed to high current velocities, displacing them from their usual habitats.

The impact of water temperature on the tadpole assemblage reflected the seasonal influence on breeding, and not direct effects on tadpole distribution. Breeding of *L. serrata* at Paluma peaked in spring and autumn in most years, and tadpole numbers most probably decreased over summer as a result of tadpoles from the spring cohort growing and metamorphosing, and due to mortality during this period. In the Tully streams, breeding peaked in spring, and tadpole abundances decreased over

winter, outside of the adult breeding periods, although the pattern was not as clear. From the model selection, water temperature was not as important in the Tully streams, although it was also indicative of seasonal effects. This was most likely because the Tully streams are in the lowlands where seasonal temperature fluctuations are lower than in the uplands, and minimum temperatures are higher.

The effect of canopy cover on tadpole distribution that was indicated by the modelling for the Paluma streams may be indirect, mediated by the effect of canopy cover on other, unmeasured variables. For example, canopy cover is likely to be related to rates of primary production, which may be important and can be independent of the standing crops of algae or leaf litter, which were also measured. The more direct exposure to sunlight in the Tully streams allowed algae to grow and accumulate during the dry season when there was less flow. Tadpoles may feed on algae (Flecker et al. 1999; Ranvestel et al. 2004), but high algal abundances can be correlated with low tadpole density and biomass (Wahbe and Bunnell 2003). This might simply reflect a lesser effect of tadpole feeding on the standing crop of algae, but could also be caused by filamentous algae impeding tadpole movements (Wahbe and Bunnell 2003) or by the dominant algae being species or growth forms that are not preferred by tadpoles. High algal cover at Tully did not affect the pool species, but was associated with lower abundances of riffle species. This indicates that the pool tadpoles may have been feeding on the algae or using it as shelter, whereas this was not the case for riffle tadpoles. Algal growth was limited at Paluma and was therefore not as important in influencing the tadpole assemblage.

The extent of tadpole influence on stream functioning depends on the stream's biophysical nature, the species present, and their abundance patterns. Tadpole abundances fluctuated substantially with season and differed among habitats, depending on the flow conditions in streams, such that life cycles varied somewhat among streams that differed in character. Breeding patterns varied within species between Paluma and Tully, and also among species within the same location. The tadpoles were influenced by different environmental factors in the two locations, and there was a strong seasonal signal for tadpole biomass at Paluma, but not at Tully. The data allowed for comparisons of tadpole populations before, during and after amphibian declines at Paluma. The riffle-species were most affected by the declines: they disappeared entirely by the end of the 1989-94 surveys and had not returned to the stream during the later surveys, whereas *M. coggeri* populations remained low and *L. serrata* showed signs of

recovery. Frog declines, associated declines in tadpole numbers, and changes in tadpole assemblage composition may therefore influence dynamics only within a particular habitat type of a stream. Future studies could combine adult and tadpole surveys to determine how adult behaviour influences tadpole assemblages in streams and to what extent environmental variables affect the terrestrial and aquatic populations.

3. Tadpole and invertebrate relationships

3.1 Introduction

The species that make up a stream assemblage have diverse functional roles (e.g., Cheshire et al. 2005), and the interactions among these organisms are important in influencing ecosystem functioning (Ranvestel et al. 2004; Whiles et al. 2006; Iwai et al. 2009). Little is known about the interactions between tadpoles and other organisms, either direct or indirect (Colon-Gaud et al. 2009). The effects of tadpoles on assemblage composition, food-web structure and stream functioning have mainly been studied by one team in streams in the Neotropics, before and after a major decline in tadpole abundance (Ranvestel et al. 2004; Whiles et al. 2006; Colon-Gaud et al. 2009). There are no other comparable studies in other regions and more information is needed to gain a better understanding of the functional role of tadpoles and their relationships with other aquatic organisms.

Invertebrates are abundant consumers in stream systems and may interact with tadpoles through competition, predation or facilitation (Richards and Bull 1990; Kiffney and Richardson 2001; Ranvestel et al. 2004; Iwai et al. 2009). Invertebrates are often grouped into functional groups based on feeding mechanisms, which describe their propensity to feed on particular food sources, depending on their morphological and behavioural adaptations (Cummins 1973; Cummins and Klug 1979). The functional groups comprise grazers, shredders, gatherers, filterers and predators. Tadpoles are generally considered to be omnivores or detritivores with broad diets (Alford 1999), and most likely feed on food sources similar to those used by grazing and detritus-collecting invertebrates, categorised as grazers and gatherers (Flecker et al. 1999; Kiffney and Richardson 2001; Colon-Gaud et al. 2009). This may result in competition between tadpoles and invertebrates, but the extent of competition depends on the species and on available resources (Kupferberg 1997).

Tadpoles may influence the population size of some invertebrates via competition, while increasing the population size of others via facilitation (Colon-Gaud et al. 2010a). For example, facilitation between tadpoles and leaf-shredding invertebrates may lead to higher rates of leaf litter breakdown than would be expected from the combined effects of their individual activities, as demonstrated in Birthday Creek by Iwai et al. (2009). Tadpoles may also benefit grazers indirectly through

bioturbation as they stir up the substratum during feeding, exposing underlying food material (Ranvestel et al. 2004). As a result of these interactions, aquatic organisms are expected to have a range of responses to changing amphibian populations, with some consumers affected more directly than others (Colon-Gaud et al. 2010a). This may lead to changes in the functional structure of invertebrate assemblages when tadpole numbers decline seasonally or disappear altogether (Colon-Gaud et al. 2009).

Ecosystem functioning is directly influenced by the functional roles of species in an assemblage (Giller et al. 2004). Some species may have overlapping roles or functional redundancy, and the loss of a species may not be obvious if others continue its function (Allan and Castillo 2007). Therefore, reduced tadpole activity could be compensated for by grazing invertebrates. There is only limited information about the functional importance of tadpoles and the extent of functional redundancy (Colon-Gaud et al. 2010a). For example, grazing invertebrates maintained resource availability two years after tadpole populations declined in a Neotropical stream (Colon-Gaud et al. 2010a). However, although invertebrate biomass remained the same, their assemblage composition changed, indicating that not all invertebrate groups responded positively to amphibian declines.

Tadpole populations in streams naturally fluctuate seasonally (Chapter 2), so the loss of species would have greatest effect at times when tadpoles are normally most abundant. Invertebrate abundances also vary with season in Wet Tropics streams and are affected by the same environmental factors as tadpoles (Rosser and Pearson 1995; Cheshire et al. 2005; Pearson 2014). Therefore, functionally similar invertebrate species may only compensate for the loss of tadpole activity in streams when invertebrate abundances are high. It is possible that the invertebrate assemblage composition in the study streams changed following amphibian declines and that the current assemblages have compensated for lower tadpole abundance and diversity. This study aimed to determine the relationships between the abundance of tadpoles and invertebrates and how they vary seasonally. This was done by comparing the seasonal abundances of invertebrates and tadpoles and identifying the environmental factors that most influenced the assemblages. Possible patterns of relative abundance of tadpoles (individual species or collectively) and invertebrates (functional groups) were:

- tadpole and invertebrate abundances follow similar trajectories, suggesting similar responses to environmental variables (e.g., season), with the possibility of facilitation between some components of the fauna;
- tadpole and invertebrate elements follow opposite trajectories, suggesting negative inter-relationships such as competition, or opposite responses to environmental variables;
- apparently random patterns of relative abundance, indicating no relationships and no similarities in response to environmental variables; or
- some combination of the above patterns, indicating seasonal changes in trajectories and interactions.

3.2 Methods

3.2.1 Sampling sites and survey methods

The sampling sites were located at Paluma and Tully, as described in Chapter 2. Tadpole surveys, using five one-minute sampling episodes at each site, are also described in Chapter 2. A further three-minute sample was used to collect invertebrates. This was done at a slower pace with a focus in riffles on turning rocks and scraping them to dislodge invertebrates attached to them, which were then collected in a dip net (0.9 x 0.3 mm mesh size) held downstream. In pools, the substratum was disturbed and the animals were collected by sweeps of the net through the water column. Invertebrates were sorted and counted live in a white tray and were identified to the lowest taxonomic level possible (variously, order, family, genus or species), and were then released back into the stream. Samples were collected every four to six weeks, between February 2012 and August 2013 at Paluma, and between October 2011 and May 2013 at Tully. The relationship between tadpoles and invertebrates was investigated by comparing their biomass (dry weights). For tadpoles, dry weights of specimens were used to obtain length-biomass relationships for the different species; for invertebrates, dry weights were estimated from animals collected for stable isotope analysis (Chapter 6).

3.2.2 Statistical Analysis

The overall abundance patterns of invertebrate taxonomic groups were similar between the two streams in each location (see Appendix 2.2 and 2.3), so the data were combined for analyses of overall seasonal abundance patterns and responses to environmental factors. Only those taxa with high enough abundances throughout the year for comparison between streams were included in analyses. Although tadpole abundance patterns were similar between the two streams in each location (Chapter 2), tadpoles were usually more abundant in one of the streams. To determine the relationship between tadpoles and each invertebrate feeding group, the streams were therefore kept separate to make it easier to detect any patterns.

To compare invertebrate assemblages between locations and habitats, non-metric multi-dimensional scaling (NMDS) ordination and permutational MANOVA/ANOVA (PERMANOVA) were carried out in PRIMER 6 Version 6.1.15 and PERMANOVA+ Version 1.0.5. Bray-Curtis similarities were used to generate the resemblance matrix (Clarke and Gorley 2006). Possible relationships between the invertebrate assemblage (using abundances of invertebrate taxa) and environmental variables were examined using distance-based linear models (DistLM) in PRIMER 6, with the selection procedure *Best* and AIC_c as the selection criterion (see Chapter 2). The combination of environmental variables that best explained the invertebrate assemblage was determined by the lowest AIC_c values. Delta AIC_c was calculated as the change in AIC_c between the model being examined and the best model, and was used to compare the models. Antecedent rainfall data were tested for 3, 7 and 14 days before the day of sampling. Water temperature for each loction was the average of the temperatures in the two streams.

The relationships between abundances of tadpoles and each invertebrate feeding group were tested using Spearman's rank correlation analyses. At Paluma, this was done only for *L. serrata* tadpoles in pools because no other species was present in sufficient numbers. At Tully, the pool species (*L. serrata*) was tested separately from the total number of tadpoles in the pools. These correlations were calculated and plotted in SigmaPlot Version 12.5, as were the invertebrate abundances in pools and riffles in each location. The relationships between the invertebrate and tadpole matrices were further tested using RELATE in PRIMER 6. This is based on a Mantel test and allows two multivariate data sets to be compared. BEST analysis in PRIMER 6 was then

applied to determine which species led to significant results. This was done separately for Paluma and Tully, and at Paluma using only the pool data because there were no tadpoles in the riffles. The analysis was run with all invertebrate feeding groups, and twice for Tully: (i) using total tadpole biomass, and (ii) for tadpoles of each species separately.

3.3 Results

3.3.1 Invertebrate abundances

Invertebrate assemblages differed between Paluma and Tully, and between pools and riffles within each location (Table 3.1). Pools in the Paluma and Tully streams clustered separately, whereas the riffle assemblages were similar, regardless of stream (Figure 3.1). The overall invertebrate abundance was lower in the Tully streams, but species adapted to high current velocities were more common than at Paluma (Appendix 2.1). The abundances of invertebrate feeding groups also differed between location and between habitats within each location (Table 3.2). NMDS of the invertebrate feeding groups in the different locations and habitats showed three main clusters: the Paluma pools were similar to each other and clustered separately from the Paluma riffles, whereas the Tully sites clustered together (Figure 3.2). The pools in Tully Stream 2 were similar to the pools at Paluma. Shredders were more abundant in pools at Paluma than at Tully, while gatherers were more abundant in the Tully pools (Appendix 2.6). The riffles clustered separately between the two locations (Figure 3.2). The riffles at Paluma had greater numbers of filterers, whereas the Tully riffles had a greater abundance of grazers (Appendix 2.6).

Table 3.1. PERMANOVA results for location (Paluma and Tully) and habitat (riffle and pool) similarities based on the abundances of invertebrates. A nested design was applied, with (1) location and (2) habitat nested in location as factors. Square root transformations and Bray-Curtis similarities were used.

Source	Df	SS	MS	Pseudo-F	P(perm)	Unique perms
Location	1	43492	43492	87.088	0.0001	9957
Habitat (Location)	2	1.2211E5	61055	122.26	0.0001	9944
Residual	317	1.5831E5	499.41			
Total	320	3.2382E5				

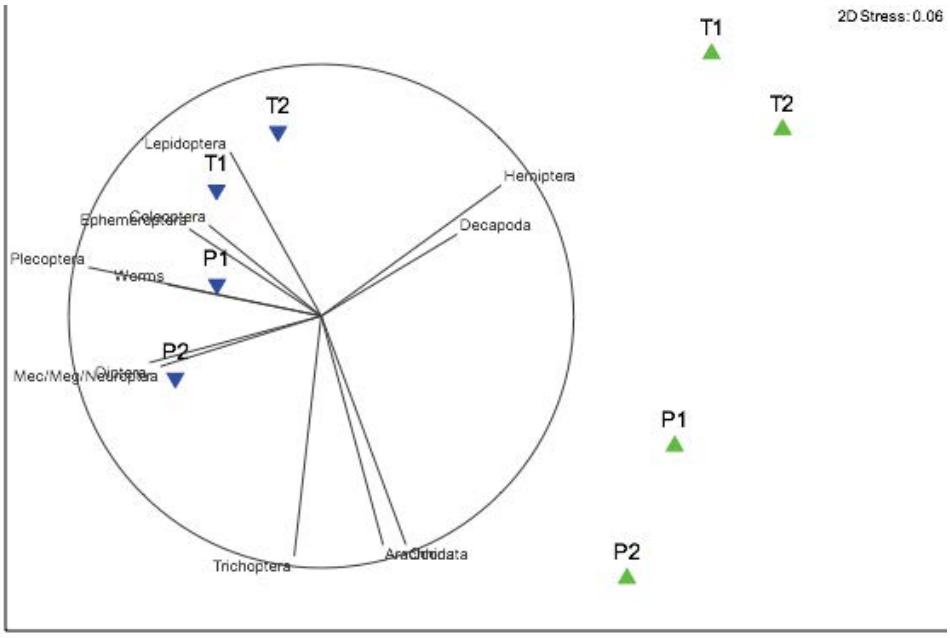

Figure 3.1. NMDS of location and habitat based on similarities among invertebrate assemblages, with vectors representing invertebrate orders. Square root transformations and Bray-Curtis similarities were used. Abbreviations: P1 = Birthday Creek, P2 = Camp Creek, T1 = Tully Stream 1, T2 = Tully Stream 2; symbols: green triangle = pool, blue triangle = riffle.

Table 3.2. PERMANOVA results for location (Paluma and Tully) and habitat (riffle and pool) similarities based on the abundances of invertebrate feeding groups. A nested design was applied, with (1) location and (2) habitat nested in location as factors. Square root transformations and Bray-Curtis similarities were used.

Source	Df	SS	MS	Pseudo-F	P(perm)	Unique perms
Location	1	22245	22245	70.171	0.0001	9938
Habitat (Location)	2	65892	32946	103.93	0.0001	9939
Residual	318	1.0081E5	317.01			
Total	321	1.8894E5				

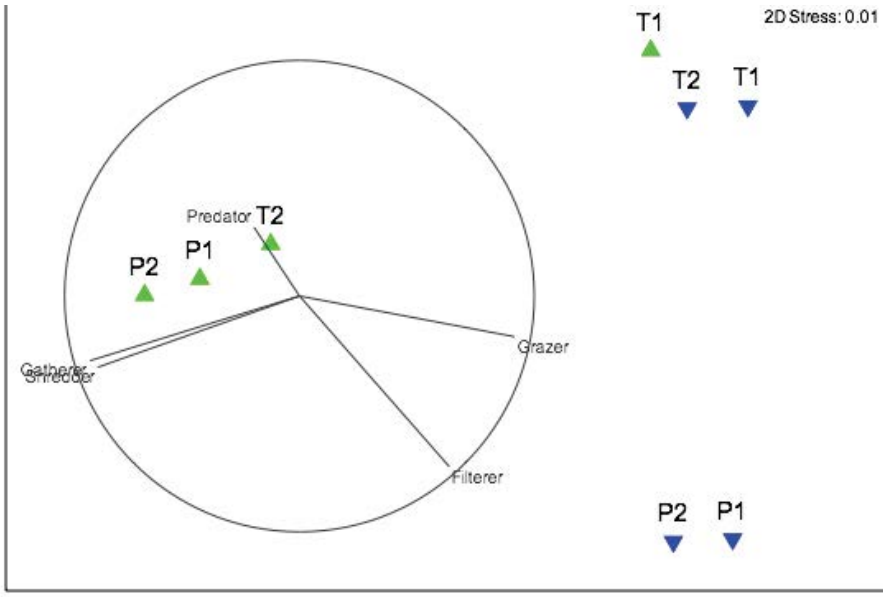

Figure 3.2. NMDS of location and habitat based on similarities among invertebrate assemblages, with vectors representing invertebrate feeding groups. Square root transformations and Bray-Curtis similarities were used. Abbreviations: P1 = Birthday Creek, P2 = Camp Creek, T1 = Tully Stream 1, T2 = Tully Stream 2; symbols: green triangle = pool, blue triangle = riffle.

Total invertebrate abundances fluctuated seasonally at Paluma and Tully and were generally lower during the winter months (Figure 3.3). Invertebrate abundances were higher in riffles than in pools at Paluma during most months, whereas abundances were more variable across habitats at Tully. Ephemeropteran larvae were the most abundant invertebrates at Paluma and Tully (Figure 3.4). In the Paluma streams, trichopterans, odonates and hemipterans were more abundant in pools than riffles, whereas ephemeropterans and dipterans were more abundant in pools. At Tully, ephemeropterans were abundant in both pools and riffles, whereas trichopterans and dipterans were most abundant in riffles, and hemipterans in pools.

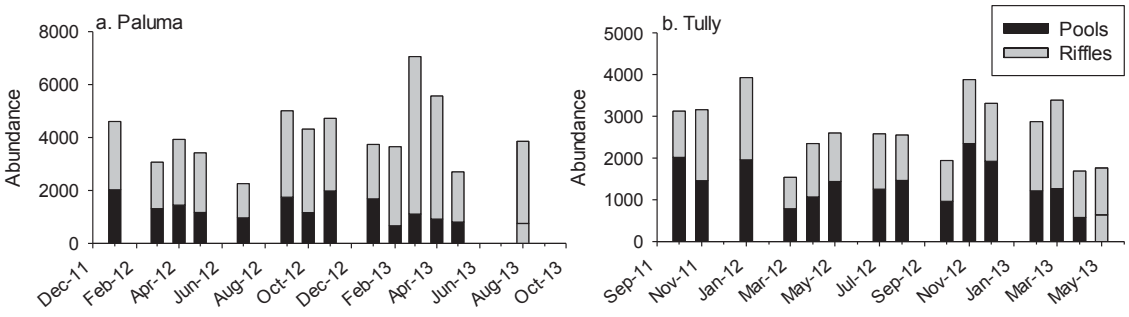

Figure 3.3. The seasonal patterns of total invertebrate abundance in pools and riffles at (a) Paluma and (b) Tully.

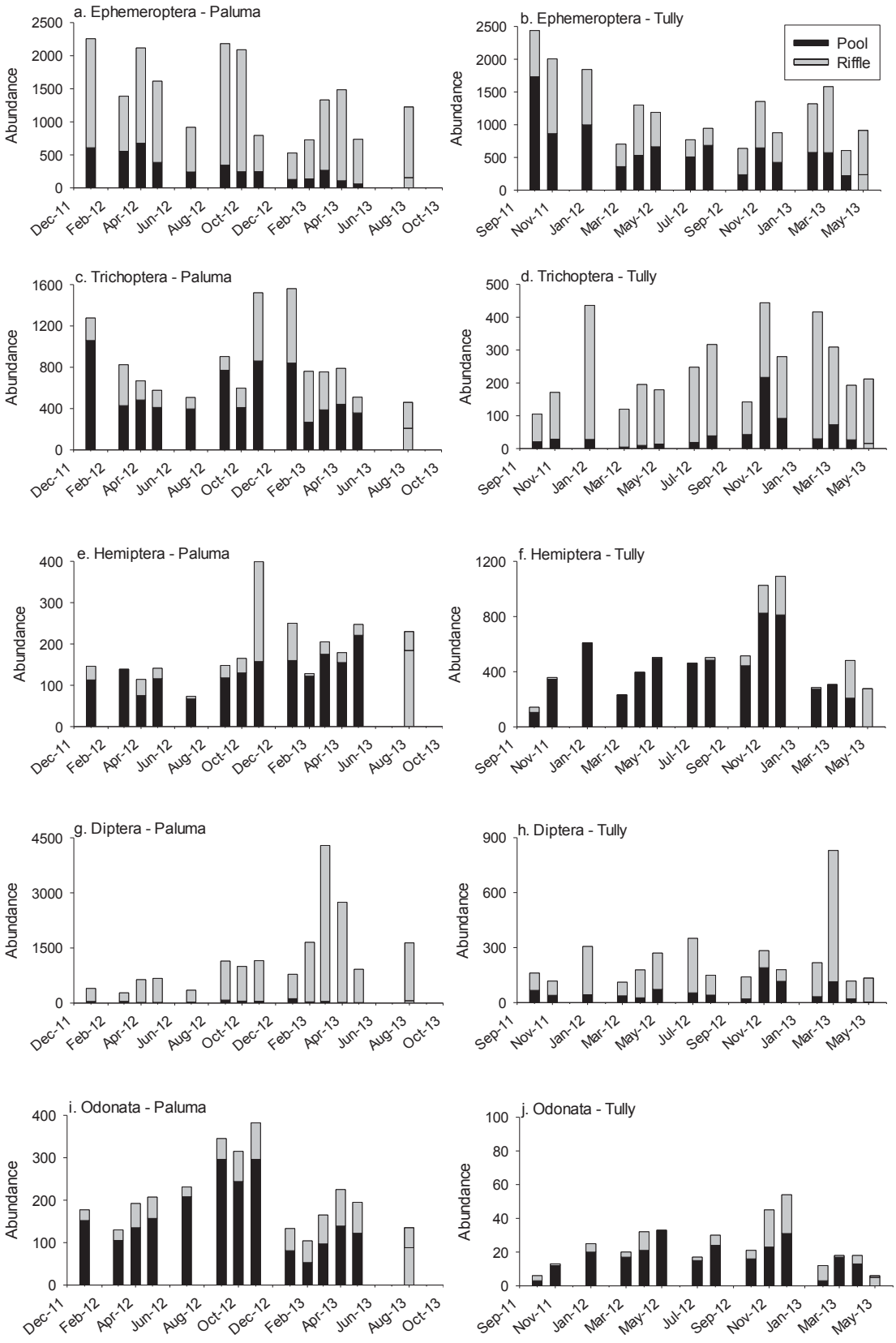

Figure 3.4. The seasonal abundance patterns of the most abundant invertebrate groups in pools and riffles at Paluma and Tully.

3.3.2 Relationships between invertebrate abundance and environmental variables

At Paluma, the best DistLM model explained 50% of the variation of total invertebrate abundance using seven environmental variables, excluding algal cover (Table 3.3a). Current velocity alone explained 40% of the variation in the abundance of the invertebrate assemblage. Combined with water temperature and 14-day antecedent rainfall, current velocity explained 46% of the variation. At Tully, the best model included all environmental variables except for leaf litter cover, and explained 47% of the variation (Table 3.3b). The best model with three environmental variables showed that 14-day antecedent rainfall, current velocity and canopy cover were most important in influencing the invertebrate assemblage, explaining 43% of the variation. Current velocity alone explained 38% of the variation at Tully.

Table 3.3. DistLM with model selection showing best model for each number of environmental variables at (a) Paluma and (b) Tully. *Best* selection procedure with AIC_c as the selection criterion was used to determine which environmental variables most influenced the invertebrate assemblage. Delta AIC_c was calculated as the change in AIC_c between the model being examined and the best (lowest AIC_c) model. RSS is the residual sum of squared deviations. Environmental variables: 1 = water temperature, 2 = 3-day rainfall, 3 = 7-day rainfall, 4 = 14-day rainfall, 5 = current velocity, 6 = canopy cover, 7 = leaf litter cover, and 8 = algal cover.

a

AIC_c	delta AIC_c	r^2	RSS	No. of variables	Variables selected
1027.1	17.8	0.40437	74129	1	5
1019.7	10.4	0.43695	70074	2	4,5
1015.5	6.2	0.45778	67482	3	1,4,5
1012.6	3.3	0.47393	65472	4	1,4,5,7
1010.4	1.1	0.48720	63821	5	1,4,5,6,7
1010.0	0.7	0.49517	62828	6	1,3,4,5,6,7
1009.3	0.0	0.50385	61749	7	1-7
1009.4	0.1	0.51006	60976	8	1-8

b

AIC_c	delta AIC_c	r^2	RSS	No. of variables	Variables selected
1144.1	16.7	0.37520	0.00001	1	5
1136.2	8.8	0.40965	0.00001	2	5,6
1133.0	5.6	0.42679	0.00001	3	4-6
1129.7	2.3	0.44436	98708	4	1,4,5,6
1128.7	1.3	0.45406	96984	5	1,4,5,6,8
1128.2	0.8	0.46230	95521	6	1,3,4,5,6,8
1127.4	0.0	0.47129	93922	7	1,2,3,4,5,6,8
1129.1	1.7	0.47281	93652	8	1-8

3.3.3 Invertebrate feeding groups and their relationships with tadpoles

Comparisons between tadpole and invertebrate biomass were made for each stream and habitat separately and showed varying degrees of correlation (Figures 3.5-3.7, Appendix 2.7). The biomass of filterers and grazers was low compared to that of tadpoles in the Paluma pools (Appendix 2.8) and there were no significant correlations between tadpoles and these invertebrate groups. Grazers and filter feeders were more abundant in riffles compared to pools at Paluma and Tully, whereas gatherers and shredders were more abundant in pools (Appendix 2.6). There was a significant positive relationship between tadpoles and predators (Spearman's rank correlation, $\rho = 0.648$, $P = 0.0155$; Figure 3.5b), and tadpoles and shredders ($\rho = 0.731$, $P = 0.0037$; Figure 3.5j) in Camp Creek at Paluma. Although there appeared to be a relationship between some invertebrate feeding groups and tadpoles for part of the year in the Paluma streams (Appendix 2.8), there were no other significant correlations.

Litoria serrata tadpoles at Tully contributed to most of the tadpole biomass in Stream 2 pools, but less so in Stream 1 (Appendix 2.9). There was a significant positive relationship between *L. serrata* tadpoles and filterers ($\rho = 0.746$, $P = 0.0009$), and between total tadpoles and filterers ($\rho = 0.658$, $P = 0.0074$) in the pools of Stream 1 (Figure 3.6c). There was also a significant positive relationship between *L. serrata* tadpoles and shredders ($\rho = 0.652$, $P = 0.0108$) and between total tadpoles and shredders ($\rho = 0.535$, $P = 0.0470$) in Stream 2 pools (Figure 3.6j). In Stream 1 riffles, total tadpoles had a significant positive correlation with gatherers ($\rho = 0.590$, $P = 0.0201$; Figure 3.7e) and near significant correlations with predators and grazers (Figure 3.7a and g). There were no significant negative relationships between tadpoles and invertebrates in any of the streams. The invertebrate and tadpole matrices at Tully were significantly correlated as shown by RELATE analysis (Table 3.4), specifically *L. nannotis* and *L. dayi* tadpoles.

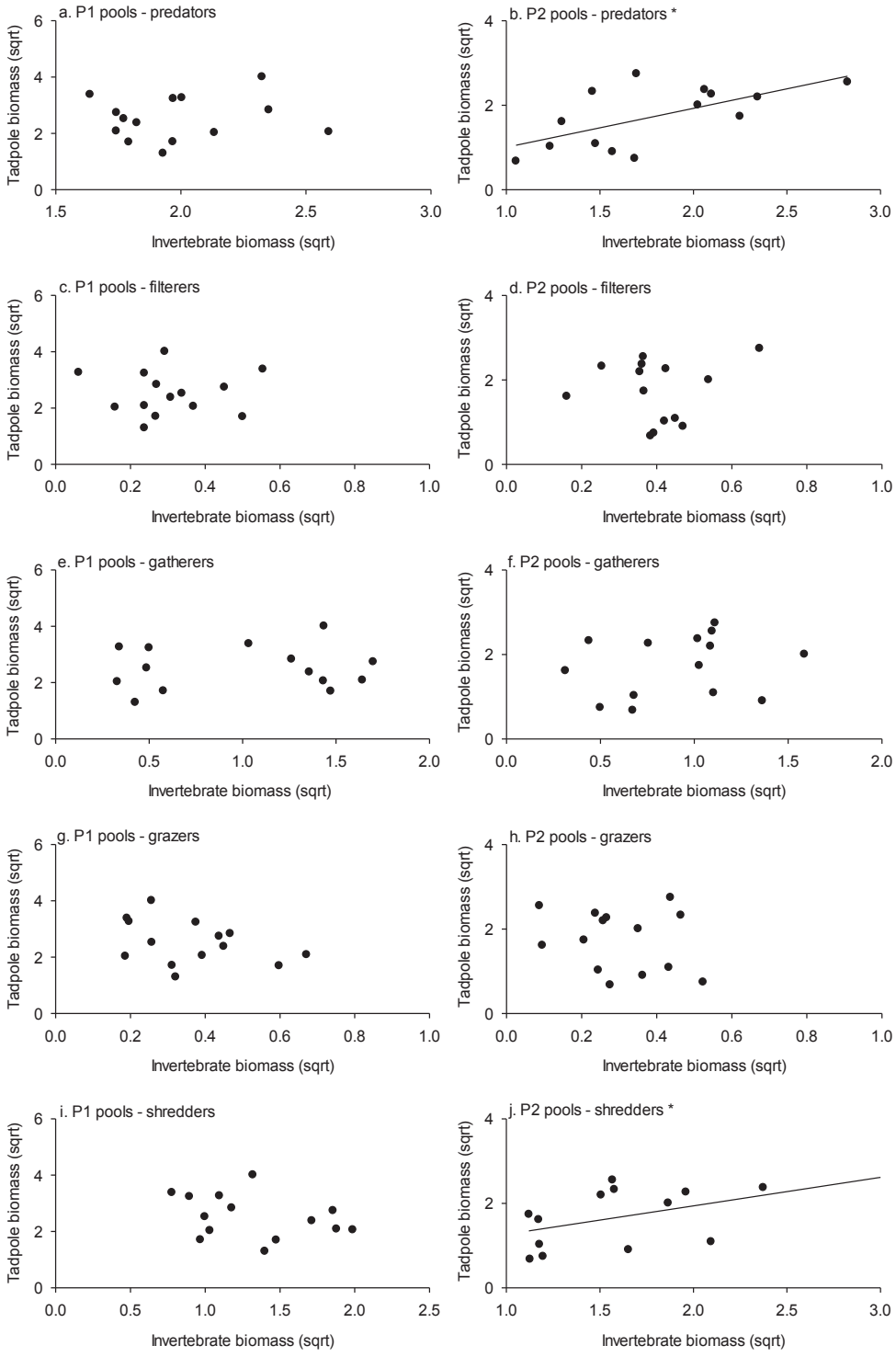

Figure 3.5. Linear regression of *Litoria serrata* tadpole biomass with invertebrate feeding group biomass in Birthday Creek (P1, left column) and Camp Creek (P2, right column) in pools at Paluma (using dry weight in mg). There was a significant positive relationship ($P < 0.05$) based on Spearman's rank correlation where indicated by *.

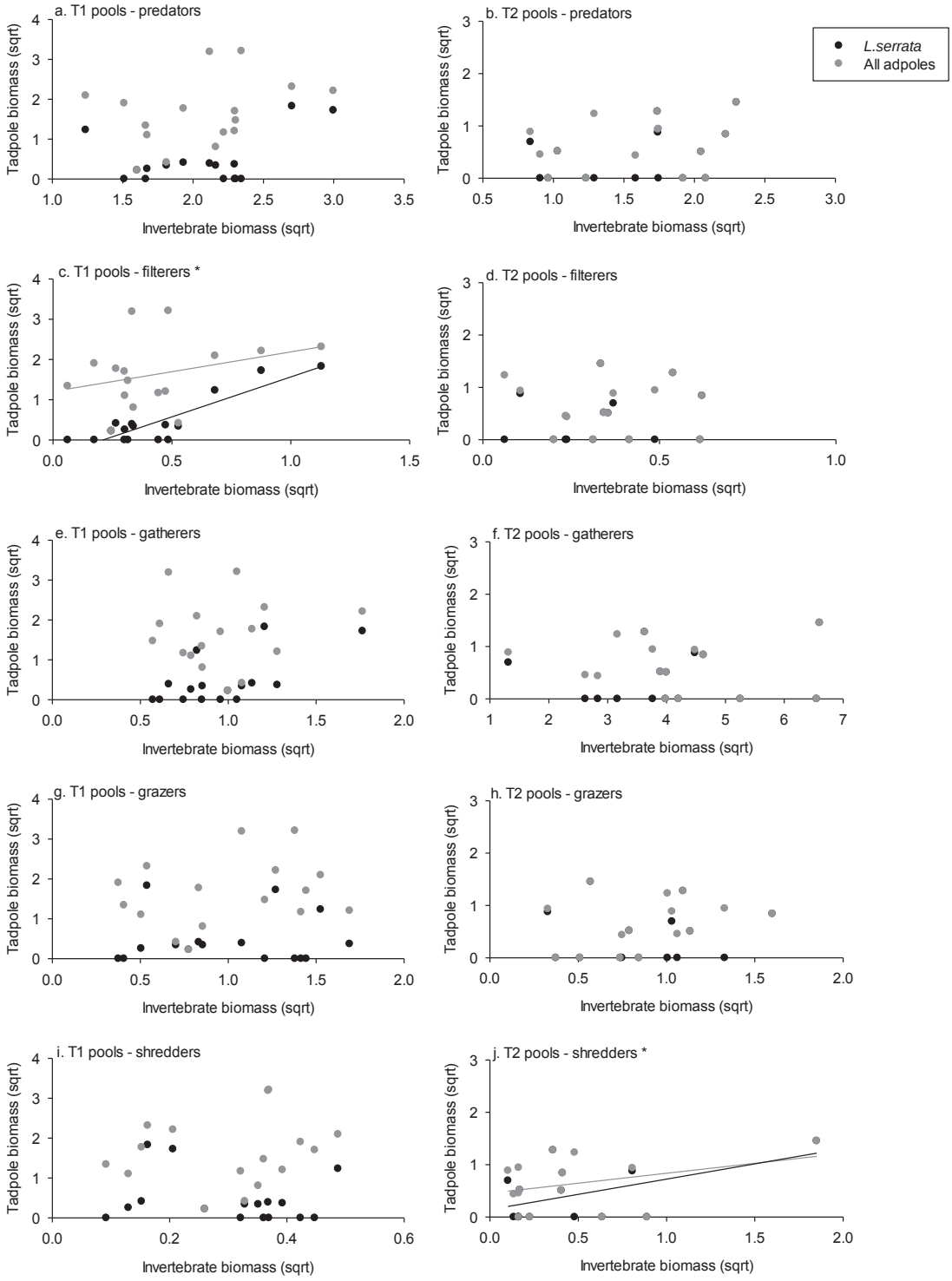

Figure 3.6. Linear regression of *Litoria serrata* tadpole and total tadpole biomass with invertebrate feeding group biomass in Stream 1 (left column) and Stream 2 (right column) in pools at Tully (using dry weight in mg). There was a significant positive relationship ($P < 0.05$) based on Spearman's rank correlation where indicated by *.

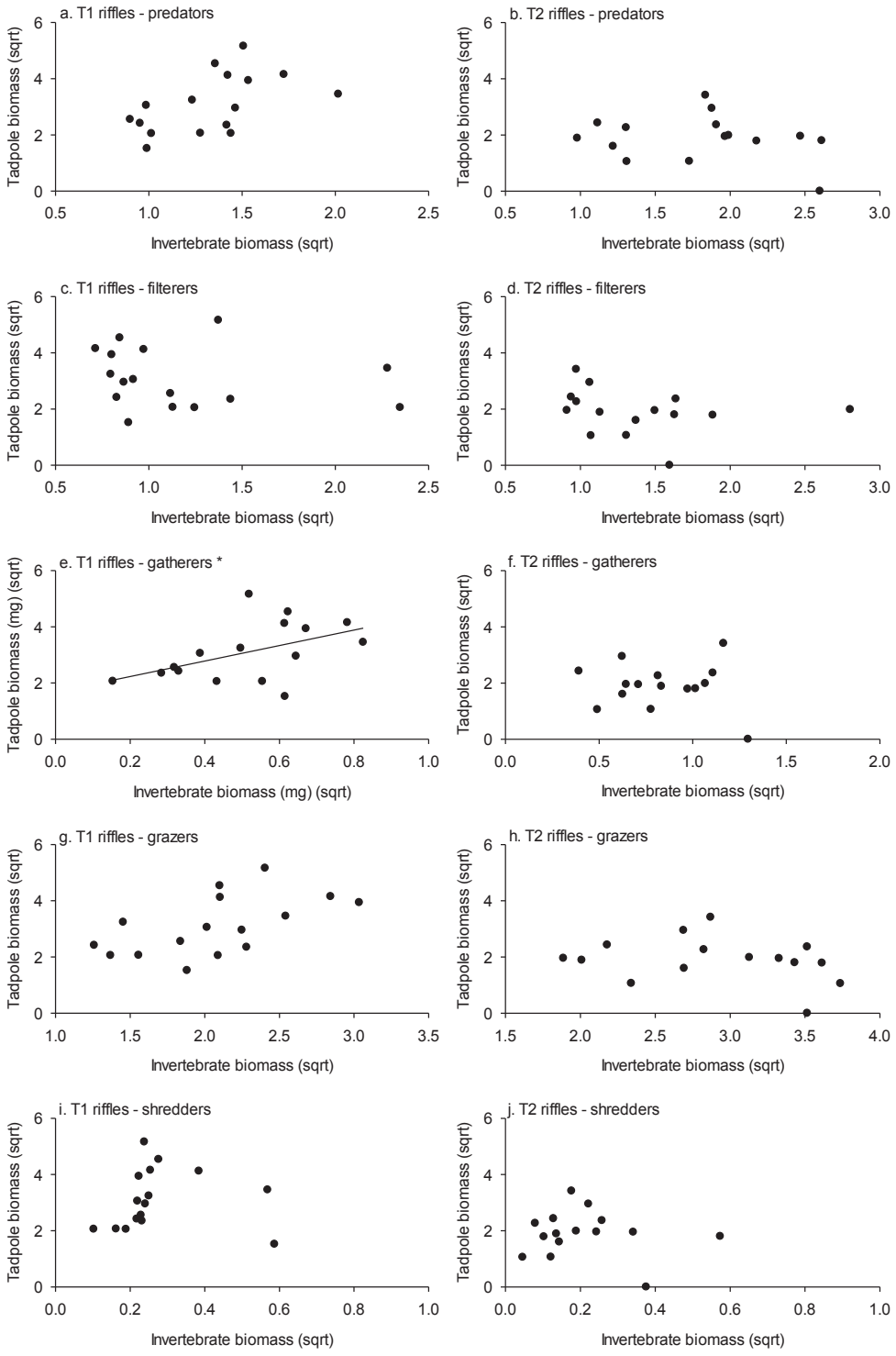

Figure 3.7. Linear regression of total tadpole biomass with invertebrate feeding group biomass in Stream 1 (left column) and Stream 2 (right column) in pools at Tully (using dry weight in mg). There was a significant positive relationship ($P < 0.05$) based on Spearman's rank correlation where indicated by *.

Table 3.4. RELATE results for all invertebrate groups using invertebrate and tadpole biomass (dry weight). At Tully the analysis was run for (i) all tadpoles combined and (ii) separately for the four species. A positive sample statistic (ρ) indicates a positive correlation. BEST results indicate which species were significantly correlated with the invertebrate assemblage.

Survey location	Invertebrate groups	Tadpoles	Test	Sample statistic (ρ)	P	BEST solution
Paluma	All groups	*L. serrata*	RELATE	0.073	0.1695	
Tully	All groups	Total tadpoles	RELATE	0.355	0.0001	
		Tadpoles separately	RELATE	0.548	0.0001	
			BEST	0.575	0.001	*L. dayi, L. nannotis*

3.4 Discussion

The invertebrate assemblages at Paluma and Tully differed in species composition and abundance, which was expected given the differences in stream characteristics. The abundances of different taxa in each location fluctuated differently between habitats, in ways that were correlated with physical factors. At Paluma, current velocity, temperature and rainfall were the most important environmental variables in explaining invertebrate abundances, whereas at Tully, the most important variables were current velocity, rainfall and canopy cover. In the Tully streams, the overall invertebrate abundance was lower, but species adapted to high current velocities were more common, than at Paluma. Most of the invertebrate feeding groups showed habitat preferences, which depended on morphology and resource availability: shredders and gatherers were more abundant in pools, whereas filterers and grazers were more abundant in riffles. Tadpoles and invertebrates mostly responded similarly to environmental variables, which may have influenced interactions such as predation, competition and facilitation between species in the streams. Where their relative abundances diverged, it appeared that rapid recruitment of tadpoles was the cause. Whether this caused increased competition with invertebrates was unclear.

Current velocity was the most important environmental influence on the invertebrate assemblages at Paluma and Tully, determining which taxa occurred in different stream sections. In turn, these effects on composition of assemblages probably influenced species interactions. Flow conditions are usually linked to rainfall, with periods of heavy rainfall leading to high current velocities and flooding (Flecker and

Feifarek 1994; Pearson et al. 2015). Such conditions decrease the abundance and diversity of benthic invertebrates due to animals being washed downstream (Bond and Downes 2003). The extent of this effect depends on in-stream habitat characteristics and the presence of flow refuges (Quinn and Hickey 1990; Bond and Downes 2003).

In both locations, 14-day antecedent rainfall was more important than short-term rainfall, probably because of the cumulative effect of rainfall on discharge, and the effects of prolonged strong flows. Long periods of heavy rainfall would have caused more invertebrates to be washed downstream and, although sites can be quickly recolonised following shorter spates (Flecker and Feifarek 1994; Rosser and Pearson 1995), large floods or prolonged high flows greatly increase the time for recolonisation (Pearson 2014). Although animals in pools are expected to be more susceptible to flood flows because they are not adapted to deal with strong currents, large spates probably cause animals from both habitats to be washed downstream (Pearson et al. 2015). The effect of heavy rainfall and increased current velocity may also differ among streams, depending on physical characteristics such as elevation and stream gradient (Flecker and Feifarek 1994).

In addition to current velocity and rainfall, water temperature was an important influence on the invertebrates at Paluma, whereas canopy cover was more important at Tully. Water temperature was linked to seasonal fluctuations in invertebrate abundances. Temperatures were lower during the winter months at Paluma, reducing invertebrate recruitment and abundance during this time (Benson and Pearson 1988), although some invertebrate groups were probably more tolerant of lower temperatures than others. The Paluma streams were generally well shaded, whereas the sites at Tully varied from relatively closed canopy cover to completely open. Canopy cover probably influenced invertebrate abundances indirectly, and its effect may depend on the season and on other environmental variables such as algal growth.

Invertebrates with different feeding modes depend on resources that may only be available in particular habitats. Shredders, for example, break down coarse particulate organic matter such as leaf litter, which is more abundant in pools (Wallace and Webster 1996; Cheshire et al. 2005). They were more abundant in pools at Paluma than at Tully, probably because of higher leaf accumulation at Paluma. The riffles at Paluma had greater numbers of filterers, whereas the Tully riffles had a greater abundance of grazers, indicating different resource availability, and reflecting the different light regimes.

Biomass of *L. nannotis* and *L. dayi* tadpoles was positively correlated with the biomass of invertebrates in the Tully streams, indicating that these species probably respond similarly to environmental variables. Tadpoles of both species are riffle specialists and they likely interacted with and influenced the invertebrates that shared the same habitat and food resources. Tadpole biomass in the Paluma streams was not significantly correlated with invertebrate biomass, and may therefore be independent of it. It is possible, however, that the tadpoles interacted directly with only certain invertebrate species, which would have not been obvious from the summed invertebrate data. There was no obvious temporal relationship between invertebrates and tadpoles, with all taxa appearing to follow individual trajectories.

Grazing invertebrates are functionally similar to tadpoles in that they scrape material from the surface of rocks (Cummins and Klug 1979; Alford 1999), but it is not known to what extent their functional roles overlap. When species are lost, the ability of the system to maintain ecosystem functions depends on the individual roles of the remaining species (Allan and Castillo 2007). One or more species of grazers at Paluma may have compensated for the absence of riffle tadpoles, but biomass data could not confirm this because no pre-decline information was available. Also, there was no evidence of interactions between the invertebrates and tadpoles present during this study. However, invertebrates are typically much smaller than tadpoles, and they may not replace tadpoles in their effects, for example, on bioturbation (Whiles et al. 2013). At a stream in Panama, there were no signs of functional redundancy eight years after amphibian declines (Rantala et al. 2014), and similarly in the Paluma streams, there may have been a permanent change in the assemblage structure and stream functioning following the disappearance of riffle-dwelling tadpoles.

Grazing invertebrates were more abundant at Tully, where the rocks were more exposed to light than at Paluma, which would encourage biofilm and algal growth (Murphy et al. 1981), supporting a greater grazer biomass. They may also have benefitted from the presence of tadpoles in riffles, as there was a positive relationship (marginally non-significant) between tadpoles and grazing invertebrates in Tully Stream 1. Although tadpoles may compete for the same resources, they can encourage invertebrate feeding by removing sediments through bioturbation (Ranvestel et al. 2004). Rugenski et al. (2012) found that biofilm production increased in the presence of tadpoles, and this may have occurred in the Tully streams. The loss of tadpoles can also cause the biofilm composition to change to predominantly larger diatoms, which may be

unsuitable for small grazers (Rantala et al. 2014). Therefore, grazers in the Paluma riffles may have had restricted access to consumable biofilm due to the absence of tadpoles. The bioturbation effect of tadpoles on invertebrates could be tested in future studies.

Facilitation among detritivorous stream invertebrates may occur between species or between whole functional groups, for example gatherers (collectors) and shredders (Heard and Richardson 1995). Gatherers typically feed on organic material on the substratum, which may consist of broken-down leaves and fine detrital material such as that produced by shredders (Cummins and Klug 1979). Pool tadpoles probably also feed on accumulated material on the substratum. In Camp Creek, *L. serrata* tadpoles and shredding invertebrates followed the same abundance trends, either because they directly influenced each other, such as during facilitation, demonstrated experimentally in Birthday Creek (Iwai et al. 2009), or because they responded to the same environmental influences.

There was no evidence for an effect of competition between tadpoles and invertebrates on the biomass of either based on the seasonal fluctuations, but it may have occurred for a few months a year when tadpole abundances were high. For example, in Birthday Creek the biomass of gathering invertebrates decreased as tadpole biomass increased during the second part of the survey period. At Tully, the gatherer biomass was high in Stream 2 pools, possibly because the low tadpole abundance reduced competition for resources. In Panama, gatherer populations increased in abundance eight years following tadpole loss, whereas filter-feeder, grazer and shredder populations decreased (Rantala et al. 2014), indicating that gathering invertebrates and tadpoles compete for either space or food for at least part of the year. More targeted experimental studies would need to be carried out to test for the prevalence of predation between tadpoles and various invertebrate predators.

There was no direct relationship between predatory invertebrates and tadpoles and any correlation between them was probably a result of similar responses to environmental factors. However, disappearance of tadpoles from an assemblage might affect predatory invertebrates indirectly. Small prey species may not be as abundant following tadpole loss, leading to a shift from smaller to larger predator species (Colon-Gaud et al. 2010b). Large predatory dragonfly larvae were common in Paluma riffles, but these were also present before riffle tadpoles disappeared (S. Richards, pers. comm.) and there is no evidence that their abundance increased since the frog declines.

In summary, tadpoles and several invertebrate feeding groups showed similar patterns of abundance (biomass), but it was not clear whether this was due to facilitation or to similar responses to environmental influences. Some invertebrate species may interact with tadpoles through similar habitat or resource requirements, but these interactions are only likely to be important for part of the year when abundances are high. Both the invertebrate and tadpole assemblages showed strong responses to current velocity, rainfall and water temperature, indicating that invertebrates and tadpoles of different species are most active during the same time of the year. In the case of tadpole loss, it is possible that trophically similar invertebrates in these streams replace tadpoles in their effects during the warmer months when tadpole activity would have been most important, but may not have equivalent influence on bioturbation. There was no compelling evidence for strong ecological interactions, positive or negative, between tadpoles and invertebrates. There may have been some weaker relationships, but this was difficult to demonstrate in the field. If present, they were likely minor compared to the influence of physical factors such as flow and season.

4. The role of tadpoles in leaf litter processing and sediment removal

4.1 Introduction

Tadpoles of different anuran species have specific food resource and habitat requirements, and may therefore have different functional roles in streams. The functional contribution of tadpoles and other aquatic organisms, together with flow and catchment features, influence stream structure and function (Boulton and Brock 1999). However, stream processes do not necessarily rely on a specific species to carry out a function, as long as the aquatic assemblage as a whole maintains the functional integrity of the ecosystem (Gessner and Chauvet 2002). Therefore, the importance of an animal group, such as the tadpoles of a particular species, is best assessed by studying its contribution to stream processes. Changes in stream processes reflect on stream condition, and are indicators of changes in the environment (Gessner and Chauvet 2002).

Leaf litter breakdown is an important stream process in rainforest streams (Gessner and Chauvet 2002). These streams are usually shaded, with limited primary production, and therefore rely largely on heterotrophic nutrient sources in the form of organic material (Graça 2001). The majority of nutrients entering forest streams come from leaf litter, making litter decomposition a good indicator process to assess stream functioning (Cummins 1974; Gessner and Chauvet 2002; Cheshire et al. 2005). Leaf processing in streams relies on microbial and invertebrate activities, as well as on physical abrasion (Graça 2001), but tadpole activity may also influence this process to some extent (Iwai et al. 2009).

Shredding invertebrates break down coarse particulate organic matter, such as leaf litter, and ensure that terrestrial input becomes available to other aquatic consumers. Shredders include species from various taxonomic groups, such as trichopterans, ephemeropterans, coleopterans, amphipods, gastropods and decapods (Cheshire et al. 2005; Yule et al. 2009; Boyero et al. 2012). These may be influenced by the activities of other stream organisms, such as microorganisms or tadpoles. Shredders prefer leaf litter that has been colonised by bacteria or fungi, which partially degrade the organic material during conditioning (Cummins and Klug 1979; Graça 2001). Tadpoles

may contribute to leaf processing through nutrient regeneration (Iwai et al. 2012), whereby they convert organic material to inorganic material through their feeding activities. The resultant nutrients boost microbial growth, increasing the nutrient quality of the leaves for invertebrates, and therefore increasing leaf breakdown rates (Iwai et al. 2012). This kind of interaction is known as facilitation.

Facilitation between organisms occurs when one or both benefit from their interaction without causing harm to either (Stachowicz 2001). Mutualism or two-way facilitation is when both organisms benefit from the interaction, and this may enhance ecosystem processes beyond the simple cumulative effect of the individual species (Iwai et al. 2012). Facilitation between tadpoles and invertebrates may occur in the direction of tadpoles to invertebrates, such as during nutrient regeneration (Iwai et al. 2012). One-way facilitation could also occur in the direction of invertebrates to tadpoles through physical breakdown of the leaves. In Birthday Creek, for example, leaf processing by shredders enabled tadpoles to feed on the smaller leaf fragments (Iwai et al. 2009). It is not known whether this occurs with other frog species in rainforest streams and whether the effect could be two-way with other species.

Facilitation may also occur between tadpoles and other invertebrate feeding groups such as grazers. Thus, in Panama, the loss of tadpoles led to a decline in grazer and detritivore abundances (Hunte-Brown 2006; Whiles et al. 2006; Colon-Gaud et al. 2009). Tadpoles may remove sediments during their foraging activities, uncovering periphyton and encouraging smaller grazers such as mayflies to feed (Ranvestel et al. 2004). In this way, tadpoles facilitate a group of invertebrates by increasing the availability of their food source.

Tadpoles may have different effects on aquatic organisms and stream functioning depending on the species' identity, their resource requirements, and abundances. If tadpoles are the dominant consumers, they may control invertebrates via competition or disturbance (Colon-Gaud et al. 2009), and may compete with each other (Flecker et al. 1999; Kim and Richardson 2000). However, tadpoles can also increase the populations of other consumers via facilitation (Colon-Gaud et al. 2010a). Tadpoles of different species may have different roles in the stream and those in similar habitats could play different roles in stream functioning.

Tadpoles occupy diverse habitats in streams, depending on their morphology: some species are adapted to living in fast-flowing waters whereas others live in slow-flowing runs or stream pools (Boulton and Brock 1999; Hoskin and Hero 2008). In the

Wet Tropics, tadpoles of two species, *Litoria serrata* and *Mixophyes coggeri*, live in pools, but occupy different microhabitats (Trenerry 1988). The roles of these two species in stream functioning may differ and, consequently, it could be important to consider species differences when evaluating the role of tadpoles and the potential effects caused by amphibian declines. However, while it is known that tadpoles can be extremely abundant during certain parts of the year in streams of the Queensland Wet Tropics, their contribution to stream functioning has not been clearly defined.

The role of tadpoles and their contribution to stream processes needs to be understood to assess the consequence of amphibian declines on stream systems. The effect of tadpoles on assemblage composition, food web structure and stream functioning in relation to other stream-dwelling organisms has mainly been studied in pre- and post-decline streams in the Neotropics (Ranvestel et al. 2004; Whiles et al. 2006; Colon-Gaud et al. 2009). There is limited knowledge on the role of tadpoles and the consequence of amphibian declines in other regions and on different species. In this Chapter, I investigated the effect of tadpoles and invertebrates on leaf litter breakdown and sediment removal using two experiments. The aim of these experiments was to understand the effects of: (1) tadpoles of different anuran species, (2) interactions between tadpoles and invertebrates (facilitation and competition), (3) tadpole density, and (4) plant species. The results of these experiments will provide information on the contribution of tadpoles to basic stream processes and give an indication on how important this contribution is for different species.

4.2 Methods

4.2.1 Artificial stream mesocosms

Two experiments were carried out in artificial stream channels located beside Birthday Creek at Paluma Range National Park, in the Wet Tropics (-18.98°, 146.17°, see Pearson and Connolly 2000). At the study site, Birthday Creek is a second order stream. Water was fed from above a small waterfall via a pipe into a header tank, which supplied 20 channels, at the foot of the fall (Figure 4.1). Each channel was 2.4 m long and 15 cm wide and was divided into three chambers (top, middle and bottom). The inlet to the header tank was covered with 1 mm mesh to prevent clogging by plant material. Separators with 63 μm mesh were placed at the upstream and downstream

ends of each channel to prevent fine suspended material from being washed into and out of the experimental chambers, and 1-mm-mesh dividers separated the three chambers. No measurements were taken from the top chambers; they served as a collection space for sediment that entered the system though the dividers, preventing the influx of sediment to the animal treatment chambers. The entire set of channels was covered with 1-mm-mesh cloth to prevent plant material from entering the channels. Temperature data-loggers (Thermochron® iButtons) were placed in three of the channels, and programmed to measure temperature every hour.

a.

b.

Figure 4.1. Artificial stream channels: (a) channels and header tank with water inlets, and (b) chambers containing leaf bags, tiles and sediment dishes.

4.2.2 Experiment 1: The effect of tadpoles on leaf breakdown and sediment accumulation

Experiment 1 investigated the effect of tadpoles and invertebrates on the amount of leaf breakdown and sediment accumulation. Tadpoles of two frog species that occur at Paluma were used: *Litoria serrata* and *Mixophyes coggeri*. As the wet biomass ratio of *M. coggeri* to *L. serrata* was approximately 4:1, for each *M. coggeri* tadpole, four *L. serrata* tadpoles were used to ensure similar total biomass of each species. Tadpole body lengths were measured from photographs taken next to a scale and the animals were weighed using a digital balance (0.1 g). All tadpoles were at Gosner stages 25-30 (Gosner 1960). The invertebrates were larvae of three caddisfly and one mayfly species: *Anisocentropus kirramus* (Calamoceratidae), *Lectrides varians* (Leptoceridae), *Triplectides gonetalus* (Leptoceridae), and *Atalophlebia* sp. (Leptophlebiidae). The caddisflies are shredders and the mayfly is a scraper and generalist shredder (Cheshire et al. 2005). There were six animal treatments, including one control, and each was replicated three times (Figure 4.2, Table 4.1).

Only the middle chambers of the channels contained animals. The purpose of these animal treatments was to investigate how tadpoles and invertebrates directly affected the leaves and sediments. The downstream effects of the animals on leaf material and fine particulate organic matter were measured in the bottom chambers. Treatments were allocated to 3 sets of 6 channels, each containing one replicate per treatment. Treatment locations within each set were randomized, subject to the constraint that the same treatment never ended up next to itself in the adjacent set.

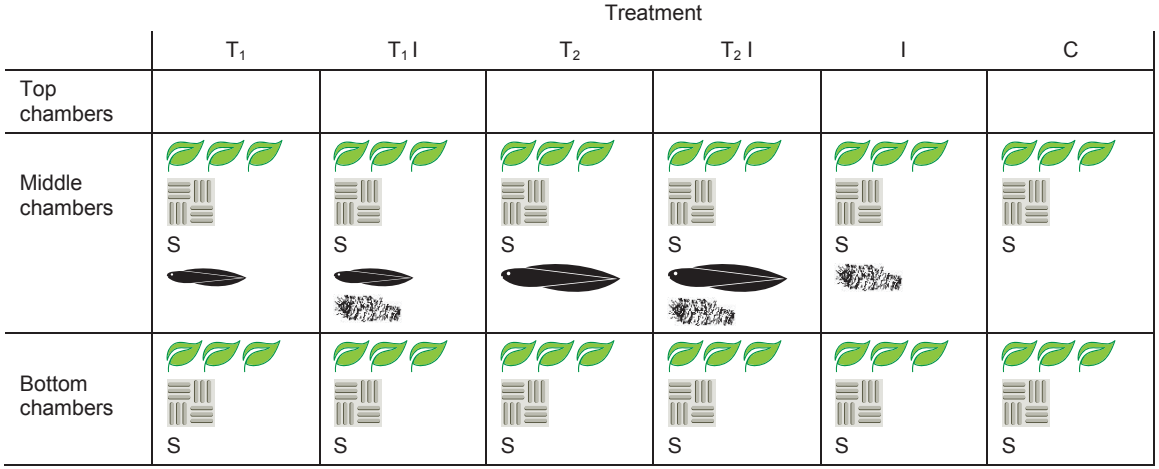

Figure 4.2. Experimental set-up showing one replicate of each animal treatment. Symbols: = leaves of 1 plant species (three leaves indicate three plant species: *Apodytes brachystylis*, *Endiandra bessaphila* and *Cryptocarya leucophylla*), = tile, S = sediment, (small) = *Litoria serrata* tadpoles, (large) = *Mixophyes coggeri* tadpoles, = invertebrates. Treatment codes represent tadpoles (T_1 and T_2), invertebrates (I) and controls (C) according to Table 4.1.

Table 4.1. Experimental design: five animal treatments and one control, with three replicates each (middle chambers). The animals were: Tadpole sp.1 = *Litoria serrata*, Tadpole sp.2 = *Mixophyes coggeri*, Invertebrates = three caddisfly and one mayfly species, as indicated.

Code	Animal treatment	Species and numbers
T1	Tadpole sp. 1	8 *L. serrata*
T1+I	Tadpole sp. 1 + Invertebrates	8 *L. serrata*, 1 *Anisocentropus kirramus*, 1 *Lectrides varians*, 1 *Triplectides gonetalus*, 1 *Atalophlebia* sp.
T2	Tadpole sp. 2	2 *M. coggeri*
T2+I	Tadpole sp. 2 + Invertebrates	2 *M. coggeri*, 1 *Anisocentropus kirramus*, 1 *Lectrides varians*, 1 *Triplectides gonetalus*, 1 *Atalophlebia* sp.
I	Invertebrates only	1 *Anisocentropus kirramus*, 1 *Lectrides varians*, 1 *Triplectides gonetalus*, 1 *Atalophlebia* sp.
C	Control	No animals

Leaves were provided for the animals as a potential food source and to measure leaf breakdown by tadpoles and invertebrates. Leaves of three common riparian plant species that are favoured by shredders (Bastian et al. 2007) were selected: *Apodytes brachystylis*, *Endiandra bessaphila* and *Cryptocarya leucophylla*. Green leaves were collected to ensure they were the correct species, and because they are frequently found in stream litter packs following rain or strong winds and are consumed by shredders (Benson and Pearson 1993; Nolen and Pearson 1993). The leaves were oven-dried for 48 hours at 60°C and 2 g of each species were weighed into separate 1-cm-mesh bags. The leaf bags were conditioned in the channels for 16 days and then randomly placed into the middle and bottom chambers, so that each chamber contained three leaf bags (2 g of each species). Sediment from the stream, provided as a second food source and to measure sediment removal, was collected by stirring up the substratum and filtering the suspended material through a 1 mm sieve. A petri dish filled with the wet filtrate (approximately 25 g dry weight) was placed into each chamber. One 10 cm x 10 cm unglazed terracotta tile was placed in each chamber to provide a fixed area for biofilm growth.

The experiment commenced on 11th October 2012 and ran for 42 days. It was monitored weekly to ensure proper water flow. Any missing or metamorphosed tadpoles or invertebrates were replacement to ensure that the number of animals in each

treatment was constant for the duration of the experiment; replacement individuals were collected from the stream as needed. At the end of the experiment, the tadpoles from each channel were removed and individuals were again photographed for measurements and weighed before releasing them back into the stream, whereas the invertebrates were counted and released. The leaf bags were removed and placed into separate zip-lock bags. The organic material on the tiles was scrubbed with a brush and rinsed into plastic jars using stream water, and sediment that had accumulated in the chambers was collected using 63-µm-mesh nets and rinsed into jars. The samples were stored on ice and frozen later the same day. In the laboratory, the biofilm and sediment samples were sorted to remove invertebrates and oven-dried at 60°C until dry, weighed and ashed in a muffle furnace at 550°C to obtain ash free dry weight (AFDW). The leaves were rinsed, invertebrates were removed, and the leaves were then were similarly oven-dried and ashed to obtain AFDW. Only *A. brachystylis* and *E. bessaphila* leaves were ashed (*C. leucophylla* leaves were dried and kept for potential nutrient analysis).

4.2.3 Experiment 2: Tadpole density effects on leaf breakdown and sediment accumulation

Experiment 2 investigated the effect of tadpole density on leaf breakdown and sediment accumulation. Only the middle chambers were used (Figure 4.3) and were separated from the bottom chambers by 63-µm-mesh separators. *Cryptocarya leucophylla* leaves were dried as described above, and 6 g were weighed into 1-cm-mesh bags. The leaves were conditioned for two weeks and then randomly placed into the middle chambers, along with a petri dish containing sediment from the stream (approximately 25 g dry weight). Each chamber contained equal numbers of caddisfly larvae: 2 *Anisocentropus kirramus*, 2 *Lectrides varians* and 1 *Triplectides gonetalus*. *Litoria serrata* tadpoles were collected from Birthday Creek and placed in the animal treatment chambers according to Table 4.2. The tadpoles were again weighed and measured before the experiment.

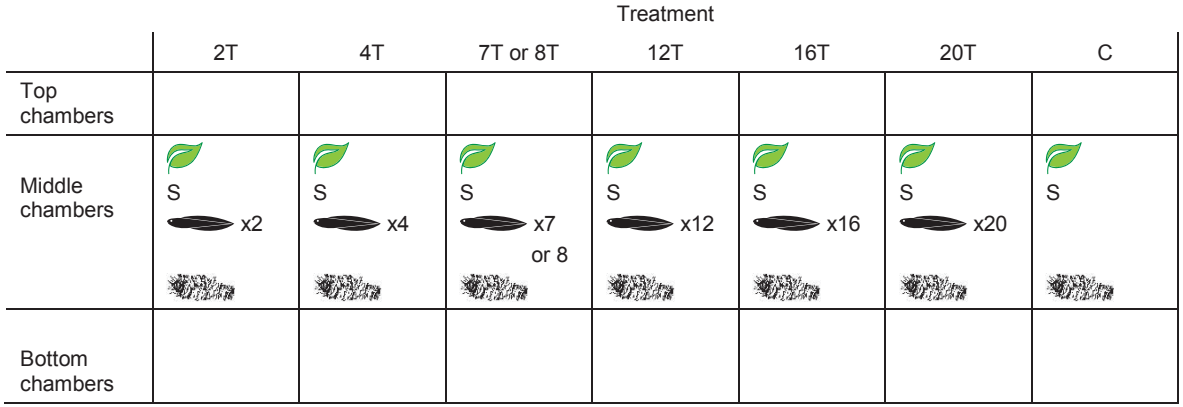

Figure 4.3. Experimental set-up showing one replicate of each animal treatment. Symbols: = *Cryptocarya leucophylla* leaves, S = sediment, = *Litoria serrata* tadpoles, = invertebrates. Treatment codes represent the number of tadpoles: 2T = 2 tadpoles, 4T = 4 tadpoles, etc.

Table 4.2. The number of *Litoria serrata* tadpoles and replicates per animal density treatment.

Animal treatment	C	2T	4T	7T	8T	12T	16T	20T
Number of tadpoles	0	2	4	7	8	12	16	20
Number of replicates	2	3	3	1	2	3	3	3

The experiment commenced on 14 December 2012 and ran for 25 days. Flow was checked regularly, but no animals were replaced, as the previous experiment indicated that invertebrate numbers would remain constant and that tadpoles were unlikely to metamorphose within this period. On finishing the experiment, the tadpoles were weighed and measured and, along with the invertebrates, released. Leaves and sediment were collected, and laboratory analysis was carried out as described for Experiment 1.

4.2.4 Statistical analysis

Data were analysed using one- or two-way ANOVAs followed by Tukey's post-hoc tests, and by linear regression analysis. Comparisons of changes in mean percentage leaf weights, sediment and biofilm AFDW and percentage tadpole biomass (wet weight) across animal treatments were made using one- or two-way ANOVAs and Tukey's tests, or a one-tailed t-test. The biofilm that accumulated on the tiles could not be separated from the sediment, so the two were analysed together. The relationship between tadpole abundance and percentage leaf weight or sediment AFDW was analysed using linear regression analysis. For Experiment 2, three channels were excluded from the analysis because at the end of the experiment one channel had dried out due to blockage in the inlet pipe, and 50% or more tadpoles were lost from two other channels. All analyses were carried out in SigmaPlot Version 12.5 and S-Plus Version 8.2.

4.3 Results

4.3.1 Experiment 1: Leaf breakdown and sediment accumulation

There was a strong plant species effect on leaf breakdown, with the leaf weights remaining significantly different for the three plant species (two-way ANOVA, $F_{2,36} = 100.14$, $P < 0.001$; Figure 4.4). There was also an animal treatment effect, with the invertebrate-containing treatments having significantly lower remaining leaf weights for all plant species than the tadpole-only treatments or the controls, regardless of anuran species ($F_{5,36} = 121.69$, $P < 0.001$; Figure 4.4). There was a significant interaction between animal treatment and plant species ($F_{10,36} = 9.11$, $P < 0.001$), indicating that the effects of the animals on the amount of leaf weight remaining differed depending on the plant species.

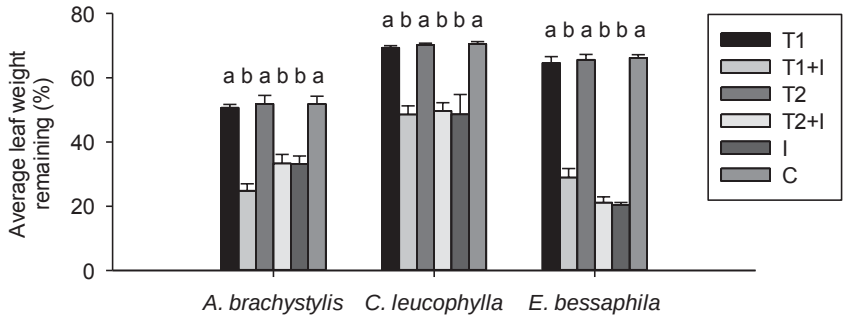

Figure 4.4. Average leaf weight remaining (mean ± s.e.) as percentage of original weight for three plant species in the treatment (middle) chambers. Significant differences between the animal treatments within plant species (indicated by Tukey's post-hoc tests, with α = 0.05) are shown by letters a and b. Treatment abbreviations: T1 = *Litoria serrata*; T2 = *Mixophyes coggeri*; I = invertebrates; and C = control.

With *M. coggeri* tadpoles present (T2 and T2+I), significantly less organic sediment material remained in the middle chambers compared to the other treatments, regardless of whether invertebrates were present or not (one-way ANOVA, $F_{5,12} = 45.01$, $P < 0.001$, Figure 4.5). When invertebrates were present with either anuran species, mean sediment AFDW was higher than without invertebrates, but the differences were not significant. The invertebrate treatment (I) had the highest mass of organic material remaining in the animal treatment chambers at the end of the experiment. There was also an animal treatment effect on AFDW in the bottom

chambers (one-way ANOVA, $F_{5,12} = 17.47$, $P < 0.001$). The *M. coggeri* and invertebrate combination (T2+I) and the *M. coggeri* only (T2) treatment left significantly more organic material in the bottom chambers than most other treatments (Figure 4.5). The sediment in the control channel likely came from the header tank through the divider.

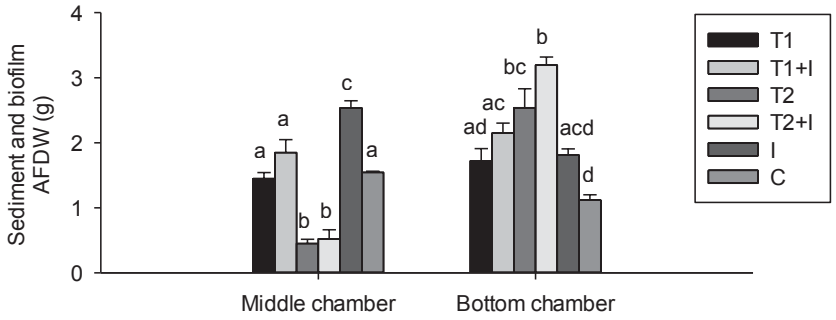

Figure 4.5. Sediment and biofilm AFDW (g) accumulation (mean ± s.e.) in the treatment (middle) and bottom chambers. Significant differences among animal treatments within chambers (indicated by Tukey's post-hoc tests, with α = 0.05) are shown by letters a – e. Treatment abbreviations: T1 = *Litoria serrata*; T2 = *Mixophyes coggeri*; I = invertebrates; and C = control.

Litoria serrata and *M. coggeri* tadpoles in the tadpole-only (T) and tadpole + invertebrate (T+I) treatments lost biomass in this experiment (Figure 4.6). The biomass loss for *M. coggeri* tadpoles was significantly greater in the treatment with invertebrates compared to the tadpole-only treatment (two-tailed t-test, $P = 0.0045$). *Litoria serrata* showed the same trend but the results were not significant (two-tailed t-test, $P = 0.152$).

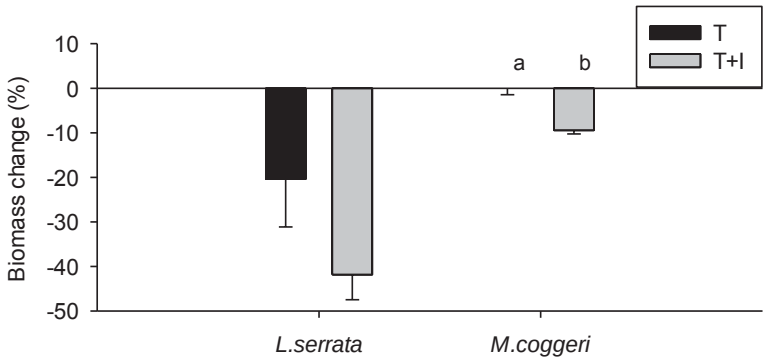

Figure 4.6. Percentage biomass change for *Litoria serrata* and *Mixophyes coggeri* tadpoles with invertebrates (T+I) and without invertebrates (T) during the experiment. A significant difference between treatments for *M. coggeri* tadpoles is shown by the letters a and b.

4.3.2 Experiment 2: Tadpole density effects

The amount of leaf litter broken down by the animals increased with tadpole density (linear regression, $F_{1,15} = 22.26$, $P < 0.001$; Figure 4.7a), but tadpole density did not have an effect on sediment accumulation ($F_{1,15} = 0.193$, $P = 0.666$; Figure 4.7b).

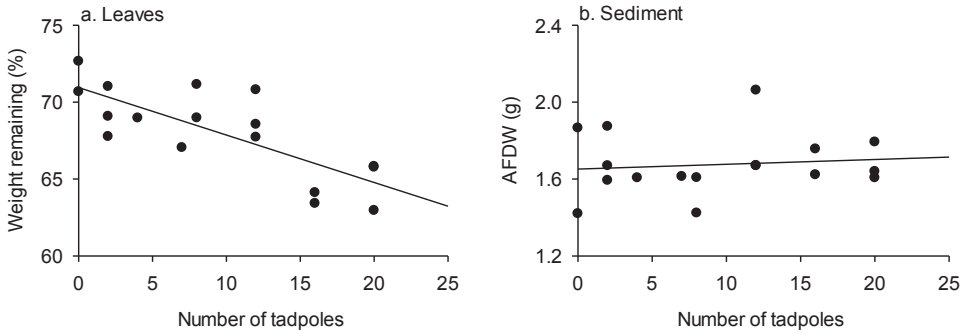

Figure 4.7. The percentage leaf weight (a) and sediment AFDW (b) remaining in 17 channels, plotted against numbers of *Litoria serrata* tadpoles at the start of the experiment. Lines of best fit are included: (a) $r^2 = 0.597$, $P < 0.001$, and (b) $r^2 = 0.013$, $P = 0.666$.

Average total gain in tadpole biomass was compared among the animal treatment densities (Figure 4.8). The tadpoles in the low density treatments with two or four tadpoles per chamber gained significantly more biomass than the treatments with higher densities (one-way ANOVA, $F_{5,119} = 48.60$, $P < 0.001$). Tadpoles in the treatment with 20 individuals lost biomass and this difference was significant when compared to all other treatments.

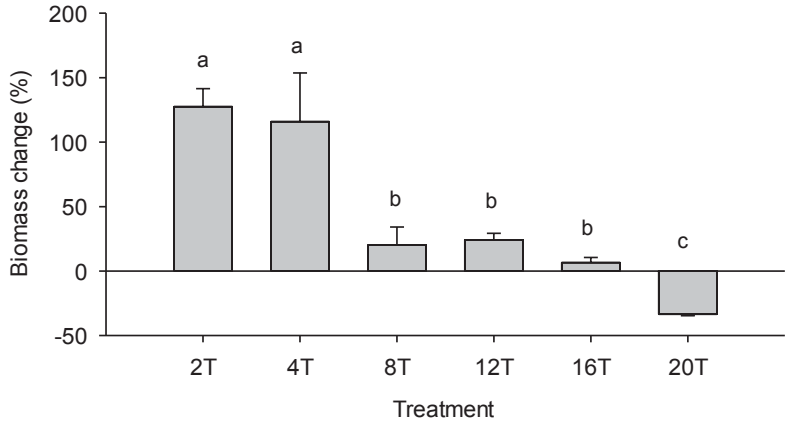

Figure 4.8. The percentage biomass change for *Litoria serrata* tadpoles during the experiment for six treatment groups with varying tadpole densities. The treatments were: 2T = 2 tadpoles, 4T = 4 tadpoles, etc. Significant differences between treatments (indicated by Tukey's post-hoc tests, with α = 0.05) are shown by the letters a – c.

4.4 Discussion

Tadpoles of two pool-dwelling species contributed differently to stream functioning, as measured by leaf litter breakdown and sediment removal. Neither *L. serrata* nor *M. coggeri* tadpoles broke down leaf material on their own, and only *L. serrata* appeared to interact with invertebrates during leaf processing. *Mixophyes coggeri* tadpoles were more efficient than *L. serrata* in removing sediments by consumption and displacement, and tadpole activity resulted in the accumulation of sediment downstream. Higher tadpole densities increased the rate of leaf litter breakdown, but did not affect sediment removal, which may be due to reduced activity at high densities.

The highest breakdown rate for *A. brachystylis* leaves occurred when *L. serrata* tadpoles and invertebrates were together, indicating facilitation. It is likely that invertebrates facilitated tadpoles, as Iwai et al. (2009) found for *Anisocentropus kirramus* leaf shredders and *L. serrata* tadpoles in the same system. However, mutual facilitation may have occurred if surface processing by tadpoles made the leaves more favourable for invertebrates. Tadpoles may not be able to feed on whole leaves due to their jaw structure and fine teeth, but Iwai et al. (2009) reported a higher organic carbon content in tadpole-processed leaves, possibly because tadpoles scraped off surface minerals, thereby increasing the proportion of organic material.

Nutrient regeneration is another way by which tadpoles can contribute to leaf processing without directly feeding on the leaves. Tadpoles may consume other sources of organic material such as biofilm and fine detritus, and release nutrients back into the stream though their excreta. In Neotropical streams, nutrient regeneration by tadpoles probably led to more nutrient-rich resources for invertebrates, and shredder production declined in the absence of tadpoles, leading to reduced breakdown of coarse particulate organic matter (Colon-Gaud et al. 2009; Colon-Gaud et al. 2010b). Iwai et al (2009) found no evidence of nutrient regeneration with *A. brachystylis* leaves; therefore any facilitation must have occurred through direct physical effects. Nutrient regeneration by *L. serrata* tadpoles was tested for in a subsequent experiment (Chapter 5).

The interaction between plant species and animal treatments indicated that the animals had preferences for specific leaves. Shredding invertebrates at Paluma feed on a broad range of leaves, but they preferred those that have been conditioned for longer and therefore have greater microbial colonisation (Bastian et al. 2007). Under laboratory conditions, however, shredders may be more selective, choosing leaves according to toughness, nutritional value or toxin content (Bastian et al. 2007). Although invertebrates in the present study were more likely to be found on *Endiandra bessaphila* leaves, there was no indication that shredders consumed more of these leaves, or of *Cryptocarya leucophylla* leaves, when tadpoles of either species were present, ruling out facilitation.

Tadpoles of different species may vary in their ability to process leaf material. *Litoria serrata* tadpoles contributed to leaf litter breakdown of one plant species, whereas *M. coggeri* tadpoles did not influence this process, despite invertebrates being present. Shredders processed leaves, and therefore it would have been more likely for tadpoles of either species to contribute to leaf breakdown in the presence of invertebrates. In a similar experiment in Panama, using in-stream closed PVC tubes, Rugenski et al. (2012) reported mutual facilitation between tadpoles and invertebrates, using one plant species and tadpoles of four species. The tadpoles had different feeding modes and therefore the contribution to leaf processing was probably not the same among the species. Centrolenid tadpoles in a different stream did not affect leaf decomposition but fed on microbes associated with leaf litter (Hunte-Brown 2006; Connelly et al. 2011). This suggests that tadpoles of different species are important at different stages of the leaf breakdown process, depending on the period of conditioning

(Bastian et al. 2007). However, they likely have unique functional roles, with some species more important than others in leaf litter processing.

Both *L. serrata* and *M. coggeri* tadpoles removed sediment from the middle chambers, in contrast to an experiment in Panama, in which tadpole treatments (with or without invertebrates) accumulated the most organic matter (Rugenski et al. 2012). This may be due to different feeding or behavioural preferences of the species; tadpoles of four feeding groups were used, any of which could have driven the results. Additionally, the Rugenski et al. (2012) experiment used closed PVC tubes rather than a flow-through system, so is not directly comparable. Sediment accumulation in the present experiment was highest in the invertebrate treatments, probably from leaf breakdown and faeces production, but it was reduced in the presence of tadpoles. *Mixophyes coggeri* tadpoles, in particular, actively removed organic material and appeared to be consuming it. However, sediment accumulation in the bottom chambers was greater for the tadpole treatments, indicating that bioturbation, causing sediments to be washed downstream, was more important than feeding.

Sediment removal may benefit invertebrate consumers by exposing underlying food resources for smaller grazers (Ranvestel et al. 2004). It can also encourage algal growth, by maximising nutrient and light availability (Connelly et al. 2008). *Mixophyes coggeri* tadpoles were more efficient at displacing sediment, probably because they are larger than *L. serrata* tadpoles and are strong swimmers (Anstis 2013), driving a stronger bioturbation effect. The tadpoles probably consumed little sediment, although Trenerry (1988) found the diet of *M. coggeri* (then *M. schevilli*) tadpoles to consist of more than 75% detritus. Nevertheless, tadpoles thought to consume detritus have been found to assimilate mainly the microbes associated with it (Hunte-Brown 2006; Altig et al. 2007). Similarly, the tadpoles in Birthday Creek may have stirred up sediment to feed on only the most nutritious parts, thereby causing the majority to be washed downstream.

Tadpoles and invertebrates may benefit from interactions during leaf litter breakdown or sediment removal, but they may also compete with each other (Morin et al. 1988). Tadpoles lost more biomass when invertebrates were present, indicating possible competition for resources (Experiment 1). Although tadpoles did not directly contribute to leaf breakdown, they may have competed with invertebrates for biofilm on leaf surfaces or other organic material that accumulated in the channels. Invertebrates may reduce biofilm or periphyton abundance, thereby decreasing food availability for

tadpoles (Morin et al. 1988). High tadpole densities may also result in intraspecific competition; thus, *L. serrata* tadpoles at low densities doubled their original biomass, whereas at high densities they either gained little or lost biomass (Experiment 2). This kind of interspecific competition has been noted in previous experiments. *Litoria serrata* and *L. dayi*, which occurred in Birthday Creek until the early 1990s, competed with each other when placed together experimentally (Trenerry 1988), and it is likely that *L. serrata* and *M. coggeri* also compete for resources in the stream. Furthermore, irrespective of the adult frog species richness, there may be an upper limit to the species of tadpoles that can co-exist at a site, that is at least partly caused by competition and limitations on resource partitioning in highly variable environments (Alford 1999).

Tadpole density affected leaf breakdown and sediment removal differently. Although tadpoles appeared to be competing with invertebrates, leaf breakdown by shredders increased as the density of tadpoles increased. Perhaps tadpoles facilitated leaf processing when present at high densities, but they themselves did not directly benefit from the FPOM produced by shredder activities, resulting in biomass loss. Tadpole density did not affect sediment accumulation in Experiment 2, even though tadpoles removed sediment from the chambers. Boyero and Pearson (2006) found that the leaf breakdown rate did not increase with higher invertebrate shredder densities, as a result of reduced activity per individual. Although there were more tadpoles present in the high density animal treatments, the individuals may have consumed less sediment due to competition, resulting in no difference between treatments.

Litoria serrata tadpoles play a role in leaf breakdown of at least one plant species in the Paluma streams, likely as a result of facilitation by invertebrates. Both *L. serrata* and *M. coggeri* tadpoles contributed to sediment and biofilm removal via consumption and bioturbation, but the large size of *M. coggeri* tadpoles allowed them to be more efficient at bioturbation than *L. serrata* tadpoles. The two species appeared to have different functional roles in the stream, with *L. serrata* tadpoles being more important in leaf processing and *M. coggeri* in sediment removal. Connelly et al. (2014) reported that amphibian declines in Panama affected stream functions to varying degrees depending on the function itself and the length of time since tadpoles disappeared. The different traits of the two species reported here suggest that species composition is also important in influencing these effects. Tadpoles and invertebrates may benefit each other during stream processes, but they also compete for space or food resources. This suggests that the relationship between tadpoles and invertebrates may

change during periods of naturally high tadpole or invertebrate densities, which could influence stream functioning.

Long-term observations are needed to fully understand the effects of amphibians on ecosystems, and any changes that might occur in their absence (Connelly et al. 2014). Long-term studies in Neotropical streams following amphibian declines showed that invertebrates did not occupy the same feeding niche as tadpoles, and they did not completely restore in-stream habitats to pre-decline condition (Barnum et al. 2013; Connelly et al. 2014). The decline of a whole tadpole assemblage is likely to have greater long-term and far-reaching effects on an entire stream system than can be estimated from experiments run over relatively short periods of time (Connelly et al. 2008). However, short-term experiments are useful in providing evidence for the mechanisms that underlay larger-scale effects, and can be used to make inferences about the potential decline of tadpoles. This study indicated that the contribution of tadpoles to stream processes depends on the species, their resource use and interactions with other stream organisms.

5. Nutrient regeneration by tadpoles in experimental streams

5.1 Introduction

The source and fate of nutrients are important indicators of stream health, because healthy nutrient cycling is essential to proper stream functioning (Bunn et al. 1999). In-stream nutrient recycling relies on organic material that enters the stream and is broken down to release nutrients. These nutrients are transported via a continuous sequence of uptake and release called nutrient spiralling (Boulton and Brock 1999; Chapin et al. 2011). This process depends on nutrient transformations by microbes or autotrophs, and on the consumption and egestion of nutrients by stream organisms (Boulton and Brock 1999). Terrestrial leaf litter is a major source of organic material entering forest streams (Cummins 1974) and is broken down by microbes and invertebrates, as well as by physical abrasion (Graça 2001). Most of the dissolved organic matter in streams has been leached from leaf litter and other detrital material (Boulton and Brock 1999), and provides the major source of carbon and nutrients for the food web.

Typically, bacteria and fungi first colonise organic material, such as leaf litter, in the stream and partially degrade it during conditioning (Cummins and Klug 1979). This makes further breakdown easier for shredding invertebrates (Graça 2001). Shredders often have preferences for certain leaves, based on colonisation by microorganisms, leaf toughness, nutrient quality and concentrations of defensive compounds (Graça 2001). They may benefit from feeding on conditioned leaves by obtaining nutrients from the partially degraded plant material, as well as from the ingested microbes (Bärlocher and Kendrick 1975; Cummins and Klug 1979). The leaf fragments and faeces produced by shredders as a result of their feeding activities are also colonised by microorganisms, and provide other invertebrates (e.g., gatherers) with nutrient-rich food (Cummins et al. 1973).

Low concentrations of nitrogen and phosphorus may limit productivity in a stream system. Connolly and Pearson (2013) found phosphorus to be the limiting factor for microbial growth in a rainforest stream in Birthday Creek. Through the microbial pathway, such nutrient limitation may also affect the physiological condition and growth of consumers such as shredding invertebrates (Connolly and Pearson 2013). Invertebrates, fishes and tadpoles may indirectly influence the nutrient concentration in

streams by bioturbation, whereby they alter their physical environment, and increase nutrient release from sediments into the water column (Vanni 2002; Ranvestel et al. 2004; Moore 2006).

Tadpoles may increase microbial activity by releasing nutrients, and may therefore facilitate microbial nutrient immobilisation during conditioning of organic material (Iwai and Kagaya 2007). Tadpoles regenerate nutrients by converting consumed organic material into inorganic material, which is then released into the stream through their excreta (Iwai and Kagaya 2007; Capps et al. 2015). Nutrient regeneration by tadpoles may therefore indirectly increase the nutrient quality of leaves in streams due to greater microbial activity. For example, the presence of tadpoles in Japanese streams lowered the carbon to nitrogen (C: N) ratio of leaves not directly exposed to tadpole feeding due to increased microbial activity (Iwai and Kagaya 2007). Also, in the Neotropics, fine particulate organic matter had higher nitrogen content in streams where tadpoles were present than where they had declined (Whiles et al. 2006; Colon-Gaud et al. 2008). This indicates that tadpoles increase the quality of coarse and fine particulate organic matter in streams, probably through nutrient regeneration.

Primary producers such as algae can also use regenerated nutrients (Iwai and Kagaya 2007), and biofilm growth may increase in the presence of tadpoles (Iwai et al. 2012). Increased algal growth on leaves increases their nutritional quality, which encourages breakdown by shredders (Abelho et al. 2005). Biofilm is also an important food source for invertebrate grazers and tadpoles. Iwai et al. (2012) found that nutrient regeneration by tadpoles increased biofilm growth, which was then consumed by the tadpoles themselves. Therefore, the tadpoles benefitted from their own nutrient regeneration by increasing the abundance of their food source. This might be a common occurrence in freshwater systems, but has not been tested in streams (Iwai et al. 2012).

Nutrient regeneration by tadpoles is thought to cause facilitation between tadpoles and invertebrates during the breakdown of leaf litter as a result of greater leaf quality. In this study I used an artificial stream experiment to test whether tadpole nutrient regeneration readily occurs in rainforest streams and whether this increases the nutrient quality of leaf litter. Further, I aimed to determine the direct and indirect effects of tadpole nutrient regeneration on sediment quality and biofilm growth, which may both be consumed by stream organisms. I also aimed to ascertain whether any extra biofilm biomass as a result of nutrient regeneration is consumed by the tadpoles

themselves or by invertebrates, which would indicate whether the presence of tadpoles benefits other stream organisms in terms of nutrient availability.

5.2 Methods

The experiment was carried out in 20 artificial stream channels, each comprising three chambers, beside Birthday Creek (Chapter 4). The top chambers were left empty and acted as a collection space for any sediment that entered the system from the header tank. Dividers with 63 μm mesh were placed at the top of the channels to minimise entry of sediments and small animals. Similar dividers were placed at the bottom of each channel to retain organic material within the channel. The middle chambers were separated from the top and bottom by 1-mm-mesh dividers to allow the flow of fine particulate organic matter and nutrients but prevent target animals from moving between chambers. To test for nutrient regeneration, I included enclosed containers to measure the effects of any increase in nutrients in the environment, while at the same time preventing direct contact with the animals (see below). The downstream effects of tadpole presence, as would be typical in a stream system, were measured from the organic material in the bottom chambers.

All middle and bottom chambers contained unglazed terracotta tiles (5 cm x 5 cm) to measure biofilm growth. To determine direct and indirect effects of tadpole presence, tiles were either 'enclosed' (indirect effect) or 'exposed' (direct effect). The enclosed tiles were placed into a plastic container with 1-mm-mesh sides to prevent animal access, whereas the exposed tiles were placed into plastic containers with open sides, allowing animals to colonise the tiles. There were two tiles in each container, and one enclosed and one exposed container per chamber, all with lids. Each middle and bottom chamber contained three leaf bags with approximately 2 g of *Cryptocaria leucophylla* leaves. The leaves of this plant species were readily available, and results from the previous experiments indicated that the animals consumed them. Two of the leaf bags were exposed to the animals (i.e., free in the chambers), whereas one leaf bag was placed in the enclosed plastic container with the tiles. These enclosed leaves were later analysed for their nutrient quality. Leaves and tiles were left in the channels to condition for a week before being randomly assigned to the various chambers. During this time, some sediment accumulated in the channels and this was left as a food source for the animals. There were two exposed leaf bags and two tiles per container so that

one could be removed half-way through the experiment if bad weather was predicted, ensuring that some data would be available if the experimental chambers were washed out before completion.

The middle chambers housed the animal treatments with different combinations of tadpoles and/or invertebrates. The downstream effects of these treatments were measured in the bottom chambers, which contained equal numbers of invertebrates but no tadpoles (Figure 5.1). There were five animal treatments and four replicates of each: (i) 8 tadpoles only (high density), (ii) 8 tadpoles (high density) and invertebrates, (iii) invertebrates only, (iv) 4 tadpoles only (low density), and (v) no animals (control). High densities of tadpoles were used to test for tadpole-invertebrate interactions to ensure that any effect would be large enough for detection. Tadpoles of one species, *Litoria serrata*, were used. The invertebrate treatments consisted of mayfly larvae (grazers) and caddisfly larvae (shredders). There were several genera of the mayfly family Leptophlebiidae, one with large larvae and several other smaller species (here simply denoted as Leptophlebiidae). The numbers and sizes of the animals put into each chamber were approximately the same. The initial numbers of invertebrates are outlined in Table 5.1. Their sizes are: small (s), medium (m) and large (L).

Tadpoles were counted approximately weekly and missing ones (metamorphosed or escaped) were replaced. Invertebrates were added periodically to replace those that moulted into the terrestrial stage; this occurred more quickly for mayflies than for caddisflies. Occasionally, insufficient numbers of replacement animals of the required size were available from the stream, particularly large *Atalophlebia* sp. In that case more animals were added the following week or whenever sufficient individuals were located. The tadpoles were weighed using a digital balance (0.1 g) and photographed alongside a scale before being randomly placed into the chambers. All tadpoles were at Gosner stages 25-30 (Gosner 1960). Invertebrates were sorted according to size and randomly placed into the chambers.

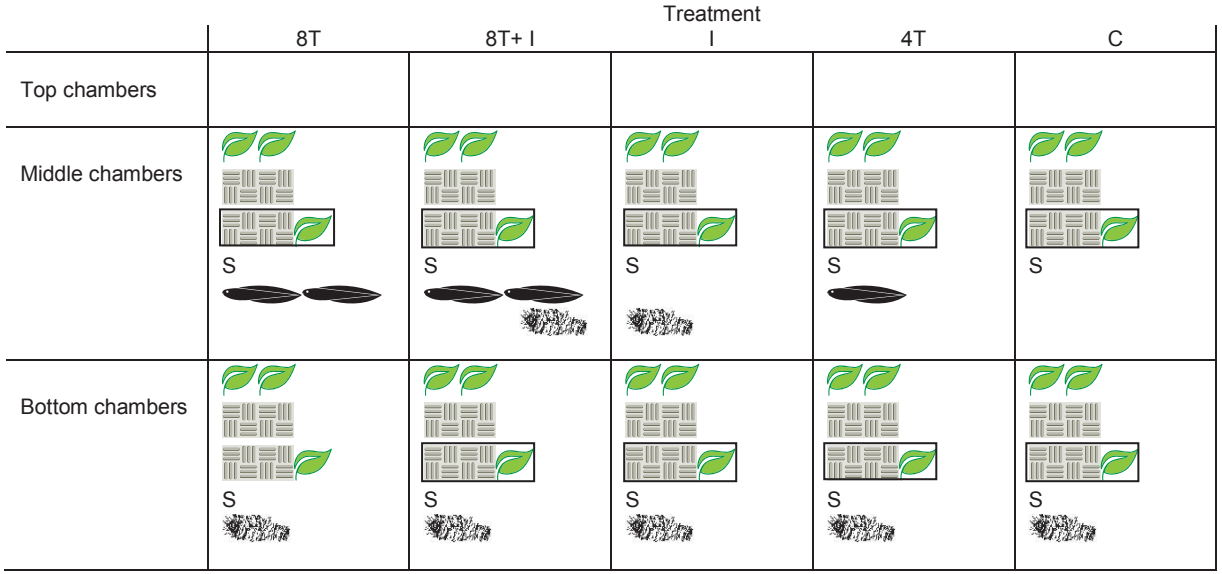

Figure 5.1. Experimental set-up showing one replicate of each treatment. Symbols: = leaves of *Cryptocarya leucophylla* (two exposed and one enclosed), = exposed tile, = enclosure, S = sediment, = 4 *Litoria serrata* tadpoles, = 8 *L. serrata* tadpoles, = invertebrates according to Table 5.1. Treatment codes represent tadpoles (T) and invertebrates (I).

Table 5.1. Invertebrate treatments including size groups small (s), medium (m) and large (L). The size ranges were approximately estimated as small = < 0.5 cm, medium = 0.5 to <1.0 cm, and large = > 1.0 cm.

	Treatment chambers	Bottom chambers
Leptophlebiidae	1m, 1s	4s
Atalophlebia sp.	1L	1L, 1m
Anisocentropus kirramus	3m	2m
Triplectides gonetalus	1L, 1m	1m
Lectrides varians	3m	2m

The experiment ran for 76 days and there was no extreme weather during this time. At the end of the experiment, the tadpoles were counted, weighed and photographed before being released. The large invertebrates were also counted and removed if this could be done without disturbing the sediment or leaf material, while the rest were collected with the other material and counted in the laboratory. The exposed and enclosed tiles from each chamber were removed separately and placed into labelled zip-lock bags. Any animals found in the enclosed containers were counted and their size noted. The leaf bags and sediment were collected as described in Chapter 4. The samples were placed on ice during transport and subsequently stored in a freezer the same day.

In the laboratory, the sediment samples were sorted under a magnifying lamp and all remaining invertebrates removed, identified and counted. Coarse particulate organic material (CPOM) was also removed and oven-dried at 60°C until completely dry and then weighed. Following this, the samples were placed in a muffle furnace and heated to 550°C to burn off the organic material. The remaining ash was weighed and used to calculate ash free dry weigh (AFDW) of the sample. The samples from the bottom chambers were dried at 60°C and sent to the University of Adelaide where they were analysed at the Waite Analytical Lab and CSIRO for their phosphorus, nitrogen and carbon content. These were represented as carbon to nitrogen or phosphorus ratios. A low C: N or C: P ratio indicates a higher nitrogen or phosphorus content respectively.

The leaf packs were washed, invertebrates removed, and the leaves then oven-dried at 60°C for 48 hours and weighed. The enclosed leaves from the animal treatment chambers were also sent to the University of Adelaide for nutrient analysis, while the exposed leaves were ground to a fine powder using a coffee grinder, and then ashed at 550°C to obtain AFDW. The two exposed leaf bags from each chamber were combined and weighed together, and percentage leaf weights remaining at the end of the

experiment were used for comparisons. The tiles were scrubbed and the accumulated biofilm and sediments washed into glass dishes. This biofilm mixture was then sorted to remove invertebrates and coarse organic material. The invertebrates removed from the samples were identified and counted. The samples were oven-dried at 60°C until completely dry and weighed before ashing at 550°C to obtain AFDW.

5.2.1 Statistical analysis

Average percentage leaf weights, sediment and biofilm AFDWs, nutrient quality, and tadpole biomass (wet weight) change in the different treatment groups were compared using one- or two-way ANOVAs and Tukey's post-hoc tests. The nutrient quality of leaves and sediment were analysed using C: N or C: P ratios. The figures were drawn in SigmaPlot Version 12.5 and the analyses were carried out in S-Plus Version 8.2.

5.3 Results

5.3.1 Leaf breakdown and nutrient analysis

At the completion of the experiment, large Leptophlebiidae larvae had entered nearly every enclosed container at some time during the experiment, thereby gaining access to the leaves. The data were not adjusted to compensate for this, but were interpreted taking into account possible mayfly feeding. The tadpoles and invertebrates in the middle animal treatment chambers influenced leaf breakdown (two-way ANOVA, $F_{4,30} = 7.11$, $P < 0.001$) and this was noticeable in the exposed leaves (Figure 5.2a). The invertebrate-only and tadpole-invertebrate treatments had the lowest leaf weights remaining. By contrast, there was only slight variation in the leaf weights in the enclosed containers (Figure 5.2a). Preventing animal access also affected leaf breakdown ($F_{1,30} = 49.69$, $P < 0.001$), and there was a significant interaction between leaf enclosure and the animal treatments ($F_{4,30} = 3.66$, $P = 0.015$). The downstream effect of the animal treatments in the middle chambers was measured in the bottom chambers. The leaf material remaining was almost the same in all the bottom chambers (Figure 5.2b), but exposed leaf weights were lower (two-way ANOVA, $F_{1,30} = 95.51$, $P <$

0.001). There was no animal treatment effect or interaction effect between the animal treatments and leaf enclosure in the bottom chambers.

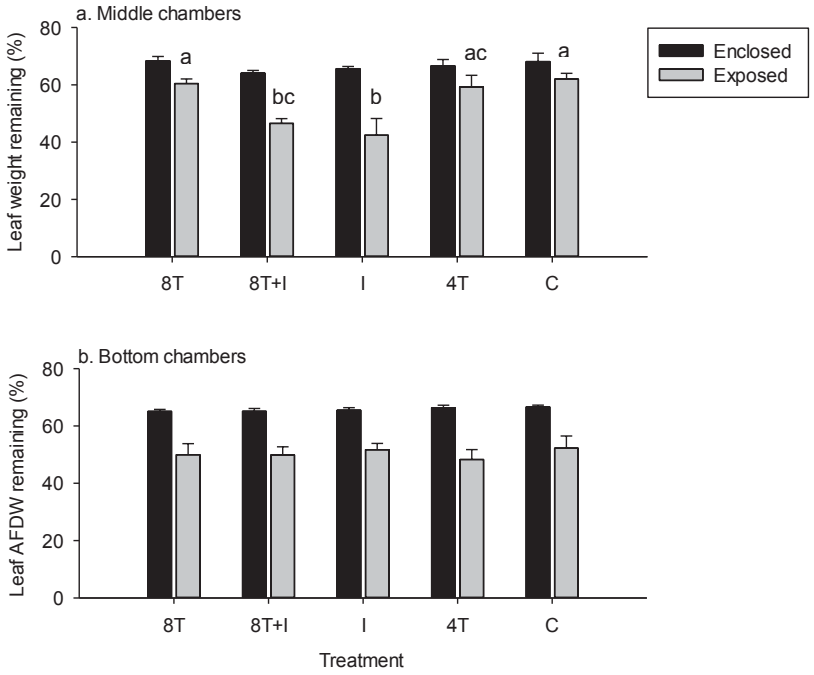

Figure 5.2. Percentage leaf weight remaining (± s.e.) of enclosed and exposed leaves in the (a) middle treatment chambers and (b) bottom chambers. The letters a – c indicate significant differences between animal treatments as shown by Tukey's post-hoc tests (α = 0.05). Treatment abbreviations: 8T = 8 *Litoria serrata* tadpoles; 4T = 4 *L. serrata* tadpoles; I = invertebrates; and C = control.

The invertebrate-only treatment had the highest C: N ratio (Figure 5.3), suggesting that the nitrogen content of leaves was higher when tadpoles were present, but the large variances made the results inconclusive (one-way ANOVA, $F_{4,15} = 0.93$, $P = 0.475$). The control had the highest phosphorus content, but there was also great variability and there were no significant differences in the C: P ratio among treatments ($F_{4,15} = 0.33$, $P = 0.851$).

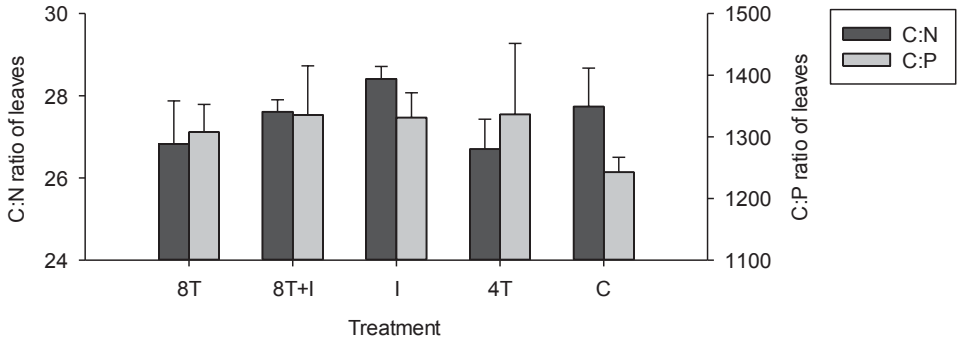

Figure 5.3. Carbon: nitrogen ratio (± s.e.) and carbon: phosphorus ratio (± s.e.) in *Cryptocarya leucophylla* leaves from the treatment chambers. Treatment abbreviations: 8T = 8 *Litoria serrata* tadpoles; 4T = 4 *L. serrata* tadpoles; I = invertebrates; and C = control.

5.3.2 Sediment accumulation and nutrient quality of organic material

There was a strong animal treatment effect on sediment accumulation in the middle chambers (one-way ANOVA, $F_{4,15} = 7.77$, $P = 0.001$; Figure 5.4), with lower sediment accumulation when tadpoles were present (Tukey's tests, $P < 0.05$).

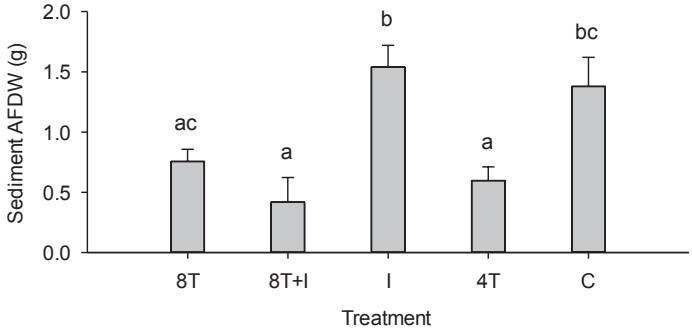

Figure 5.4. Ash free dry weight of sediment (± s.e.) accumulated in the treatment chambers. The letters a – c indicate significant differences between treatments as shown by Tukey's post-hoc tests (α = 0.05). Treatment abbreviations: 8T = 8 *Litoria serrata* tadpoles; 4T = 4 *L. serrata* tadpoles; I = invertebrates; and C = control.

There was an animal treatment effect on the C: N ratio in the sediment (one-way ANOVA, $F_{4,15} = 5.00$, $P = 0.009$) and the Tukey's post-hoc tests showed that the control had a lower C: N ratio than the tadpole-only and the two invertebrate treatments (Figure 5.5). The control had the lowest C: P ratio, but there was no obvious animal treatment effect ($F_{4,15} = 2.87$, $P = 0.060$).

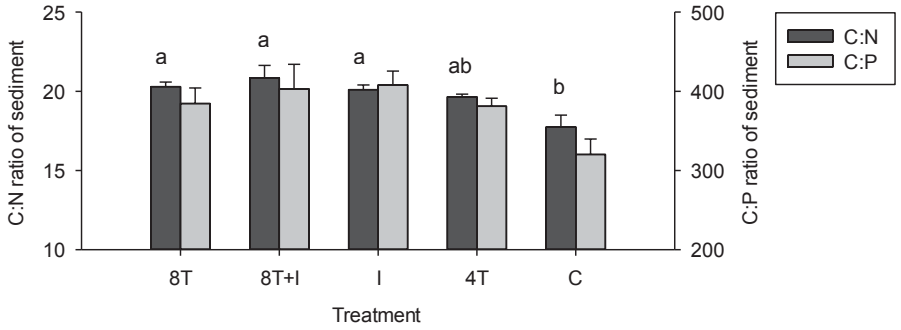

Figure 5.5. Carbon: nitrogen ratio (± s.e.) in sediment from the bottom chambers. The letters a and b indicate significant differences between treatments (for C: N) as shown by Tukey's post-hoc tests (α = 0.05). The treatments are those in the middle chambers, each upstream of a bottom chamber: 8T = 8 *Litoria serrata* tadpoles; 4T = 4 *L. serrata* tadpoles; I = invertebrates; and C = control.

5.3.3 Biofilm growth

Biofilm growth was measured on exposed and enclosed tiles in the middle treatment and bottom chambers as AFDW. There was great variability in the results for biofilm growth in the treatment chambers, especially on the enclosed tiles (Figure 5.6a). Overall, there were no treatment, tile enclosure, or interaction effects (two-way ANOVA; Treatment: $F_{4,30} = 0.21$, $P = 0.931$, Exposure: $F_{1,30} = 2.82$, $P = 0.104$, Interaction: $F_{4,30} = 0.50$, $P = 0.735$), although there was less biofilm accumulation on the exposed tiles in all treatments than in the control. Analysis of the exposed tiles only suggested a weak treatment effect (one-way ANOVA, $F_{4,15} = 2.70$, $P = 0.07$). In the bottom chambers, the biofilm AFDW was highly variable for both the exposed and enclosed tiles (Figure 5.6b). There were no animal treatment, tile enclosure or interaction effects (two-way ANOVA; Treatment: $F_{4,30} = 0.33$, $P = 0.885$, Exposure: $F_{1,30} = 0.002$, $P = 0.964$, Interaction: $F_{4,30} = 0.64$, $P = 0.636$).

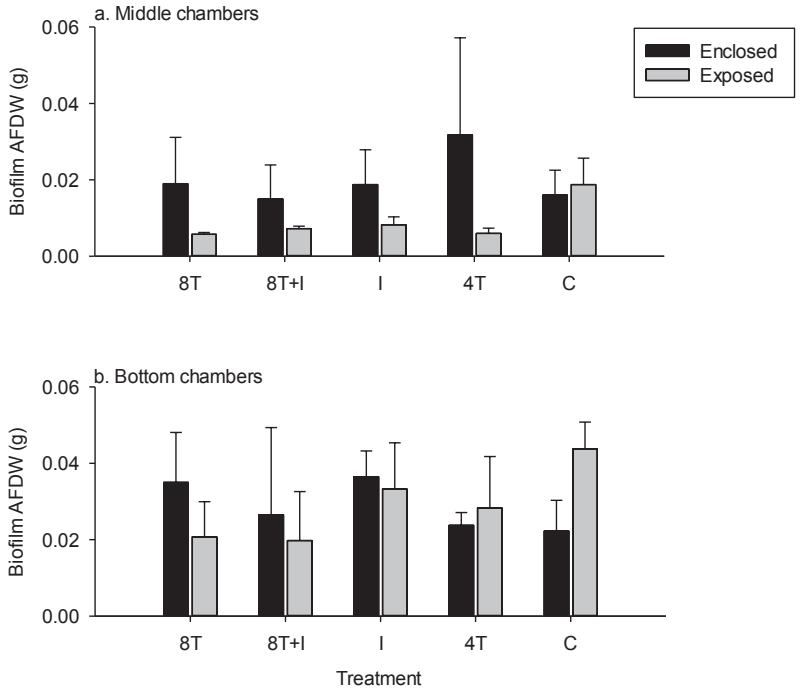

Figure 5.6. Mean biofilm AFDW (± s.e.) on exposed and enclosed tiles in the (a) middle treatment chambers and (b) bottom chambers. Treatment abbreviations: 8T = 8 *Litoria serrata* tadpoles; 4T = 4 *L. serrata* tadpoles; I = invertebrates; and C = control.

5.3.4 Tadpole biomass

The gain in tadpole biomass was significantly greater in the four-tadpole treatment (4T) than in the eight-tadpole (8T) and tadpole-invertebrate (8T+I) treatments (one-way ANOVA, $F_{2,8} = 11.10$, $P = 0.005$; Figure 5.7). In the two treatments with eight tadpoles, the tadpole biomass was lower when invertebrates were present, but the difference was not significant.

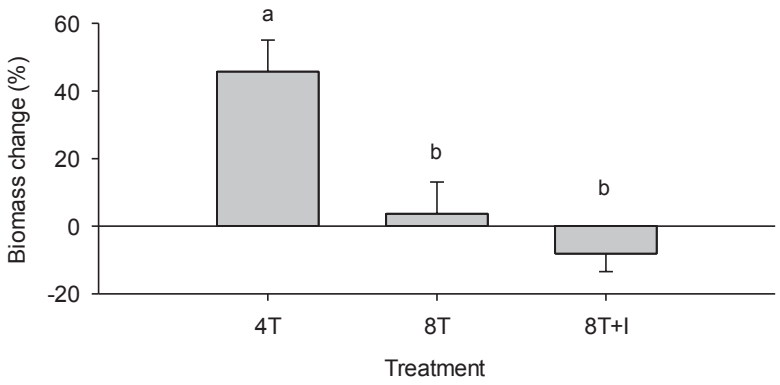

Figure 5.7. The percentage biomass change for *Litoria serrata* tadpoles for three treatment groups. The treatments were: 4T = 4 tadpoles, 8T = 8 tadpoles, and 8T+I = 8 tadpoles with invertebrates. Significant differences between treatments according to Tukey's post-hoc tests (α = 0.05) are shown by the letters a and b.

5.4 Discussion

Litoria serrata tadpoles were not important in adding nutrients to the system in this study. There was no evidence of measurable nutrient regeneration by *L. serrata* tadpoles and they did not influence the nutrient quality of leaves and sediment, or biofilm growth. An increase in nutrients may encourage microbial activity on organic material such as leaf litter, and lead to nutrients being released during conditioning (Iwai and Kagaya 2007; Rugenski et al. 2012). This may benefit other organisms that break down the leaf material (Pearson and Connolly 2000). Although tadpoles did not change the nutrient quality of the organic material, they actively removed sediment from the channels.

Tadpoles did not feed on *C. leucophylla* leaves without invertebrates, and there was no evidence of tadpoles facilitating invertebrates during leaf breakdown. The leaf weight in the bottom chambers remained constant across the treatments, indicating that shredder activity was not affected by tadpoles or invertebrates upstream. This suggests that nutrient levels and microbial colonisation were similar downstream of the treatments. Microbial colonisation on leaves, as a result of tadpole activity, may depend on the particular frog and plant species, and the period of conditioning. Higher nutrient quality in leaves was taken as an indication of greater microbial activity, and this was expected to increase leaf litter breakdown rates by shredders. However, nutrient enrichment may instead positively influence other invertebrate characteristics, such as

their growth rate and condition (Pearson and Connolly 2000; Connolly and Pearson 2013).

There was substantial variability in the nutrient quality of *C. leucophylla* leaves, and there was no evidence that leaves exposed to tadpoles had significantly higher nutrient content than the invertebrate or control treatments. It is possible that the food sources available to the tadpoles were not nutritious enough for high levels of nutrient regeneration to occur (Iwai and Kagaya 2007). The amount of nutrients introduced into a system depends on the nutrient quality of the organic matter and the species involved in its breakdown (Vanni 2002). Furthermore, the N: P ratio of an animal's body tissue affects the N: P ratio of the nutrients it releases. Therefore, when an organisms feeds on nutrient-rich food, less nutrients will be required to maintain a constant N: P ratio and more nutrients will be excreted (Vanni 2002). The lack of evidence of nutrient regeneration by *L. serrata* tadpoles may be partly due to *C. leucophylla* leaves being too low in nutrients for tadpoles to release measurable amounts of nitrogen or phosphorus. Any nutrients regenerated by the tadpoles were probably washed out from the experimental system because of the flow-through nature of the system. An experiment that is more sensitive to nutrient regeneration effects would be necessary to measure small nutrient inputs by tadpoles.

It is likely that sediment loss in the tadpole treatments of the middle chambers came from tadpoles stirring up fine particulate organic matter (FPOM), causing it to be washed downstream. In the previous study (Chapter 4), although it appeared that tadpoles were feeding on the organic material, they were actually actively displacing a large portion of it. Whether by consumption or bioturbation, the results agree with other studies that found tadpoles to remove or consume sediment in streams (Flecker et al. 1999; Ranvestel et al. 2004). Invertebrates, on the other hand, added material through their feeding activities and egestion, concurring with Rugenski et al. (2012), who found that the accumulated particulate organic matter comprised primarily materials egested by the animals. Although I did not test by what means FPOM was removed, the sediment accumulation measured in the treatment chambers suggests that, in a particular stream section, invertebrates are important in creating FPOM, whereas tadpoles are important in its removal.

The presence of tadpoles and invertebrates in the treatment chambers reduced the nutrient quality of the organic material downstream, again concurring with Rugenski et al. (2012). They found that tadpole treatments had a higher C: N ratio (lower nitrogen

content) than the invertebrate-only treatment or control, indicating that tadpoles may be lowering the nutrient quality of the sediment. They suggested that tadpoles assimilated nutrients while carbon-rich faecal matter accumulated in the sediment, resulting in a high C: N ratio. The lower nutrient quality in downstream sediment indicates that tadpoles and invertebrates fed on high-quality fine particulate organic matter and stirred up the rest of the material, causing it to be washed downstream.

When nutrient regeneration takes place, tadpoles enrich the stream environment despite their feeding activities. For example, tadpoles in Panama lowered the C: N ratio of fine seston in streams (Colon-Gaud et al. 2008), and organic matter had a higher nitrogen content where tadpoles were abundant compared to where they had declined (Whiles et al. 2006). The next step in this work is to examine nutrient regeneration with different species in the Paluma streams, such as *Mixophyes coggeri* tadpoles. *Mixophyes coggeri* tadpoles are much larger than *L. serrata* tadpoles and their activities may amplify any small effects detected with *L. serrata*, as was the case in sediment mobilisation (Chapter 4).

Future research should also measure nutrient regeneration in a different stream system, where food sources might be more nutrient rich (e.g., algae). Iwai and Kagaya (2007) showed that tadpoles fed on different diets reduced the C: N ratio of leaves to varying degrees depending on the quality of the food source, which in turn benefited invertebrate detritivores. Furthermore, habitat preference may influence feeding. Riffle or pool tadpoles may have different capacities for nutrient regeneration depending on their feeding preferences. Clearly, complexities such as diversity of litter and of consumers can have important effects on trophic processes (e.g., Bastian et al. 2007). While the narrow focus of this mesocosm experiment precludes general conclusions about in-stream trophic processes involving tadpoles and invertebrates, it seems clear that nutrient regeneration by tadpoles is not important in some stream systems, and there may be other mechanisms by which tadpoles influence stream processes.

Primary producers such as algae may also use nutrients regenerated by tadpoles (Iwai and Kagaya 2007). Therefore, the presence of tadpoles may influence biofilm growth, which is an important food source in streams. The amount of biofilm in this experiment also depended on fine sediment accumulation, which formed a biofilm-sediment layer on the tiles. This suggests that in slow-flowing rainforest streams (or stream sections), as simulated with the artificial stream channels, biofilm does not

'grow' as much as it 'accumulates' from FPOM; there is little sunlight at the site and algal growth is restricted (Pearson and Connolly 2000).

Tadpoles and invertebrates probably fed on the biofilm-sediment layer on the exposed tiles in the animal treatment chambers. Although tadpoles reduce the biomass of biofilm through consumption, they may also encourage its growth through nutrient regeneration (Rugenski et al. 2012). Iwai et al. (2012) found that tadpoles themselves benefited from the extra nutrients produced during nutrient regeneration in a pond-based experiment, by feeding on the additional algae. In the present study, increased biofilm growth on the enclosed tiles would have indicated that nutrients were entering the system; variability of the data precluded any definitive interpretation. There were no obvious indications that biofilm growth on the enclosed tiles was greater in the tadpole treatments and therefore it was not clear whether there was surplus biofilm that could have been consumed by either tadpoles or invertebrates. The mayflies that entered the enclosed containers probably grazed on the biofilm and probably contributed to the variability in the data.

There was evidence of intraspecific competition among tadpoles. In the high density treatments, tadpoles gained significantly less biomass than in the low density treatment. This indicates that tadpoles competed with each other and with the invertebrates for resources. There was no difference in the amount of leaves consumed and sediments removed by tadpoles between the low density and high density treatments, so it is possible that tadpoles were less active at higher densities (Boyero and Pearson 2006).

These results suggest that nutrient regeneration by tadpoles may not be important in Australian rainforest streams, depending on the species present and the food sources. There was no evidence of measurable nutrient regeneration by *L. serrata* tadpoles in this ecosystem. This result is probably associated with the quality of the food sources available to the tadpoles, particularly the lack of algae. The limited sunlight at Birthday Creek led to restricted algal growth, likely a common occurrence in rainforest streams. It is also possible that tadpoles of different species respond differently to the available food sources. Other stream-dwelling tadpoles may have had a greater nutrient regeneration effect with the available resources. Also, the flow-through design of the experiment may have not been sensitive enough to measure small amounts of nutrient input. However, evidence of facilitation between tadpoles and invertebrates in the same system (Chapter 4) indicates that tadpoles of this species may

influence stream processes through other mechanisms. Tadpoles also actively removed sediment accumulation that may benefit other organisms. Tadpole-invertebrate interactions are therefore complex and tadpoles may still play an important role in streams.

6. The trophic status of tadpoles in Wet Tropics streams

6.1 Introduction

To assess the importance of tadpoles in streams, their trophic interactions and place in the food web need to be known. Tadpoles of many species are assumed to be herbivores or detritivores, but their feeding ecology and nutritional needs are poorly understood (Altig et al. 2007). Tadpoles are known to feed on algae, sediments, detritus and other animals (Flecker et al. 1999; Ranvestel et al. 2004; Whiles et al. 2006), but they may not assimilate all of these food sources in the same proportions as they are consumed. For example, it is possible that tadpoles do not obtain their required nutrients from the plant material they consume, but from the microbes associated with the biofilm (Hunte-Brown 2006; Altig et al. 2007). Altig et al. (2007) suggest that tadpoles are most likely to be omnivorous, with diets that vary over time and space.

Food resources for tadpoles may vary among streams or among habitats within streams depending on food availability (Whiles et al. 2010). Leaf litter and other organic material, for example, is abundant in shaded upland streams, whereas primary producers such as filamentous algae or diatoms may be more important in open areas (Anderson and Sedell 1979). Leaf litter is a significant source of organic matter in forest streams (Cummins 1973; Wallace and Webster 1996; Gessner and Chauvet 2002), including in the Wet Tropics (Cheshire et al. 2005) and microbes and shredding invertebrates process this material, releasing nutrients available for other organisms (Pearson et al. 1989; Graça 2001). Tadpoles in pools may graze on leaf surfaces or sandy and rocky substrata, feeding on detritus and algae (Trenerry 1988).

Tadpoles adapted to different stream conditions may use different food sources. In Birthday Creek, for example, the riffle species *Litoria nannotis* and *L. dayi* consumed similar amounts of algae, whereas *Mixophyes coggeri* (then *M. schevilli*), a pool species, consumed primarily detritus (Trenerry 1988). The size of the particles may also vary among species: the two riffle species at Paluma had the highest dietary overlap and the detrital material they consumed consisted mainly of fine particles, whereas the pool species, *M. coggeri* and *L. serrata,* consumed both fine and coarse particles (Trenerry 1988). Biofilm or periphyton found on the surfaces of rocks can also be an important food source (Whiles et al. 2010; Frauendorf et al. 2013) and tadpoles in riffles have

been observed scraping this material from rocks in Wet Tropics streams (Trenerry 1988; Cashins 2009).

Tadpoles of some species may feed at a single trophic level (regardless of season or size class; i.e., specialists), whereas omnivorous feeders change their main food source depending on resource availability, season or ontogenetic diet shifts (Hocking and Babbitt 2014). Food webs link basal sources and consumers within an ecosystem, and provide information on biotic interactions, transport of organic materials and energy transfer within the system (Allan and Castillo 2007). Food web energy flows via specific pathways and this is often controlled by a few taxa, and the loss of an important taxon can affect ecosystem structure (Allan and Castillo 2007). As relatively large and abundant organisms in small streams, it is possible that tadpoles have an important influence on the food web, at least periodically, whatever their main source of food (see Chapter 2).

Traditionally, gut content analyses have been used to determine species' diets and how they are related to those of other organisms in the food web (e.g., Trenerry 1988; Cheshire et al. 2005; Regester et al. 2008). The gut contents of animals provide information on what was recently consumed and, therefore, only gives a short-term account of food materials ingested (Winemiller et al. 2011). This method identifies ingested material but does not indicate digestibility or assimilation, so the food components that are identified do not necessarily represent the material that the animals assimilate within their tissues (Allan and Castillo 2007; Altig et al. 2007). More recently, the use of stable isotope techniques has become important in ecological studies, addressing some of the shortcomings of gut content analysis (Post 2002; Boecklen et al. 2011), and consequently were applied in the present study.

Stable isotope analysis examines the proportional composition of the animal's body by isotopes of elements such as carbon or nitrogen with different atomic weights. Different food sources yield different ratios. The stable isotope composition of an animal thus depends largely on its diet and reflects the material that has been assimilated within the tissues (Peterson and Fry 1987). Stable isotope techniques therefore provide an indication of what the animal has been eating over the long term, and also incorporates information on its short-term diet (Peterson and Fry 1987), and allows the animal's main sources of assimilated material to be identified (Dodd 2010). However, stable isotope analysis has its own problems, and is probably best used to complement gut content analyses in food web studies to more accurately determine

short- and long-term food sources (France 1998; Unrine et al. 2007; Davis et al. 2012; Blanchette et al. 2014).

Stable isotope analyses have been applied to assess the trophic status of aquatic invertebrates (e.g., Dudgeon 2008; Blanchette et al. 2014; Jardine 2014) and fishes (e.g., Jepsen and Winemiller 2002; Davis et al. 2012) and have also been applied to assess the effects of environmental disturbances on food web structure (e.g., Bunn et al. 1999). Studies that have used stable isotopes to focus on the effects of tadpole loss on stream systems were carried out in Panama, where amphibian declines were monitored over several years (Hunte-Brown 2006; Whiles et al. 2006; Verburg et al. 2007; Barnum et al. 2013). However, few studies have measured the stable isotope content in tadpoles to determine their trophic position and importance in the food web (Verburg et al. 2007; Barnum et al. 2013; Francis 2013; Huckembeck et al. 2014).

Previous research that focused on the role of tadpoles in stream food webs (e.g., Verburg et al. 2007; Colon-Gaud et al. 2010a; Winemiller et al. 2011; Barnum et al. 2013; Frauendorf et al. 2013) was mostly conducted in the Neotropics and there are no similar studies on Australian species. The present study used stable isotope analysis to help determine the position of tadpoles in stream food webs in relation to basal food sources and other aquatic consumers. The aims of the study were to: (1) determine the main food sources for tadpoles at Paluma and Tully, (2) ascertain whether tadpoles are specialist or generalist feeders, (3) assign the trophic status of tadpoles, and (4) determine food web structure in three stream reaches at Paluma. The food web structure of invertebrates in Birthday Creek at Paluma has been described using gut content analysis (Cheshire et al. 2005), and this study added data on tadpoles and larger predators.

6.2 Methods

6.2.1 Study sites and sample collection

Sampling for stable isotope analysis was conducted at Paluma in September 2012 and November 2013, and at Tully in November 2013. Three stream reaches were chosen at Paluma: Birthday Creek road crossing ("road crossing", -18.98°, 146.17°), Birthday Creek at "artificial streams" site (Pearson and Connolly 2000, -19.00°, 146.18°) and Camp Creek (-18.97°, 146.17°). Two pools and two riffles were sampled

along each stream reach, giving a total of 12 sampling sites at Paluma. At Tully, three pools and three riffles were sampled at each of two stream reaches (see Chapter 2): Tully Stream 1 (-17.77°, 145.65°) and Tully Stream 2 (-17.75°, 145.61°), again giving a total of 12 sampling sites.

A Hydrolab Quanta was used to measure pH, conductivity and dissolved oxygen of the water. Current velocity was measured using a flow meter (Owen's River Hydroprop), and depth, canopy cover and substratum composition were also noted in each riffle and pool. In September 2012, invertebrates, tadpoles and basal sources were collected in the Paluma stream reaches. The following year (2013), only tadpoles were collected at the Paluma stream reaches, and tadpoles and basal sources at the Tully stream reaches.

Dip-net sweeps were used to sample tadpoles and invertebrates in riffles and pools (see Chapters 2 and 3), and among any vegetation growing on or hanging from the side of the banks. Sampling was carried out without a time limit to ensure enough animals (minimum 1 mg dry weight per sample specified by the analyst) were caught and tadpoles extra to requirements were released back in the stream. The tadpoles that were collected were between Gosner stages 25 and 31 (Gosner 1960). In *Litoria nannotis*, *L. rheocola* and *L. dayi,* the hind limbs of tadpoles develop under a sheath (Davies and Richards 1990; Cashins 2009) and therefore exact Gosner stages could not be determined. It is not clear whether tadpoles of different sizes within this range of Gosner stages represent different tropho-taxa, and size classes are often arbitrarily assigned to tadpoles according to body length (Richards 2002; Cashins 2009). In this study, tadpoles were grouped into three size classes (Table 6.1), similar to those proposed by Richards et al. (2002) for tadpoles at Paluma.

Tadpoles of different species were kept separate and were euthanised in a solution of 0.02% MS-222 (200 mg/L) buffered with sodium bicarbonate (Braunbeck et al. 2007) and placed on ice. Invertebrates were sorted to order live in the field, rinsed with distilled water where possible, placed into plastic containers or zip-lock bags on ice, and returned to the laboratory. A Smith-Root Model 12B backpack electrofisher was used to catch fishes and large crayfish. Eels were fin-clipped, then released. Only small individuals of other fish species were caught, which were put on ice and returned to the laboratory.

Table 6.1. Size categories for *Litoria serrata*, *L. nannotis, L. rheocola, L. dayi* and *Mixophyes coggeri* tadpoles according to body length measurements.

Size category	*M. coggeri*	*L. serrata, L. nannotis, L. rheocola* and *L. dayi*
1 – large	≥ 22	≥ 12
2 – medium	≥ 10 to < 22	≥ 7.5 to < 12
3 – small	< 10	< 7.5

The following potential basal sources were collected at each sampling site: leaves accumulating in pools and riffles, filamentous algae, biofilm, periphyton, coarse and fine particulate organic matter (CPOM and FPOM respectively), and an iron matrix layer (Blanchette et al. 2014), which was present only in one pool in Camp Creek at Paluma (Appendix 3.1). The terms 'biofilm' and 'periphyton' are often used interchangeably (Rasmussen 2010; Ishikawa et al. 2012; Bunn et al. 2013), with some studies referring to 'periphyton-dominated' biofilm (Jardine et al. 2012; Jardine 2014). In this study, biofilm and periphyton represent two different potential basal sources. Periphyton comprised a large proportion of algae and was recognisable by its green colour, whereas biofilm was not always obvious (due to limited algae). The biofilm matrix most likely consisted of small amounts of algae, fine detritus and microorganisms. Periphyton was collected from the surface of the sandy substratum in the shallow edge of the pool using a zip-lock bag. Biofilm was collected from pools and riffles by scrubbing rocks and washing the material into plastic containers with distilled water. This was done until a 500 ml jar had been filled. CPOM and FPOM did not accumulate in the riffles, so benthic substratum was collected from pools and elutriated in a bucket. The elutriate was then sieved using 1 mm mesh for CPOM and 250 μm mesh for FPOM, collecting about 500 ml of each. All the material was placed on ice and then frozen as soon as possible.

6.2.2 Sample processing for stable isotope analysis

In the laboratory, all samples were rinsed with distilled water before further processing. Tadpoles were dissected and their guts were removed for gut content analysis. For the small tadpoles, whole bodies (excluding the gastrointestinal tract) were used for isotopic analysis, whereas for the large individuals only the tail muscle was analysed (Caut et al. 2013). Some individuals had to be combined because the sample

was too small, but species were kept separate. Material from the tadpole guts was removed, mixed with a drop of water, placed on a glass slide and observed under a microscope. The proportion of each type of food particle was estimated as a percentage of the total volume of particles present (Hyslop 1980), to the nearest 5%.

The invertebrates were sorted under a dissecting microscope (Appendix 3.4). They were kept separate by species where possible because different species may vary in their isotopic nitrogen composition despite being raised on the same diet (Deniro and Epstein 1981). However, depending on body size and abundance, families from the same feeding group and habitat within an order were combined if necessary for adequate sample size (Merritt and Cummins 1984; Gooderham and Tsyrlin 2002; Cheshire et al. 2005). For most invertebrates, the whole body was analysed because individuals were small and abundances were low. For crayfish, only the tail muscles were analysed. Eel fin clips were used whole, whereas for small fish, the bones, scales and guts were removed, and the whole individual was analysed.

CPOM and FPOM samples were rinsed and filtered to remove any unwanted particles such as stones and invertebrates. Whole leaves from the same site were washed with distilled water, then blended and homogenised; a subsample of this mixture was used to represent leaf litter at a particular pool or riffle. The biofilm scraped from rocks contained fine detritus, which was included as part of the matrix, as there was insufficient material to analyse the components separately. All the samples were oven-dried at 60°C for at least 48 hours and ground to a fine powder using a stone mortar and pestle, except for some very small samples, which were analysed intact.

Analysis for $\delta^{15}N$, $\delta^{13}C$, %N and %C was carried out by the Stable Isotope Laboratory at the University of Hong Kong. The samples were analysed using a continuous flow stable isotope ratio mass spectrometer (Nu Instruments, Perspective series) connected to an elemental analyser (Eurovector EA3028). Isotope values were normalised with a certified acetanilide reference standard. Vienna Pee Dee Belemnite and atmospheric nitrogen were used as standard references for carbon and nitrogen respectively (Peterson and Fry 1987).

6.2.3 Data analyses

The raw stable isotope data are presented as the proportion of isotope composition in the sample to the proportion in the relevant standard (above) and expressed in parts per thousand (‰): $\delta X = [(Rsample/Rstandard) - 1] \times 10^3$, where X is ^{15}N or ^{13}C, and R is the ratio of the heavier to lighter isotope, $^{15}N/^{14}N$ or $^{13}C/^{12}C$ (Peterson and Fry 1987). The $\delta^{13}C$ measure does not change much from the basal food source to the consumer and is therefore an indication of a consumer's food source (Peterson and Fry 1987; Winemiller et al. 2011). The $\delta^{15}N$ measure usually increases from food source to consumer, and therefore indicates a consumer's trophic position (Peterson and Fry 1987).

Mean $\delta^{15}N$ and $\delta^{13}C$ ratios of basal sources and consumers were plotted across stream reaches at both Paluma and Tully using raw isotopic data (Whiles et al. 2006). The $\delta^{13}C$ values are shown on the x-axis and indicate the range of food sources, whereas the $\delta^{15}N$ values are shown on the y-axis and represent the vertical trophic levels (i.e., basal sources at the bottom, followed by primary consumers, and secondary consumers at the top). Consumers sampled included invertebrates, tadpoles and fishes at Paluma, and only tadpoles at Tully. Invertebrates were grouped taxonomically and according to feeding mode, tadpoles were grouped according to species and size class, and fishes were separated into small fish (*Morgurnda adspersa*) and eels (*Anguilla reinhardtii*). The C: N ratio of basal sources was calculated to determine the comparative nutritional quality of the various food sources available to consumers (Iwai and Kagaya 2007). A lower C: N ratio generally indicates a higher quality food source (Gulis et al. 2004; Iwai et al. 2012).

6.2.3.1 Lipid correction

In animals, lipid-rich tissues have a lower proportion of the heavier carbon isotope (^{13}C) compared to other tissues (DeNiro and Epstein 1977), and the differences in lipid content among individuals may make comparisons of isotopic signatures less reliable (Post et al. 2007). Corrections for the different carbon ratio of lipid-rich tissues can be made by removing lipids before analysis, or by applying mathematical models after laboratory analysis (Post et al. 2007; Logan et al. 2008). If the C: N mass ratio in the tissues of aquatic organisms is less than 3.5, lipid correction is not necessary (Post et al. 2007).

For tissues with a C: N ratio greater than 3.5, Post et al. (2007) proposed the following equation to correct for high lipid concentrations of aquatic organisms: $\delta^{13}C_{normalised} = \delta^{13}C_{untreated} - 3.32 + 0.99$ (C: N_{bulk}). *Normalised* refers to tissues that are lipid-extracted and *untreated* refers to bulk tissues. This equation was obtained by comparing physical lipid extraction with mathematical normalisation on a range of aquatic animals, which consisted mainly of different fish species (Post et al. 2007). This equation was applied when C: N ratios were greater than 3.5 for the fish samples in this study. For tadpoles with a C: N ratio greater than 3.5, lipid correction was carried out on the isotopic carbon values using the equation $\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 1.11 + 0.37$ (C: N_{bulk}) proposed by Caut et al. (2013), which was tested specifically on tadpoles. Lipid correction was not conducted for invertebrates (following Blanchette et al. 2014) because variation in lipid content of invertebrates does not usually differ between corrected and uncorrected samples (Kiljunen et al. 2006; Logan et al. 2008).

6.2.3.2 Basal source contribution model

The relative contribution of basal sources to consumer isotopic signature was modelled using the statistical package SIAR (Stable Isotope Analysis in R, Parnell et al. 2010). The Bayesian methods in SIAR allow the user to incorporate prior information in the analysis, including different discrimination factors (see below) and standard deviations for each source (Parnell et al. 2010; Bond and Diamond 2011). The model can simultaneously analyse various basal sources to produce the most likely dietary scenarios, incorporating uncertainty in the data and variability associated with the natural system (Parnell et al. 2010). SIAR comes with caveats, some of which are common to other mixing models: (1) SIAR can only provide probable solutions, (2) SIAR assumes that the variance of sources and trophic enrichment factors is normally distributed, (3) SIAR assumes that consumers assimilate isotopes equally, and (4) SIAR will always try to fit a model, even if some sources lie outside of the isotopic mixing space (Parnell et al. 2010).

To minimise the caveats in SIAR, conservative reporting parameters were used (discussed below). The SIAR model was run using one of two commands depending on the number of data points, with 500,000 iterations, of which the first 50,000 were discarded. Siarmcmcdirichletv4 is a command that runs the mixing model when multiple data points are available for each consumer taxon, whereas siarsolomcmcv4

runs the model for single data points. The stream reaches in each location were kept separate during the analysis: road crossing, artificial streams and Camp Creek at Paluma, and Streams 1 and 2 at Tully. Sources were averaged across the riffle and pool samples from each stream reach. The animals within each reach were pooled for analysis and only basal sources found in that reach were included in the analysis.

Standard deviations for modelling basal source contribution in SIAR were calculated from samples within each stream where possible, and where only one sample of a particular source was found in the stream, standard deviations were calculated based on all the samples of that source within each location (either Paluma or Tully). The iron matrix was only found at one site (a pool in Camp Creek) and therefore standard deviations could not be calculated for this sample. Microorganisms can support consumers in some systems (Opsahl and Chanton 2006; Roach et al. 2011), and bacteria associated with the iron matrix may have therefore provided some consumers with a temporary food source. A conservative standard deviation of 0.2 was used, based on calculations for the other basal sources (Appendix 3.2), so that the iron matrix could be included in the model as a basal source.

Basal sources within a stream reach were combined if the carbon isotope signatures were within 0.5‰ of each other. Other studies have used a threshold of 2.0‰ (e.g., Blanchette et al. 2014), but the carbon signatures of most sources in this study were within this range, so a more conservative measure was appropriate. However, allochthonous food sources were kept separate from autochthonous food sources, maintaining source fidelity; for example, leaf litter was not combined with biofilm or filamentous algae (Appendix 3.3). Where basal sources were combined, the average isotopic signatures and standard deviations were used for the mixing models.

The trophic enrichment factor (TEF), also known as the discrimination or fractionation factor, represents the change in ratio of heavy to light isotopes from resource to consumer (Peterson and Fry 1987). The isotopic ratio in a consumer may not match that of its resource (Caut et al. 2013), and SIAR requires the input of TEF values to place the consumers within the source geometry. Animals are usually enriched in $\delta^{15}N$ compared to their food source, whereas they are similar to their food sources in $\delta^{13}C$ (DeNiro and Epstein 1978; Peterson and Fry 1987; Post 2002). Enrichment factors obtained from controlled laboratory experiments specific to the diet and consumer tissue are more accurate than estimates obtained from the literature or field (Caut et al. 2008), but this was beyond the scope of this study.

In this study, tadpole TEF values (3.80 ± 0.46 ‰ for $\Delta^{15}N$ and 1.19 ± 0.31 ‰ for $\Delta^{13}C$) were obtained from Caut et al. (2013). These were determined from controlled diet experiments using two anuran species and four food sources: macrophytes, zooplankton, algae and dead tadpoles. Bunn et al. (2013) obtained $\Delta^{15}N$ enrichment factors for invertebrates and fishes from a range of streams and rivers in various climatic regions in Australia and New Guinea. The overall mean values for invertebrate $\Delta^{15}N$ were 0.6 ± 1.7 ‰ for herbivores and 1.2 ± 1.3 ‰ for predators and these values were applied in this study. Two fish species were caught in the Paluma reaches: *Mogurnda adspersa,* which feeds mainly on invertebrates, and *Anguilla reinhardtii,* which feeds on invertebrates or fishes (Sloane 1984; Hortle and Pearson 1990; Pusey et al. 2010). A $\Delta^{15}N$ value of 3.7 ± 2.2 ‰ for predatory fishes was used for both (Bunn et al. 2013). The TEF value for $\Delta^{13}C$ was taken to be 0.4 ± 1.3‰ for invertebrates and fishes (Post 2002; Blanchette et al. 2014).

SIAR was first used to plot the raw isotopic data of basal sources and consumers, adding TEF values to sources. The basal sources were plotted with standard deviations, and these standard deviations were connected to produce the source mixing space. Consumers that fell outside of this isotopic mixing space were removed before analysis and the models of those consumers were taken to be unresolved (see Blanchette et al. 2014). All other consumers were then analysed using the SIAR basal source contribution models. The results for these models were reported as 95% confidence intervals (Blanchette et al. 2014). Boxplot outputs from R were used to determine the source contribution for each consumer group following Blanchette et al. (2014). A source with a minimum contribution of greater than or equal to 20% was considered a 'likely contributor', and a source with a minimum contribution of greater than 0% but less than 20%, and a maximum of greater than or equal to 50% was considered a 'possible contributor'. If the minimum contribution was 0%, the source was not considered to be a contributor and the model was unresolved. This may be due to omnivory, whereby consumers assimilate a variety of food sources, which precludes the identification of one main source (Blanchette et al. 2014). The models were also unresolved if the isotopic signatures of the basal food sources overlapped, making it difficult to identify the source that was assimilated.

6.2.3.3 *Determination of trophic position using stable isotope data*

Baseline $\delta^{15}N$ and $\delta^{13}C$ in sources may vary depending on the habitat (Vander Zanden and Rasmussen 1999); this makes it impossible to accurately assign animals to trophic levels. The $\delta^{15}N$ variability in basal sources was standardised by obtaining a baseline relationship between $\delta^{15}N$ and $\delta^{13}C$ for primary consumers, which could then be used to calculate isotopic trophic position for higher consumers (Vander Zanden and Rasmussen 1999; Blanchette et al. 2014). The baseline equation was obtained from the invertebrate primary consumers at Paluma (grazers, gatherers, filterers or shredders). Tadpoles are most probably not exclusively primary consumers (Alford 1999) and were therefore not included in the equation estimate. The baseline equation for primary consumers was $\delta^{15}N_{base} = 14.224 + (0.344* \delta^{13}C)$, $r^2 = 0.388$, $n = 33$, $P < 0.0001$ (Figure 6.1).

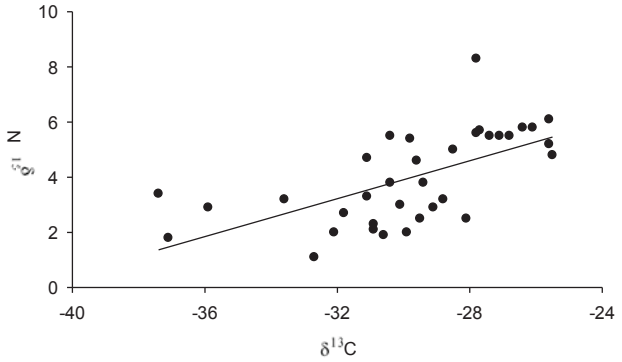

Figure 6.1. The baseline relationship between $\delta^{13}C$ and $\delta^{15}N$ for the invertebrate primary consumers at Paluma. The equation of the regression line is $y = 14.224 + 0.344x$, $r^2 = 0.228$, $n = 33$, $P < 0.0001$.

Consumer Isotopic Trophic Position (ITP) was calculated using the equation: $ITP = [(\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta^{15}N] + 2$ (Winemiller et al. 2011), where $\delta^{15}N_{consumer}$ is the isotopic measure of the consumer in question, $\delta^{15}N_{base}$ is calculated from the $\delta^{13}C$ of the consumer using the baseline equation (above), and $\Delta^{15}N$ is the mean trophic fractionation of $\delta^{15}N$ between basal sources and consumers. For $\Delta^{15}N$, a value of 3.8‰ was used for tadpoles (Caut et al. 2013), 3.7‰ for predatory fishes (Bunn et al. 2013), and an average of 1.2‰ for invertebrates (Bunn et al. 2013). ITPs were compared among consumer groups using Kruskal-Wallis one-way nonparametric analysis of variance (ANOVA) followed by Dunn's pairwise comparison tests. Samples were

pooled from all sites within each group for tadpoles, herbivorous and predatory invertebrates, and fishes. Consumers with ITPs approximating integer values were taken to occupy a specific trophic level: ITPs close to 2 (1.9 - 2.1) were considered to be primary consumers, and those with trophic levels close to 3 (2.9 - 3.1) were categorised as secondary consumers (Thompson et al. 2007). Consumers with ITPs that were not centred on an integer were most likely omnivorous (Thompson et al. 2007).

6.2.3.4 Stream food webs

Food webs were used to show the links (trophic interactions) between the various consumers (nodes) and basal sources for the three stream reaches at Paluma in 2012. Invertebrates and large predators were not collected at Tully, precluding the construction of complete food webs. Invertebrate consumers were categorised according to their feeding behaviours: gatherers, grazers, filterers, shredders and predators (Merritt and Cummins 1984; Gooderham and Tsyrlin 2002; Cheshire et al. 2005; Whiles et al. 2013), whereas the tadpoles were grouped together in the food webs.

The relative size of the consumer boxes in the food webs is based on the average biomass of the animals in the streams during the month of sampling (obtained from the survey data, Chapters 2 and 3). Basal sources were included in the food web if there was enough material for stable isotope analysis. Up to seven basal sources were included, which varied among stream reaches. Basal sources were used for the construction of food webs in both habitat types, regardless of whether they were collected in riffles or pools. Other predators such as kingfishers or platypus were rare and were not included in the food webs.

Trophic positions were indicated by the stable isotope data for consumers, and links were added based on the basal source contribution data from the SIAR analysis. The isotopic trophic positions for invertebrates were variable and were therefore used in combination with information from the literature to determine trophic positions for the food web link calculations. Grazers, filterers and shredders were considered to be omnivores, feeding primarily on autochthonous and allochthonous material. Gatherers and tadpoles likely consumed a combination of plant and animal material, and were therefore linked with basal sources and the above-mentioned invertebrate groups. Predatory invertebrates and fishes formed the top trophic level and were linked to all other consumers. Food web complexity was measured by connectance, which is the

number of actual trophic links divided by the number of possible links (Pimm 1984; Pimm et al. 1991). The number of links was estimated as the number observed in each stream, and the maximum number of links as (S(S-1)/2), where S is the number of basal sources and consumers in the food web (Cheshire et al. 2005; Blanchette et al. 2014).

6.3 Results

6.3.1 Biophysical stream variables

The in-stream habitats varied between the stream reaches at Paluma and Tully (Table 6.2). The substratum at Tully consisted of a larger proportion of boulders compared to that at Paluma and the stream reaches were generally more shaded at Paluma (Chapter 2). At the time of sampling (spring), water levels were low in both locations and flow conditions were therefore comparable between the two locations, but the riffles were generally deeper at Tully. The habitats sampled for the stable isotope analysis had similar biophysical variables such as pH, dissolved oxygen and conductivity.

Table 6.2. Stream characteristics within sampling reaches at Paluma in 2012 and Tully in 2013. The substratum size distribution is presented as proportions (%) of sand/gravel, cobbles and boulders/bedrock, with percentages averaged across riffles or pools.

Stream characteristics	PALUMA						TULLY			
	Road crossing		Camp Creek		Artificial streams		Stream 1		Stream 2	
	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle
Current velocity (ms^{-1})	0.01	0.46	0.01	0.48	0.01	0.36	0.07	0.45	0.00	0.52
Canopy cover (%)	25-50%	50-75%	75-100%	0-25%	25-50%	25-50%	25-50%	0-25%	0-25%	50-75%
Substratum composition										
Sand/gravel: cobbles: boulders/bedrock (%)	40:15:45	52:18:30	87:5:8	82:18:0	72:15:13	72:15:13	16:22:62	10:28:62	16:34:50	10:20:70
Stream depth (cm)	62	13	59	9	24	10	37	29	50	22
Conductivity (μScm^{-1})	0.03	0.03	0.03	0.03	0.03	0.03	0.03		0.04	
Dissolved oxygen (mgL^{-1})	7.95	8.15	7.73	7.80	7.31	7.48	8.12		7.93	
Dissolved oxygen (% saturation)	84.20	85.85	80.45	81.05	76.70	78.45	96		88.7	
pH	6.70	6.70	6.35	6.40	6.35	6.45	8.4		8.1	

6.3.2 Raw isotopic values of consumers and basal sources across sites

The isotopic composition of most basal sources was similar among stream reaches within an location, but differed between Paluma and Tully (Figure 6.2). At Tully, the carbon signature of biofilm was generally more enriched compared to allochthonous basal sources (Figure 6.2b), whereas the biofilm carbon signatures at Paluma overlapped with most allochthonous sources (Figure 6.2a). Leaf and FPOM samples were more $\delta^{15}N$ enriched at Tully than at Paluma, but leaves were generally less nitrogen-enriched compared to most of the other samples in both locations. Filamentous algae and biofilm had the most variable isotopic signatures within and among stream reaches. CPOM, periphyton and the iron matrix were not found in the Tully reaches (Figure 6.2b), and some sources were only found at one site in a stream reach (Appendix 3.1).

At both Paluma and Tully, consumers were generally $\delta^{15}N$ enriched compared to the basal sources (Figure 6.3). At Paluma, most of the tadpole and invertebrate groups had $\delta^{13}C$ signatures similar to the basal sources (Figure 6.3a), whereas at Tully the carbon signatures of tadpoles overlapped only with the autochthonous sources (Figure 6.3b). *Mogurnda* and *Anguilla* had the highest $\delta^{15}N$ measure at Paluma, with *Anguilla* being the most carbon enriched. The nitrogen isotopic composition of invertebrates and tadpoles overlapped at Paluma, although most tadpoles clustered on one trophic level.

Mean isotopic signatures for tadpoles of different species and sizes were examined separately. The tadpole isotopic composition at Paluma varied from one year to the next, with tadpoles from 2013 more $\delta^{15}N$ depleted compared to the same groups in 2012, although there was some overlap (Figure 6.4a). The $\delta^{13}C$ values, on the other hand, were similar among the various species and size classes. Tadpoles from 2012 clustered together (apart from small *Litoria serrata* tadpoles), whereas 2013 tadpoles generally exhibited more variability in isotopic signatures among species and size classes (Figure 6.4b). Invertebrate taxa were also separated according to their feeding modes (Appendix 3.4). At Paluma, the isotope signatures of most invertebrates were highly variable and predators and herbivores overlapped in their $\delta^{15}N$ and $\delta^{13}C$ measures, but gatherers were generally more enriched in the nitrogen isotope compared to grazers (Figure 6.5).

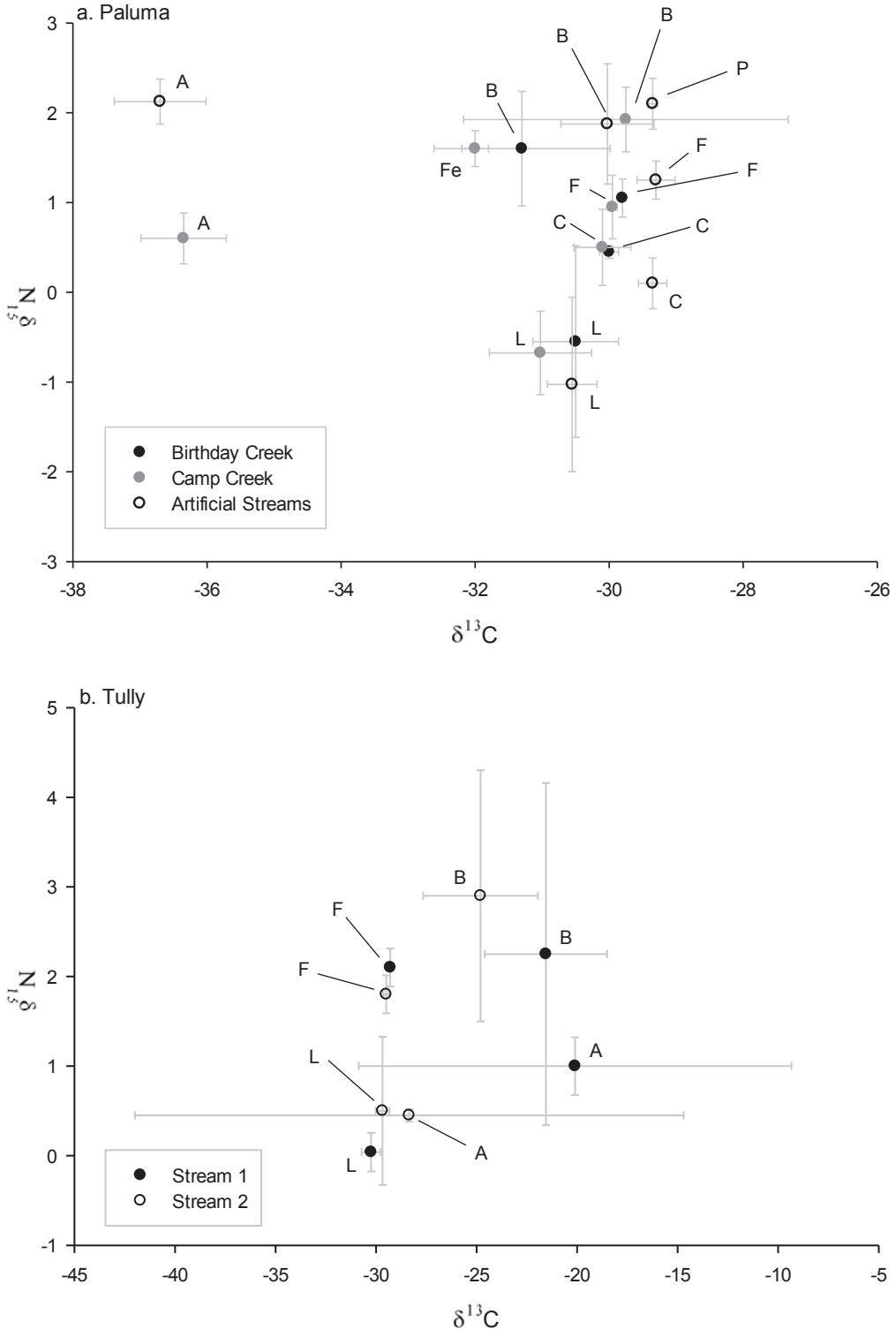

Figure 6.2. Mean (± s.d.) $\delta^{15}N$ and $\delta^{13}C$ ratios of basal sources for each stream at (a) Paluma in 2012 and (b) Tully in 2013. Basal source codes: F = FPOM, C = CPOM, L = leaves, B = biofilm, P = periphyton, A = filamentous algae, and Fe = iron matrix.

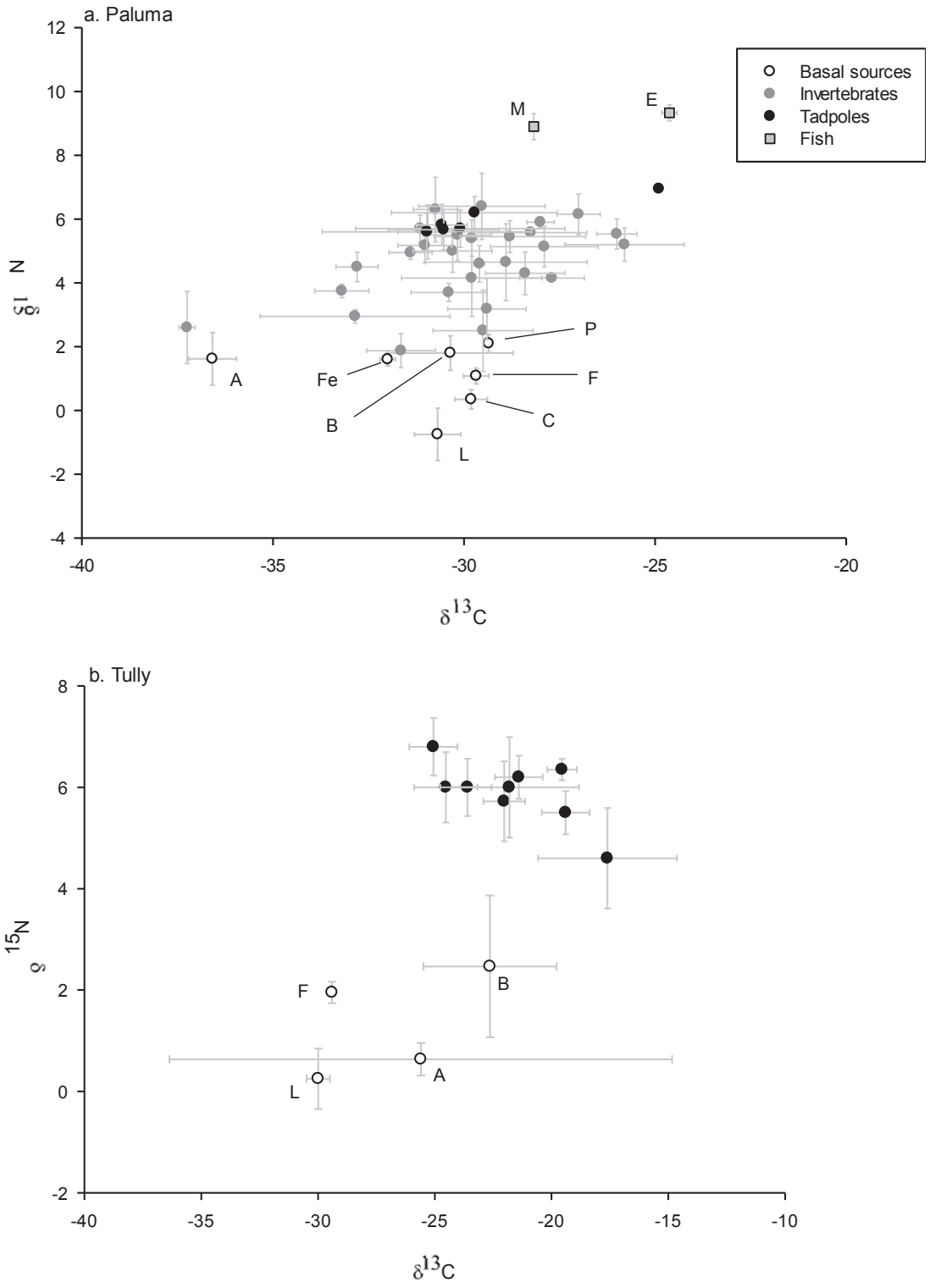

Figure 6.3. Mean (± s.d.) $\delta^{15}N$ and $\delta^{13}C$ ratios of (a) basal sources, invertebrates, tadpoles, and fishes (M = *Mogurnda adspersa*, E = *Anguilla reinhardtii*) for stream reaches at Paluma in 2012, and for (b) basal sources and tadpoles for stream reaches at Tully. Each consumer point represents a taxon of a specific feeding group for invertebrates, a species for fishes, and a specific size class of a species for tadpoles. Basal source codes: F = FPOM, C = CPOM, L = leaves, B = biofilm, P = periphyton, A = filamentous algae, and Fe = iron matrix.

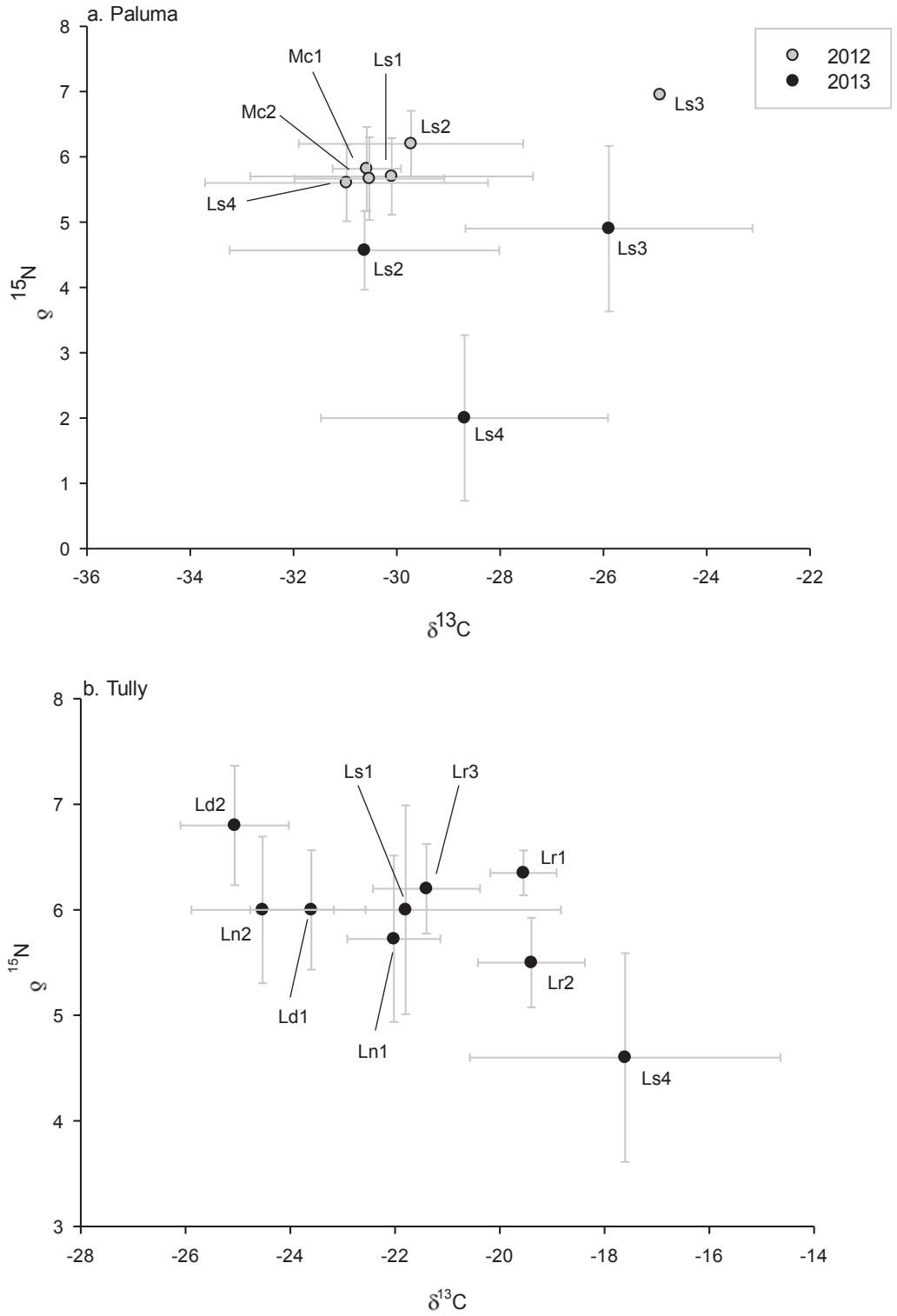

Figure 6.4. Mean (± s.d.) $\delta^{15}N$ and $\delta^{13}C$ ratios of tadpoles at (a) Paluma in 2012 and 2013, and (b) Tully in 2013. Each point represents a specific size class of a species. Species codes: Ls = *Litoria serrata,* Ln = *L. nannotis,* Lr = *L. rheocola,* Ld = *L. dayi,* Mc = *Mixophyes coggeri.* Size categories: 1 = large, 2 = medium, 3 = small, 4 = small and medium tadpoles combined.

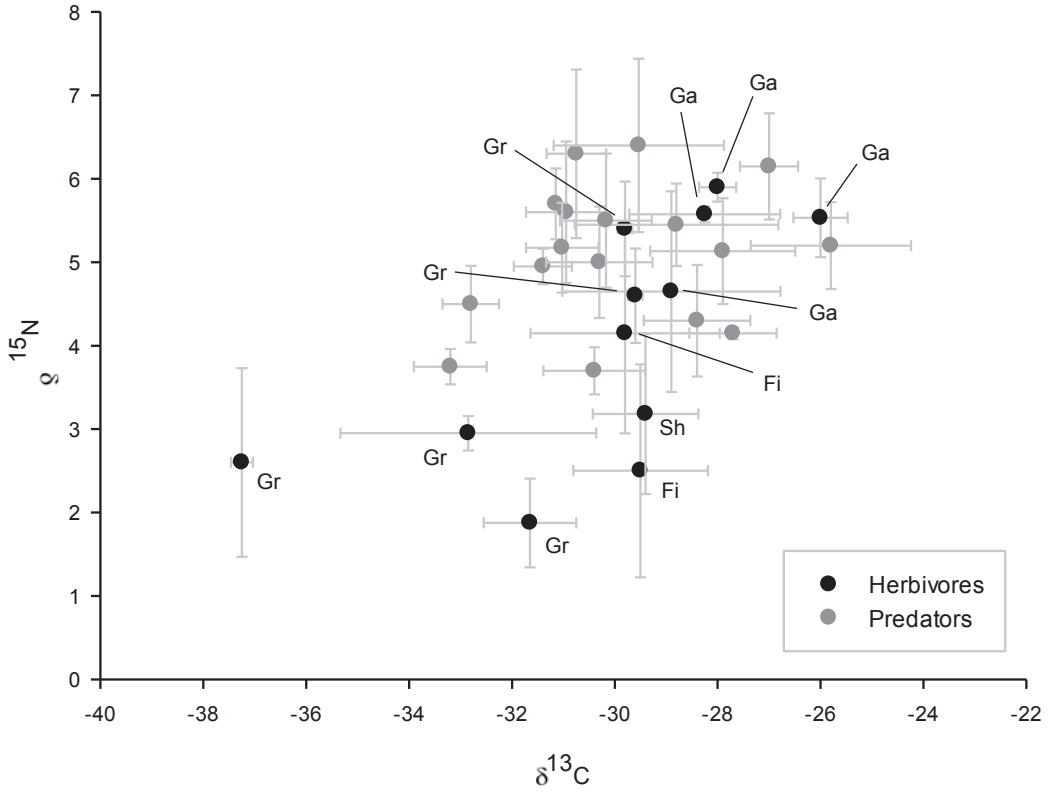

Figure 6.5. Mean (± s.d.) $\delta^{15}N$ and $\delta^{13}C$ ratios of invertebrate herbivores and predators at Paluma in 2012. Each point represents a taxon of a specific feeding group. Feeding group codes for herbivores: Fi = filterer, Ga = gatherer, Gr = grazer and Sh = shredder.

6.3.3 Source contribution to consumer diets at Paluma and Tully

Biofilm was an important food source for tadpoles at Paluma and Tully, but use of this resource varied, depending on stream and the year of sampling (Table 6.3). Biofilm was a possible or likely contributor for tadpoles at the road crossing, whereas tadpoles in the other Paluma reaches assimilated a variety of basal sources. At Tully, most tadpoles obtained their nutrients from more than one source. Biofilm, alone or in addition to another source, was identified as an important contributor in Stream 1, whereas filamentous algae and a combination of leaves and FPOM were the main sources in Stream 2. Biofilm and filamentous algae had the highest nutritional quality of the basal sources available for consumers, as shown by the C: N ratio (Table 6.4). The iron matrix was not part of the tadpoles' diet in Camp Creek, the only site where it was found (Table 6.3). FPOM was a more important basal source at Tully than at Paluma, being a possible contributor for several tadpole groups at Tully. Leaf material was present at all the sites in the study, but was only consumed by a few tadpoles. Leaves

had the highest C: N ratio (44 – 69), indicating low nutritional quality (Table 6.4). Periphyton found at the artificial stream reach had lower nutrient quality compared to biofilm on rocks in the same stream reach and was closer to FPOM and CPOM in nutrient quality.

Comparing the gut content analysis of tadpoles with the results from the stable isotope models suggested that the material assimilated did not correspond closely to what was consumed (Table 6.5). High proportions of FPOM or CPOM were found in tadpole guts, with some algae or diatoms present. However, the isotope analysis revealed that they were actually assimilating more biofilm and filamentous algae, depending on availability, and to a lesser extent FPOM, CPOM or leaf material. The gut content analysis also indicated that some *Litoria nannotis* tadpoles consumed invertebrates, identified as trichopteran larvae.

The majority (63%) of SIAR mixing models for invertebrates at Paluma were unresolved (Table 6.6). There was high level of source fidelity across taxa within the road crossing reach, with most invertebrates at this site assimilating biofilm. The majority of mixing models in Camp Creek and the artificial streams were unresolved so it was unclear if site-specific source fidelity occurred in these stream reaches. This meant that some invertebrate groups changed their use of a particular source among sites; for example, psephenid larvae (Coleoptera) consumed mainly biofilm in the road crossing reach, but filamentous algae in the artificial streams (where filamentous algae were the dominant source in the mixing models). CPOM, FPOM and periphyton were also identified as possible sources for some grazers and shredders. Leaf material was only important for lepidopteran larvae, whereas caddisfly shredders did not assimilate substantial amounts of leaf litter compared to other basal sources.

Several animals were outside of the source mixing space and therefore had unresolved source contributions. These included small *L. serrata* tadpoles from the road crossing, *Anguilla* from the road crossing, and a number of invertebrates from all three stream reaches at Paluma. For some consumers, the models were unresolved despite the animals being within the source mixing space; for example, tadpoles at Camp Creek and large *Mixophyes coggeri* tadpoles at the artificial streams, all in 2012 (Table 6.3). This was also the case for several invertebrate groups, especially at Camp Creek and the artificial streams, where there were more basal sources available (Table 6.6).

Table 6.3. Stable isotope mixing model results for tadpoles at Paluma in 2012 and 2013, and Tully in 2013. Basal source abbreviations: B = biofilm, A = filamentous algae, C = coarse particulate organic matter, F = fine particulate organic matter, L = leaves, P = periphyton, and Fe = iron matrix. Highlighted source = likely source contribution (minimum contribution ≥ 20%), regular type = possible contribution (minimum contribution > 0% and maximum contribution ≥ 50%), nr = unresolved (equal source contribution or isotopic source overlap), and nr[1] = consumer outside the basal source mixing space (not analysed using SIAR). Tadpole size categories were according to Table 6.1.

Species	Size class	PALUMA						TULLY	
		Road crossing		Camp Creek		Artificial streams		Stream 1	Stream 2
		2012	2013	2012	2013	2012	2013	2013	2013
L. serrata	Large	**B**	-	-	-	-	-	B	-
	Medium	**B**	B, L	nr	A	B	C-F	-	-
	Small	nr[1]	**B**	-	-	-	-	-	-
	Mix	-	-	nr	**L**	-	-	-	A, L-F
M. coggeri	Large	**B**	-	nr	-	nr	-	-	-
	Medium	**B***	-		-	P	-	-	-
L. nannotis	Large	-	-	-	-	-	-	B	A, L-F
	Small/ medium	-	-	-	-	-	-	B, F	-
L. rheocola	Large	-	-	-	-	-	-	B, A	-
	Medium	-	-	-	-	-	-	B, A	-
	Small	-	-	-	-	-	-	B**	
L. dayi	Large	-	-	-	-	-	-	B, L	-
	Small/ medium	-	-	-	-	-	-	**B**, F	-

* Sample composed of specimens obtained from the road crossing and Camp Creek

** Sample composed of specimens obtained from Tully Stream 1 and Stream 2

Table 6.4. The C: N ratio of basal sources in the stream reaches at Paluma and Tully. A lower C: N ratio indicates a higher nutrient quality.

Basal source	PALUMA			TULLY	
	Road crossing	Camp Creek	Artificial streams	Stream 1	Stream 2
Biofilm	10.5	10.2	12.5	10.6	10.3
Filamentous algae	-	8.9	9.2	11.6	7.8
FPOM	25.2	25.6	25.5	20.3	18.8
CPOM	31.8	33.0	31.4	-	-
Leaves	53.0	45.2	44.5	68.7	53.9
Periphyton	-	-	27.8	-	-
Iron matrix	-	19.6	-	-	-

Table 6.5. Gut contents of tadpoles at Paluma collected in 2012 and 2013 and Tully collected in 2013. The proportions of the various sources are presented as a percentage of overall gut content. Source abbreviations: FPOM = fine particulate organic matter, and CPOM = coarse particulate organic matter. Algae and diatoms were not differentiated. Tadpole size categories were according to Table 6.1.

Species	Size	Location	FPOM (%)	CPOM (%)	FPOM/ CPOM (%)	Algae/ diatoms (%)	Others
L. serrata	Large	Paluma			100		
		Tully			60	40	
	Medium	Paluma	65	25		10	
		Tully			75	25	
	Small	Paluma			90	10	
		Tully			75	25	
M. coggeri	Large	Paluma		75	20	5	
	Medium			75	25		
L. nannotis	Large	Tully	60	20		20	Invertebrates
	Small/medium	Tully	85	10		5	
L. rheocola	Large	Tully	50	30		20	
	Medium	Tully	60	30		10	
	Small	Tully	60	20		20	
L. dayi	Large	Tully	80	10		10	
	Small/medium	Tully	80	10		10	

Table 6.6. Stable isotope mixing model results for invertebrates and fishes at Paluma in 2012. Basal source abbreviations: B = biofilm, A = filamentous algae, C = coarse particulate organic matter, F = fine particulate organic matter, L = leaves, P = periphyton, and Fe = iron matrix. Highlighted source = likely source contribution (minimum contribution ≥ 20%), regular type = possible contribution (minimum contribution > 0% and maximum contribution ≥ 50%), nr = unresolved (equal source contribution or isotopic source overlap), and nr[1] = consumer outside the basal source mixing space (not analysed using SIAR).

Taxon	Family	Feeding group	Road crossing	Camp Creek	Artificial streams
Diptera	Simuliidae	Filterer	-	nr	nr
	Mixed	Filterer	B, C-F	-	-
"Worms"	Mixed	Gatherer/filterer	B	-	nr[1]
Parastacidae	Large	Gatherer	nr[1]	nr[1]	nr[1]
	Medium	Gatherer	nr[1]	nr[1]	nr
	Small	Gatherer/predator	nr[1]	nr[1]	nr[1]
Palaemonidae	Medium	Grazer	-	nr	-
Coleoptera	Psephenidae	Grazer	**B**	-	**A**
Ephemeroptera	Leptophlebiidae	Grazer/shredder/gatherer	B	nr	A
	Mixed	Grazer	**B**	A	nr
Trichoptera	Mixed	Grazer/gatherer/filterer	-	-	nr
	Philopotamidae	Grazer/gatherer/filterer	nr	-	-
	Mixed	Shredder	nr	nr	P
Lepidoptera		Grazer/shredder	B, L, C-F	-	-
"Worms"	Mixed	Predator	B	-	nr
Coleoptera	Dytiscidae	Predator	B	nr	-
	Mixed	Predator		-	nr
Ephemeroptera	Ameletopsidae	Predator	B	-	A
Plecoptera	Mixed	Predator/grazers	-	-	**A**
	Gripopterygidae	Predator/grazers	**B**	A	-
Hemiptera	Gelastocoridae	Predator	nr[1]	-	-
	Mixed	Predator	nr	B	nr[1]
Megaloptera	Corygalidae	Predator	-	nr	nr[1]
Arachnida	Pisauridae	Predator	-	nr[1]	nr[1]
Zygoptera	Synlestidae	Predator	B	-	nr[1]
	Mixed	Predator	nr	nr	n[1]
Epiproctophora	Gomphidae	Predator	nr	-	-
	Synthemistidae	Predator	B	-	-
	Telephlebiidae	Predator	B	-	-
	Mixed	Predator	-	nr	nr
Trichoptera	Mixed	Predator	B	-	nr
Fishes	*Mogurnda adspersa*	Predator	-	B	-
	Anguilla reinhardtii	Predator	nr[1]	-	-

6.3.4 Isotopic trophic position for consumers at Paluma and Tully

Tadpoles fed across trophic levels, and the ITPs (1.4 to 2.6) indicated that tadpoles were either primary consumers or omnivores (Table 6.7). At Paluma in 2012, all tadpoles were categorised as omnivores, whereas in 2013 some of them were categorised as primary consumers (ITP near 2). Most tadpoles at Tully were primary consumers with ITPs close to 2, although some were omnivores. Both fishes at Paluma were secondary consumers with ITPs of 3 or greater (Table 6.8). The ITPs for the invertebrates at Paluma were more variable (0.4 to 3.2 for invertebrates generally thought to be herbivores and 1.5 to 4.8 for predators), both spatially and taxonomically (Table 6.8). Most of the herbivores were classified as omnivores with non-integer trophic levels, whereas a small proportion of herbivores were classified as either primary consumers (ITP near 2) or secondary consumers (ITP near 3). Most of the parastacids and palaemonids were found to be secondary consumers. The majority of predators were classified as secondary consumers, whereas some fed as omnivores or primary consumers.

The ITPs differed among the four consumer groups ($H = 24.70$, $df = 3$, $P < 0.001$; Figure 6.6). Fish and predatory invertebrate ITPs were significantly higher than those of "herbivorous" invertebrates and tadpoles (Dunn's pairwise comparison, $P < 0.05$). There were no significant differences between fishes and predatory invertebrates or between "herbivorous" invertebrates and tadpoles. Generally, most tadpoles and invertebrates were omnivores, whereas fishes were secondary consumers.

Table 6.7. Isotopic trophic positions (ITPs) for tadpoles at Paluma in 2012 and 2013, and Tully in 2013. The tadpoles were categorised as primary consumers if ITP was close to 2 (1.9 - 2.1), as secondary consumers if ITP was close to 3 (2.9 – 3.1), and as omnivores if ITPs were not centred on an integer. Tadpole size categories were according to Table 6.1.

Species	Size class	PALUMA						TULLY	
		Road crossing		Camp Creek		Artificial streams		Stream 1	Stream 2
		2012	2013	2012	2013	2012	2013	2013	2013
L. serrata	Large	2.5	-	-	-	-	-	1.8	-
	Medium	2.4	2.1	2.6	2.6	2.8	2.0	-	-
	Small	2.3	1.9	-	-	-	-	-	-
	Mix	-	-	2.5	1.4	-	-	-	1.1
M. coggeri	Large	2.6	-	2.5	-	2.7	-	-	-
	Medium	2.5*	-		-	2.5	-	-	-
L. nannotis	Large	-	-	-	-	-	-	1.8	1.6
	Small/ medium	-	-	-	-	-	-	2.1	-
L. rheocola	Large	-	-	-	-	-	-	1.7	-
	Medium	-	-	-	-	-	-	1.5	-
	Small	-	-	-	-	-	-	1.8**	
L. dayi	Large	-	-	-	-	-	-	2.0	-
	Small/ medium	-	-	-	-	-	-	2.3	-

* Sample composed of specimens obtained from the road crossing and Camp Creek

** Sample composed of specimens obtained from Tully Stream 1 and Stream 2

Table 6.8. Isotopic trophic positions (ITPs) for invertebrates and fishes at Paluma in 2012. Invertebrates were categorised as primary consumers if ITP was close to 2 (1.9 - 2.1), as secondary consumers if ITP was close to 3 (2.9 – 3.1), and as omnivores if ITPs were not centred on an integer.

Taxon	Family	Feeding group	Road crossing	Camp Creek	Artificial streams
Diptera	Simuliidae	Filterer	-	1.8	2.5
	Mixed	Filterer	0.7	-	-
"Worms"	Mixed	Gatherer/filterer	2.0	-	2.6
Parastacidae	Large	Gatherer	2.5	2.5	1.9
	Medium	Gatherer	2.7	2.8	3.4
	Small	Gatherer/predator	3.2	3.0	3.1
Palaemonidae	Medium	Grazer	-	5.0	-
Coleoptera	Psephenidae	Grazer	2.3	-	3.7
Ephemeroptera	Leptophlebiidae	Grazer/shredder/gatherer	1.0	0.8	0.4
	Mixed	Grazer	2.4	2.2	1.3
Trichoptera	Mixed	Grazer/gatherer/filterer	-	-	3.2
	Philopotamidae	Grazer/gatherer/filterer	2.5	-	-
	Mixed	Shredder	1.5	0.4	0.9
Lepidoptera		Grazer/shredder	0.5	-	-
"Worms"	Mixed	Predator	3.0	-	4.3
Coleoptera	Dytiscidae	Predator	1.9	2.0	-
	Mixed	Predator	-	-	1.5
Ephemeroptera	Ameletopsidae	Predator	3.3	-	3.3
Plecoptera	Mixed	Predator/grazers	-	-	3.3
	Gripopterygidae	Predator/grazers	2.5	3.1	-
Hemiptera	Gelastocoridae	Predator	1.9	-	-
	Mixed	Predator	3.1	1.9	2.3
Megaloptera	Corygalidae	Predator	-	3.1	2.8
Arachnida	Pisauridae	Predator	-	3.3	2.4
Zygoptera	Synlestidae	Predator	3.6	-	4.8
	Mixed	Predator	3.5	3.9	4.4
Epiproctophora	Gomphidae	Predator	1.9	-	-
	Synthemistidae	Predator	3.0	-	-
	Telephlebiidae	Predator	3.4	-	-
	Mixed	Predator	-	3.5	3.3
Trichoptera	Mixed	Predator	3.6	-	4.1
Fishes	*Mogurnda adspersa*	Predator	-	3.2	-
	Anguilla reinhardtii	Predator	3.0	-	-

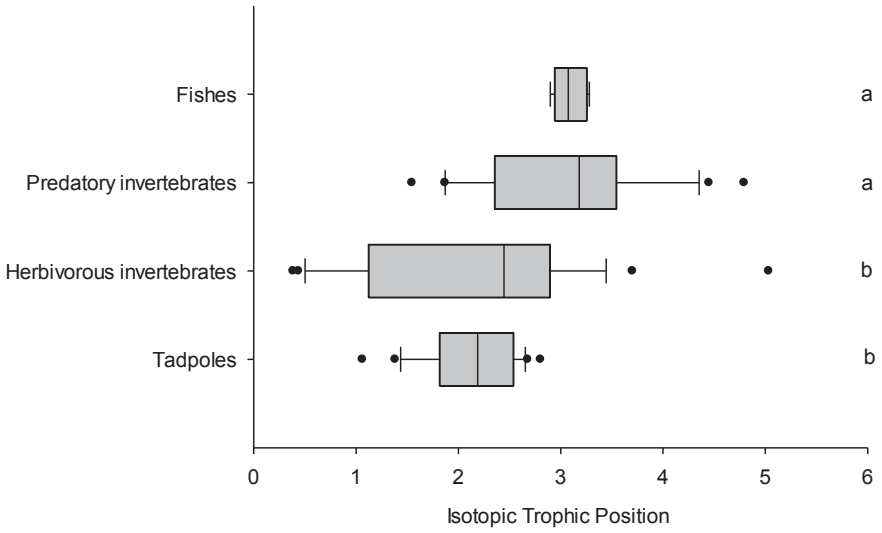

Figure 6.6. Isotopic trophic positions (ITPs) of tadpoles, "herbivorous" invertebrates, predatory invertebrates and fishes across all sites at Paluma and Tully in 2012 and 2013. Significant differences between ITPs for the various consumer groups (indicated by Dunn's test with α = 0.05) are shown by letters a and b.

6.3.5 Food webs

The food web structure was more complex in pools than in riffles, leading to a higher connectance in pools for all three stream reaches at Paluma (Figure 6.7, Table 6.9). Tadpoles were found only in pools in all the reaches, whereas *Mogurnda* and *Anguilla* were present in pools at the road crossing and Camp Creek respectively, leading to more links in the pool food webs (Figure 6.7). Shredders and gatherers were more abundant in pools, filterers and grazers were more abundant in riffles, and predatory invertebrates were represented equally in both habitat types (according to biomass). The food web structure also differed among the three stream reaches. The road crossing had fewer basal sources (four) than the other two stream reaches (each with six), although food web connectance in this study was highest in the pools (C = 0.56) and riffles (C = 0.47) of the road crossing (Table 6.9). Although the artificial streams did not have top vertebrate predators (fishes), the pool food web had a similar connectance as that at Camp Creek (C = 0.48 and 0.49 respectively). Where fishes were present, their biomass was greater at the road crossing (three eels with average length of 80 cm) compared to Camp Creek (six fish with average length of 8 cm). Tadpole biomass (average biomass = 10.9 g) was greater than that of the invertebrate consumer groups (average biomass = 2.2 g) in the pools of all three stream reaches. Omnivory was

prevalent in the food webs, with primary consumers feeding on various food sources, and secondary consumers feeding within and across trophic levels.

Table 6.9. Food web components of the stream reaches at Paluma separated by habitat, showing links and connectance. Links = actual number of links, S = total number of basal sources and consumers, Max. links = (S(S-1)/2), and Connectance = links/ Max. links.

Site	Habitat	Links	S	Max. links	Connectance
Road crossing	Pools	31	11	55	0.56
	Riffles	17	9	36	0.47
Camp Creek	Pools	38	13	78	0.49
	Riffles	21	11	55	0.38
Artificial streams	Pools	32	12	66	0.48
	Riffles	21	11	55	0.38

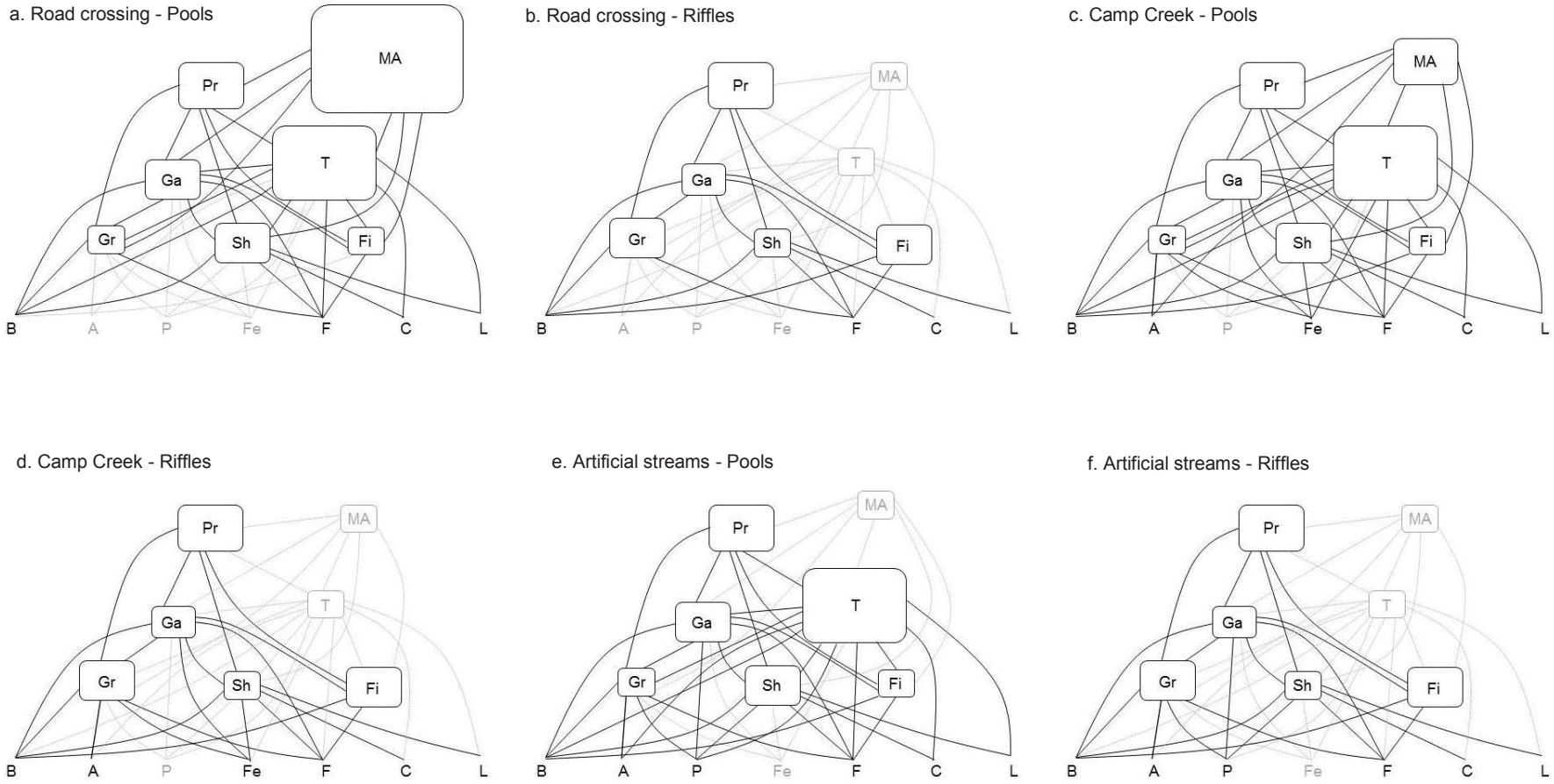

Figure 6.7. Food web structures for stream reaches at Paluma. The boldface letters on the bottom of the figure represent basal sources that were collected from a particular site and analysed for $\delta^{15}N$ and $\delta^{13}C$. The boldface letters in the boxes represent consumer groups that were present, with the size of the box representing the relative biomass of the consumers. Basal sources: B = biofilm, A = filamentous algae, P = periphyton, Fe = iron matrix, F = FPOM, C = CPOM, and L = leaf litter. Invertebrate consumer groups: Gr = grazers, Sh = shredders, Fi = filter feeders, Ga = gatherers, and Pr = predators. Other consumers: T = tadpoles, and MA = fishes.

6.4 Discussion

The main source of assimilated food for consumers in Paluma and Tully stream reaches was biofilm and algae. However, tadpoles also consumed other sources, including allochthonous material such as fine particulate organic matter, depending on the site and year. Tadpoles were therefore generalist feeders, most likely choosing available high quality food sources. They also fed across trophic levels, and were therefore omnivores. Resource use overlapped between tadpoles and invertebrates in the Paluma reaches, indicating that they may have competed for food, depending on animal densities and food availability. The riffle food webs at Paluma were simpler than those in pools due to the absence of riffle tadpoles and top predators such as fishes. Although tadpoles added complexity to the food webs in pools at Paluma, it is not known to what extent the food webs in riffles were different when tadpoles were present.

6.4.1 Food sources and trophic positions

The isotopic composition of each basal source was similar among stream reaches within each location (Paluma or Tully), but there were differences between the two locations, especially for algae and biofilm. There was limited algal growth in the Paluma reaches, whereas at Tully, algae accumulated in some pools during the dry season, most likely because the Tully stream reaches were more open (less canopy cover) and received more light than those at Paluma. The differences in stream environments, such as flow conditions and substratum composition, may have resulted in the growth of different algal species, contributing to the variability in isotopic composition between the two locations. The difference in isotopic signatures of biofilm between the two locations may have been due to a greater algal component at Tully.

Among consumers, herbivores are generally depleted in $\delta^{15}N$, predators are enriched, and omnivores have variable $\delta^{15}N$ signatures (Minagawa and Wada 1984; Fry 1988). Omnivores may obtain their nutrition from various basal sources, as well as from other organisms (Lancaster et al. 2005), and this makes trophic classification difficult (Polis and Strong 1996). Overall, the basal sources at Paluma and Tully were the most $\delta^{15}N$ depleted, invertebrates and tadpoles were intermediate, and fishes were the most enriched, similar to the findings of other studies (e.g., Blanchette et al. 2014). Omnivory

was important in the tadpole and invertebrate assemblages, indicating that the animals fed on more than one trophic level (Pimm and Lawton 1978), allowing them to feed in a wide range of habitats and maximise resource use (Lancaster et al. 2005). This may be more common in tropical streams where food sources vary with seasonal rainfall (Frauendorf et al. 2013).

Although biofilm was an important resource for tadpoles, they were opportunistic feeders and varied their diets. For example, medium *L. serrata* fed on biofilm, leaves, filamentous algae, or a mixture of CPOM and FPOM, depending on the stream reach and the year of sampling. At some sites, two possible food sources for tadpoles were identified, indicating that the tadpoles fed on both, perhaps depending on availability. Microorganisms in biofilms associated with the basal sources were probably an important part of the tadpole diet. Biofilm consists of a complex bacterial and algal matrix (Lock et al. 1984), and is the basis of energy pathways in many freshwater systems (Lear et al. 2008). Microorganisms also colonise other basal sources, such as leaf litter, during conditioning (Cummins and Klug 1979). In this study, tadpoles did not feed directly on leaf material (Chapter 4), and leaf signatures probably represented assimilation of microorganisms associated with the leaves (Hunte-Brown 2006; Altig et al. 2007) or FPOM.

Heterotrophic streams are those in which allochthonous resources are the major food source for consumers, but algae and other autotrophic foods may still play an important role (Bunn et al. 1999; Mantel et al. 2004; Dudgeon et al. 2010). Although the small, shaded stream reaches at Paluma were most likely heterotrophic, biofilm and algae were more important to tadpoles and invertebrates than allochthonous food sources. Tadpoles consumed substantial amounts of FPOM and CPOM, but they only assimilated a small proportion of these sources. The degree to which aquatic organisms assimilate various types of food sources varies with the quality of the resources and their availability, and may vary among sites (Frauendorf et al. 2013; Blanchette et al. 2014). Many consumers may assimilate food sources that are composed of both autochthonous and allochthonous material. For example, the carbon signature of biofilm in two Paluma study reaches was similar to that of FPOM, which was probably caused by fine detritus within the biofilm matrix (Bunn et al. 2013).

The quality of the basal sources varied, as indicated by their C: N ratios. Biofilm and filamentous algae had the lowest C: N ratios of the available basal sources, making them the highest quality. Periphyton had an isotopic signature similar to that of biofilm,

but its nutritional quality was lower than that of biofilm. In this study, leaves had the highest C: N ratio, indicating that they were the lowest quality food source. Periphyton and FPOM in streams usually have a higher nutrient quality than terrestrially derived leaf litter (Cross et al. 2005), although the quality of detritus (which was abundant at Paluma) varies widely and high-quality detritus may be limited (Trenerry 1988; Allan and Castillo 2007). The results indicate that animals generally selected food sources with high nutrient quality, depending on availability.

Slight variations in isotopic composition among tadpoles of various species and size classes may have resulted from different food preferences or different food availability in a particular microhabitat. At Tully, *L. serrata* and *L. rheocola* tadpoles were generally more carbon enriched than most *L. nannotis* or *L. dayi* tadpoles. Gut content analysis of tadpoles in Birthday Creek in a previous study showed that *L. dayi* and *L. nannotis* overlapped in diet as a result of their similar feeding behaviours in riffles (Trenerry 1988). The trophic positions of tadpoles differed between the two locations. Most tadpoles at Paluma were omnivores, whereas the majority of the tadpoles at Tully were closer to being primary consumers. In addition to the plant-based basal sources, tadpoles at Paluma may have assimilated dead or decaying animal material (Heinen and Abdella 2005; Altig et al. 2007) that accumulated in pools, leading to their higher trophic position.

Biofilm and algae were also important food sources for invertebrates in this study, as has been found in other stream systems (e.g., Jardine et al. 2012; Frauendorf et al. 2013). These results indicate that invertebrates of various feeding groups use resources similar to those of tadpoles, which may lead to competition between tadpoles and invertebrates. There was no clear distinction between feeding mode and food source, and previous studies of gut content analysis showed that most invertebrates at Birthday Creek were generalist feeders (Cheshire et al. 2005). Although shredders in this system consume mainly leaf material (Cheshire et al. 2005), they likely assimilate a range of sources. Many predators had strong biofilm signatures, probably because they consumed other invertebrates that fed on biofilm. However, predators may consume non-animal material when densities are high and food sources are limited, or as an additional high-quality energy source (Lancaster et al. 2005).

Invertebrate trophic levels did not always correspond to the traditional feeding groups from the literature. Predatory invertebrates were expected to feed mainly on other animals (Cheshire et al. 2005), but their isotopic compositions overlapped with

those of many herbivores. Although the grazers and filterers were generally $\delta^{15}N$ depleted compared to the predators, it is likely that some of the predators were partly omnivorous. Frauendorf et al. (2013) also found that omnivory was common among predators in a Neotropical stream, and some omnivorous predators in a temperate stream assimilated substantial amounts of algae (Lancaster et al. 2005). Other predators, such as crayfish, can function as omnivores and are important in processing organic matter (e.g., Parkyn et al. 2001), including in Birthday Creek (Coughlan et al. 2010). Although it may be common for predators to feed on plant- or detritus-based resources, they do not usually contribute to depleting these resources (Polis and Strong 1996). Most of the invertebrates generally thought to be herbivorous fed as primary consumers or omnivores, but a few clearly fed as secondary consumers, particularly palaemonids and parastacids. Similar results were found in Australian dryland rivers, where decapods had ITPs between 3.2 and 5.1 (Blanchette et al. 2014). These results indicate that they most likely feed on dead and decaying animal material in addition to plant- or microbial-based sources.

Of all the consumers, 27% had unresolved models due to source overlap and/or omnivory, and 22% were outside the source mixing space. Many of the invertebrate models were unresolved, most likely due to similar source contributions (a total of 63%, of which 30% were outside the source polygon). The basal source signatures were very similar within a stream reach, many within 2.0‰ of each other. Basal sources may have similar isotopic signatures if one is derived from the other, making it difficult to differentiate between them. For example, in Hong Kong streams, FPOM and periphyton had similar carbon signatures, and a large proportion of the FPOM was probably originally present as periphyton (Lau et al. 2009). Some of the basal sources at Paluma and Tully were likely similar for the same reason. A large proportion of the fine and coarse particulate organic matter could have been derived from leaf litter in these stream reaches. Bunn et al. (1999) also found that the isotopic carbon signature of fine and coarse particulate organic matter was similar to that of the riparian vegetation.

The stream reaches with more basal sources had higher occurrences of unresolved models. In one study of Australian dryland rivers, more than 70% of the invertebrates had unresolved models, of which only 10% were outside the source polygon (Blanchette et al. 2014). These rivers have many potential basal sources, including phytoplankton and terrestrial grasses, which are not present in rainforest streams, making identification of the main food source more difficult. Most of the

models from the road crossing indicated a possible or likely source, probably because there were fewer sources available for the consumers. It is possible that consumers in the other stream reaches assimilated similar amounts of various food sources, and therefore did not rely on a single source that could have been identified with the mixing model. On the other hand, food sources assimilated by consumers may have not been included in the analysis. Animals such as fishes, which can be long-lived and migratory, may obtain their food sources from a wide range of habitats and are not restricted to the stretch of stream or river where they are found (Blanchette et al. 2014), and it is possible that *Mogurnda* and *Anguilla* obtained their food sources from outside the sampled stream reach.

6.4.2 Food webs

The spatial distribution of consumers and basal sources among and within the stream reaches led to variations in food web structure. Food web complexity depends on the connectance within the system and represents the trophic interactions among consumers and basal sources. Higher connectance makes a system more resilient to species loss (Barnum et al. 2013), and may be more important than species richness and omnivory in maintaining ecosystem robustness (Dunne et al. 2002). At Paluma, the food webs were more complex in pools than in riffles. The greater number of links in pools was due to the presence of tadpoles and top predators (fishes). The drifting and active movement of consumers can connect different habitats within a stream; however, despite this exchange, food webs and predation pathways may vary with habitat depending on the presence of apex predators (Worischka et al. 2014).

The food webs in this study represent periods when tadpoles are present in the streams (i.e., spring and summer). During winter, when tadpole abundances are low, the links and connectivity in pools may be similar to those in riffles. Although the overall abundance of consumers is low during winter, tadpoles may be absent for a period of time while invertebrates are still present (Chapters 2 and 3). Therefore, variation in the links among tadpoles, invertebrates and basal sources may lead to seasonal changes in food web structure. Although tadpoles may only be abundant for parts of the year, their biomass was greater than that of the various invertebrate feeding groups, indicating that they most likely influenced source availability and, therefore, invertebrate assemblage structure.

The food web structure differed between Tully and Paluma because tadpoles were present in pools and riffles at Tully and basal source availability differed between the two locations. Frauendorf et al. (2013) predicted that amphibian declines would lead to a greater reliance on autochthonous food sources in streams, with more algae being available without tadpoles, and grazing invertebrates compensating for the decline in tadpoles. Therefore the detrital pathway may become less important (Frauendorf et al. 2013) and food web structure may become simplified when tadpoles decline (Hunte-Brown 2006). It was not possible to compare food webs between Paluma and Tully to determine any difference in energy pathways based on the tadpole assemblages. Future studies could investigate the entire food web in Tully stream reaches, including invertebrates and top predators, to determine how sources and consumers are linked.

Although the effects of amphibian declines on stream food webs at Paluma could not be measured, the loss of tadpoles from riffles clearly affected food web structure. The dominant invertebrate groups in riffles were predators, filterers and grazers, and tadpoles would add an extra layer of complexity to these food webs (as they did in the pools). Although tadpoles are expected to have resource use similar to that of many grazers in these stream reaches, they feed between trophic levels, and would have increased the number of links among consumers and basal sources. However, Barnum et al. (2015) found that food web structure and complexity did not change as much as expected after amphibian declines in a Neotropical stream. Connectance decreased by less than 3% due to new linkages being formed and the presence of new invertebrate genera, which restructured the stream food web in the absence of tadpoles. Nevertheless, the weight of linkages may change as a result of tadpole loss, indicating changes in food sources by consumers (Barnum et al. 2015).

The food webs in this study are simple representations of the more complicated food webs that actually exist in nature due to the high prevalence of omnivory among tadpoles and invertebrates. Thompson et al. (2007) stated that food webs are only accurate up to the primary consumer level, after which they consist of a tangled web of omnivores. Many studies have used gut content analysis to draw detailed food webs, which enables identification of prey species as well as autochthonous or allochthonous food sources (Mantel et al. 2004; Cheshire et al. 2005; Barnum et al. 2015). These food webs therefore incorporate consumers at more detailed taxonomic levels, but the food webs do not accurately show what the consumers assimilate. Stable isotope analysis did not allow for identification of prey species, and many invertebrates had to be combined

into broader taxonomic groups to obtain enough biomass for stable isotope analysis, but the results provided a more accurate overview of the food sources the various consumer groups assimilated. However, it should be noted that some dietary items may be important as a source of energy and not assimilated, therefore not showing up in stable isotope analysis.

Polis and Strong (1996) argue that food webs depicting trophic levels in a linear manner, as done in this study, do not accurately represent the complexities of the interactions between sources and the large number of consumers present in nature. The links in a system typically vary in strength and include interactions within and between habitats (Polis and Strong 1996). Also, consumers and producers cannot simply be categorised into trophic levels due to the occurrence of omnivory, ontogenetic and environmentally induced changes in diet, and spatial and temporal effects on diet (Polis and Strong 1996). Although incorporating more detail into the food webs was beyond the scope of this study, the patterns recorded provide sufficient information to draw the broad conclusions presented here.

6.4.3 Summary

To understand the importance of tadpoles in stream systems, it is necessary to determine their trophic interactions. The results of this study indicate that tadpoles are not specialist feeders and that they change their main food source depending on availability. This omnivory may depend on the density of the tadpoles and competition with conspecifics or other aquatic consumers, such as tadpoles of other species or grazing invertebrates. The tadpoles' choice of basal sources is also likely related to the quality of the food sources. Biofilm and algae had higher nutrient quality compared to allochthonous sources, whereas leaf litter was the lowest-quality food source available to consumers. The results show that even in shaded, largely heterotrophic streams, biofilm and algae are important food sources. As generalist feeders, tadpoles feed at several trophic levels, and their loss may therefore affect the interaction of species across feeding groups. However, this also indicates that trophic linkages are not fixed (Barnum et al. 2015) and can change in response to altered resource availability and assemblage composition. Comparing food web structure between pools and riffles showed that the presence of tadpoles and top predators in pools made the food webs

more complex, and it is likely that the loss of tadpoles from Paluma riffles has led to simplified connections among consumers and basal sources.

7. General Discussion

The aim of this research was to investigate the role of tadpoles in the ecology of rainforest streams in the Australian Wet Tropics. Its results show that tadpoles may play an important role in stream systems, depending on their temporal and spatial occurrence, trophic status, contribution to stream functioning, and interactions with other organisms, especially aquatic invertebrates. In this chapter, I provide an overview of the research findings, present a conceptual model of the likely extent and timing of tadpole influence on stream systems, and discuss the implications of the research. I conclude by making suggestions for future research that would further advance the knowledge of stream tadpoles.

7.1 Summary of research findings

Populations of various stream-breeding frogs have declined or disappeared in many regions throughout the world (Alford 2010) and this has led to a decline in the abundance and diversity of stream-dwelling tadpoles. Studies from the Neotropics have shown that the loss of tadpoles may lead to changes in the structure and functioning of stream systems (e.g., Colon-Gaud et al. 2010a; Barnum et al. 2013; Whiles et al. 2013). These studies examined the effect of tadpole loss on assemblage composition, food web structure and stream processes, but there are no similar studies from the Australian tropics. Although pre-decline data are largely unavailable for tadpoles in Australian streams, experimental techniques and inferences from natural populations can be used to investigate the role of tadpoles in Wet Tropics streams.

7.1.1 Tadpole and invertebrate population dynamics

Tadpole abundances fluctuated seasonally and were generally highest during spring and summer, the main frog breeding periods. However, the timing of the breeding episodes and the influx of tadpoles varied among species, which probably helped to reduce competition for space and food sources (Altig and Johnston 1989; Bertoluci and Rodrigues 2002). Species occurrence and abundance differed between riffles and pools, and depended on the adaptations of the species, especially their

feeding mode and tolerance of high current velocities. Three of the four species at Tully were riffle dwellers, with differences in their tolerances of strong flows that may have caused partitioning of microhabitats and reduced the likelihood of competition. At Paluma, tadpoles were predominantly present in pools in the recent surveys; two riffle species that were present in the late 1980s disappeared from the streams in 1990 and 1991. Current tadpole influence on stream ecosystems at Paluma may therefore only be important in pools, where tadpoles have persisted.

The tadpole and the invertebrate assemblages showed similar strong responses to flow, rainfall and water temperature, indicating that they are likely to be most dense and active during the same time of the year. Interactions between tadpoles and invertebrates are probably most important during periods of high densities, and may occur in the form of predation, competition or facilitation. Invertebrate gatherers and grazers are most likely to compete with tadpoles for food because of similar feeding habits (Flecker et al. 1999; Kiffney and Richardson 2001; Colon-Gaud et al. 2009), while facilitation may take place between shredders and tadpoles during leaf litter processing (Iwai et al. 2009). I found a positive relationship between tadpoles and grazers in one Tully stream; this indicated that grazers may have benefitted from the presence of tadpoles. However, there were no other clear relationships between tadpoles and invertebrates, and positive correlations were most likely because of similar responses to environmental influences.

Although invertebrate grazers are generally thought to be functionally similar to tadpoles (Cummins and Klug 1979; Alford 1999), they may or may not be functionally redundant. Invertebrates are typically much smaller and are unlikely to replace tadpoles in their effects, for example, on bioturbation (Whiles et al. 2013). The trends I found in consumer abundance did not clearly indicate the presence of functional redundancy between the major taxa. It was difficult to detect interactions between tadpoles and invertebrates in the field; any interactions were probably minor compared to the influence of physical factors such as flow and temperature.

7.1.2 The role of tadpoles in ecosystem processes

Experiments at Paluma showed that the roles of tadpoles in leaf litter processing and sediment removal differed between the two pool species. Tadpoles of *Litoria serrata*, but not of *Mixophyes coggeri*, interacted with shredders to increase the rate of

leaf litter breakdown of *Apodytes brachystylis* leaves, indicating facilitation. The direction of facilitation was most likely from invertebrates to tadpoles via direct physical effects on leaves (Iwai et al. 2009). There was no evidence of nutrient regeneration by *L. serrata* tadpoles in this system, possibly because the experimental system did not allow for the detection of small amounts of nutrient regeneration.

Although there was evidence of facilitation, tadpoles and invertebrates also competed with each other, as shown by tadpole biomass loss. Tadpoles may have competed with invertebrates for biofilm on leaf surfaces or for other organic material that accumulated in the channels. High tadpole densities also resulted in intraspecific competition, as *L. serrata* tadpoles at low densities doubled their original biomass over the period of the experiment, whereas at high densities they either gained little or lost biomass. Although tadpoles competed with invertebrates, leaf breakdown by shredders increased as the density of tadpoles increased, indicating that tadpoles may be important in leaf processing when present at high densities.

Sediment accumulation was highest in the invertebrate experimental treatments, probably from leaf breakdown and faeces production, but it was reduced in the presence of tadpoles. *Mixophyes coggeri* tadpoles were more efficient than *L. serrata* at removing sediments by consumption and displacement. Although tadpoles feed on fine particulate organic matter (Trenerry 1988), most sediment was removed through displacement. They may have stirred up sediment to feed on only the most nutritious material, thereby causing the majority to be washed downstream.

7.1.3 The trophic position of stream tadpoles

Gut content and stable isotope analyses showed that tadpoles at Paluma and Tully were generalist feeders and could change their food source depending on availability. Autotrophic sources were important, despite their apparent scarcity in the stream, especially under low light intensities at Paluma. Tadpoles assimilated mainly biofilm and filamentous algae, which had the highest nutritional quality, indicating a preference for high quality food sources when these were available. Microorganisms associated with the basal sources were most likely an essential part of the tadpoles' diets, and under low light conditions, the microbial component in biofilm may have been more important than the algae. Microorganisms, including protists and microinvertebrates, also colonise particulate organic matter (Cummins and Klug 1979;

Pearson et al. 1989), so it is probable that tadpoles assimilated material derived from microorganisms when feeding on leaf litter, FPOM or CPOM (Hunte-Brown 2006; Altig et al. 2007).

Similarly to tadpoles, many invertebrates were omnivores, making trophic classification difficult. They obtained their nutrition from various basal sources, as well as from other organisms, enabling them to feed on more nutritious food available at a particular site and time. Like tadpoles, invertebrates assimilated mainly biofilm and algae; this may have led to competitive interactions. Grazers were expected to have overlapping diets with tadpoles, but other feeding groups, including predators, also assimilated biofilm, indicating that the presence or absence of tadpoles may influence invertebrates of various feeding groups.

Although tadpoles may not be present in high densities throughout the year, they influence food web structure and complexity when they are present, and the absence of tadpoles from riffles clearly altered food web structure. During periods of high tadpole abundance at Paluma, food webs were more complex in pools than in riffles, mainly because of the presence of tadpoles and top predators (fishes). The food webs in this study were simpler than those found in some other habitats because of the high prevalence of omnivory among tadpoles and invertebrates.

7.1.4 Conceptual model of tadpole roles in stream systems

The surveys provided baseline information on the seasonal abundance patterns of tadpoles at Paluma and Tully. Physical factors such as flow and season were more important in affecting assemblage structures than were interactions among consumer groups. Therefore, any changes to the external environment, either anthropogenic or caused by extreme weather conditions, may affect abundance patterns and, as a result, interactions among consumers. The functional roles of different species are important to consider when assessing the role of tadpoles in streams. Although some species may persist or recover after amphibian declines, they may not have the same functional roles as the species that declined, therefore altering stream functioning. Furthermore, loss of species with different functional roles may lead to complex interactive effects (Jabiol et al. 2013).

The results of this study provide the basis of a conceptual model of tadpole contributions to ecosystem processes in Wet Tropics streams (Figure 7.1). It shows the

likely interactions of tadpoles with other aquatic consumers and the relative importance of these interactions in different seasons and stream conditions. The model is not quantitative, given that materials and energy budgets were not estimated; however, this study provides sufficient information to indicate relative contributions among taxa. The model highlights the nature of each of the more important interaction by which the organisms affect each other and stream processes. Together with the food webs (Chapter 6) it provides substantial insight into ecological processes and the associated role of tadpoles in Wet Tropics streams.

The likely levels of competition between tadpoles and invertebrates depend on the functional feeding group to which each belongs and the availability of food sources. As biofilm and algae are the preferred food choice for both tadpoles and grazing invertebrates, they are likely to compete with each other for these resources. Gathering invertebrates, on the other hand, are more likely to compete with tadpoles for food sources such as fine and coarse particulate organic matter. This probably occurs when the more nutritious food sources are in limited availability and tadpoles therefore resort to less nutritious foods. Although I found no evidence of facilitation through nutrient regeneration by *Litoria serrata* tadpoles, it may be more common with tadpoles of other species, and may operate at larger scales than I could experimentally detect. It was therefore included in the model as being potentially important when consumer abundances are high. There was evidence of facilitation between tadpoles and shredders during leaf litter breakdown, but this was species dependent. Most of the interactions that are likely to be important when tadpole abundances are high are probably less so when abundances are scarce. However, the influence of the interactions on the stream ecosystem during periods of high abundances is likely to be substantial and may well carry over to the rest of the year.

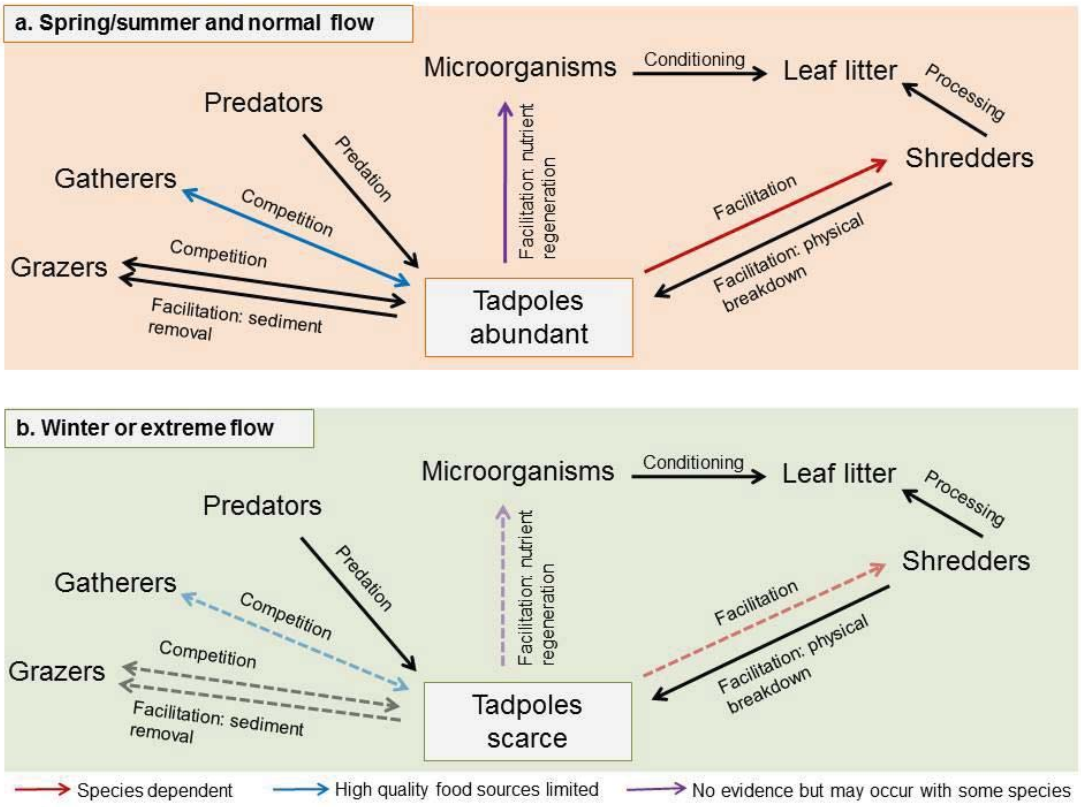

Figure 7.1. Conceptual model of the interactions between tadpoles and other consumers across pools and riffles in Wet Tropics streams during (a) spring or summer and normal flow conditions when tadpoles are abundant, and (b) winter or extreme flow conditions when tadpoles are likely to be scarce. The arrows highlight the likely processes by which the consumers interact, with the dashed arrows indicating processes that are unlikely to be important under the given conditions.

7.2 Future research needs

Future studies could combine adult and tadpole surveys to determine how adult behaviour influences tadpole assemblages in streams and to what extent environmental variables affect the terrestrial and aquatic populations. It would also be useful to carry out periodic tadpole and adult surveys over an extended period. At Paluma, *Litoria nannotis* disappeared from Birthday Creek and Camp Creek, but they are currently present in nearby Little Crystal Creek and Ethel Creek. The upper reaches of Ethel Creek are close to the artificial streams of Birthday Creek and it is possible that *L. nannotis* adults will recolonise Birthday Creek. It would be useful to monitor this process and investigate its impacts, if any, on current assemblages and stream system processes.

As nutrient regeneration is an important process that can benefit other aquatic consumers and enhance stream processes, it would be interesting to devise an experiment that might be more sensitive to its effects, given the flow-through nature of the system. Such an experiment could be used to determine whether *Litoria serrata* and other species have nutrient regeneration effects. Also, a range of other food sources could be provided to determine whether the nutrient quality of a source influences the tadpoles' ability to regenerate nutrients. Similarly, future studies could use other species, or different size classes of one species, to test for the contribution of tadpoles in leaf breakdown and sediment removal, regardless of whether nutrient regeneration occurs. The direction of facilitation could not be determined in this study, but this could be done using methods described by Iwai et al. (2009). Sediment removal by tadpoles was tested using two species at Paluma, but its effect on invertebrate grazers was not studied. The effects on various invertebrate groups of sediment removal could be tested by measuring protein, lipid and carbohydrate content as indicators of invertebrate condition (Pearson and Connolly 2000; Connolly and Pearson 2013).

The trophic enrichment factor (TEF) values used for the stable isotope analysis were obtained from the literature because of time and cost constraints. This may have led to inaccuracies that contributed to the failure to resolve some models. Trophic enrichment can vary depending on the species and environmental influence (Caut et al. 2013), and it is important to have accurate $\delta^{13}N$ and $\delta^{13}C$ estimates (Bond and Diamond 2011). No studies have experimentally determined TEF values for Australian rainforest amphibians and it would be useful to carry out controlled diet experiments on tadpoles of various Wet Tropics species to obtain more accurate estimates for future stable isotope work in this region.

The stable isotope results provide information on source use and trophic position of consumers for a specific time of the year. Sources at Paluma were only collected in 2012, due to cost constraints, and the data were used in the analyses for 2012 and 2013. It was assumed that the source isotopic composition did not differ much between years during the same season. Isotopic composition and resource use can vary seasonally (Salas and Dudgeon 2003; Lau et al. 2009), as does food web structure (Cheshire et al. 2005). Future research could collect samples for stable isotope analysis over several years to determine seasonal and annual changes in basal source availability and contributions to the food web. In this study, invertebrates and vertebrate predators were only collected from Paluma, and it would be useful to include all the food web

components from other locations, including Tully, enabling comparisons, especially given the differences in amphibian and invertebrate assemblage compositions that occur between streams in different parts of the Wet Tropics, such as Paluma and Tully.

7.3 Implications and concluding remarks

The influences of tadpoles on the ecology of Wet Tropics streams are complex and depend in a complex manner on which species are present, their population dynamics, their feeding activities and their interactions with the physical environment and other species, as described in this thesis. Tadpoles can be some of the largest animals in the stream benthos and, when abundant, may compose a large part of the biomass. At such times their influence is likely to be major. At other times, outside the breeding season, lower densities may reduce their influence. This thesis shows something of the dynamics of this interaction between tadpoles and their environment, although to fully quantify the influence of tadpoles would require inclusion/exclusion experiments over an annual cycle, at least. The alternative would be before/after studies such as those that were undertaken in Central America (Whiles et al. 2006; Whiles et al. 2013), but this opportunity was not available in the Wet Tropics.

The likelihood of species loss is high under scenarios of land-use and climate change. A foretaste of such loss has been provided by the disease chytridiomycosis, which has reduced frog species diversity, for example at Paluma. It is apparent that such loss can cause substantial shifts in ecosystem processes. In the Neotropics, amphibian declines resulted in changes to the assemblage composition and structure of invertebrate grazer communities (Colon-Gaud et al. 2010a). Amphibian diversity is high in these systems, and about 20 species may co-occur in a stream (Lips et al. 2006; Whiles et al. 2013), so the effects of tadpole loss on stream structure and functioning may be less pronounced in the Australian tropics where diversity is lower. However, loss of all species from a particular habitat, as occurred at Paluma, or of a single species that has strong effects on the ecosystem, such as *M. coggeri*, will have more influence than loss of scarce or more physically benign species; that is, the identity of species that are lost is likely to be more important than the number of species lost. Further, other taxa may also be lost, which will produce even greater changes in species interactions, ecosystem processes and food webs (Boyero et al. 2006; Boyero et al. 2012).

Studies on system energetics and carbon budgets could further elucidate effects of tadpole population changes on both aquatic and terrestrial systems. When abundant in the Wet Tropics, tadpoles contribute substantially to stream ecosystem processes, and after metamorphosis, presumably also contribute substantially to terrestrial food webs via predation (as prey and predator); but when scarce their effects are minor. Both scenarios can occur at a single site, depending on season and other factors. The answer to the main question raised in this thesis, therefore, is that the roles of tadpoles in Wet Tropics streams vary qualitatively and quantitatively depending on species identity, time of the year, habitat and food availability and the presence of other interacting species.

8. References

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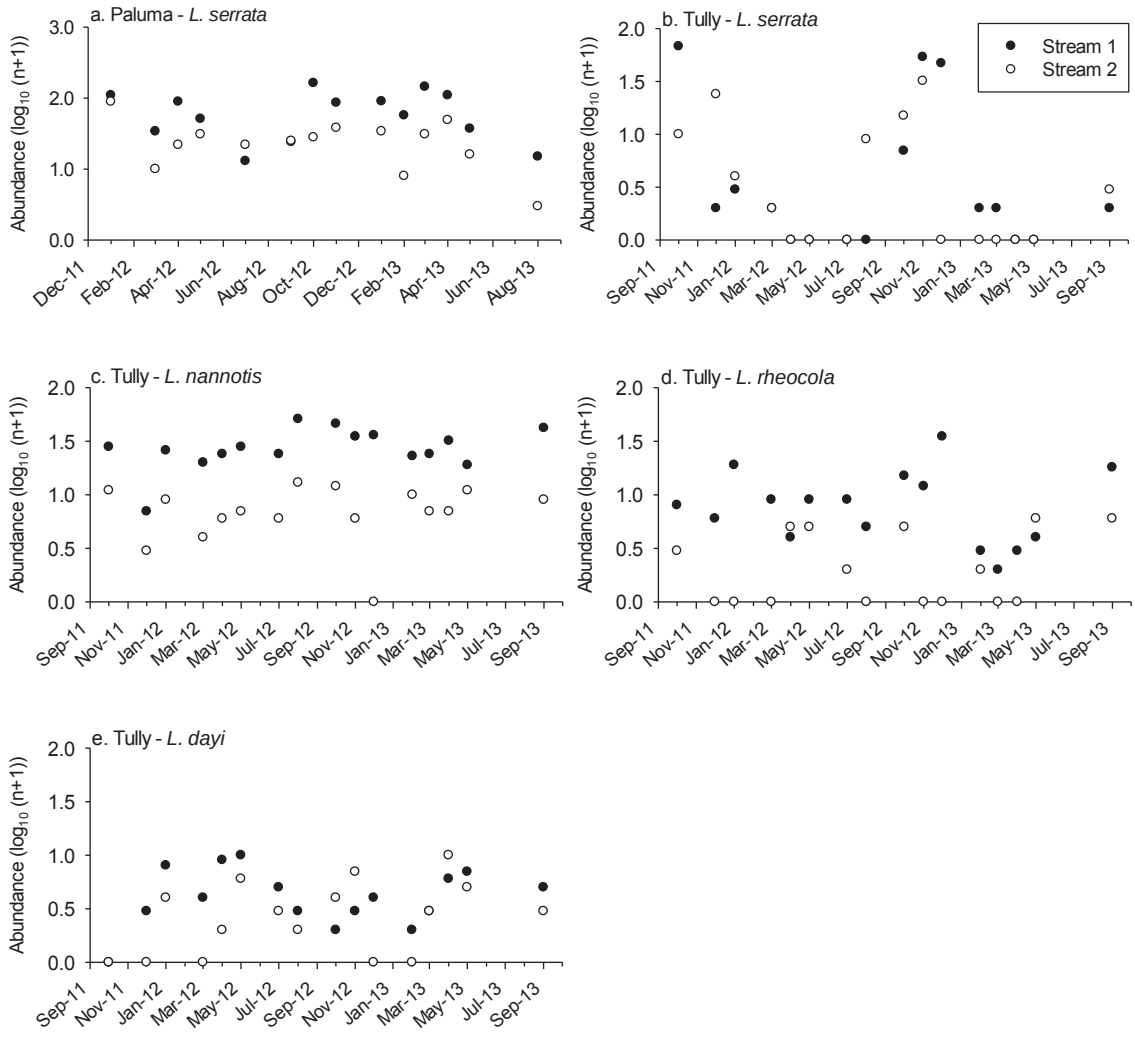
9. Appendices

Appendix 1: Tadpole population dynamics



Appendix 1.1. Tadpole abundances in Birthday Creek (Stream 1) and Camp Creek (Stream 2) at Paluma, and Stream 1 and Stream 2 at Tully, for (a) *Litoria serrata* at Paluma, and (b) *L. serrata,* (c) *L. nannotis*, (d) *L. rheocola* and (e) *L. dayi* at Tully. Tadpole abundance for September 2013 in Tully Stream 2 was estimated using data from Stream 1.

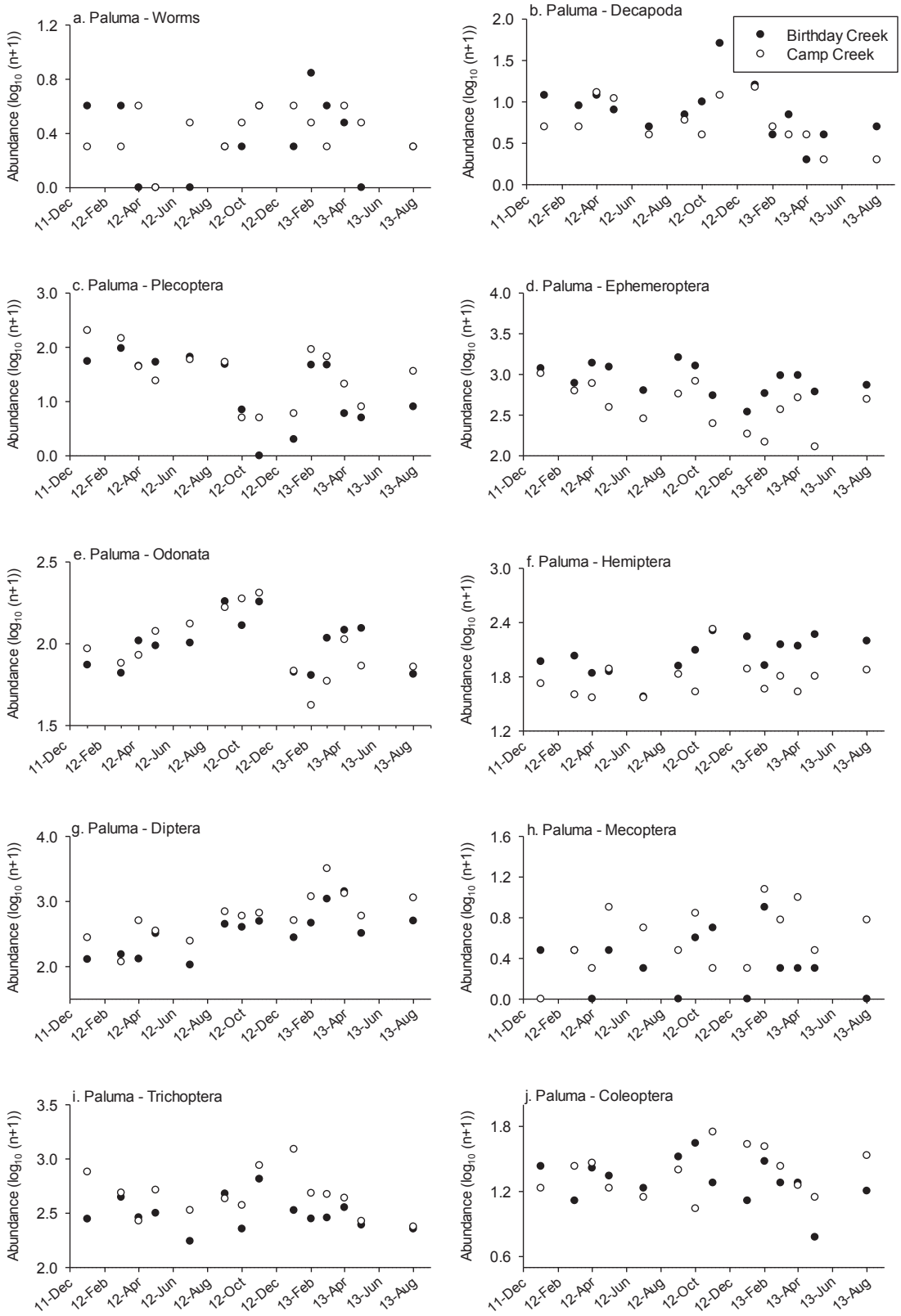
Appendix 2: Tadpole and invertebrate relationships

Appendix 2.1. Invertebrate taxa and their total abundances in stream samples from Paluma (February 2012 to August 2013) and from Tully (October 2011 to May 2013).

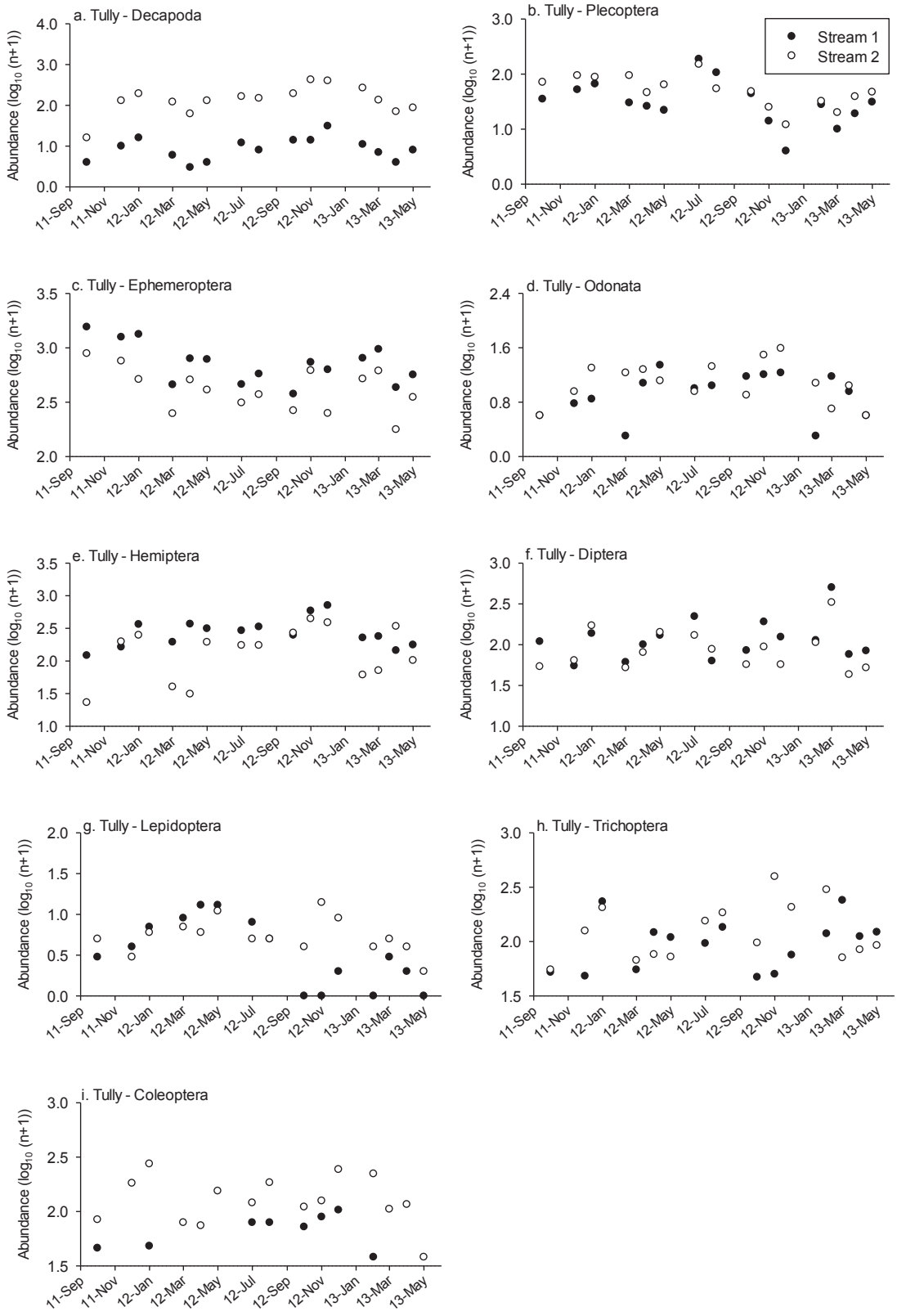
Phylum/order	Class/ Family	Genus	Birthday Creek	Camp Creek	Tully Stream 1	Tully Stream 2
Nematoda			1	1	0	0
Nematomorpha	Gordioidea		14	17	0	0
Platyhelminthes			7	7	1	38
Annelida	Oligochaeta		2	0	1	2
Arachnida			3	6	3	6
Arachnida	Tetragnathidae		0	0	0	2
Acarina			340	538	132	40
Decapoda	Parastacidae (<2cm)		61	22	0	0
Decapoda	Parastacidae (<4cm)		56	41	0	0
Decapoda	Parastacidae (2-4cm)		21	12	0	0
Decapoda	Palaemonidae		0	1	6	449
Decapoda	Atyidae		0	2	135	2081
Plecoptera	Gripopterygidae		469	748	736	835
Plecoptera	Eustheniidae		2	8	20	34
Ephemeroptera			11428	5663	11108	6499
Ephemeroptera	Leptophlebiidae		1287	868	784	143
Ephemeroptera	Leptophlebiidae	*Kirrara*	0	0	59	22
Ephemeroptera	Leptophlebiidae	*Atalophlebia*	29	23	1	1
Ephemeroptera	Ameletopsidae		61	17	3	6
Ephemeroptera	Caenidae		14	1	132	103
Odonata - Epiproctophora			57	89	64	56
Odonata - Epiproctophora	Telephlebiidae		182	264	17	29
Odonata - Epiproctophora	Lindeniidae/Gomphidae		57	49	10	23
Odonata - Epiproctophora	Hemicorduliidae, Urothemistidae, Libellulidae		39	16	8	16
Odonata - Zygoptera	Synlestidae/ Chorismagrionidae		998	878	8	23
Odonata - Zygoptera	Diphlebiidae		134	173	40	60
Hemiptera			0	0	5	285
Hemiptera	Corixidae/ Naucoridae		5	15	2344	295
Hemiptera	Gerridae		339	61	12	14
Hemiptera	Veliidae		873	618	2334	2105
Hemiptera	Notonectidae		436	225	21	30
Hemiptera	Gelastacoridae		0	0	2	4
Hemiptera	Hydrometridae		5	2	0	0

Appendix 2.1. (Continued)

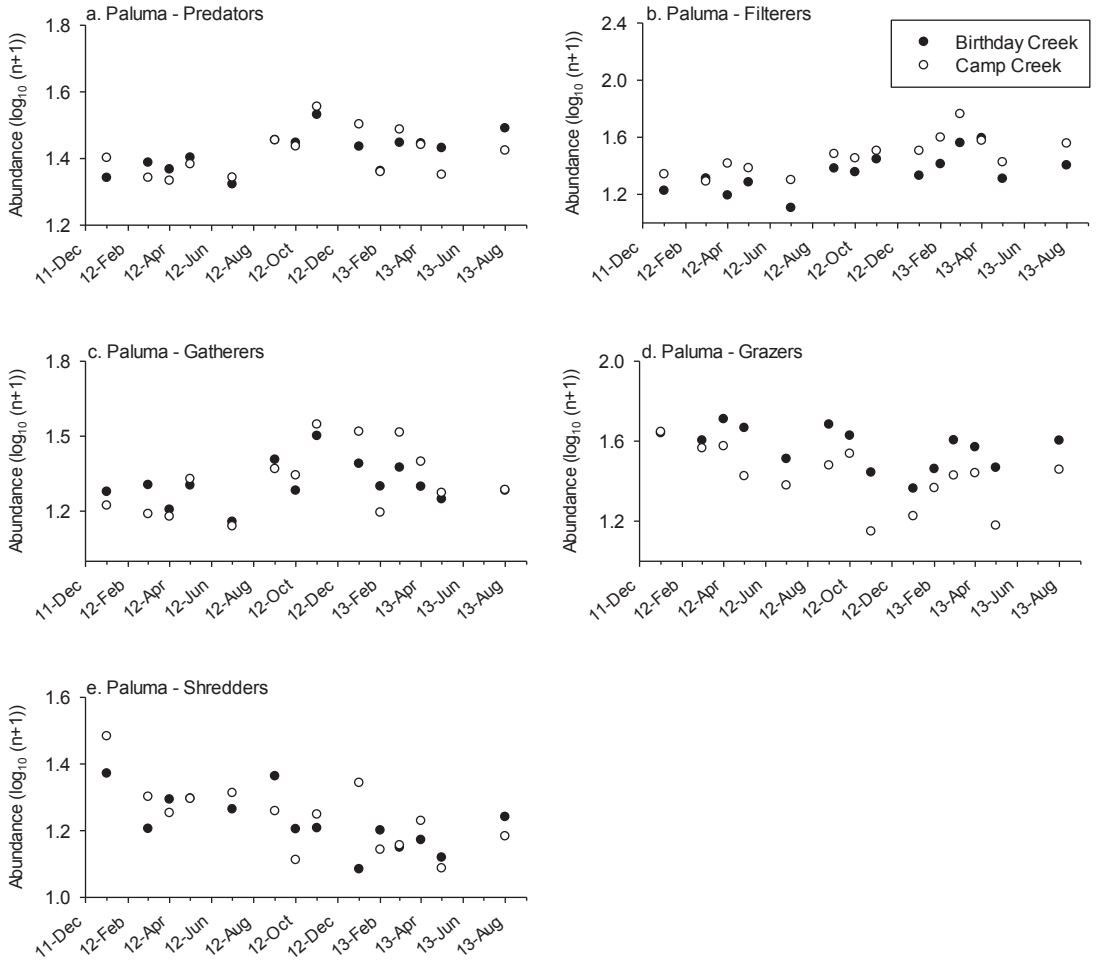
Phylum/order	Class/ Family	Genus	Birthday Creek	Camp Creek	Tully Stream 1	Tully Stream 2
Diptera			71	55	72	43
Diptera	Athericidae		2	0	0	0
Diptera	Culicidae		0	2	191	2
Diptera	Dixidae		11	14	0	1
Diptera	Simuliidae		4570	8377	563	493
Diptera	Chironomidae		1569	2926	665	586
Diptera	Ceratopogonidae		27	29	6	5
Diptera	Blephariceridae		0	0	610	374
Diptera	Chaoboridae		0	3	0	0
Mecoptera			1	0	0	2
Megaloptera			14	36	6	4
Neuroptera	Neurorthidae		9	20	0	0
Lepidoptera			9	4	61	75
Trichoptera	Philopotamidae		977	1658	286	296
Trichoptera	Hydropsychidae		68	71	849	1295
Trichoptera	Polycentropodidae		72	139	8	5
Trichoptera	Hydrobiosidae		106	210	1	0
Trichoptera	(sand case)		117	147	38	30
Trichoptera	(plant case)		348	393	15	14
Trichoptera	(silk case)		318	1165	3	14
Trichoptera	Leptoceridae	*Triplectides*	295	268	27	64
Trichoptera	Leptoceridae	*Lectrides*	476	1416	28	10
Trichoptera	Leptoceridae		267	263	7	10
Trichoptera	Calamoceratidae	*Anisocentropus*	551	629	24	317
Trichoptera	Conoesucidae		0	0	290	88
Trichoptera	Helicopsychidae		77	358	17	12
Trichoptera	Hyrdroptilidae		898	431	98	15
Coleoptera	(adult)		140	159	396	1351
Coleoptera	Psephenidae		14	21	294	569
Coleoptera	Ptilodactylidae (larvae)		13	6	0	5
Coleoptera	Elmidae (larvae)		49	49	39	145
Coleoptera	Scirtidae		35	39	7	7
Coleoptera	Gyrinidae (adult)		37	77	10	5
Coleoptera	Gyrinidae (larvae)		0	2	0	0
Coleoptera	Hydrophilidae (larvae)		2	6	1	13



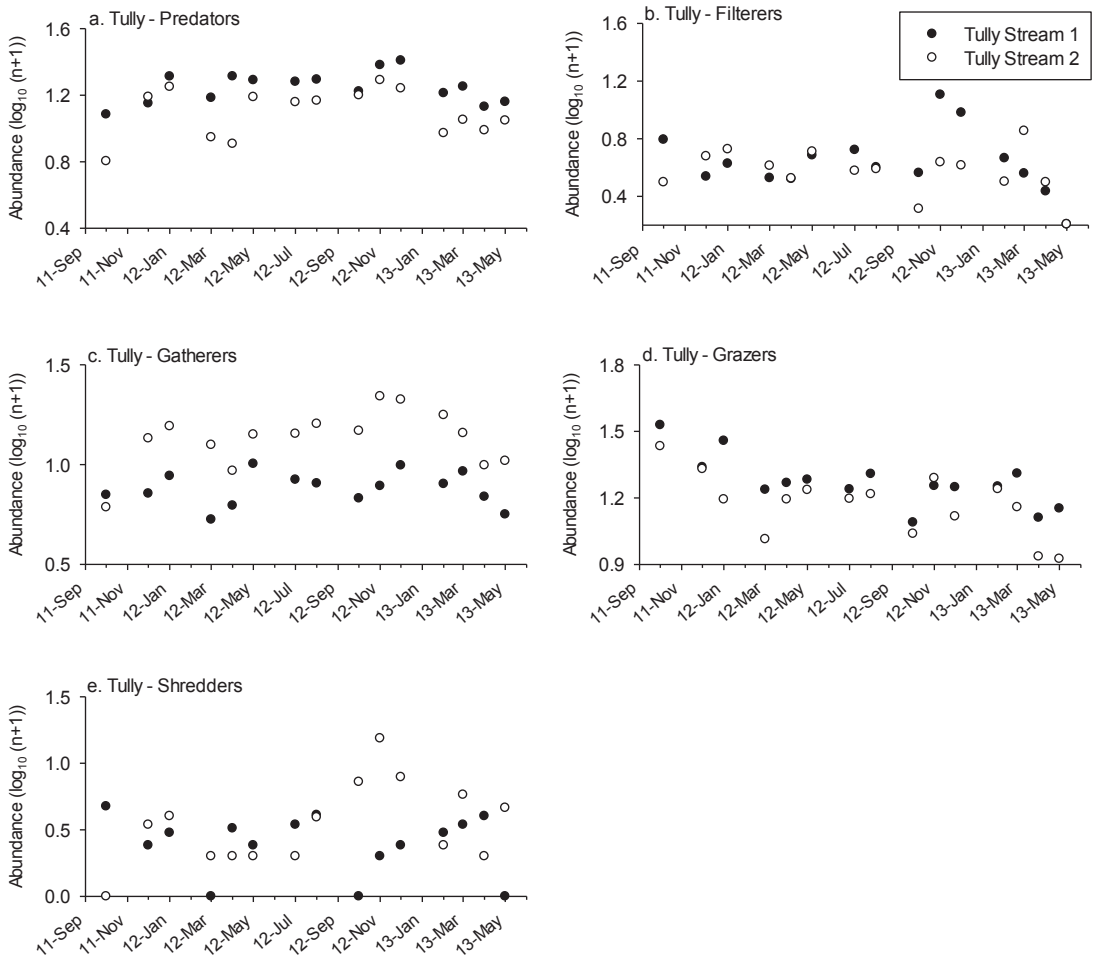
Appendix 2.2. Total invertebrate abundances in pools and riffles at Paluma from January 2012 to August 2013, for 10 taxonomic groups: (a) worms, comprising segmented and unsegmented worms, (b) Decapoda, (c) Plecoptera, (d) Ephemeroptera, (e) Odonata, (f) Hemiptera, (g) Diptera, (h) Mecoptera, Megaloptera and Neuroptera (i) Trichoptera and (j) Coleoptera.



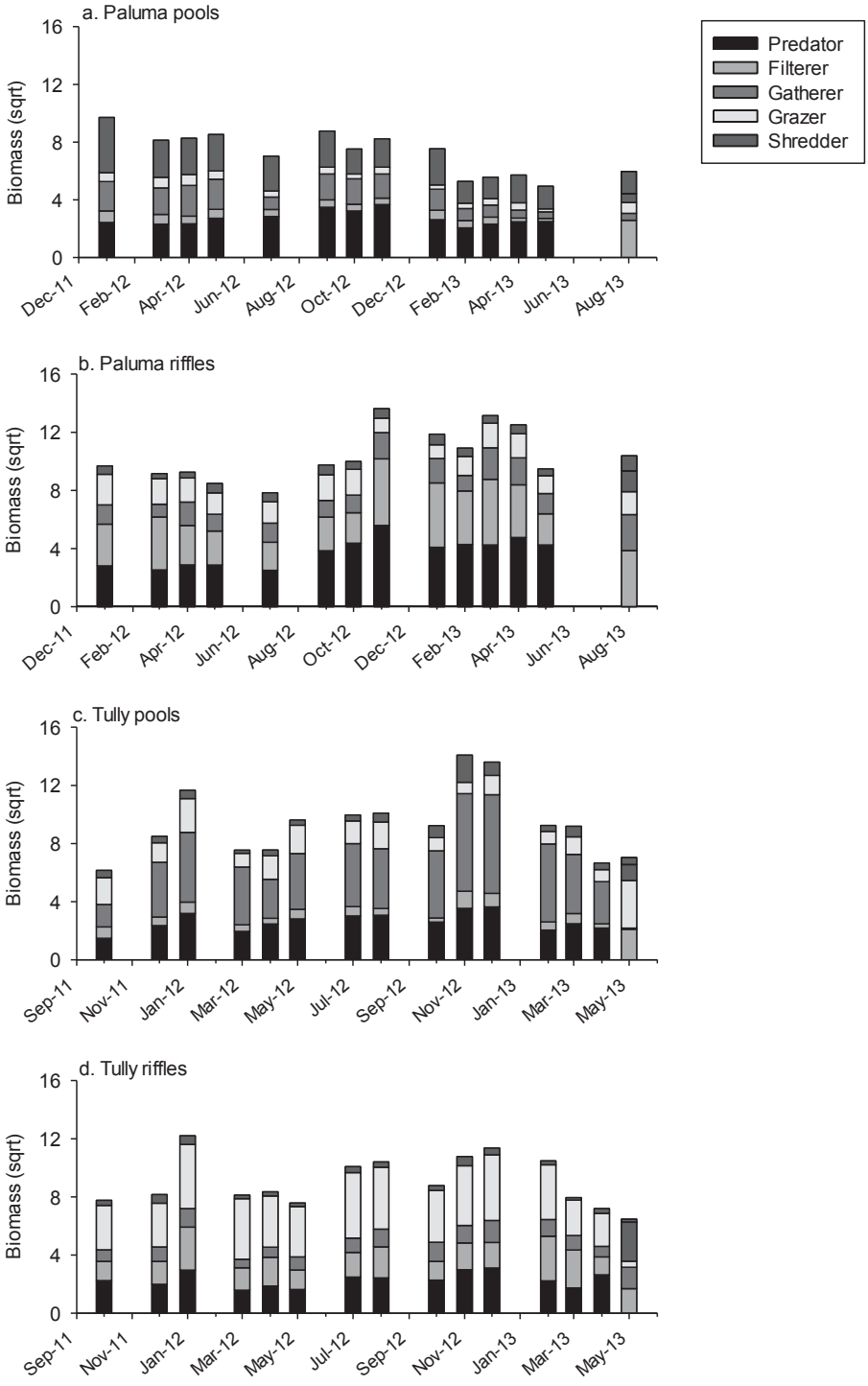
Appendix 2.3. Total invertebrate abundances in pools and riffles at Tully from October 2011 to May 2013, for nine taxonomic groups: (a) Decapoda, (b) Plecoptera, (c) Ephemeroptera, (d) Odonata, (e) Hemiptera, (f) Diptera, (g) Lepidoptera (h) Trichoptera and (i) Coleoptera.



Appendix 2.4. Total invertebrate abundances at Paluma from January 2012 to August 2013, for five feeding groups: (a) predators, (b) filterers, (c) gatherers, (d) grazers, and (e) shredders.



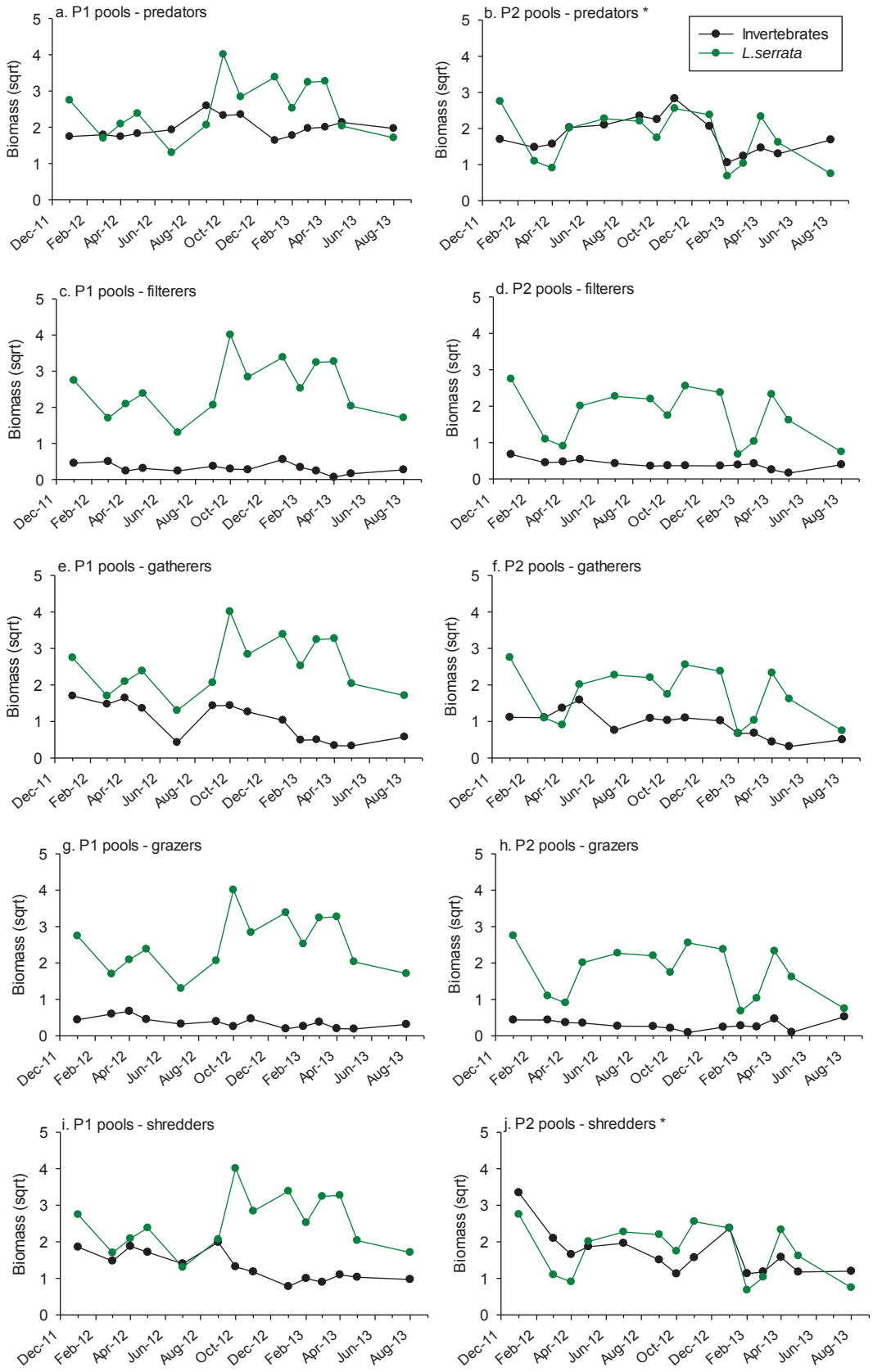
Appendix 2.5. Total invertebrate abundances at Tully from October 2011 to May 2013, for five feeding groups: (a) predators, (b) filterers, (c) gatherers, (d) grazers, and (e) shredders.



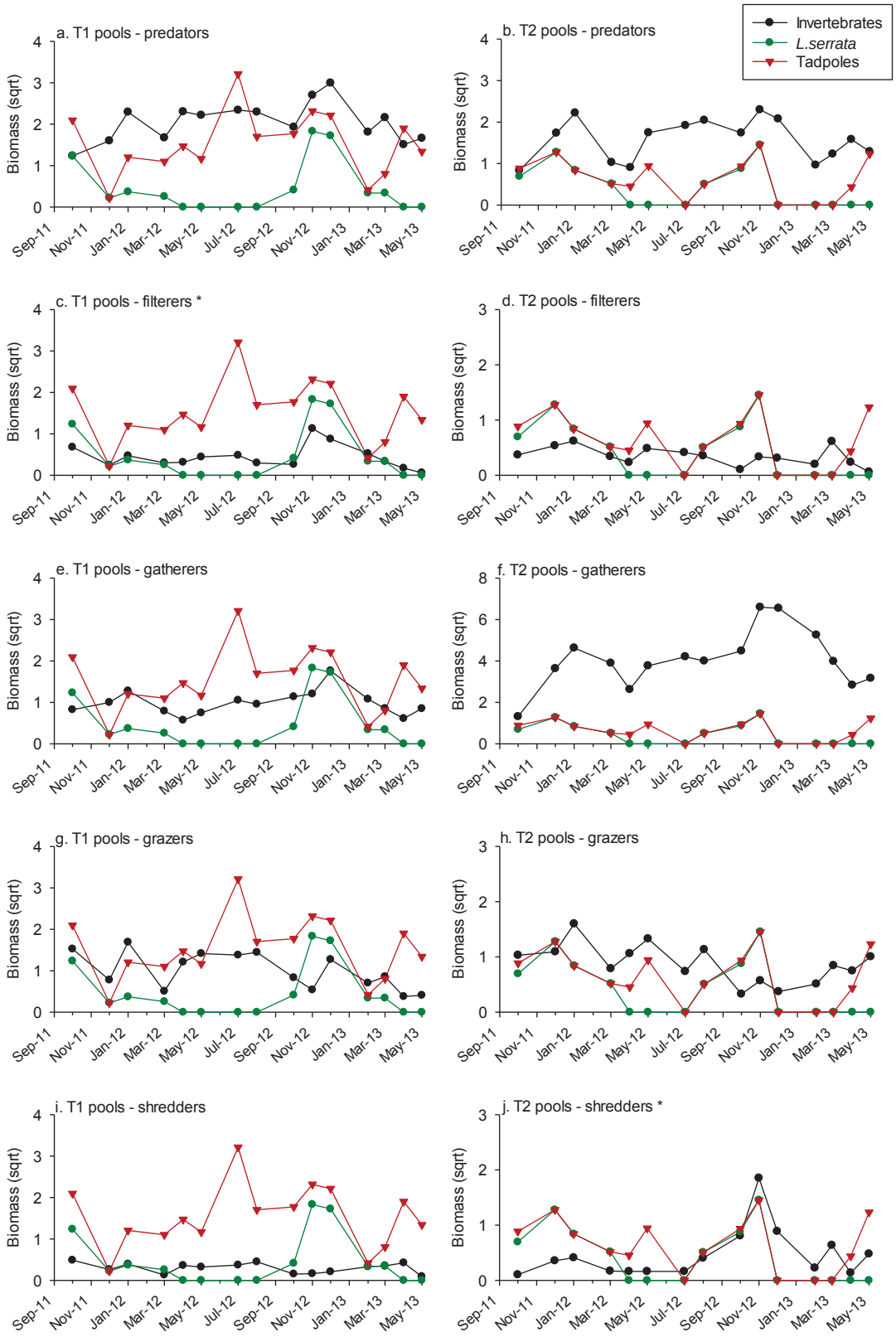
Appendix 2.6. Invertebrate feeding group biomass (square root) in riffles and pools at Paluma and Tully. Biomass refers to the dry weight of the animals in mg.

Appendix 2.7. Spearman's rank correlations between invertebrate feeding groups and tadpoles (*Litoria serrata* tadpoles only or all tadpoles combined), showing the correlation coefficient (ρ) and P value.

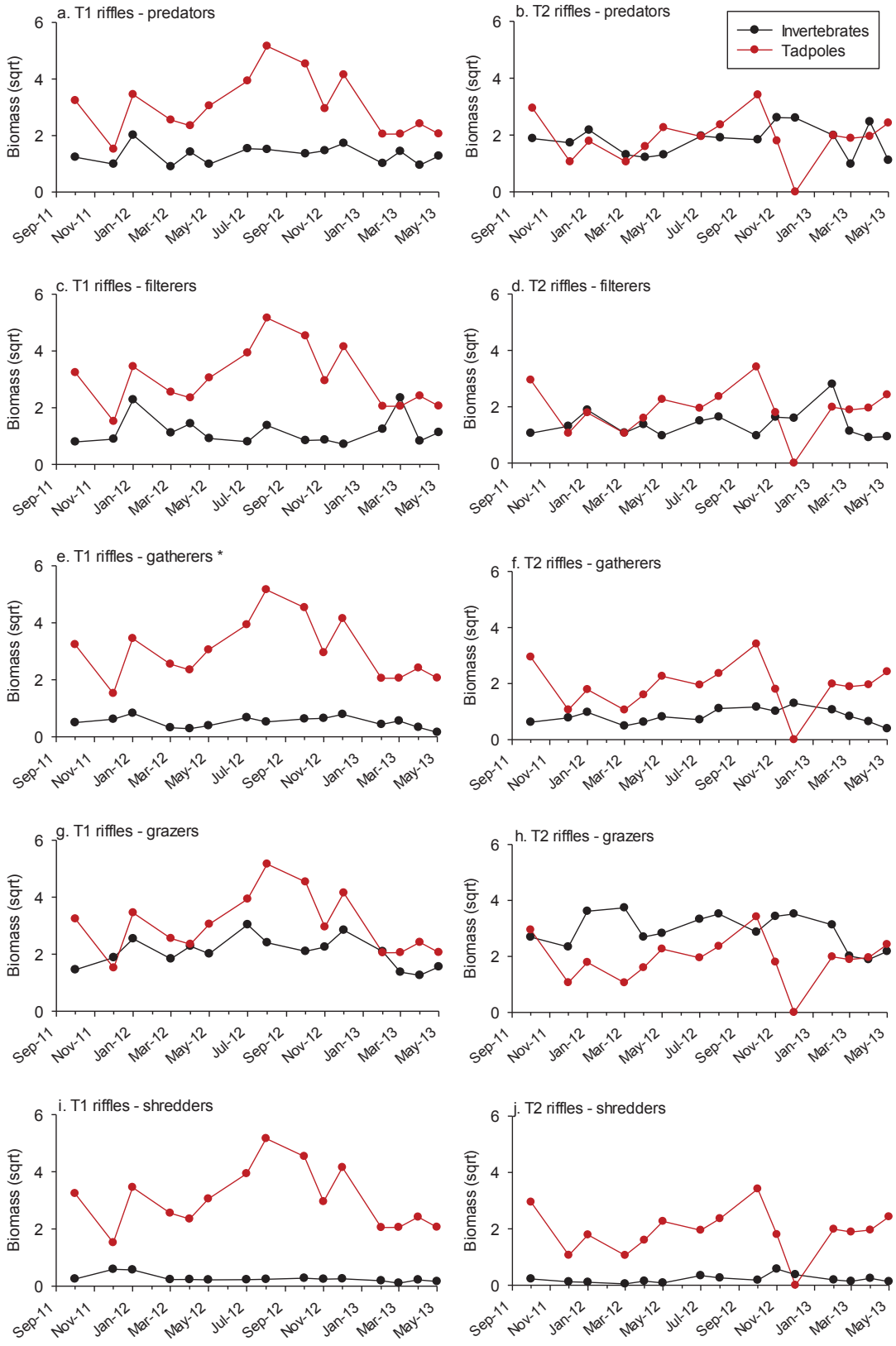
		P1 pools	P2 pools	T1 pools	T2 pools	T1 riffles	T2 riffles
Predator	*L. serrata*	ρ = -0.094, P = 0.738	ρ = 0.648, P = 0.016	ρ = 0.422, P = 0.113	ρ = 0.368, P = 0.189		
	All tadpoles			ρ = 0.300, P = 0.269	ρ = 0.209 P = 0.463	ρ = 0.500, P = 0.056	ρ = -0.095, P = 0.738
Filterer	*L. serrata*	ρ = 0.116, P = 0.682	ρ = -0.055, P = 0.849	ρ = 0.746, P = 0.001	ρ = 0.198, P = 0.482		
	All tadpoles			ρ = 0.658, P = 0.007	ρ = 0.008, P = 0.976	ρ = 0.070, P = 0.793	ρ = 0.116, P = 0.682
Gatherer	*L. serrata*	ρ = 0.015, P = 0.952	ρ = 0.137, P = 0.643	ρ = 0.367, P = 0.171	ρ = 0.450, P = 0.101		
	All tadpoles			ρ = 0.061, P = 0.822	ρ = 0.217, P = 0.444	ρ = 0.590, P = 0.020	ρ = 0.015, P = 0.952
Grazer	*L. serrata*	ρ = -0.141, P = 0.615	ρ = -0.094, P = 0.751	ρ = 0.257, P = 0.346	ρ = -0.008, P = 0.976		
	All tadpoles			ρ = 0.286, P = 0.293	ρ = 0.284, P = 0.316	ρ = 0.504, P = 0.054	ρ = -0.141, P = 0.615
Shredder	*L. serrata*	ρ = -0.150, P = 0.594	ρ = 0.731, P = 0.004	ρ = -0.107, P = 0.695	ρ = 0.652, P = 0.011		
	All tadpoles			ρ = 0.370, P = 0.167	ρ = 0.535, P = 0.047	ρ = 0.309, P = 0.252	ρ = -0.150, P = 0.594



Appendix 2.8. Biomass (dry weight in mg) of invertebrate feeding groups and *Litoria serrata* tadpoles in pools of Birthday Creek (P1, left column) and Camp Creek (P2, right column) at Paluma. Significant positive relationships, as indicated by Spearman's rank correlations, are indicated by *.



Appendix 2.9. Biomass (dry weight in mg) of invertebrate feeding groups, *Litoria serrata* tadpoles (pool specialists) and all tadpoles (pool and riffle specialists) in pools of Stream 1 (T1, left column) and Stream 2 (T2, right column) at Tully. Significant positive relationships, as indicated by Spearman's rank correlations ($P < 0.05$), are indicated by *.



Appendix 2.10. Biomass (dry weight in mg) of invertebrate feeding groups and total tadpoles in riffles of Stream 1 (T1, left column) and Stream 2 (T2, right column) at Tully.

Appendix 3: The trophic status of tadpoles

Appendix 3.1. The number of basal source samples collected in each stream reach at Paluma and Tully.

Source	PALUMA			TULLY	
	Road crossing	Camp Creek	Artificial streams	Stream 1	Stream 2
Biofilm	4	4	4	2	1
Algae	-	2	4	1	2
Periphyton	-	-	2	-	-
FPOM	2	2	2	1	1
CPOM	2	2	2	-	-
Leaves	4	4	4	6	5
Iron matrix	-	1	-	-	-

Appendix 3.2. Standard deviations for $\delta^{15}N$ and $\delta^{13}C$ of basal sources in the stream reaches at Paluma and Tully.

Source	Paluma						Tully			
	Road crossing		Camp Creek		Artificial streams		Stream 1		Stream 2	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Biofilm	0.64	1.31	0.36	2.42	0.67	0.69	1.91	3.04	1.40	2.85
Algae	-	-	0.28	0.64	0.25	0.68	0.32	10.76	0.07	13.65
Periphyton	-	-	-	-	0.28	0.07	-	-	-	-
FPOM	0.21	0.00	0.35	0.07	0.21	0.28	0.21	0.14	0.21	0.14
CPOM	0.07	0.14	0.42	0.42	0.28	0.21	-	-	-	-
Leaves	1.07	0.64	0.46	0.76	0.97	0.37	0.22	0.48	0.83	0.34
Iron matrix	-	-	0.20	0.20	-	-	-	-	-	-

Appendix 3.3. Basal sources present in the stream reaches at Paluma and Tully. Sources were combined when the $\delta^{13}C$ measures were within 0.5‰ of each other.

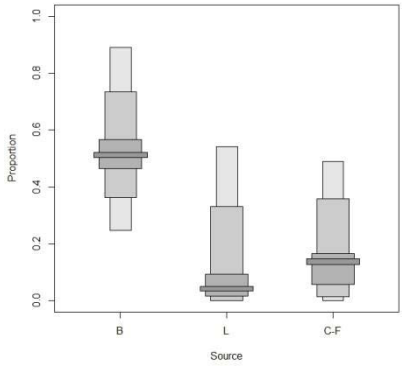
Location	Stream	Basal sources
Paluma	Road crossing	Biofilm, leaves, CPOM-FPOM
	Camp Creek	Biofilm, filamentous algae, iron matrix, leaves, CPOM-FPOM
	Artificial streams	Biofilm, filamentous algae, periphyton, leaves, CPOM-FPOM
Tully	Stream 1	Biofilm, filamentous algae, leaves, FPOM
	Stream 2	Biofilm filamentous algae, leaves-FPOM

Appendix 3.4. Feeding modes for each invertebrate group used in the stable isotope analyses at Paluma.

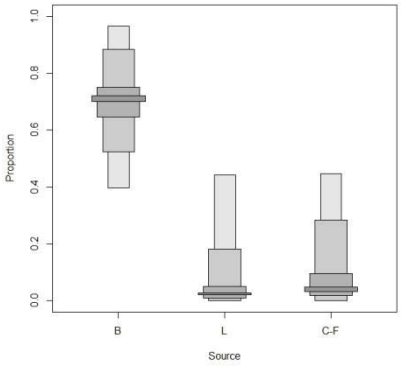
Consumer code	Feeding group	Consumer taxon	Family (if applicable)
Fi1	Filterer	Diptera	Simuliidae

Fi2	Filterer	Diptera	Mix
Ga1	Gatherer/filterer	"Worms"	mix
Ga2	Gatherer	Parastacidae	Large
Ga3	Gatherer	Parastacidae	Medium
Ga4	Gatherer/predator	Parastacidae	Small
Gr1	Grazer	Coleoptera	Psephenidae
Gr2	Grazer/shredder/gatherer	Ephemeroptera	Leptophlebiidae
Gr3	Grazer	Ephemeroptera	mix
Gr4	Grazer/gatherer/filterer	Trichoptera	mix
Gr5	Grazer/gatherer/filterer	Trichoptera	Philopotamidae
Sh1	Shredder	Trichoptera	mix
Pr1	Predator	"Worms"	mix
Pr10	Predator	Hemiptera	Gelastocoridae
Pr11	Predator	Hemiptera	mix
Pr12	Predator	Megaloptera	Corygalidae
Pr13	Predator/grazers	Plecoptera	mix
Pr14	Predator/grazers	Plecoptera	Gripopterygidae
Pr15	Predator	Trichoptera	mix
Pr16	Predator	Zygoptera	Synlestidae
Pr17	Predator	Zygoptera	mix
Pr2	Predator	Arachnida	Pisauridae
Pr3	Predator	Coleoptera	Dytiscidae
Pr4	Predator	Coleoptera	mix
Pr5	Predator	Ephemeroptera	Ameletopsidae
Pr6	Predator	Epiproctophora	Gomphidae
Pr7	Predator	Epiproctophora	Synthemistidae
Pr8	Predator	Epiproctophora	Telephlebiidae
Pr9	Predator	Epiproctophora	mix

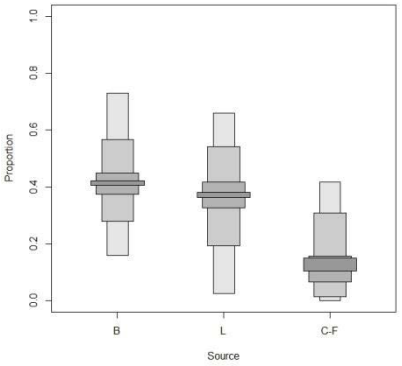
L. serrata (large) – RC 2012



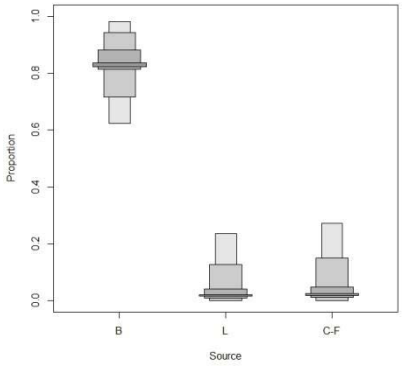
L. serrata (medium) – RC 2012



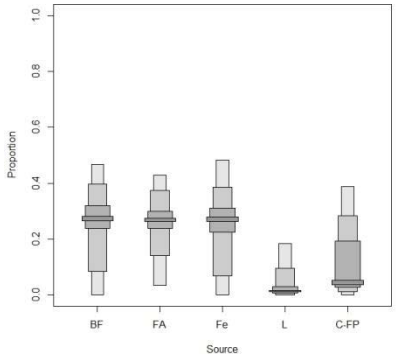
L. serrata (medium) – RC 2013



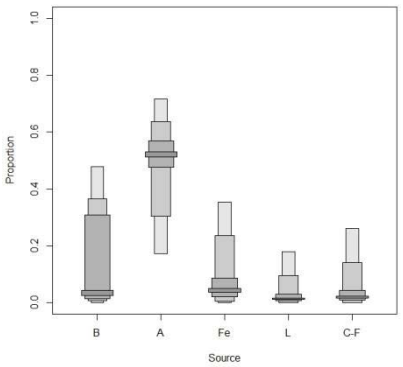
L. serrata (small) – RC 2013



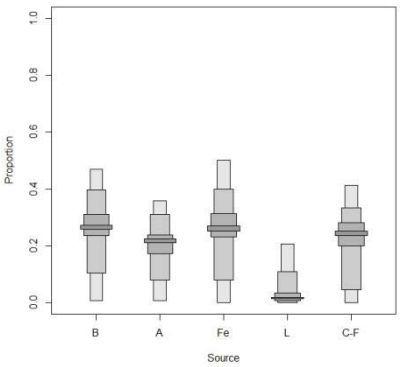
L. serrata (medium) – CC 2012



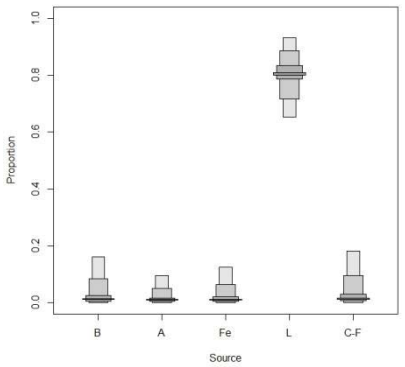
L. serrata (medium) – CC 2013



L. serrata (mix) – CC 2012



L. serrata (mix) – CC 2013



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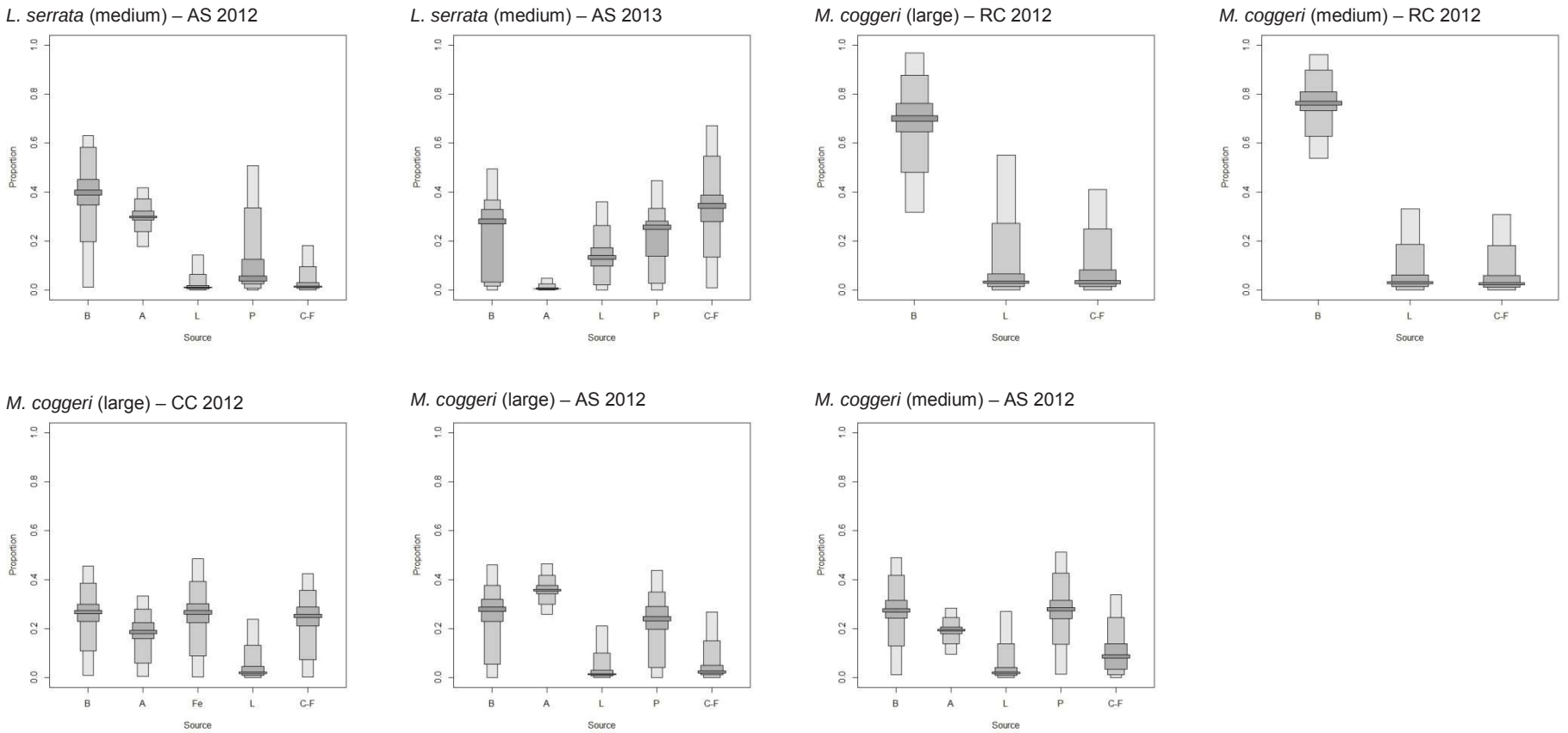
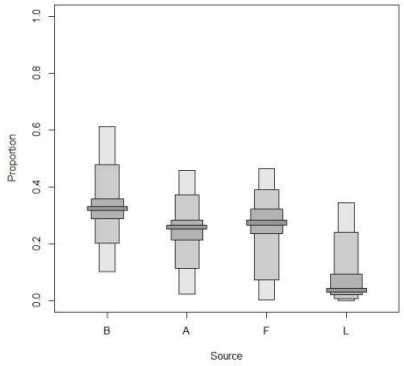
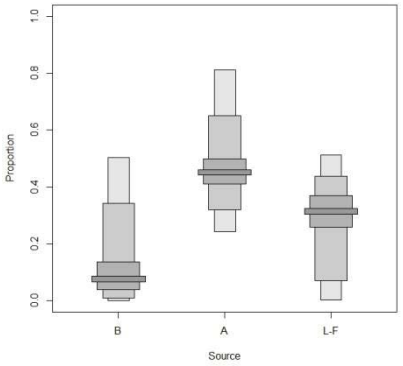


Appendix 3.5. The basal source contributions to tadpole diet for *Litoria serrata* and *Mixophyes coggeri* at Paluma in 2012 and 2013. Site abbreviations: RC = Road crossing, CC = Camp Creek, and AS = Artificial streams. Basal sources: B = biofilm, A = filamentous algae, C = coarse particulate organic matter, F = fine particulate organic matter, L = leaves, P = periphyton, and Fe = iron matrix. The boxplots represent confidence intervals, in the order from light grey to dark grey: 5%, 25%, 75% and 95%.

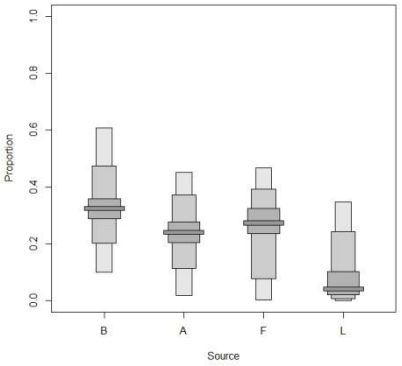
L. serrata (large) – T1 2013



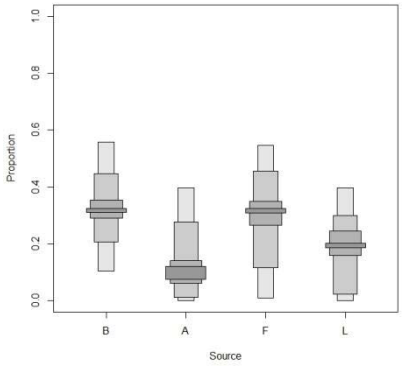
L. serrata (mix) – T2 2013



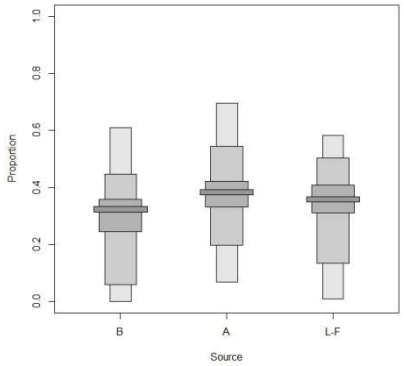
L. nannotis (large) – T1 2013



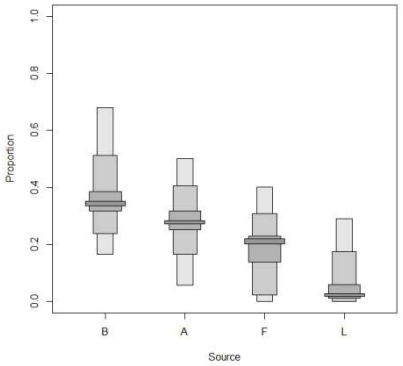
L. nannotis (small/medium) – T1 2013



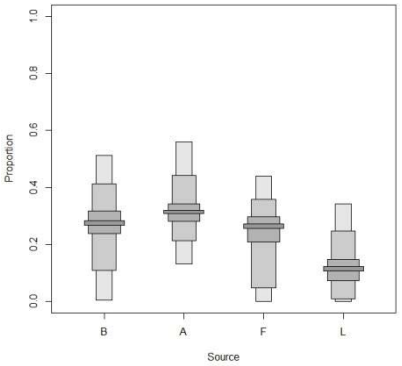
L. nannotis (large) – T2 2013



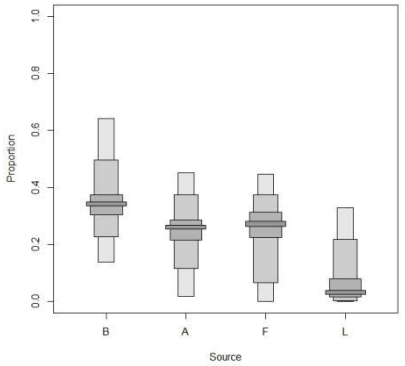
L. rheocola (large) – T1 2013



L. rheocola (medium) – T1 2013

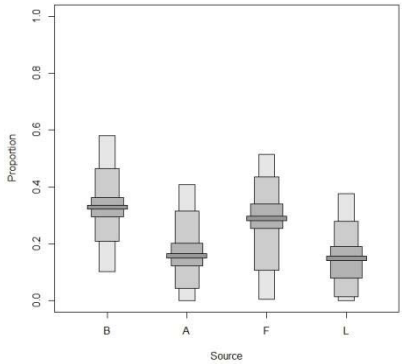


L. rheocola (small) – T1 2013

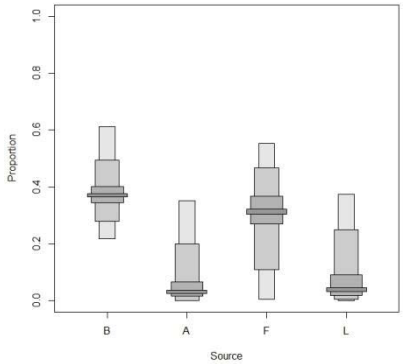


Appendix 3.6. (Continued on next page)

L. dayi (large) – T1 2013

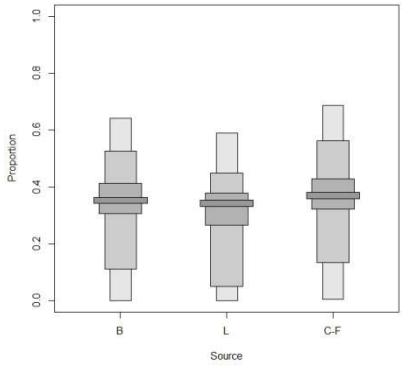


L. dayi (small/medium) – T1 2013

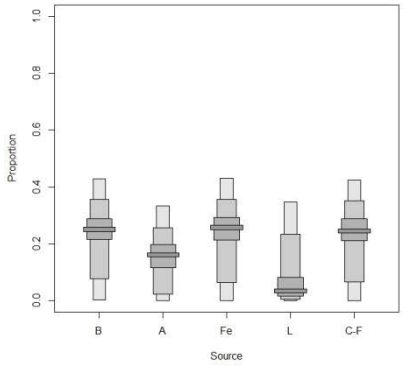


Appendix 3.6. The basal source contributions to tadpole diet for *Litoria serrata, L. nannotis, L. rheocola* and *L. dayi* at Tully in 2013. Site abbreviations: T1 = Tully Stream 1, and T2 = Tully Stream 2. Basal sources: B = biofilm, A = filamentous algae, F = fine particulate organic matter, and L = leaves. The boxplots represent confidence intervals, in the order from light grey to dark grey: 5%, 25%, 75% and 95%.

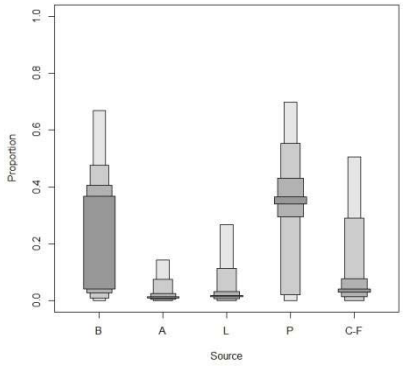
Diptera (mix) – RC



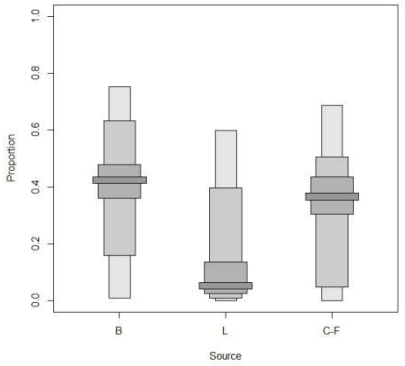
Diptera Simuliidae – CC



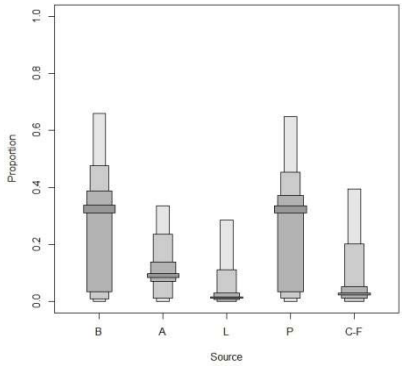
Diptera Simuliidae – AS



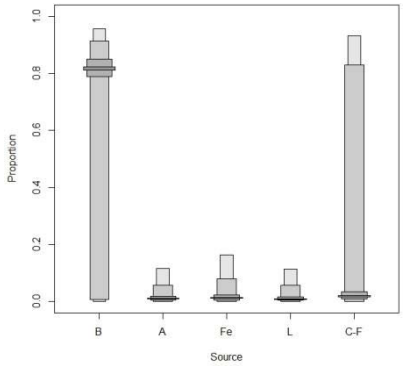
“Worms” (mix) – RC



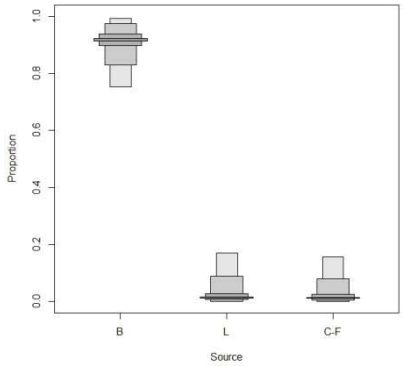
Parastacidae (medium) – AS



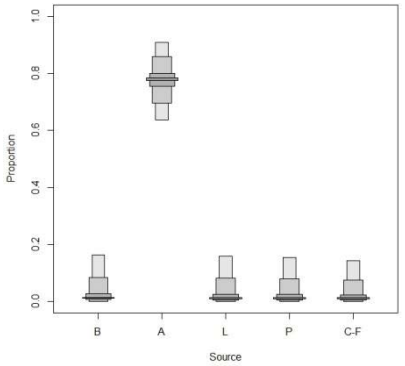
Palaemonidae (medium) – CC



Coleoptera Psephenidae – RC



Coleoptera Psephenidae – AS



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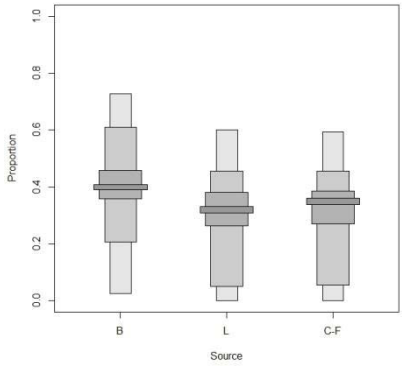
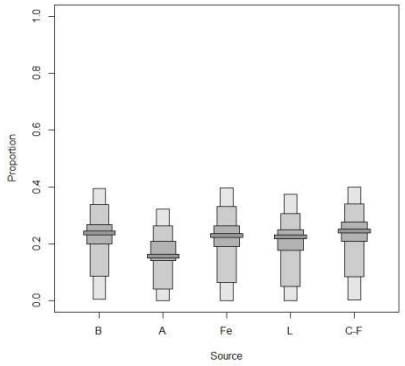
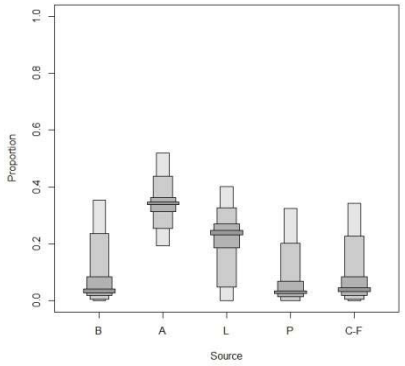
Ephemeroptera Leptophlebiidae – RC



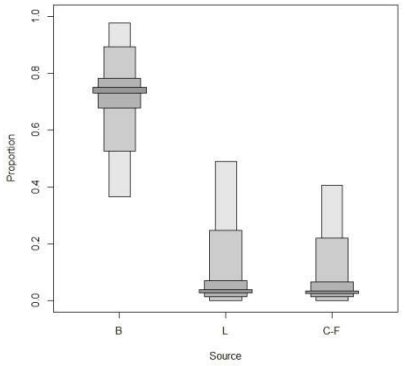
Ephemeroptera Leptophlebiidae – CC



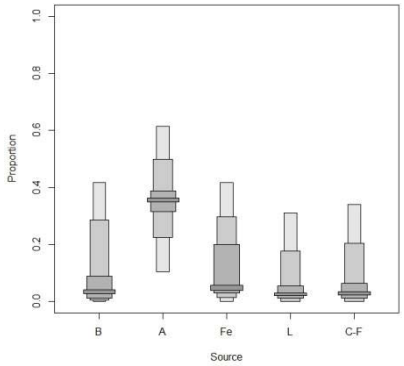
Ephemeroptera Leptophlebiidae – AS



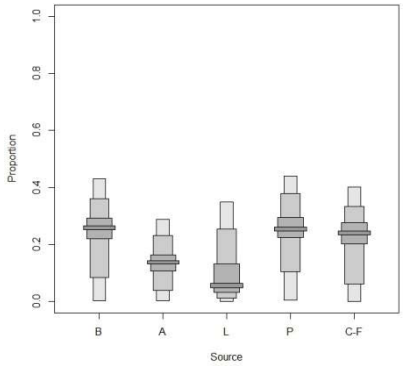
Ephemeroptera (mix) – RC



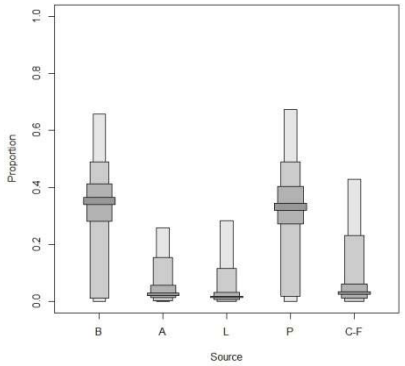
Ephemeroptera (mix) – CC



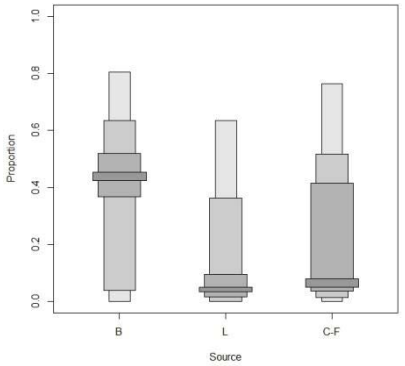
Ephemeroptera (mix) – AS



Trichoptera (mix) – AS



Trichoptera Philopotamidae – RC



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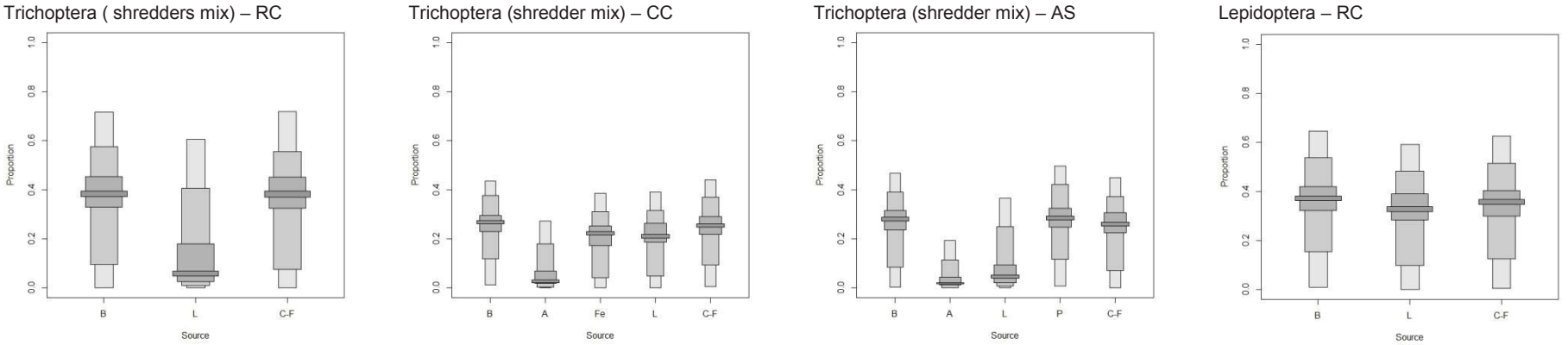
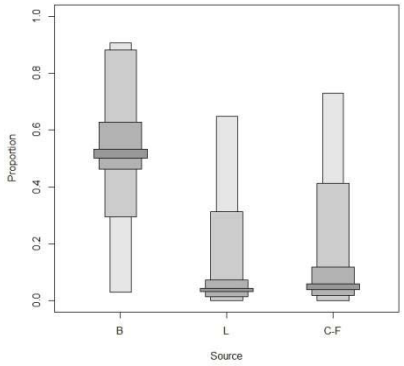
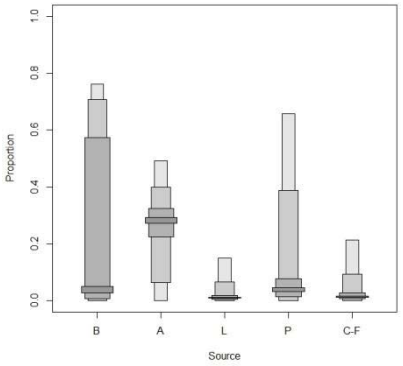


Appendix 3.7. The basal source contributions to herbivorous invertebrates at Paluma in 2012. Site abbreviations: RC = Road crossing, CC = Camp Creek, and AS = Artificial streams. Basal sources: B = biofilm, A = filamentous algae, C = coarse particulate organic matter, F = fine particulate organic matter, L = leaves, P = periphyton, and Fe = iron matrix. The boxplots represent confidence intervals, in the order from light grey to dark grey: 5%, 25%, 75% and 95%.

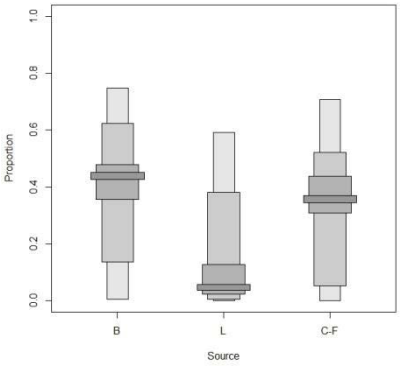
“Worms” (mix) – RC



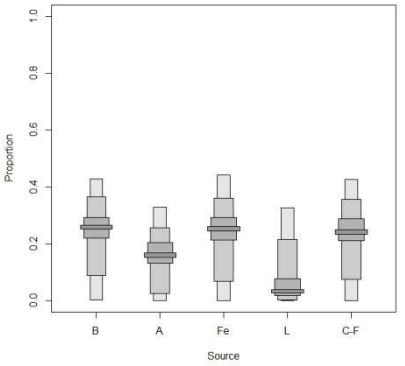
“Worms” (mix) – AS



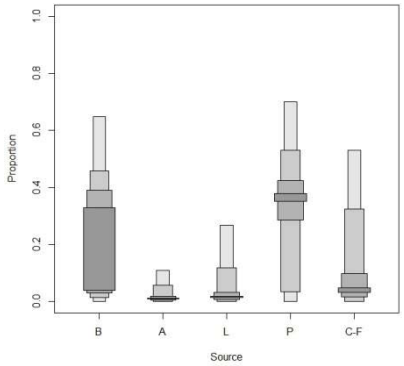
Coleoptera Dytiscidae – RC



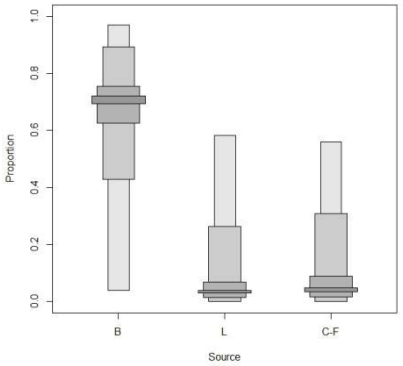
Coleoptera Dytiscidae – CC



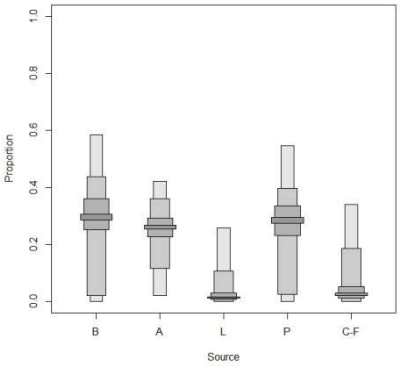
Coleoptera (mix) – AS



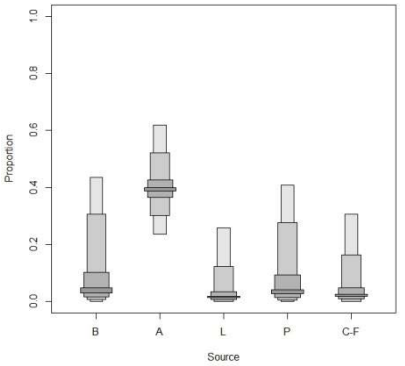
Ephemeroptera Ameletopsidae – RC



Ephemeroptera Ameletopsidae – CC

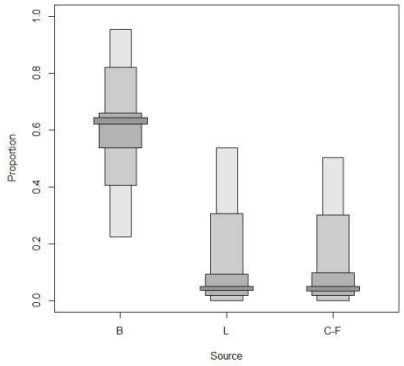


Plecoptera (mix) – AS

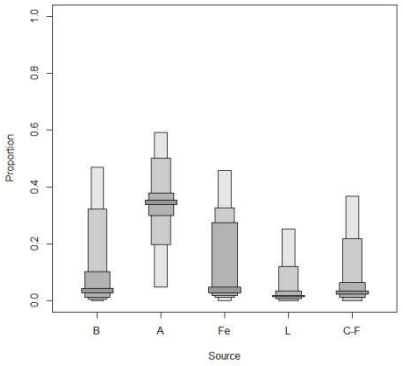


Appendix 3.8. (Continued on next page)

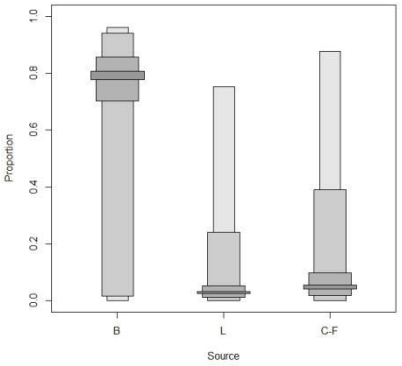
Plecoptera Gripopterygidae – RC



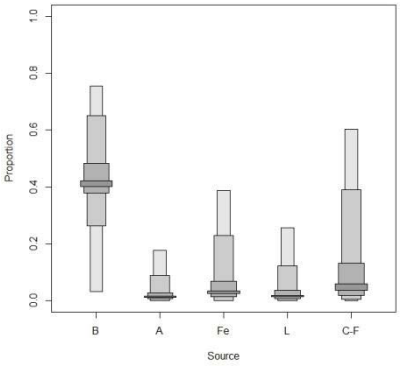
Plecoptera Gripopterygidae – CC



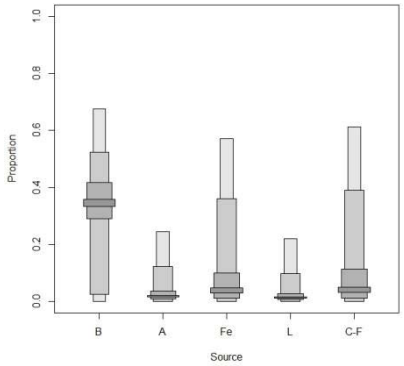
Hemiptera (mix) – RC



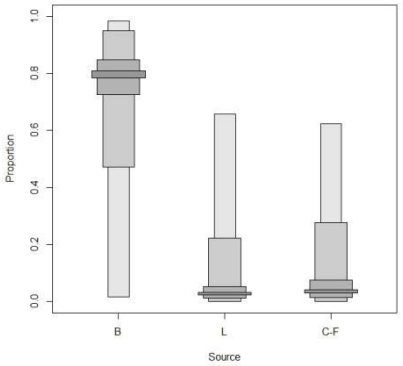
Hemiptera (mix) – CC



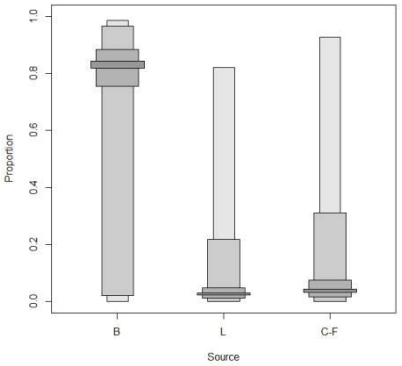
Megaloptera Corygalidae – CC



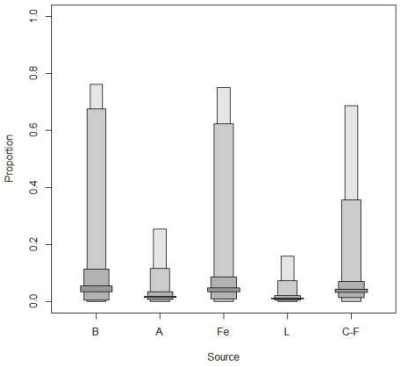
Zygoptera Synlestidae – RC



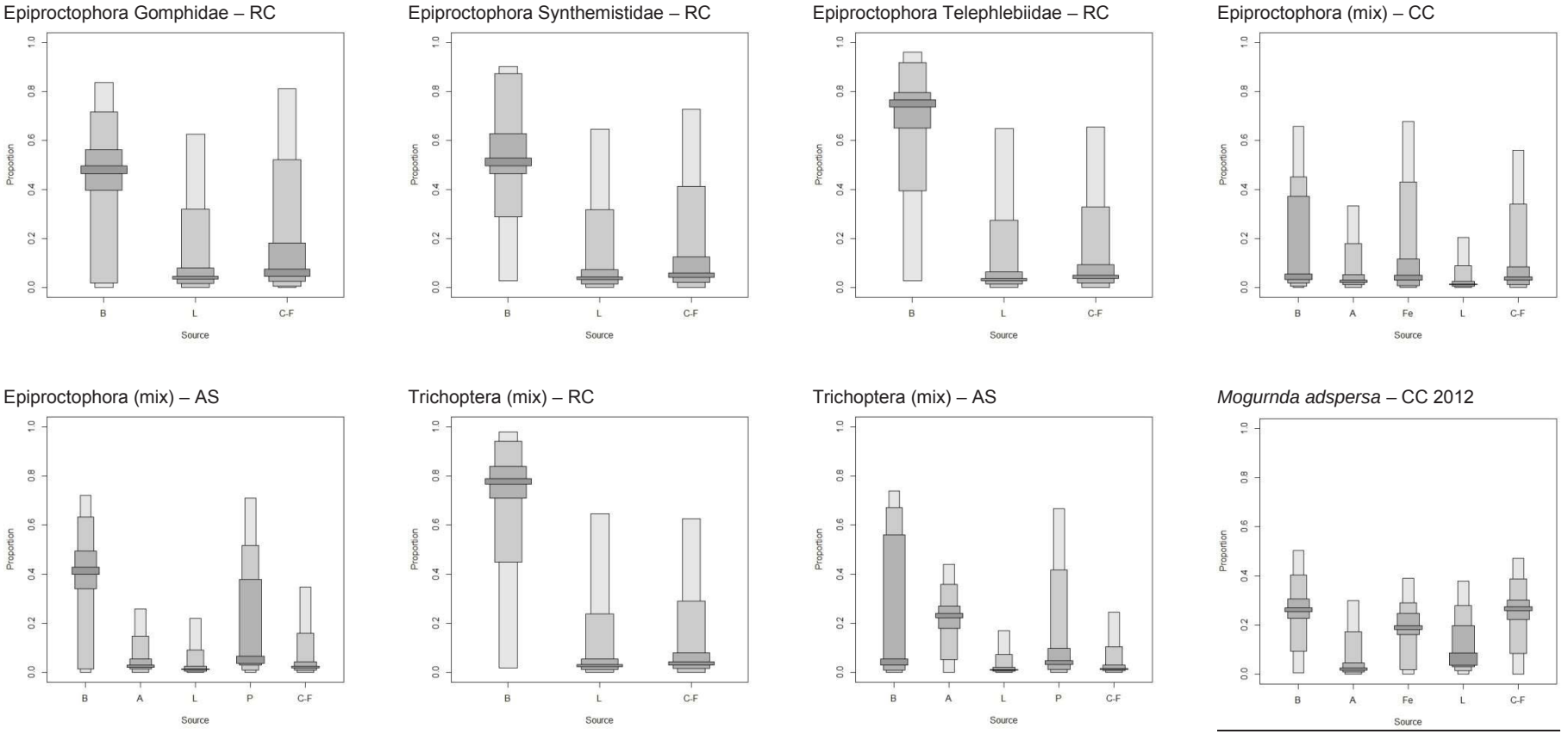
Zygoptera (mix) – RC



Zygoptera (mix) – CC



Appendix 3.8. (Continued on next page)



Appendix 3.8. The basal source contributions to predatory invertebrate diet and fishes at Paluma in 2012. Site abbreviations: RC = Road crossing, CC = Camp Creek, and AS = Artificial streams. Basal sources: B = biofilm, A = filamentous algae, C = coarse particulate organic matter, F = fine particulate organic matter, L = leaves, P = periphyton, and Fe = iron matrix. The boxplots represent confidence intervals, in the order from light grey to dark grey: 5%, 25%, 75% and 95%.