

Life history and impact of non-native brown tree frog, *Litoria ewingii*, on New Zealand pond ecosystems

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Abstract

Freshwater ecosystems worldwide are under increasing pressure from multiple threats, including invasive species and climate change. Some ponds naturally dry, but these threatened wetland ecosystems are vulnerable to increases in the magnitude of drying events associated with climate warming. To invade these dynamic habitats, especially those drying unpredictably, invaders like the Australian brown tree frog (*Litoria ewingii*) would be advantaged by both flexibility and tolerance. *L. ewingii* was introduced to New Zealand in 1922 and has since spread across the country, breeding in both urban and undisturbed ponds. Native pond ecosystems may be especially vulnerable to *L. ewingii* tadpoles because native frogs do not produce aquatic tadpoles, so pond organisms lack co-evolution with tadpoles. *L. ewingii* has several traits that may enhance invasive success, but this species has received little attention in New Zealand. I repeatedly surveyed ponds spanning a pond-permanence gradient in the Canterbury high country to determine whether drying limited their spread, and to evaluate factors that may regulate their densities or development. High tadpole densities (50/m²) occurred throughout the year, with a spring peak, in fishless ponds varying widely in conditions, suggesting wide tolerance (except to fish). Tadpoles also increased their rate of metamorphosis to more quickly escape pond drying, with a cost to size at metamorphosis. All of these traits mean *L. ewingii* could have strong ecological impacts. To determine the functional role of *L. ewingii* tadpoles and to uncover potential influences on pond community structure and function, I conducted two mesocosm experiments. The first crossed tadpole presence with predatory invertebrate presence. Tadpoles grazed down algae and macrophytes, and reduced the number of two invertebrate taxa (Culicidae and Chironomidae). However, predatory invertebrates did not affect tadpoles, so *L. ewingii* probably lack any top-down control in fishless ponds. I evaluated effects of tadpole density on ecosystem processes, in a second mesocosm experiment that also manipulated abiotic conditions. Half the mesocosms were shaded to simulate permanent ponds, since shade stabilised temperature fluctuations typical of temporary ponds. Phytoplankton increased with higher tadpole densities in unshaded mesocosms, possibly because nutrient release from tadpole excretion enhanced algal growth when sunlight was plentiful. Higher densities of tadpoles also decreased dissolved oxygen – potentially an indirect effect of grazing reducing photosynthesis. Overall, tadpoles are likely to have large influences on ecosystem processes like nutrient cycling in small lentic habitats, especially at the high densities they appear in. In light of their many invasive traits and potential effects on pond ecosystems, the spread of these frogs in New Zealand should be more carefully considered.

Chapter 1: Introduction

Invasions to dynamic systems in a changing world

Natural ecosystems worldwide are under increasing pressure from multiple threats, with invasive species and climate change being some of the largest (Early et al., 2016; Engel, Tollrian, & Jeschke, 2011; Suen, 2010). Invasive animals on their own can alter ecosystems directly, through mechanisms such as predation and competition (Innes et al., 2010), and can also alter ecosystem function indirectly through trophic cascades (Pagnucco, Remmal, & Ricciardi, 2016), or alterations of the physical habitat (Bajer et al., 2016; Bajer & Sorensen, 2014). Freshwater habitats are affected disproportionately by climate change and invasion (Moorhouse & Macdonald, 2014; Woodward, Perkins, & Brown, 2010), but they present good opportunities to investigate trophic consequences of invasions because they occupy discrete areas. Climate change can facilitate these harmful invasions in a multitude of ways, through temporal range shifts, altitudinal range shifts (Freeman et al., 2018), or geographic range shifts (Régnière, Nealis, & Porter, 2009). Furthermore, resilient ecosystems can resist invasions (Downing et al., 2012), but climate change may reduce resilience when abiotic conditions are altered, making ecosystems more susceptible to establishment invader establishment (Côté & Darling, 2010). Thus, understanding invasions is important for mitigating harm on ecosystems in a change climate.

Climate change is characterised by altered temperatures and other abiotic alterations to environments (Suen, 2010), but some environments naturally experience large fluctuations in abiotic conditions (Hornbach, Schilling, & Kundel, 2020). These ecosystems are dynamic systems, going through extreme changes naturally over short or long-time scales, such as to temperature, oxygen, or water availability. Marine intertidal zones, estuaries, and small ponds generally exemplify such systems, and can fluctuate on an hourly to seasonal basis. Alterations in the climate mean that the fluctuations that characterise these systems will be altered, and they may no longer follow the natural regime (Suen, 2010). For example, the severity of rainfall events and temperature extremes are predicted to increase in many countries (NIWA, 2020b; Suen, 2010; WMO, 2013). These changes might affect ponds and small lakes disproportionately, because ponds are highly dynamic and rely heavily on rainfall to be filled (Greig & Galatowitsch, 2016). Relative to other waterbodies like lakes and rivers, ponds have little water to regulate heat or resist drought, which results in high dynamism (Frisch & Santer, 2004). Being lentic, they are also more prone to extreme fluctuations in dissolved oxygen (Hornbach et al., 2020). There is also dynamism between ponds – some are permanently filled, whereas some dry frequently (i.e. are temporary), and so pond communities differ along an environmental gradient (Greig & Galatowitsch, 2016; Wellborn, Skelly, & Werner, 1996). In the



Figure 1.1. An example of a dynamic system: a kettle hole tarn in the Canterbury high country (Kettle Tarn) that went from fully dry (top, May) to flooded (bottom, July) within a period of three months, whereas in periods of more consistent rainfall it was usually only moderately filled (middle, October), emergent macrophytes demonstrating usual water level.

short-term, ponds usually experience large temperature fluctuations (Székely et al., 2017), and in the long-term they may go through cycles of drying and filling. Organisms inhabiting these dynamic ecosystems have adapted to manage and survive in the natural abiotic fluctuations (Frisch & Santer, 2004; Wyngaard, Taylor, & Mahoney, 1991). Despite flexibility and tolerance to the range of conditions in dynamic ponds, new extremes due to climate change may alter systems beyond the limits of those organisms.

To live in dynamic pond systems, especially those that dry periodically, organisms must have adaptations to succeed despite the abiotic stresses (Wellborn et al., 1996; Wyngaard et al., 1991). Aquatic pond inhabitants are often resistant to desiccation (Stenert et al., 2017), or have an accelerated developmental period in order to escape drying (Frisch & Santer, 2004). Life history flexibility is particularly useful to survive unpredictable stressors. Ponds with unpredictable hydrological regimes, however, may be susceptible to drying quickly (Bauder, 2005), and so many species that live in these ponds exhibit flexible or generalist life history. For example, in New Zealand, nymphs of the damselfly *Xanthocnemis zelandicus* are able to accelerate development in more temporary ponds to escape drying by moving to terrestrial life stages (Galatowitsch & McIntosh, 2016a). Communities in these unpredictably drying ponds may be resilient because of the frequency of generalist traits, but at abiotic limits they may be especially vulnerable. Furthermore, for an invasive species to succeed in these environments they must either be tolerant of a wide range of physico-chemical conditions or likewise adapted for flexibility. For example, invasive zooplankton *Daphnia lumholtzi* have become prevalent in North America, with success attributed to a wide thermal tolerance and phenotypic plasticity (Engel et al., 2011). Brown trout (*Salmo trutta*) also has flexible life history, aiding worldwide invasion (Valiente et al., 2010). Thus, animals with flexible life histories will likely have an advantage when invading dynamic systems. Moreover, because climatic regimes are predicted to change in the future, pond animals adapted for particular conditions may be disadvantaged compared to more tolerant or flexible invasive animals.

Pond invasions

Much of the focus on invasions to freshwater systems has been on rivers and lakes, and pond invasions have been reported less often (Hill et al., 2021). However, ponds and small lakes make up almost half of the total area occupied by continental waterbodies (Downing et al., 2006). Proportionately, ponds can absorb more organic carbon than almost any other ecosystem (Taylor et al., 2019). When corrected for their small size, they collectively sequester about half of the amount of carbon buried by coniferous woodlands in the UK, and so globally, are important for carbon sequestration (Taylor et al., 2019). Ecologically, ponds tend to be richer in species and possess more

rare species than other waterbodies (Biggs et al., 2005; Greig & Galatowitsch, 2016). Community composition differs along environmental gradients between ponds as well. Generally, species not tolerant or flexible to drying inhabit only permanent ponds (Cook, Heppell, & Garcia, 2013), whereas temporary pond specialists tend to be tolerant or flexible (Wissinger et al., 2006). Generalist species can inhabit both, but may be outcompeted by temporary pond specialists (Galatowitsch & McIntosh, 2016a), so preserving pond heterogeneity is also important for species protection. Overall, the importance of pond ecosystems means they are worth protecting, and so alterations via invasions should be investigated.

Pond invasions can alter pond communities and entire ecosystems, and many documented pond invasions are by fish. For example, *Gambusia holbrooki*, introduced intentionally as a means to control disease-bearing mosquitoes, has now invaded several countries (Azevedo-Santos et al., 2017). Their ecological impacts include predation of tadpoles (Morgan & Buttemer, 1996; van Tol, 2001), plankton communities (Margaritora, Ferrara, & Vagaggini, 2001), and large impacts on other fishes (Arthington, 1989). New Zealand, in particular, has been negatively affected by *G. holbrooki*, as well as other introduced freshwater fish (NIWA, 2020a). Grass carp introductions have also altered the planktonic communities in New Zealand ponds (Branford & Duggan, 2017). As well as influences on organisms, prolific and high density invasive species can alter physical habitat structure, by altering primary production which can alter light availability in the water column (Bajer et al., 2016), or increase suspended solids in the water column through bioturbation (Bajer & Sorensen, 2014). Often the management response to remove invasive fish is to drain the pond (Pollard et al., 2017). While successful for protecting native animals against exotic fish, this technique will not work for invasive amphibians that can tolerate drying, so preventing ecological harm from amphibian invasions may be challenging compared to fish.

Many examples of significant pond invasions are caused by amphibians in particular, because many amphibian species are adapted for pond life (Cabrera-Guzmán, Crossland, & Shine, 2013; Kruger et al., 2019; Measey, 2001). Invasive amphibians often put native amphibians at risk at both tadpole and adult stages (Berger et al., 1998). Invading tadpoles can exert strong competitive pressure on native tadpoles, especially in high densities, evidenced by bullfrog invasion to California (Kupferhiirci, 1997), and cane toad invasion to Australia (Cabrera-Guzmán et al., 2013). These exotic tadpoles are advantaged by exhibiting anti-predator responses to local predators, as is the case in the *Xenopus laevis* invasion to France (Kruger et al., 2019). Additionally, fast life history traits in amphibians can enhance invasive success, especially to ephemeral ponds (Allen, Street, & Capellini, 2017). These invasive, generalist anurans can push at-risk pond systems towards more unstable states and increase the risk for loss of ecosystem function and extinctions (Wainright et al., 2021). With the

exception of the recent invasion by the Italian alpine newt, to which eradication is being attempted due to proximity to native frog populations (Burns et al., 2017), naturalised amphibian populations in New Zealand have generally not been a concern (Bishop, 2008; Burns et al., 2017; NIWA, 2020a). However, generalist frogs with fast life history traits and extensive spread may be a concern and should be investigated.

History of frogs in New Zealand

New Zealand has no more than four species of native frog (Bell, 2010; Easton et al., 2018), and three species of frog introduced approximately 150 years ago from Australia (Thomson, 1922). The number of extant endemic species is debated, specifically as to whether *Leiopelma pakeka* and *Leiopelma hamiltoni* are distinct. Genetic differences indicate they may not be separate, so there may effectively be three species (Burns et al., 2017; Easton et al., 2018). After human colonization, native frog populations declined drastically, and at least three species have gone extinct (Bell, 2010; Shaw et al., 2010). The remaining species are now all restricted to small ranges or predator free islands (Bell, 2010). *Leiopelma hamiltoni* is classified as Threatened: Nationally Vulnerable, and both *Leiopelma archeyi* and *Leiopelma hochstetteri* as At Risk – Declining (Burns et al., 2017). All introduced frogs in New Zealand are classified as Introduced and Naturalised (Burns et al., 2017). Two of the introduced species, *Ranoidea raniformis* and *R. aurea*, are generally common in low wetlands and ponds across much of New Zealand, contrasting their status as endangered and vulnerable, respectively, in their home country of Australia (Bishop, 2008; Burns et al., 2017; Shaw et al., 2014). However, the other introduced species, *Litoria ewingii* or brown tree frog, is classified as Least Concern in Australia. In New Zealand it also appears to be thriving, appearing across much of the country, in both urban and very remote areas (Alderton, 1985; Cousineau, 1990; Shaw et al., 2014), even above the treeline (Barclay, 2020).

Litoria ewingii was initially introduced to Greymouth in 1875 from a population in Tasmania, Australia (Thomson, 1922), and has become widespread in New Zealand (Figure 1.2; Shaw et al., 2014). Since their introduction, they have been spread by people to various South Island cities, so populations were soon established in Otago, Christchurch, and towns along the West Coast. They were not established in the North Island until 1948, when they were introduced to the Manawatu (Alderton, 1985; Gill, 1978). However, it took some time to spread to more remote regions like the high country of Canterbury. They were not mentioned in a comprehensive list of taxa associated with the Cass basin in 1977 for example (Stout, 1977), and were first recorded there in the early 1980s (Cree, 1984). It is unknown whether they were introduced to the area or if they spread naturally. Other alpine occurrences have been recorded, sometimes in tarns as high as 1500 m

(Barclay, 2020). In these tarns they appear to be successful, with one trumper saying, “there were so many tadpoles in the tarns that it was hard to exclude them from the bottles,” after collecting drinking water from an alpine tarn (Christchurch Tramping Club, 2003). So, although *L. ewingii* are not a new addition Aotearoa, there may be remote ecosystems and ponds which have been more recently colonized, and perhaps some are yet to be reached.

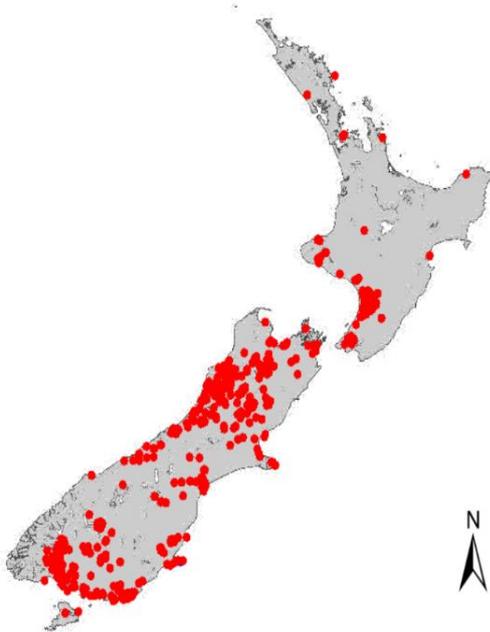


Figure 1.2. Recordings of *L. ewingii* since 1988 in New Zealand, indicated by red circles. Adapted from the Department of Conservation’s “Atlas of the amphibians and reptiles of New Zealand”.

<https://www.doc.govt.nz/our-work/reptiles-and-frogs-distribution/atlas/atlas-details/?SpeciesID=13197>

New Zealand is naturally depauperate in many taxonomic groups, including Amphibia, so an addition of high densities of a novel amphibian species may have had large effects. Of the four native amphibian species, three are terrestrial and do not have an aquatic tadpole stage, emerging from eggs with well-developed hind legs, and being carried dorsally by the father (Bell, 2010). Only *L. hochstetteri* could be described as semi-aquatic, with a range limited to the warm north of the country, small clutch sizes, and tadpoles do not spend long in water (Bell, 2010). In contrast, *L. ewingii* has a strong aquatic tadpole stage (Figure 1.3), emerging from eggs at a very early developmental stage, and is capable of breeding in a variety of pond conditions across a large thermal range (Cousineau, 1990; Cree, 1984; Lauck, Swain, & Barmuta, 2005a). Moreover, they can have clutches as large as 500 eggs (Anstis, 2017). Generally, concern towards their presence in New Zealand has been related to possible interactions with native frogs (Bishop, 2008), because *L. ewingii* is known to carry the *Chytridiomycosis* fungal disease which has contributed heavily to frog decline

around the world (Berger et al., 1998; Shaw et al., 2010; Whiles et al., 2013). However, because sympatric overlap is deemed unlikely due to their differing distributions, it is often assumed that *L. ewingii* have no ecological impacts here (Bishop, 2008). Furthermore, they are not mentioned in a 2020 report on invasive freshwater species (NIWA, 2020a). However, it is likely that they do have ecological impacts on pond communities, because unlike any native vertebrate, they are found in high densities in temporary aquatic systems (Cousineau, 1990). Fauna composition of New Zealand ponds is dominated by freshwater invertebrates, many of which are already at risk (Grainger et al., 2018), lacking other amphibians like frogs and salamanders which characterise pond ecosystems overseas (Biggs et al., 2005). Consequently, native pond organisms with no coevolution with *L. ewingii* may be especially vulnerable, so pond ecosystems in New Zealand could be affected more strongly than most documented frog invasions, potentially leading to ecosystem disruption.

Thesis outline

Overall, I aimed to increase understanding of *L. ewingii* life history in New Zealand pond systems and uncover trophic relationships between the larvae and native pond organisms, to determine whether they are harmful, invasive and affect ecosystem functions. I have written Chapters Two and Three with the intention of publishing them as two stand-alone papers, and so they may share some material, and there may be some overlap with the introductory and concluding chapters. However, all references from all chapters are collated and can be found at the end of the thesis. I have provided a wider overview of the need to study *L. ewingii* in New Zealand in Chapter One, and a general synthesis in Chapter Four to enrich the conclusions from the two empirical chapters and provide a starting point for management.

Chapter Two examines the life history of *L. ewingii* in ponds across a water permanence gradient, from permanent to highly ephemeral ponds, to investigate if tadpoles exhibited a plastic response to different pond conditions. I also assessed breeding, larval densities, and larval body sizes throughout the year in this range of ponds to determine when and where they were most prolific and may have the biggest impacts. These aspects of life history were important to investigate, because certain traits are associated with invasiveness and knowledge of them could inform management.

Much literature on the ecological consequences of frog invasion is concerned with impacts on frogs native to the invaded areas. In New Zealand however, it is unlikely native frogs will be affected and so there appears to have been little to no other research on the effects of larval *L. ewingii* in pond ecosystems. Thus, in Chapter Three I studied the ecological impacts of *L. ewingii* larvae on New Zealand pond ecosystems using mesocosm experiments. Specifically, I investigated trophic interactions between tadpoles and native pond organisms, by looking at abundance of small

invertebrates, biomass of macrophytes and algae in treatments with or without varying densities of tadpole, as well as investigating whether larger predatory invertebrates could reduce tadpole densities.

My final chapter, Chapter Four, includes a synthesis of chapters Two and Three, to outline likely current and future impacts of larval *L. ewingii* on New Zealand pond ecosystems, taking both life history and trophic linkages into consideration. I also discuss directions for future research and recommendations for management of this species.



Figure 1.3. *Litoria ewingii* at various life stages: two large tadpoles are beginning metamorphosis (top), a froglet with a tail emerges from the water (middle), and an adult frog rests on a leaf (bottom).

Chapter Two: Life history and larval densities of introduced brown tree frog, *Litoria ewingii*, across a range of pond-permanence.

INTRODUCTION

Invasive species often benefit from a certain combination of traits and circumstances when introduced, such as production of numerous offspring (Allen et al., 2017), habitat generalisation (Dammhahn, Randriamoria, & Goodman, 2017), life history flexibility (Allen et al., 2017; Valiente et al., 2010), and escape from natural predators and competitors (Kupferhiirci, 1997; Menéndez et al., 2008). High fecundity, especially large clutch sizes mean that even when a small initial group of animals are introduced, the population can grow exponentially (Avery et al., 2014). A species which possesses high fecundity as well as ecological competence (having flexibility to new habitats) may be more likely to be invasive (Pereira et al., 2017), and the addition of dispersal ability would likely enable them to reach areas they are generally tolerant of. The larvae of *Litoria ewingii*, the brown tree frog, introduced to New Zealand from Australia in 1875 (Thomson, 1922), likely exhibits many of these traits, (Anstis, 2017; Lauck et al., 2005a) and yet they have largely slipped under the radar as a non-native species. Therefore, it is useful to understand how the life history characteristics of *L. ewingii* have affected its colonization of New Zealand ponds.

Amphibian invasions are interesting because frogs are often adapted for producing large numbers of offspring in a short time period. However, there are only a few invasive species, and in contrast many amphibian species are in decline (Berger et al., 1998). Amphibians are unusual compared to many invading species, because they have complete life cycles, with only part of their life is spent in the freshwater environment. Unlike many freshwater invaders such as fish or mussels, (Azevedo-Santos et al., 2017; Pagnucco et al., 2016), frogs with tadpoles have a terrestrial stage and so can disperse relatively easily between disconnected freshwater habitats. Having a terrestrial stage means they can inhabit both permanent and temporary waterbodies. Species that are adapted for growth in temporary ponds may complete their larval stage quickly and leave the pond, in a boom and bust cycle before pond drying. Therefore, life history characteristics of non-native frog species with invasive potential should be investigated in a novel country.

Invasive species impacts on freshwater systems also likely depend on climate change, which in some cases can facilitate invasion (Elmhagen et al., 2015; George & Harris, 1985; Hellmann et al., 2008). Therefore, there is a need to investigate how life history traits may benefit invaders and harm ecosystems under climate change. Freshwater systems are particularly vulnerable, because they are highly dependent on local rainfall (Srivastava et al., 2020). Pond ecosystems – especially ephemeral

ponds – rely on drying and filling cycles for species within to persist, and so extreme rainfall events such as droughts or floods will impact them (Osborne & McLachlan, 1985). Moreover, increased temperatures typical of climate change can also alter trophic patterns in ponds (Shurin et al., 2012), potentially reducing resilience to invading animals (Côté & Darling, 2010). In general, increasing temperatures driven by climate change can also increase the range of a cold-limited invader through poleward or altitudinal range expansion (Menéndez et al., 2008). Overall, if invading animals have flexible life histories, they may be able to tolerate climate-driven changes to a higher degree than some native organisms.

To persist in both a changing climate and a novel environment, traits like flexible life history and phenotypic plasticity are very useful (Engel et al., 2011; Knop & Reusser, 2012). Phenotypic plasticity is often implicated in invasive species establishment (Knop & Reusser, 2012; Valiente et al., 2010), and it could be especially important in ponds due to their fluctuating temperatures and water levels (Engel et al., 2011; Frisch & Santer, 2004). Many frog species are able to speed up larval development to escape predation or pond drying, but trade-off on their adult fitness (Altwegg & Reyer, 2003; Newman, 1992; Pechenik, 2006; Wilson, Seymour, & Williams, 2014). These fitness consequences often cause decreased adult size, reducing reproductive fitness by delaying sexual maturity (Altwegg & Reyer, 2003; Smith, 1987), or require compensatory growth which can increase likelihood of genetic errors later in life (Hector, Bishop, & Nakagawa, 2011). Thus, life history flexibility may enable invasive frogs to inhabit different pond types, but the fitness consequences of their flexibility may slow their spread.

Litoria ewingii are non-native, widely distributed in a range of habitats (Alderton, 1985; Shaw et al., 2014), and their life history is flexible (Lauck et al., 2005a). In their native Australian habitat, *L. ewingii* respond to pond drying by accelerating growth, and also show plasticity to shade, altitude, and presence of predators (Anstis, 2017; Lauck et al., 2005a; Lauck, Swain, & Barmuta, 2005b). Thus, they can complete metamorphosis in temporary ponds, allowing them to breed in more waterbodies. Alternately, in permanent ponds that cool during winter, they can delay metamorphosis, meaning their larval period can range between four weeks and seven months depending on environment (Lauck et al., 2005a). Predation usually accounts for a large portion of tadpole mortality (Wilson et al., 2014), but in a novel environment with historically no aquatic tadpoles, *L. ewingii* may escape predator-driven fitness trade-offs if predators are naïve. Overall, these adaptations likely enable *L. ewingii* to breed in a wide variety of ponds and areas in a novel and changing environment, despite likely trade-offs affecting adult fitness. Therefore, investigating potential phenotypic plasticity in *L. ewingii* in New Zealand is important to determine factors driving their success.

Climate change in New Zealand will likely negatively affect biodiversity in temporary ponds (Greig & Galatowitsch, 2016), and it is not well understood the extent to which life history flexibility has allowed *L. ewingii* to invade those systems in more remote parts of the country. Most studies of *L. ewingii* breeding in New Zealand took place over 30 years ago, primarily in the North Island or West Coast, in relatively urban, low altitude areas, or focused primarily on presence and absence rather than densities or developmental patterns (Alderton, 1985; Cousineau, 1990; Cree, 1984; Gill, 1978). Consequently, there is little understanding of *L. ewingii* life history in more remote New Zealand ponds. Despite their introduction to New Zealand in 1875 (Thomson, 1922), *L. ewingii* was probably new to the Canterbury high country at the time of those studies. For example, it started appearing in the 1980s around Cass (Cousineau, 1990; Stout & Winterbourn, 2008). Perhaps this appearance was due to warming-related range expansion, and recent reports predict further warming (NIWA, 2020b, 2022). Additionally, in parts of New Zealand, rainfall is predicted to increase (Ministry for the Environment, 2008), which may result in more temporary pond habitats. If permanent ponds begin to periodically dry, fish could be excluded from more ponds, opening up yet more potential habitat to *L. ewingii*. Moreover, even without drying, increased temperatures may exclude temperature-sensitive fish (Stout & Winterbourn, 2008; Van Zuiden & Sharma, 2016). *Litoria ewingii* tadpoles, on the other hand, are extremely tolerant of a range of temperatures, having been observed in daytime temperatures ranging from 6 – 34 °C (Chapter Three; Cree, 1985). Those increasing temperatures may also harm native biodiversity in temporary ponds, where many species tend to be understudied and declining (Grainger et al., 2018; Greig & Galatowitsch, 2016). If *L. ewingii*, a temporary pond specialist, reaches high larval densities in these temporary ponds, the generalist biodiversity could be outcompeted (Galatowitsch & McIntosh, 2016a). Climate change necessitates that we understand drivers on *L. ewingii* larval densities and life history in remote areas of New Zealand where more rare native species may occur, and especially their proclivity to different forms of temporary ponds.

Current knowledge suggests *L. ewingii* is phenotypically plastic, breeding in different pond types in Australia, and could potentially use these traits to reach high larval densities in New Zealand, yet their density and life history are not well understood in these new habitats. Therefore, the objectives of this study were to increase understanding of *L. ewingii* life history and breeding success in New Zealand. Overall, I hypothesized that this introduced frog would exhibit flexibility to a range of pond permanence in New Zealand, driving invasive success. I expected that they would reach high larval densities, even in colder high country areas, because of their adaptations for cold temperatures (Bazin, Wharton, & Bishop, 2007; Cree, 1984; Rexer-Huber, Bishop, & Wharton, 2015). Additionally, I predicted that they would occur in fishless ponds across the pond permanence

gradient, and would exhibit phenotypic plasticity and increase developmental rate in drying ponds, with a trade-off of reduced size at metamorphosis to survive shorter pond hydroperiods. I also expected continual breeding throughout the year in Canterbury due to their wide temperature tolerance and the unpredictable hydrological regime of the area (Greenland, 1977). It is important to describe the spread, larval densities, and fecundity of *L. ewingii* in New Zealand for monitoring and for future management.

METHODS

Initial presence/absence survey

Litoria ewingii are present in urban areas and often kept as tadpoles (Alderton, 1985; Shaw et al., 2014), but records of their presence in the Canterbury high country are sporadic (Alderton, 1985; Barclay, 2020; Greig, 2008). Therefore, I surveyed ponds in the Canterbury high country to determine which types of ponds were inhabited by *L. ewingii* tadpoles. I focused on tadpole presence in the Cass area, a well-known part of the upper Waimakariri catchment with many ponds ranging from permanent to ephemeral (Galatowitsch & McIntosh, 2016a). Sites were selected and initially categorised using information from past research in the area (Galatowitsch & McIntosh, 2016a; Greig, 2008), talking to landowners, and looking for ponds on maps.

I surveyed twenty ponds in summer, in late January or early February, and they were sampled using D-net sweeps to detect larval *L. ewingii*. To ensure detection of tadpoles if present, I used the dipnet protocol specified by Gunzburger (2007), in which net sweeps in a lentic waterbody are continued until the desired species is found, or if not, sweeping is continued until five sweeps return no new species. Due to the varying sizes of the ponds I sampled, this was appropriate to ensure consistency and maximise likelihood of tadpole detection.

Tadpole abundance and life history over time

To measure changes in tadpole abundance, growth rates, and evidence of breeding in relation to pond permanence, eleven ponds were further selected for monthly sampling over a 14-month period. These ponds differed in size, land use, and substrate, but because *L. ewingii* tadpole presence had been confirmed at all of these sites during the previous survey, they were suitable for monitoring continuous tadpole presence. To observe *L. ewingii* responses to pond permanence, selecting ponds spanning a range of hydroperiod from permanent to ephemeral was important, and hydroperiod information for many of the sites was available from previous research (Galatowitsch & McIntosh, 2016a; Greig, 2008).

To measure monthly tadpole abundance, I visited each of the eleven ponds once a month starting in October of 2020, and measured tadpole abundance, the size and stage of each tadpole, as well as pond surface area, depth, temperature, pH, dissolved oxygen, and specific conductivity. Surface area was recorded by using the 'Area Calculation' feature of a GPSMAP 62s, which involved walking around the pond edge so the device could calculate the area (Garmin, Kansas, USA). Dissolved oxygen (mg/L) was recorded with an EcoSense ODO200 probe, and pH, temperature, and specific conductivity were measured with a Pro1030 pH and conductivity meter (YSI, Yellow Springs, OH, USA).

To measure tadpole abundance, I conducted six 'sweeps' per pond with a D-net. One 'sweep' covered an area approximately one m², by sweeping the net (30 cm wide) over a one metre span three times, moving 30 cm forward each time. This covered up to 300 L per sweep, but not in drying ponds where sweeps were shallower. The six sweeps were taken from stratified points along the pond shorelines to ensure they were random, within 4 m of the shore, because tadpoles appeared to be less common in the middle of ponds, and because of sampling depth constraints. Despite different pond sizes, six sweeps were optimal to have the same unit effort at all ponds, and to reduce time spent searching through samples. Rather than focus on the benthos, I swept through the water column to avoid clogging the net with substrate and subsequent tadpole escape, which for sampling *L. ewingii* was appropriate because they tend to utilise the entire water column (Peterson, Bull, & Wheeler, 1992). The contents of each sweep were put into a large white tray and every tadpole (living and dead) was placed into a smaller tray next to a 30 cm ruler. Dead tadpoles presumably died during collection, and so were counted. Collected tadpoles were photographed next to the ruler and subsequently returned to the pond. The ruler was used to calibrate the image, so snout-vent-lengths (SVLs) and stages of all tadpoles could be digitally measured using Acrobat DC (Adobe, California, USA). I also recorded presence of *L. ewingii* eggs by walking the perimeter of the pond to search for spawn near the edge, to determine when and where breeding was taking place regardless of appearance of larvae. Frog calls heard while surveying were also recorded, for evidence of sexually mature frog presence at a site.

Data analysis

Periods of breeding were defined as presence of eggs or, in their absence, recently hatched (<10 mm SVL) tadpoles. Tadpoles with visible legs were classed as metamorphosing, i.e. past developmental stage 33 (Gosner, 1960). All tadpoles regardless of stage were used for density estimates.

To determine how permanent or ephemeral each pond was, I created a pond permanence metric, calculated from:

$$\text{pond permanence} = \frac{\% \text{ months filled}}{\% \text{ area lost}}$$

where percent months filled was how many months ponds had water divided by total months sampled, and percent area lost was difference between maximum and minimum surface area divided by maximum surface area for each pond during this period. Although 14 months were sampled, for consistency only 12 months were used for this calculation, omitting the first two sampled. Thus, the proportion of months filled was calculated out of the 12. Area lost was a proportion so area fluctuation between difference pond sizes was standardised. Large fluctuations, or a large proportion of surface area lost indicated, ponds that were closer to drying. Using this formula, every pond had a discrete non-0 permanence value, which reflected how long they spent dry from the proportion of months they were filled, as well as how large fluctuations were, determined by proportion area lost, meaning ponds that did and did not dry fully could both be represented.

Using this pond permanence metric, I was able to compare pond permanence to tadpole size at metamorphosis. Tadpoles were removed from analysis if they were beyond stage 42, because their SVLs began to decrease as they become frogs (Gosner, 1960). Normality of the SVLs was confirmed using a Shapiro-Wilk's normality test, as well as visually assessing a quantile-quantile plot and density plot. Using pond permanence as the predictor, the response of size at metamorphosis was assessed using a linear regression, with ponds as replicates. Modelled means and 95% confidence intervals for the developed tadpole by pond permanence regression were calculated using the emmeans package (Lenth, 2021). Data were analysed using R, version 4.04 (R Core Team, 2021), and all figures were produced using ggplot2 (Wickham, 2016).

RESULTS

Presence/absence survey

In the first survey, I found *L. ewingii* tadpoles present in a range of ponds in the Cass basin. Tadpoles were detected in many different pond types: forested, unshaded, soft-bottomed, stony-bottomed, ponds accessible to live-stock, and ponds with low and high macrophyte densities. Of the twenty ponds surveyed, *L. ewingii* were present at only eleven (Table 2.1). However, continuous sampling in the second survey revealed that tadpole presence was sporadic, and in many ponds, they were absent for much of the year (Figure 2.2), so recording an absence from one observation was not conclusive. So, if all twenty ponds were re-visited, recordings of tadpole presence may increase, but overall, they were not restricted to one type of pond habitat.

To investigate whether tadpoles and fish co-occurred, I compared tadpole presence in ponds known to contain fish (Greig, 2008), to those without. The ponds where *L. ewingii* were not found tended to be larger and more permanent, and several (3-5) had fish present, consisting of upland bullies, *Gobiomorphus breviceps*, and rainbow trout, *Oncorhynchus mykiss* (Table 2.1; Greig, 2008). However, larger lakes were not sampled. Tadpoles didn't occur when fish were present in all but one pond sampled (Table 2.1). The only instance where a tadpole was recorded with fish was in one sampling period in one pond (pond d in Table 2.1 and Figure 2.2). Furthermore, these tadpoles were small and young, and tadpoles did not reappear despite pond permanence. In contrast, ponds consistently containing tadpoles did not have fish present. Moreover, having sampled each pond over twelve times, I am confident there were no fish present. Therefore, it is likely that tadpoles do not occur in ponds with fish in my study area.

Tadpole abundance and life history over time

Most evidence of frog spawning was seen in spring. Eggs appeared in September across several ponds, with high levels of breeding in October and November as well, evident from egg appearance and the quantity of small tadpoles across most ponds (Figures 2.1-3). In most ponds, tadpoles were absent by late summer (February), and they primarily remained absent over winter (Figure 2.1). However, there was evidence of continuous spawning across the year at low levels in Dragonfly Tarn, a permanent forested pond (Figure 2.1; pond a). Despite observing very few eggs between November 2020 and July 2021, young tadpoles were present throughout much of that time period in this pond (Figure 2.2; pond a). Because *L. ewingii* tadpoles can hatch in as little as 4 days after eggs are laid (Anstis, 2017), eggs were likely missed by monthly sampling. There was continual spawning in Dragonfly Tarn and calls confirmed adult presence throughout the year in

Table 2.1. Characteristics and position of pond sites surveyed for tadpole presence in Canterbury high country, visited summer of 2020 or 2021, or visited repeatedly for ponds a-k above the line. Additional measurements were taken at those repeatedly visited; dashes indicate no measurement. Permanence classifications are defined as: temporary ponds dried down completely, semi-permanent (semi-perm) ponds did not fully dry, but had severe reductions in water volume, and permanent ponds did not fluctuate much in size. Fish presence also indicated permanence.

Site (a-k surveyed repeatedly; others surveyed once)	Tadpoles detected (Y/N)	Permanence	Months filled (count)	Area (range max - min area, m ²)	Latitude (NZ WGS 84)	Longitude (NZ WGS 84)	Fish present (Y/N)	Avg pH	Avg DO ₂ (mg/L)	Avg sp. conductivity (µS/cm at 25 °C)	Temp range (max-min, °C)
(a) Dragonfly Tarn	Y	Permanent	12	350-530	-43.0350587	171.6436323	N	5.6	7.3	23.4	20.1-6.1
(b) Flock hill	Y	Permanent	12	250-350	-43.132488	171.769738	N	7.0	11.4	42.6	19.8-5.2
(c) Hawden ridge	Y	Semi-perm	12	1500-3975	-43.101857	171.854582	N	6.0	10.6	46.8	19-5.8
(d) North Hawden	Y	Permanent	12	500-1100	-43.099084	171.842136	Y	6.6	9.8	45.1	18.1-6.6
(e) Goldney	Y	Semi-perm	12	170-815	-43.0123471	171.7441943	N	6.0	9.2	37.5	21.2-6.1
(f) Beehive 2	Y	Semi-perm	12	90-880	-43.065552	171.796174	N	6.1	10.0	64.6	20.8-7.1
(g) Gooseberry	Y	Semi-perm	12	230-3500	-43.064116	171.867242	N	6.1	9.3	35.7	22.1-4.4
(h) Beehive 1	Y	Temporary	11	0-360	-43.066003	171.794590	N	6.0	10.7	37.2	18.5-6.8
(i) Kettle tarn	Y	Temporary	7	0-2500	-43.054204	171.785274	N	5.7	9.9	20.9	20.4-8.4
(j) Railway	Y	Temporary	6	0-4100	-43.038300	171.757078	N	6.4	9.7	83.2	13.2-6.4
(k) Rhemus	Y	Temporary	5	0-2300	-43.044432	171.758366	N	6.1	11.9	37.4	20.5-6.8
Vagabonds Inn	N	Permanent	-	16750	-43.138849	171.857028	N	-	-	-	-
Little vagabonds	N	Semi-perm	-	3325	-43.141209	171.863594	N	-	-	-	-
Mystery pond	N	Permanent	-	1475	-42.9979754	171.8020181	N	-	-	-	-
Lyndon tarn	N	Permanent	-	6800	-43.3249793	171.6851392	Y	-	-	-	-
Drying Lyndon	N	Temporary	-	0-120000	-43.2888683	171.7069656	?	-	-	-	-
Waimakariri flat	N	Semi-perm	-	240	-43.0084268	171.7376193	N	-	-	-	-
Flock hill willows	N	Semi-perm	-	2270	-43.130117	171.771601	?	-	-	-	-
Lake Sarah	N	Permanent	-	220000	-43.0486111	171.7766667	Y	-	-	-	-
Hawden marsh	N	Permanent	-	11630	-43.099670	171.844904	Y	-	-	-	-

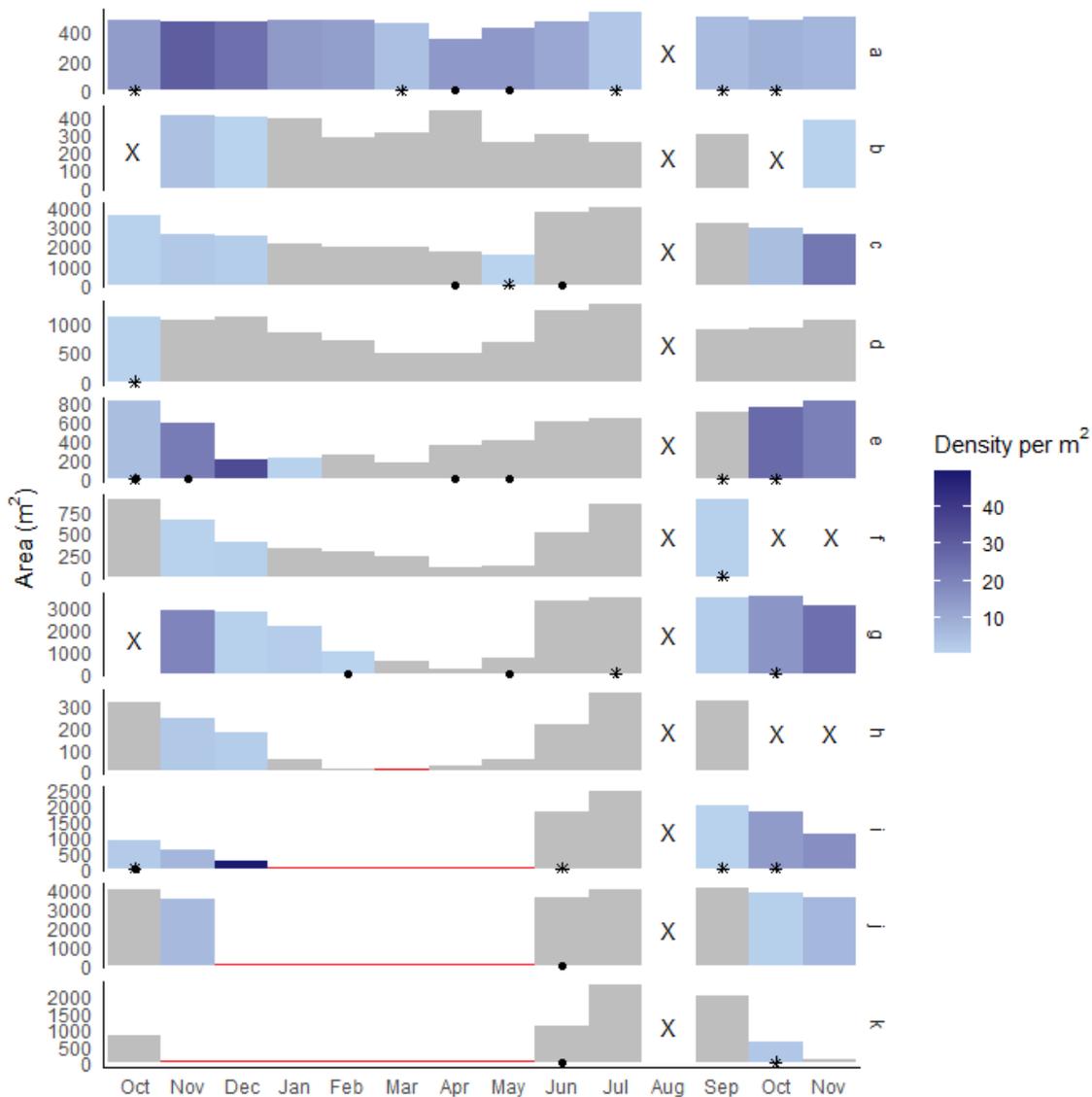


Figure 2.1. Changes in density per m² of *L. ewingii* tadpoles in relation to surface area of 11 different ponds ranging from most to least permanent (top to bottom, a-k; Table 2.1) between October 2020 and November 2021. Darker blue indicates higher larval density of *L. ewingii*, whereas pale blue indicates lower larval density. Grey indicates ponds with water where tadpole presence was not detected, red lines indicate dried ponds, and crosses indicate periods where the pond was not sampled. Egg presence (stars) and calling (circles) were also recorded, as well as metamorphosing tadpoles.

others (Figure 2.1). However, for the most part a pond being filled did not necessarily result in spawning or new tadpoles, as evidenced by the lack of detected tadpoles in winter even in ponds that never dried (Figure 2.1).

Tadpole density fluctuated in ponds across the permanence gradient, in ponds with a hydroperiod as short as five months, to fully permanent ponds (Figure 2.1). Overall, over 2,500 tadpoles were captured and measured. Their densities fluctuated greatly, ranging from a single tadpole caught, to a maximum of approximately 60 tadpoles/m². They tended to be most common in ponds with a longer

hydroperiod but reached the greatest densities in drying ponds as surface area decreased (Figure 2.1, Table 2.1). While the highest densities were observed when ponds were drying down, not all tadpoles in these drying ponds were metamorphosing. A few small tadpoles were also observed right before ponds dried down entirely, so it was unlikely they metamorphosed in time to escape (Figure 2.1, Figure 2.2; ponds i and k). Moreover, although they could be dense, tadpoles often had a patchy distribution within the habitat. For example, in one pond I caught two tadpoles in one sweep, and 111 in the next. Thus, their densities range between and within ponds, but could reach very high numbers in both permanent and temporary ponds.

To investigate growth rates of tadpoles in relation to the time of year, I observed tadpole SVLs throughout the year. However, very fast growth made it apparent that the monthly sampling rate was not sufficient for detailed growth measurements or cohort analysis (Figure 2.2). Pond c (Figure 2.2) demonstrates the fast growth, because in the first October sample all tadpoles were small, and at the next sampling period in November there were tadpoles at the maximum SVL, so intermediate cohorts were missing (Figure 2.2). Additionally, because of continual spawning in the most permanent pond (Figure 2.1; pond a), it was difficult to separate out discrete cohorts (Figure 2.2). Many ponds had a variety of large and small tadpoles at different months (Figure 2.3d), except in October, where most ponds had small tadpoles only (Figure 2.2). Because of the small tadpole period, I could tease out a minimum growth rate – after a month, some tadpoles were large enough to metamorphose, meaning they can almost fully develop in four weeks (Figure 2.2; ponds a and c).

To investigate whether pond permanence affected the size tadpoles reached at metamorphosis (i.e. visible legs) I compared tadpole size across the pond permanence gradient. In ponds that were more permanent, tadpoles tended to reach a larger size before or at metamorphosis, compared to more temporary ponds where metamorphosing tadpoles tended to be smaller (Figure 2.4; Regression: $F_{(1,4)} = 10.09$, $p = 0.034$, Multiple $R^2 = 0.716$). Consistently, the largest tadpoles with legs were found in a permanent, shaded pond, whereas the smallest tadpoles with legs were found in a temporary pond (Figure 2.2; ponds a and i, respectively). However, I only observed metamorphosing tadpoles in six ponds across the entire sampling period. Due to the volume of tadpoles sampled and the low number observed undergoing metamorphosis, it is likely that many metamorphosed in the time between sampling and were missed. Therefore, they must be capable of rapid growth and metamorphosis, with tadpoles likely to be larger at metamorphosis in permanent ponds compared to temporary ponds.

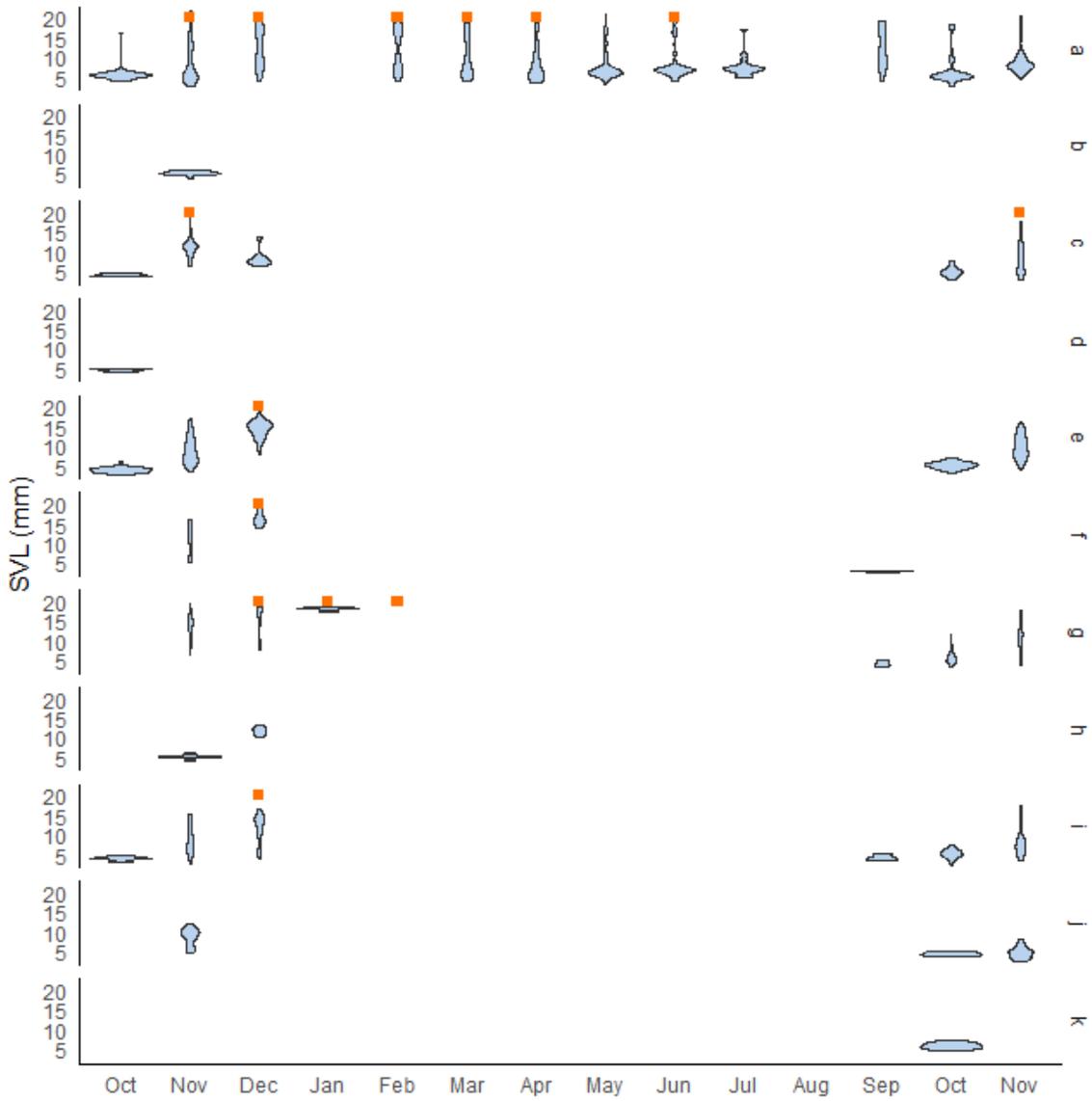


Figure 2.2. Distributions of tadpole snout-vent-lengths (SVL) across different sampling periods, at different sites ranging from most to least permanent (top to bottom, a-k; Table 2.1). Orange squares represent presence of metamorphosing tadpoles. SVL measurements were lost in January (apart from site g), and ponds could not be sampled in August due to lockdown, otherwise, absences indicate very few or no tadpoles.

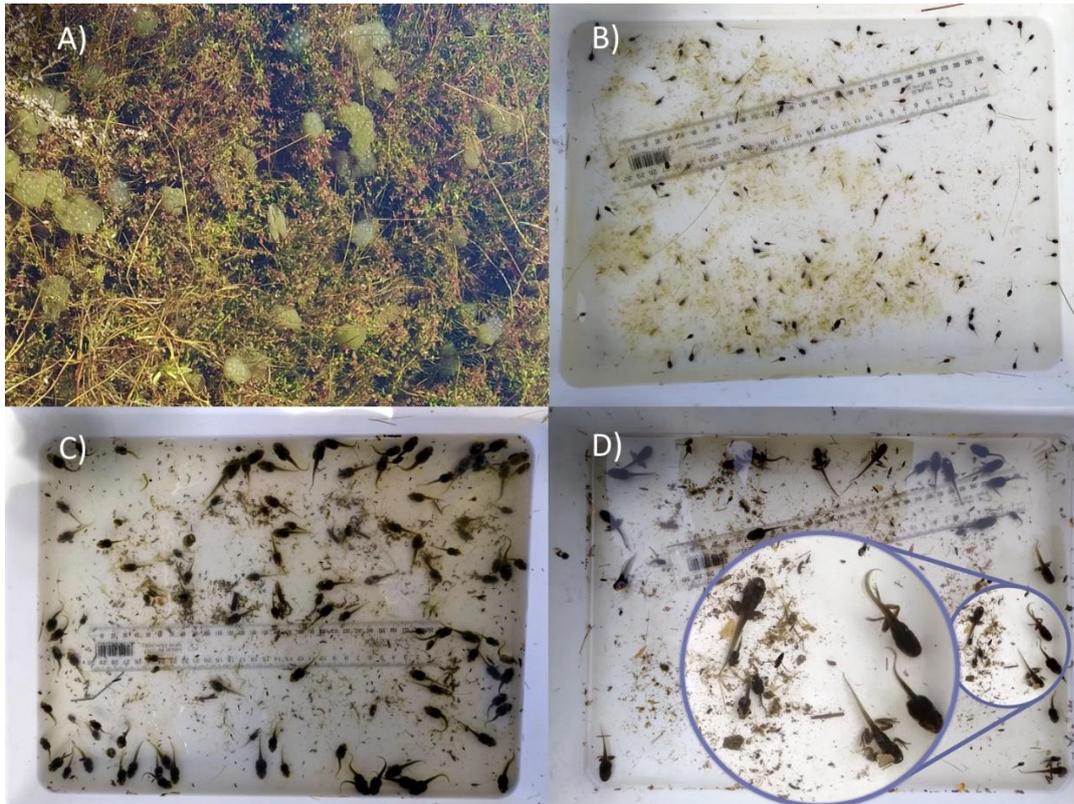


Figure 2.3. Images showing different life stages of *L. ewingii* at high densities against 30 cm ruler, showing a high density of egg masses (pale green masses) in a small area of one pond (A); a high density of small size class tadpoles, which have recently hatched (B); and a high density of older tadpoles at a larger size (C); as well as a mixture of tadpoles at different developmental stages, including metamorphosing tadpoles, shown by leg growth (D).

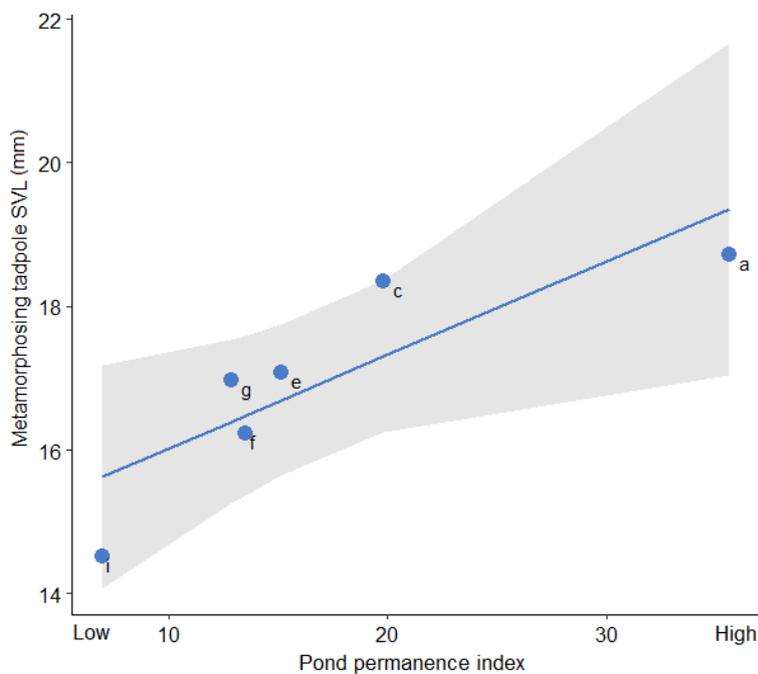


Figure 2.4. Modelled slope of relationship between average snout-vent-length (SVL) of metamorphosing tadpoles across pond permanence gradient, pond permanence being proportion months filled divided by proportion of surface area lost over twelve months for each pond, with modelled 95% confidence interval in grey. Letters refer to specific pond sites from Table 2.1.

DISCUSSION

My objective was to elucidate pond type preferences, densities within ponds, breeding seasonality, life history, and developmental responses to different levels of pond permanence of larval Australian brown tree frog *L. ewingii* in New Zealand, because most ecological research has taken place in their native range (Lauck et al., 2005b). Their breeding was continuous throughout the year, and only partially influenced by season shown by a peak in egg and larvae appearance in spring. Sexually mature frogs were present at many ponds throughout the year, evidenced by frog calls, so environmental conditions rather than adult dispersal must have limited breeding. The ponds the tadpoles occupied varied in many parameters such as hydroperiod, shade, and land use, so larval *L. ewingii* must be opportunistic, tolerant and flexible. In these ponds they were temporarily excluded by fish and by pond drying, so in a permanent fishless pond they were capable of persisting and continually spawning year-round. Therefore, their continual spawning and tolerance of multiple pond types explains their wide spread thus far, and implies their spread will continue as long as there are ponds available.

Despite their tolerance of multiple pond types, not all ponds were available to *L. ewingii*: fish presence was likely to be one of the strongest controls on their distribution. In almost all sampling instances, fish and tadpoles never co-occurred, which implies exclusion. There was one exception, which is likely explained by opportunistic spawning of *L. ewingii*. Small tadpoles appeared in a pond inhabited by small upland bullies (*G. breviceps*), but were not detected again, suggesting the fish prevented metamorphosis and any further colonization. While some frogs are selective about avoiding fish presence for spawning (Binckley & Resetarits Jr, 2003; Pollard et al., 2017), in this case it is less likely because spawning occurred in a pond with fish. If *L. ewingii* are capable of fish discrimination, perhaps they are less able to recognise these novel predators, as is often the case with interactions between tadpoles and unfamiliar predators (Gomez-Mestre & Díaz-Paniagua, 2011; Polo-Cavia et al., 2010; Romagnoli, Ficetola, & Manenti, 2020). Thus, they may be excluded through predation by fish, to which *L. ewingii* has little defence (van Tol, 2001). Regardless of the mechanism, fish appear to largely exclude *L. ewingii* tadpoles from waterbodies, so they are unlikely to colonise large permanent lakes. Permanent waterbodies like lakes can support top predators such as fish, which control community composition through predation, leaving species that can better tolerate drying stresses to colonise ephemeral ponds, as predicted in the predator-permanence model (Wellborn et al., 1996). However, despite tadpole exclusion by fish presence, there are many fishless waterbodies in the landscape, especially temporary ponds, able to be colonised by this exotic frog.

In the absence of fish, the tadpoles reach high densities in ponds across the permanence gradient, demonstrating their tolerance of different conditions. Notably high densities were associated with two factors. Firstly, ponds drying down increased in tadpole density relative to pond size. A portion of the many tadpoles in drying ponds may not have survived because young tadpoles far from metamorphosis were observed the month prior to drying. However, the majority of tadpoles were large or beginning metamorphosis, so many would successfully complete metamorphosis. The second factor, spawning, resulted in high larval densities appearing quickly, showing that breeding of *L. ewingii* could lead to frequent 'boom and bust' cycles of tadpole presence for pond ecosystems, especially as breeding was infrequent for most ponds. At 'boom' densities, it is likely that larval *L. ewingii* had the highest animal biomass in the ponds, so they likely impact ecosystem processes. This is asserted in Chapter Three. These high densities are unlikely to cause intraspecific competition strong enough to limit the number reaching metamorphosis, because Gill (1978) found that 40 *L. ewingii* froglets emerged per m of pond periphery, which is similar to the highest densities of larvae I recorded. Moreover, the 'bust' part of this cycle is caused by tadpole exodus rather than population collapse, so frog populations can continue to grow. Additionally, the speed at which tadpole densities increase has implications for invasiveness: a small number of breeding adults could produce a large number of offspring in a short period of time, as is the case with invasive bullfrogs (Urbina et al., 2020). Therefore, even if populations decline, they may spring back quickly. Alternately, high survivorship could lead to large numbers of adult frogs, which can have further ecological impacts (Beard, Price, & Pitt, 2009). Like many amphibian species that have become invasive (Allen et al., 2017; Cabrera-Guzmán et al., 2013; Rebelo et al., 2010), the large investment in offspring likely contributes to *L. ewingii*'s success in New Zealand thus far, and shows that they are not merely tolerating different pond conditions, but thriving in them.

Litoria ewingii may be able to succeed in a range pond hydroperiods at such high densities due to phenotypic plasticity. Increased temperatures typical of drying ponds might increase food availability and result in larger tadpoles, but the reverse was true; metamorphosing tadpoles were largest in permanent ponds. This could be explained by the findings of Lauck et al. (2005b), where food availability did not limit growth for ephemeral nor permanent ponds for *L. ewingii*, perhaps because their generalist diet increases the amount of available food (Chapter Three; Cousineau, 1990). If food availability is not controlling size at metamorphosis, it must be rate of development. Because I observed smaller sizes at metamorphosis in temporary ponds, *L. ewingii* larvae must detect pond drying and increase their rate of development to escape, like many frog species (Altwegg & Reyer, 2003; Székely et al., 2017). While this plasticity likely enables higher survivorship, it involves a trade-off with fitness later in life, because they will emerge as smaller frogs and have a reproductive

disadvantage (Altwegg & Reyer, 2003; Pechenik, 2006). This life history plasticity is an adaptation to survive and exploit temporary ponds, found in many frogs (Lauck et al., 2005a; Peterson et al., 1992). Being able to exploit temporary ponds is advantageous for frog larvae, because without fish, predation on tadpoles is generally less severe (Pollard et al., 2017). Moreover, as drying stress increases in ponds (i.e. they are less permanent), larger predators may be excluded (Wellborn et al., 1996), so the ability to avoid drying stress allows breeding in temporary pond habitats while avoiding heavy predatory stress. Thus, although *L. ewingii* spawning in temporary ponds may result in less fit adult populations, they are able to survive in a range of ponds along the pond permanence gradient, increasing potential spawning habitat.

Sampling techniques

To record abundances of these tadpoles in different ponds, using an appropriate technique was important, but in New Zealand there are no native aquatic frogs, so there was no precedent for this. The sampling technique used was mostly effective, but many factors can affect whether tadpoles will be detected. Firstly, the temporal variation shown by Figure 2.2 indicates that to detect *L. ewingii* presence in a particular waterbody, searching in spring would maximise chances of survey efforts coinciding with sporadic tadpole appearance. In the short term, tadpole behaviour could bias results, because their swimming ability and microhabitat use may change as they grow. Smaller tadpoles are poorer swimmers, so are easier to catch, but were often observed in very shallow reaches where sweeping can be difficult. Many small tadpoles that were caught died. Larger stages can be very strong swimmers and sometimes dart down into the substrate when disturbed. Therefore, to avoid bias between tadpoles sizes, sweeps with the D-net must be fast to create a strong current, and must be taken from a range of depths. Thick algae and mud can occlude the net and nullify any current, so avoiding sweeping through sediment is helpful. Low light and low temperature could reduce tadpole activity and result in missed detection. Tadpoles likely rest nearer to the sediment when it is dark or especially cold, so sampling during the day is important. When sampling for eggs I noticed no trend with microhabitat: they were often found attached to vegetation at the surface, but sometimes as deep as 1 m underwater, and varied in distance from the pond margin. Their attachment to vegetation was not strong, so they were often caught in sweeps. The use of Gee minnow traps may be more effective when tadpoles are sparse, as demonstrated by Lauck et al. (2005a), but requires setting up beforehand, so sweeping may be preferable when time is limited. Overall, this general sweeping technique enabled capture of all developmental stages of *L. ewingii* tadpoles in a range of ponds, and aided the uncovering of their life history in New Zealand as documented above.

Conclusions and Implications

Occurrence of *L. ewingii* in a large variety of pond types in Canterbury high country reflects that they are capable of breeding in many different conditions. However, conditions will change under climate change, which may alter future distributions. Increasing temperatures could allow their range to expand to higher altitudes. This may already be happening since *L. ewingii* tadpoles were found by Barclay (2020) in high alpine tarns (1500 m). Alternatively, altered rainfall and higher temperatures may increase pond drying frequency. In escaping drying, tadpoles may incur costs on adult fitness from a hastened larval period, which may further reduce the fitness of wild populations (Pechenik, 2006). However, any consequence on adult fitness is likely outweighed by the benefits of evading death, so their life history flexibility ultimately allows them greater success, even in the face of increased drying frequency. Additionally, consequences from altered filling cycles of temporary ponds may impact the native fauna before heavily restricting *L. ewingii*, because temporary pond communities in New Zealand are dominated by generalists which have some flexibility to drying (Galatowitsch & McIntosh, 2016b), but *L. ewingii* could be considered a very flexible temporary pond specialist. Furthermore, fast life history traits tend to benefit invasive amphibians disproportionately to other taxa (Allen et al., 2017). Thus, life history flexibility may enable *L. ewingii* to persist under climate change and possibly outcompete native fauna, especially when other advantageous traits are considered.

Through this research I have shown that *L. ewingii* has a suite of life history traits potentially associated with invasiveness: flexibility and tolerance to conditions, habitat generalisation, and rapid reproduction and fast development. While ‘invasive’ traits alone cannot predict species that will threaten ecosystems (Kolar & Lodge, 2001), they indicate invasive potential (Allen et al., 2017; Fournier et al., 2019; Kolar & Lodge, 2001). Other amphibians which demonstrate similar traits have become invasive, such as the cane toad (*Rhinella marina*) and African clawed frogs (*Xenopus laevis*), which breed in a wide range of aquatic habitats (Evans, Yaber, & Hero, 1996; Moreira et al., 2017). This evidence of multiple ‘invasive’ traits in naturalised populations of *L. ewingii* in New Zealand, in conjunction with their known wide spread (Shaw et al., 2014), continued propagule pressure through human releases (Bishop, 2008), and potentially naïve biodiversity, highlight the potential for *L. ewingii* invasion and ecological harm. Thus, managers of aquatic habitats should be more concerned about *L. ewingii* spread, and to explore this issue further I investigated ecological impacts of different densities of *L. ewingii* tadpoles (Chapter Three).

Chapter Three: Invasion of a non-native anuran likely disrupts pond ecosystems.

INTRODUCTION

Worldwide, invasions present ecological problems that disrupt trophic interactions and lead to species loss and altered ecosystem processes (Jackson et al., 2017; Pagnucco et al., 2016). In particular, trophic interactions can be disrupted if a new source of top-down control is introduced (Azevedo-Santos et al., 2017; Woodward & Hildrew, 2001), potentially leading to indirect cascading effects on other organisms (Pagnucco et al., 2016). Alternately, invasion by primary consumers can disrupt systems due to competition, exemplified by *Potamopyrgus antipodarum* invasions around the world (Alonso & Castro-Díez, 2008; Zaranko, Farara, & Thompson, 1997), and by rerouting energy to local predators and sustaining them at unnaturally high levels (Inger et al., 2010). In addition to direct and indirect effects via cascading interactions, invasive species can alter nutrient transfer between ecosystems. For example, rat predation on seabird colonies in northern New Zealand reduced nutrient subsidies from the ocean, decreasing local invertebrate abundance (Townes et al., 2009). Generalists such as rats can have multiple impacts on terrestrial ecosystems simply by being polyphagous; rat invasions have negatively affected primary producers, primary and secondary consumers (Bell, Bell, & Merton, 2016). The larvae of the brown tree frog, *Litoria ewingii*, introduced to New Zealand, are habitat generalists in aquatic systems (Lauck et al., 2005a; Thomson, 1922), and occur at high densities in ponds across all but the northern regions of New Zealand (Chapter Two; Shaw et al., 2014). *Litoria ewingii* diets are not well known; they may eat vegetation, algae, detritus, and invertebrates (Anstis, 2017; Cousineau, 1990; Cree, 1984). Because of their high densities and likely generalist diet, they could disrupt New Zealand ponds by altering pond community structure and ecosystem processes via all three mechanisms above.

Ecosystems and the organisms that they are made up of may also be under increasing stress due to climate change, and invasion could exacerbate this (Jackson et al., 2017; Woodward et al., 2010). Freshwaters are predicted to be especially vulnerable to climate warming, because they rely on rainfall that will be increasingly subjected to extreme events (i.e. floods and droughts; Woodward et al., 2010). For example, increased temperatures can decrease pH and oxygen in water, as well as increasing available phosphorous, all of which could be stressful to pond inhabitants (McKee et al., 2003). These potential climate change-related physical alterations can affect communities because more sensitive species may be affected disproportionately, meaning systems may be more susceptible to invasion.

Climate change and invasion may compound to doubly threaten pond biodiversity. Vulnerability to invasion of a fast-growing, high-density tadpole is expected because New Zealand organisms likely have not coevolved with anything similar, because the only native anurans (frogs) are largely terrestrial (Bell, 2010). Furthermore, New Zealand faces projections of warming, with freshwater ecosystems especially projected to be immediately impacted, because warming can drive algal blooming, and ranges of thermal-limited animals may decrease due to warmer waters (Lundquist et al., 2011). Pond systems can naturally dry periodically depending on rainfall, but climate change may increase drying frequency, which may impact some communities disproportionately due to adaptations for permanent (i.e. non-drying) ponds being unsuitable. Therefore, New Zealand pond ecosystems already under increasing stress from climate change may be especially vulnerable to a novel invader like *L. ewingii*.

Litoria ewingii occur across the pond-permanence gradient and are habitat generalists (Chapter Two; Lauck et al., 2005a), and could have different impacts depending on pond drying frequency. Permanently filled ponds can host fish, which generally prey upon tadpoles to the point of exclusion (Heyer, 1976; Petranka, Kats, & Sih, 1987; van Tol, 2001). Therefore, it is likely that *L. ewingii* breed primarily in fishless ponds, meaning the majority of potential larval impacts would be on temporary pond communities. Drying pond water volume can fluctuate seasonally (Chapter Two), but they also fluctuate in temperature daily, because a decreasing volume of water will heat and cool more readily. Thus, temporary pond communities tend to be more affected by abiotic stressors, have less diversity in predators, yet have strong top-down pressure, making them inherently stressful environments (Galatowitsch & McIntosh, 2016b; Greig, Wissinger, & McIntosh, 2013). When a pond is drying down, organisms will also be under increased competitive pressure due to reduction in habitat size (Petranka & Kennedy, 1999; White, McHugh, & McIntosh, 2016). Effects on temporary ponds are also somewhat cyclical – after drying, the freshwater community will disappear, and after filling they rely on colonization from nearby waterbodies or dormant life history stages. Because *L. ewingii* have a flexible life history, they are able to occupy temporary ponds by decreasing time to metamorphosis to escape drying (Chapter Two; Lauck et al., 2005a). Furthermore, full drying is unlikely to eliminate *L. ewingii* from ponds, because the frogs can quickly recolonise (Chapter Two; Gill 1978). The increased abiotic stress in conjunction with heavy predatory and competitive pressure means drying pond systems may be particularly vulnerable to a high-density addition of an animal not naturally represented in that system.

Litoria ewingii, nor any other frog species with aquatic tadpoles, were historically present in New Zealand, so where do the larvae fit in pond trophic interactions after being present for approximately 150 years (Thomson, 1922)? Their small larval size (snout-vent-length, SVL, ranging

from 1 – 20 mm, Anstis, 2017), means early stages could be consumed by predatory invertebrates raising potential for control by top-down interactions from native predators. However, later stage *L. ewingii* tadpoles could plausibly consume small invertebrates due to their increased gape size. Although they may be small, elsewhere high densities of tadpoles can dominate waterbody biomass, with large structural effects on pond ecosystems (Dickman, 1968; Whiles et al., 2013). Additionally, temporary ponds tend to lack larger vertebrate predators (Greig et al., 2013), and some species have less pronounced responses to predators (Wissinger et al., 2006), so *L. ewingii* tadpoles could be an influential predator in these systems. Alternatively, high larval densities could affect bottom-up energy transfer by reducing primary production through grazing (Dickman, 1968). Moreover, through excretion and respiration, they could indirectly influence ecosystem processes. Many native pond species feed on algae, and so could potentially be affected by competition with tadpoles (Berg, 1995). Therefore, high larval densities and their generalist diet likely mean *L. ewingii* potentially could have a wide range of direct and indirect effects on different pond ecosystems.

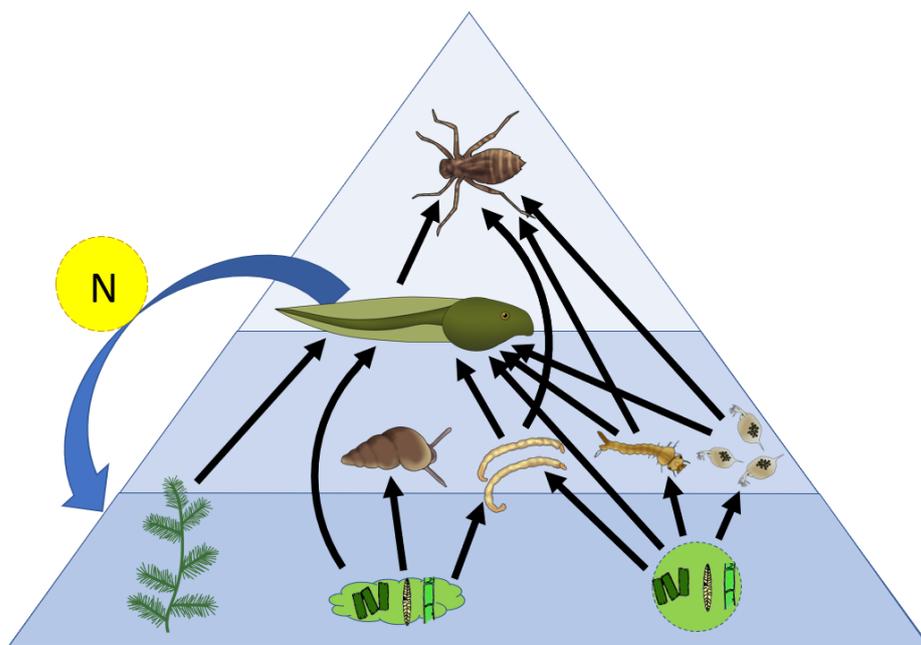


Figure 3.1. Hypothetical trophic placement of *L. ewingii* in a simplified temporary pond trophic web pyramid, with black arrows indicating direct energy flow to higher trophic levels, and the blue arrow representing nutrient return (N, nitrogen) to lower levels. Primary producers (left to right) are macrophytes, benthic algae, and phytoplankton. Invertebrates pictured (clock-wise, from top): a dragonfly nymph representing larger predatory invertebrates; and zooplankton, Culicidae larva, Chironomidae larvae, and snails all representing small invertebrates.

Overall, I hypothesised that a non-native generalist like *L. ewingii* added to a vulnerable ecosystem like New Zealand temporary ponds lacking that guild of organisms (i.e. native tadpoles) are likely to have large ecosystem-wide effects. Specifically, I predicted that *L. ewingii* tadpoles would enter the

middle of the food web as a primary and secondary consumer, by consuming from a range of trophic levels and being consumed in turn by local predatory invertebrates (Figure 3.1). I expected they would consume primary producers such as macrophytes, algae, phytoplankton, and primary consumers such as small pond invertebrates. Additionally, I predicted that their presence may affect physico-chemical aspects of pond systems, such as dissolved oxygen levels and nitrogen processing. Since *L. ewingii* has a highly plastic life history and behaviour, I also expected they may increase growth rate and therefore rate of consumption in more temporary compared to permanent ponds (Lauck et al., 2005b), so the above effects may be exacerbated in drying compared to permanent ponds.

METHODS

General mesocosm setup

I tested these hypotheses using experiments conducted at the University of Canterbury's Cass field station, in the high country of Canterbury. Two mesocosm experiments were conducted first in January and February of 2020 and then in the same months of 2021; some methods used were identical but deviations in methodology are detailed below. Both experiments used the same 54 L plastic tanks as mesocosms. The (L 675 x W 410 x D 255 mm) tanks had holes drilled for drainage 10 cm from the top edge, sealed with 700 µm mesh, meaning they held approximately 45 L for the duration of the experiments. Tanks were refilled periodically to maintain consistent depths.

To replicate local temporary pond communities, each mesocosm was filled with D-net 'sweeps' from two different nearby lentic waterbodies; each waterbody contributed two discrete 'sweeps' to each mesocosm to minimise variability in invertebrates stocked. To ensure consistent predatory invertebrate assemblages and to reduce confounding predation effects, all large (>10 mm body length) predatory invertebrates were picked out of the sweeps before introduction. The remaining small predators did not present a predatory threat to tadpoles, and this consistent assemblage of small predators meant that the effects of large predators could be manipulated as described in Experiment 1 below.

To provide a potential food source and to measure tadpole grazing, each tank had two macrophyte fragments devoid of invertebrates added (*Myriophyllum* sp.). Each tank had two ceramic tiles placed on the floor, both to anchor macrophyte roots, and to act as a substrate for algal growth (and to facilitate its measurement). Natural pond systems have diverse assemblages of bacteria, fungi, and other microbes (Finlay et al., 1988; Nho et al., 2018), so to ensure these were present, approximately 0.5 L of pond mud and detritus was added to each mesocosm. To ensure there was

representation of invertebrate grazers, 4 *Physa* spp. snails approximately 8 mm in length were also added to each tank. Two plastic aquarium plants were also added to each tank, to provide more structure and refugia for tadpoles and other organisms.

Tadpoles were collected from a permanent fishless forested pond at the Arthur's Pass Wilderness Lodge with a D-net and were carefully transported via aerated bucket to the experiment site.

Tadpoles with visible legs were excluded to reduce likelihood of emergence during the experiment.

Predator by tadpole presence manipulation - Mesocosm Experiment 1

To investigate functional roles of tadpoles in pond ecosystems, and especially whether predatory invertebrates exerted any top-down influences, I conducted a two by two crossed factorial design mesocosm experiment crossing *L. ewingii* tadpole presence and predatory invertebrate presence. Twenty-eight tanks were laid out in a randomized block design; blocking was important to control for any potential shade effects of nearby trees. Tadpoles were collected as described above, their total length (TL) was measured with a fish measuring board, and they were sorted into three categories (tiny, ≤ 20 mm; small, >20 mm - < 38 mm; and medium, ≥ 38 mm). To allow the full range of possible size-based interactions, 5 tiny, 10 small, and 5 medium tadpoles were added to each tadpole-treatment tank (14 tanks), totalling 20 tadpoles per tank, or 0.44 larvae/L. This standard high density of 0.44 larvae/L falls within the range (0.25-0.65 larvae/L) of several tadpole mesocosm experiments with similar sized tanks (Kehr et al., 2014; Morgan & Buttemer, 1996), and within ranges of densities I observed in the field in New Zealand (Chapter Two).

Mesocosms were set up as described above for this experiment, and in addition 20 partially rotted willow leaves were collected from a nearby pond and added to each tank to investigate whether tadpoles aid in detrital breakdown. After the leaves, macrophytes, invertebrate community, and mud were added, and the invertebrates and algae were allowed to establish for two days before tadpoles were added.

To investigate potential native predatory invertebrate effects on tadpoles, a range of larger predatory invertebrates from ponds were added to half of the tanks (14 tanks). These consisted of two *Rhantus* beetle larvae, 4 *Austrolestes* damselfly nymphs, two *Procordulia* dragonfly nymphs, and one adult predatory Dytiscidae beetle, all large (i.e. ≥ 20 mm total length).

Experiment 1 ran for three weeks before it was taken down and all response variables were collected. Dissolved oxygen was measured in each tank using a YSI Ecosense ODO 200 probe after a week, in the afternoon. Algae was measured on each ceramic tile using a BenthosTorch, which can effectively estimate algal biomass by measuring chlorophyll a fluorescence in $\mu\text{g}/\text{cm}^2$ (Kahlert &

McKie, 2014). Different algal types can be detected by different fluorescences, so BenthosTorch measurements of green algae, cyanobacteria, and diatoms, were added together for a combined algae abundance measurement. Macrophytes and terrestrial leaves were counted out from each tank and frozen for transport to the laboratory. Macrophytes were later rinsed to remove external detritus, and 15-cm sections were taken from the top of the macrophyte fragment, to standardise variations in length. They were then dried at 50 °C for a minimum of 48 hours and weighed. Terrestrial leaves were later thawed, rinsed, dried at 50 °C for a minimum of 48 hours, and weighed. Tadpoles were caught, measured using a fish measuring board, euthanised in a fatal dose of fish anaesthetic MS-222 (500 mg/L) buffered with equal parts baking soda, and then preserved in 70% ethanol. The remaining tank contents were then poured through a 250 µm sieve, to drain the water but preserve coarse particulate organic matter and most invertebrates. The filtrate was then preserved in 70% ethanol.

Once samples were returned to the laboratory, one quarter subsamples of invertebrates were counted and identified to low taxonomic levels, usually genus or family, using Winterbourn, Gregson, and Dolphin (2006). Initially, easily visible invertebrates were picked, and then the remainder of the sample was picked out in a Bogorov tray under a stereo microscope at up to 40x magnification.

Shade by tadpole density manipulation - Mesocosm Experiment 2

To investigate how tadpole influences on pond ecosystems differed over a range of larval densities and between permanent and temporary ponds, I conducted a second mesocosm experiment with four tadpole densities crossed with shaded and open treatments. The purpose of the shading was to simulate permanent rather than temporary pond conditions, as shade would reduce temperature fluctuations typical of drying ponds. This time, 40 tanks were laid out, and half were covered with shade cloth suspended roughly half a meter above the tanks, attached to metal waratahs. Shaded treatments were grouped into ten blocks of four tanks each; one block consisted of one shade cloth suspended above four tanks, with treatments randomly allocated within a block of four tanks.



Figure 3.2. Layout of Experiment 2, showing the mesocosms. Green shade clothes were stretched across blocks of four for the shaded treatments, totalling half of the tanks.

The mesocosms were set up and inoculated as explained above, except half (randomly selected in shaded and unshaded) had a HOBO data logger installed on the tank bottom to measure temperature hourly across treatments. In addition to the two *Myriophyllum* spp. macrophytes added to each tank, two *Elodea* spp. macrophytes were added, to investigate if *L. ewingii* would graze on a range of macrophytes. The *Elodea* macrophytes were subjected to the same invertebrate removal as the *Myriophyllum*. Due to variations in lengths and bifurcation of macrophyte branches, we removed extra branches to leave one primary strand, and differences in lengths were roughly standardised per tank (i.e. longer macrophytes were matched with shorter macrophytes).

Tadpoles were collected as in Experiment 1 and sorted into three sizes, with average snout-vent-lengths (SVL) taken from 10 of each size, rather than the TL used in Experiment 1. These were 6.5 mm, 10.7 mm, and 16.3 mm, for tiny, small, and medium tadpoles, respectively. Different sized tadpoles were included in each density treatment (Table 3.1) to represent wild populations, and to ensure I examined the full range of potential effects on pond systems associated with different tadpole stages.

Table 3.1. Density treatments and the tadpole sizes they incorporated for the shade by density mesocosm experiment, with a conversion to larval density per L, with 45 L per tank.

Density treatments (total tadpoles)	Tadpole sizes			tadpoles/L
	tiny	small	medium	
0	0	0	0	0
6	1	2	3	0.13
12	3	4	5	0.27
18	4	7	7	0.40

The experiment was taken down after three weeks. Tadpoles were caught, transferred to a white tray and photographed for digital measurement of SVLs using Adobe Acrobat DC, with tadpole developmental stages assessed according to Gosner (1960). Tadpoles were euthanized as per Experiment 1.

All macrophytes were removed from tanks and frozen before later drying and weighing as in Experiment 1. Some *Elodea* spp. macrophytes were less than 15 cm in total, so instead, total dry weight was used. For *Myriophyllum* sp. macrophytes, 15 cm sections were cut, but to be consistent and comparable to the *Elodea* measurements, the weights of the 15 cm sections were added to the weights of the corresponding offcuts to produce total weight.

Specific conductivity and pH were measured using a YSI Pro1030 meter in the morning on the final day of the experiment before mesocosms were disturbed for takedown, and were consistent across all tanks (averages \pm SE; pH: 7.46, \pm 0.04; specific conductivity in μ S/cm: 44.20, \pm 4.66). Dissolved oxygen was measured across several afternoons during the experiment, as well as at the end using a YSI Ecosense ODO 200 probe.

To determine if suspended particulate matter visible in some tanks was organic, and by extension determine if tadpoles affected suspended phytoplankton, 400 mL of water was filtered (47 mm Whatman GFC Glass Microfiber Filter Papers) per tank, and these filters were frozen for later laboratory analysis. Chlorophyll was extracted from filters using procedures described in Biggs and Kilroy (2000), and measured with a UV-1800 Shimadzu UV Spectrophotometer. In addition, after chlorophyll extraction, filters were dried, weighed, ashed for four hours at 550°C and weighed again, to determine mass of the remaining matter on the filters and if it was organic.

Ammonia laboratory experiment

I suspected that nutrients excreted by tadpoles may have stimulated phytoplankton in Experiment 2, but had difficulty obtaining field nutrient measures from the mesocosms. Thus, to investigate ammonia excretion in a controlled environment, I conducted an experiment to measure ammonia excretion of individual tadpoles using a procedure used previously to measure excretion of NH₃-N in tadpoles (Ramamonjisoa et al., 2021). I chose to measure ammonia because it is the primary form of nitrogenous waste excreted by *L. ewingii* tadpoles before leg development (Cree, 1985). First, tadpoles were collected from Dragonfly Tarn and transported back to the laboratory and fed with boiled lettuce in a 10 L bucket 24h prior to the ammonia measurement, to ensure they had proteins to digest so any nitrogenous waste excretion could be measured. To measure ammonia excretion, tadpoles were removed from the bucket and placed in 100 mL of distilled water and left for one

hour. Their SVL was only measured with a fish measuring board after this procedure, as extra handling may have stressed the tadpoles and affected excretion rates. Ammonia-nitrogen $\text{NH}_3\text{-N}$ was determined using the salicylate method with a HACH DR 900 portable colorimeter, and readings were converted to ammonia NH_3 by multiplying with constant 1.21589 (HACH, 2020).

Data analyses

Data analysis was conducted using R, version 4.04 (R Core Team, 2021), and figures were produced using ggplot2 (Wickham, 2016). Modelled means and 95% confidence intervals in all instances were calculated using the emmeans package (Lenth, 2021). For all response variables, normality was confirmed using Q-Q plots and Shapiro-Wilk's tests, and treatment in cases of departure from normality is detailed below.

Experiment 1 – Predator x tadpole presence

The predator by tadpole mesocosm experiment had a two by two factorial design with seven randomized blocks, and so to test the relationship tadpole and predatory invertebrate presence on macrophyte mass, benthic algae, particulate organic matter, detrital breakdown of terrestrial leaves, and dissolved oxygen (Table 3.3) a two-way blocked ANOVA was used.

For analysing invertebrate and tadpole counts in relation to tadpole and predatory invertebrate treatments, I used quasipoisson analysis of deviance to correct for overdispersion in the data. This analysis was used for all invertebrate taxa individually such as for chironomids (full list Table 3.2). Final predatory invertebrate and tadpole counts were not over-dispersed, so Poisson analysis for dispersion was used for these to detect treatment-related changes.

To analyse whether the invertebrate community in the mesocosms shifted due to the treatments (tadpole and predatory invertebrate presence and absence), a Bray-Curtis non-metric multidimensional scaling (NMDS) ordination were performed using vegan (Oksanen et al., 2020). Bray-curtis was the most appropriate NMDS approach because of the variation of taxa present in different tanks, causing an abundance of zero values in the data. Even so, rare species (present in <10 mesocosms, and/or present in average densities of <3) were removed from analysis. To determine if community shifts were related to treatments, I conducted a PERMANOVA in vegan on Bray-Curtis distances.

Experiment 2 – Shade x tadpole density

The shade by tadpole density mesocosm experiment had two levels of shade, and four levels of density, a continuous variable in a split-plot design. The tanks allocated to ten plots of four tanks per shading treatment, and so to test the relationship between treatments and *Elodea*, *Myriophyllum*,

dissolved oxygen, suspended organic matter, and phytoplankton, I used analysis of covariance (ANCOVA). Normality was checked using Q-Q plots and Shapiro-Wilk's tests, and all but phytoplankton data were normal. One phytoplankton sample was missing due to having been spilled, and another outlier was removed due to a mistake in extraction procedure. To correct for the unbalanced data, means of respective treatments were used to replace the missing data. Although replacing missing values with means increases bias towards a Type I error, both of these data points were samples from the shaded treatment, which when tested showed no significant influence on phytoplankton, meaning no Type I error was made (Table 3.3, Figure 3.9).

Ammonia experiment

To investigate whether tadpoles excreted ammonia and if this changed based on body size, I used a linear regression to test the relationship between ammonia and tadpole SVL, with individual tadpoles as replicates and SVL as predictor.

RESULTS

In Experiment 1, the predatory invertebrates crossed by tadpole presence and absence manipulation, mesocosms provided sufficient structure and resources for inhabitants to survive, including tadpoles (average \pm SE; 18.21 ± 0.57 remaining after 3 weeks from 20), over the duration of the experiment. This was also true in Experiment 2, reductions in tadpole numbers at the end of the experiment could be partially attributed to cannibalism; I observed this happening occasionally. There was visual evidence of some reduction in predatory invertebrates from damselfly exuviae and deceased *Rhantus* larvae, but these reductions were not affected by tadpole presence ($z=-0.665$, $p=0.506$). Plant and animal communities were established successfully – all plants were alive at the end of the experiment, and small invertebrate communities were diverse, with 24 different groups detected. Benthic algae grew on surfaces within the mesocosm, including both of the tiles and on the tank walls.

In Experiment 2, the shade by tadpole density experiment, shading was also effective. At the warmest part of the day, 14:00, average temperatures ($n = 4$ days) were 17.98 °C (± 0.20 SE) and 23.23 °C (± 0.15 SE), for the shaded and open tanks, respectively. At the coolest time, 02:00, average temperature still differed but only by approximately one degree (11.79 °C ± 0.11 SE, for shaded; 12.72 °C ± 0.11 SE, for open). On the warmest hour of the experiment, the average temperatures of the shaded tanks and open tanks were 27.33 °C and 34.36 °C, respectively.

Experiment 1 - Predatory invertebrate influence on tadpoles

To investigate if predatory invertebrates were reducing the abundance of the tadpoles, tadpole counts were compared in tanks with and without predatory invertebrates. The final number of tadpoles did not differ depending on predatory invertebrate presence or absence ($z=-0.063$, $p=0.95$, Figure 3.3), and tadpoles did not affect the number of predatory invertebrates either ($z=-0.665$, $p=0.506$).

Tadpole influence on invertebrate community

Tadpoles significantly changed the invertebrate communities, shown by a shift towards the left on NMDS axis 1, and this relationship between tadpole presence and community composition was identified with PERMANOVA ($F_{1,24} = 4.33$, $p < 0.01$; Figure 3.4). Predators did not affect community composition, nor was there an interaction effect. Abundances of other invertebrates were not totally standardised at the start of the experiment by counting, so some variation in communities was expected, which could explain some community scatter among mesocosms (Figure 3.4). Nevertheless, there was a large community shift. The only single taxa significantly reduced by tadpoles were Chironomidae and Culicidae, with the latter also significantly reduced by predatory invertebrates (Table 3.2, Figure 3.5a & c). Chironomids were the most numerous taxa, often with several hundred counted per subsample, meaning tadpoles were reducing them by a large extent (Figure 3.5b). Culicidae were abundant in tadpole-absent tanks but were almost entirely eliminated when tadpoles were present (Figure 3.5a). There were no significant tadpole by predator interaction effects for any taxa, and zooplankton, *Anisops*, Dytiscidae, Oligochaeta, Nematoda, and Gastropoda were not affected by tadpoles or predators (Table 3.2, Figure 3.5b & d). Therefore, tadpoles caused declines in some small invertebrates, much more so than the effect of predatory invertebrates, and overall community structure was affected.

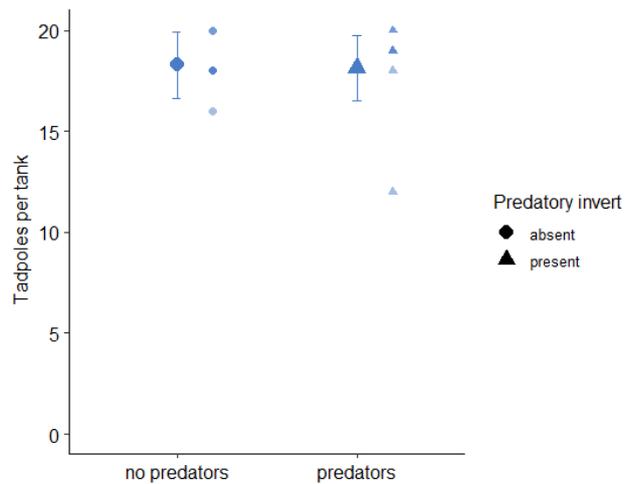


Figure 3.3. Modelled means and 95% confidence intervals of final tadpole count per tank at the end of the experiment in relation to presence (triangle) or absence (circle) of predatory invertebrates. Smaller points indicate raw data points, and darkness indicates overlapping points.

Table 3.2. Results of quasipoisson analysis of deviance of tadpole or predator effects (predictions) for different invertebrate taxa (responses). All interaction effects were not significant. Rare taxa were removed if present in <5 mesocosms (*Microvelia*, *Pycnocentroides*, Sphaeriidae, Acari).

Invertebrate per tank	Predictor	df	Deviance	Resid. Deviance	F	p
Chironomidae	Tadpole	1, 26	566.56	1864.1	9.436	<0.01
	Predator	1,25	3.51	1860.1	0.058	0.812
Culicidae	Tadpole	1,26	304.69	827.32	10.175	<0.01
	Predator	1,25	182.83	644.5	6.106	0.024
zooplankton *	Tadpole	1,26	4.152	293.26	0.542	0.471
	Predator	1,25	6.036	287.22	0.787	0.387
<i>Anisops</i>	Tadpole	1,26	0.091	92.064	0.029	0.867
	Predator	1,25	0.364	91.7	0.116	0.737
<i>Sigara</i>	Tadpole	1,26	7.063	86.131	2.203	0.155
	Predator	1,25	10.479	75.652	3.268	0.087
Dytiscidae †	Tadpole	1,26	0.964	29.058	1.129	0.302
	Predator	1,25	0.491	28.567	0.575	0.458
Oligochaeta	Tadpole	1,26	0.015	61.373	0.007	0.933
	Predator	1,25	2.618	58.756	1.253	0.278
Nematoda	Tadpole	1,26	0.311	52.989	0.294	0.594
	Predator	1,25	1.707	51.283	1.616	0.220
Gastropoda ‡	Tadpole	1,26	0.067	239.12	0.008	0.931
	Predator	1,25	0.024	239.09	0.003	0.959

* (Cladocera & Copepoda & Ostracoda) † (*Antiporus* & *Liodes* & small *Rhantus*)
‡ (*Potamopyrgus* & *Physa*)

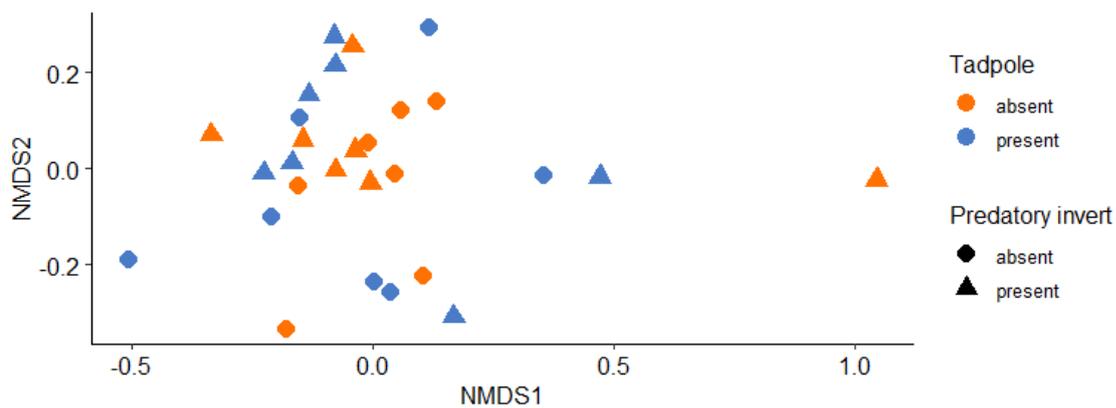


Figure 3.4. Non metric dimensional scaling plot describing invertebrate communities in relation to tadpole presence (blue) or absence (orange) and predator presence (triangle) or absence (circle), with points representing the invertebrate community of individual mesocosms.

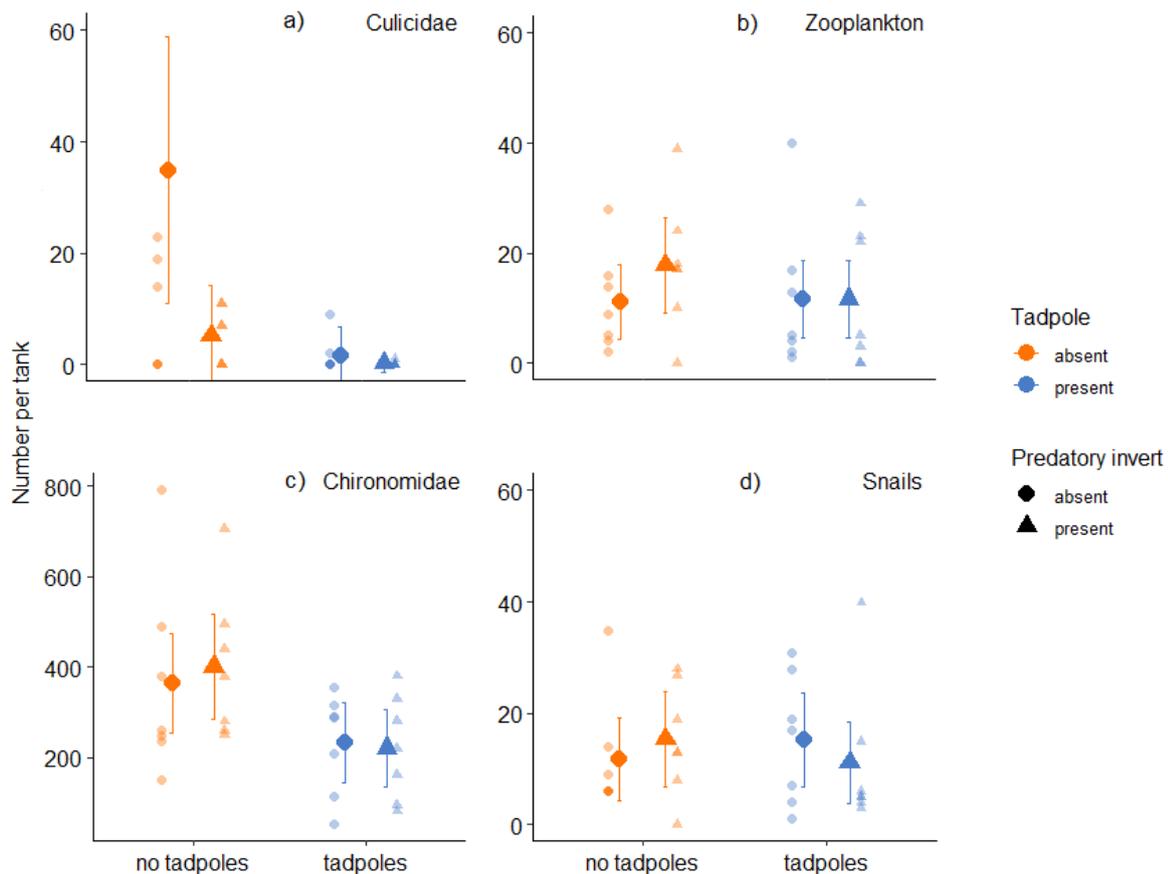


Figure 3.5. Modelled mean of abundance per tank of four key invertebrate taxa in relation to tadpole presence (blue) or absence (orange), and predatory invertebrate presence (triangle) or absence (circle). Error bars indicate 95% confidence intervals. Smaller points indicate raw data points of invertebrate counts for each mesocosm, and darker points indicate overlap. Culicidae, a; zooplankton, b; Chironomidae, c; and snails, d. For other invertebrate groups not shown refer to Table 1.

Tadpole influence on primary production

To investigate if tadpoles functioned as shredders, I compared macrophyte dry weight in tadpole present and absent treatments in Experiment 1, and also as tadpole density increased in shaded and open treatments in Experiment 2. Neither predators nor the predator by tadpole interaction affected plant weight in Experiment 1 (Figure 3.6b, Table 3.3). When *Myriophyllum* was the only macrophyte available in Experiment 1, tadpoles significantly reduced *Myriophyllum* dry plant weight (Figure 3.6b, Table 3.3). However, in Experiment 2 when both *Elodea* and *Myriophyllum* macrophytes were available, *Elodea* dry weight declined as tadpole density increased, yet tadpole density had no effect on *Myriophyllum* dry weight (Figure 3.6a & c, Table 3.3). Shade had no effect on macrophyte dry weight regardless of species, and there was no interaction effect (Figure 3.6a & c, Table 3.3). Therefore, tadpoles had a negative effect on macrophytes, reducing their biomass, regardless of shade and predators.

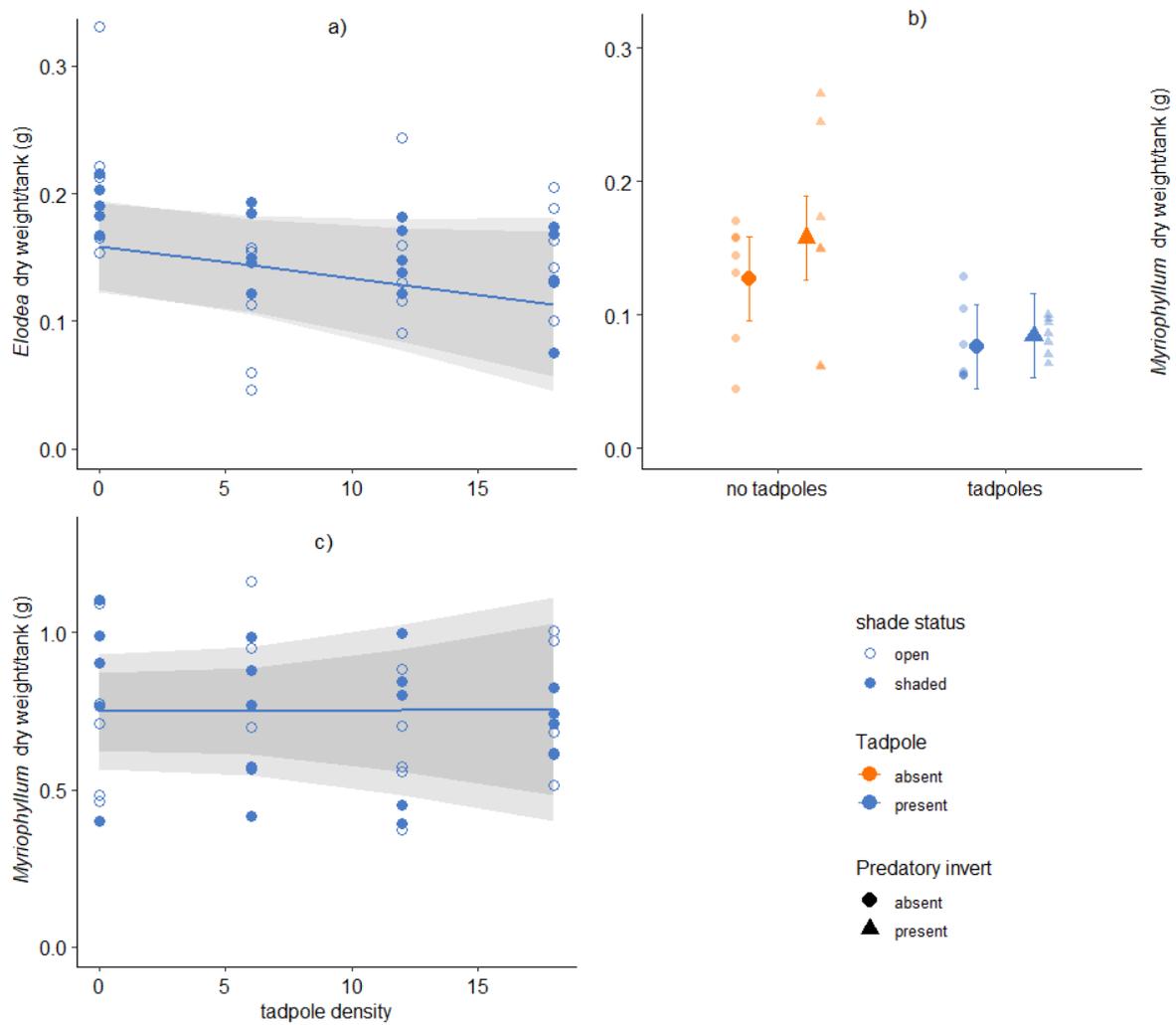


Figure 3.6. Dry weight of two macrophyte species in relation to tadpole density (a, c) or tadpole presence or absence (blue and orange, respectively, plot b), and predatory invertebrate presence (triangle) or absence (circle). *Myriophyllum* spp shown in (b,c), and *Elodea* spp shown in (a). All plots have 95% confidence intervals indicated by error bars or shaded area, plots (a,c) show modelled slope and plot (b) shows modelled means. Smaller points indicate raw data points, and darker point shading indicates overlap.

Table 3.3. Results of ANOVA and ANCOVA tests for each variable in relation to tadpole presence/absence, predator presence/absence, tadpole density, and shade, as well as interactions. Degrees of freedom, sums of squares, mean squares, F values, and p values for each response variable are shown.

Response	Experiment	Test	Predictor	Df	MS	F	p
Final tadpole per tank	2	Ancova	Tadpole density	1,18	638.4	394.92	< 0.01
			Shade	1,8	6.533	2.761	0.135
			Interaction	1,8	0.500	0.278	0.604
15 cm dry <i>Myriophyllum</i>	1	2x2 Anova	Tadpole density	1, 18	0.027	17.121	<0.01
			Predator	1,18	0.003	1.650	0.215
			Interaction	1,18	9 x 10 ⁻⁴	0.574	0.459
<i>Elodea</i> whole plant	2	Ancova	Tadpole density	1,28	0.011	5.725	0.024
			Shade	1,8	4 x 10 ⁻⁴	0.009	0.929
			Interaction	1,8	2.5 x 10 ⁻⁴	0.128	0.724
<i>Myriophyllum</i> whole plant	2	Ancova	Tadpole density	1,28	2 x 10 ⁻⁴	0.004	0.949
			Shade	1,8	0.038	0.427	0.532
			Interaction	1,8	6 x 10 ⁻⁴	0.011	0.916
Phytoplankton	2	Ancova	Tadpole density	1,26	3 x 10 ⁻⁴	6.132	0.02
			Shade	1,7	2.7 x 10 ⁻⁶	0.061	0.812
			Interaction	1,7	4.5 x 10 ⁻⁴	8.158	<0.01
Dissolved oxygen	1	2x2 Anova	Tadpole	1,18	1.472	16.825	<0.01
			Predator	1,18	0.002	0.022	0.884
			Interaction	1,18	0.035	0.4	0.535
Benthic algae	1	2x2 Anova	Tadpole	1,18	0.034	7.034	0.016
			Predator	1,18	8 x 10 ⁻⁵	0.014	0.901
			Interaction	1,18	0.005	0.849	0.369
Dissolved oxygen	2	Ancova	Tadpole	1,28	1.328	13.721	<0.01
			Shade	1,8	0.324	2.225	0.174
			Interaction	1,8	0.027	0.278	0.602
Pelagic organic mass	2	Ancova	Tadpole density	1,28	0.003	2.287	0.142
			Shade	1,8	8 x 10 ⁻⁴	0.695	0.429
			Interaction	1,8	0.003	2.134	0.155
Terrestrial leaves	1	2x2 Anova	Tadpole	1,18	0.011	2.648	0.121
			Predator	1,18	0.008	0.429	0.521
			Interaction	1,18	1.9 x 10 ⁻⁴	0.048	0.83

I investigated the effects on tadpoles on benthic algae in Experiment 1 and phytoplankton in Experiment 2. In Experiment 1 I compared the mass of algae between the tadpole and predator treatments. Tadpoles reduced algal mass, whereas predators and the tadpole by predator interaction did not have an effect on algae (Table 3.3, Figure 3.7). Grazing scars in tadpole tanks indicated they were directly consuming the benthic algae (Figure 3.8). In Experiment 2, to investigate if higher densities of tadpoles had a role in filter feeding, and if algal growth caused by unhindered sunlight negated this, I compared chlorophyll concentration as a measure of phytoplankton biomass across densities and in the shaded and open treatments. There was an interaction between shading and tadpole density, so as tadpole density increased, phytoplankton mass significantly increased, but only in the open treatment (Table 3.3, Figure 3.9). In the shaded treatment, there was no relationship between tadpole density and phytoplankton (Figure 3.9, Table 3.3). Thus, tadpoles increased phytoplankton in the water column in sunny conditions, but in shaded conditions they had no effect, and they decreased benthic algae independently of predator presence.

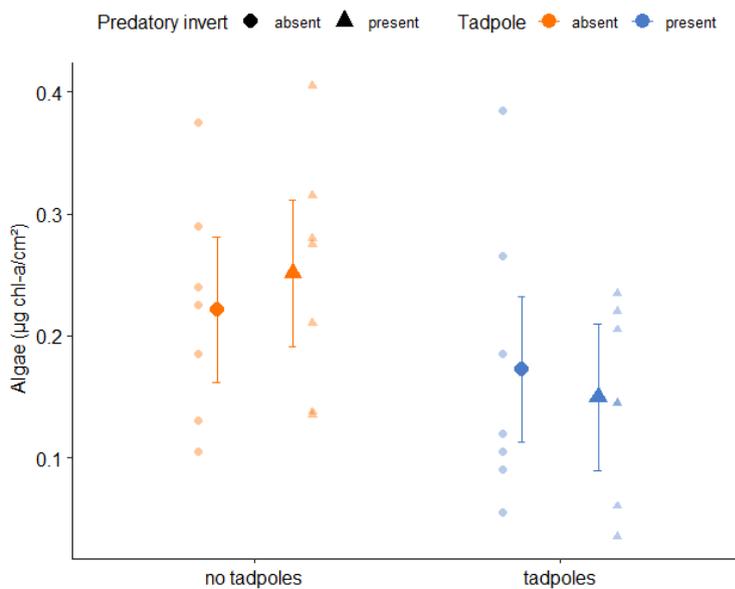


Figure 3.7. Modelled mean of chlorophyll a mass of benthic algae per tank in relation to tadpole presence (blue) or absence (orange), and predatory invertebrate presence (triangle) or absence (circle). Error bars indicate 95% confidence intervals. Smaller points indicate raw data points of invertebrate counts for each mesocosm, darker points indicate overlap.



Figure 3.8. Grazing scars in benthic algae growing on the side of a yellow mesocosm, very likely caused by *L. ewingii* tadpole grazing, with a tadpole swimming on the bottom right.

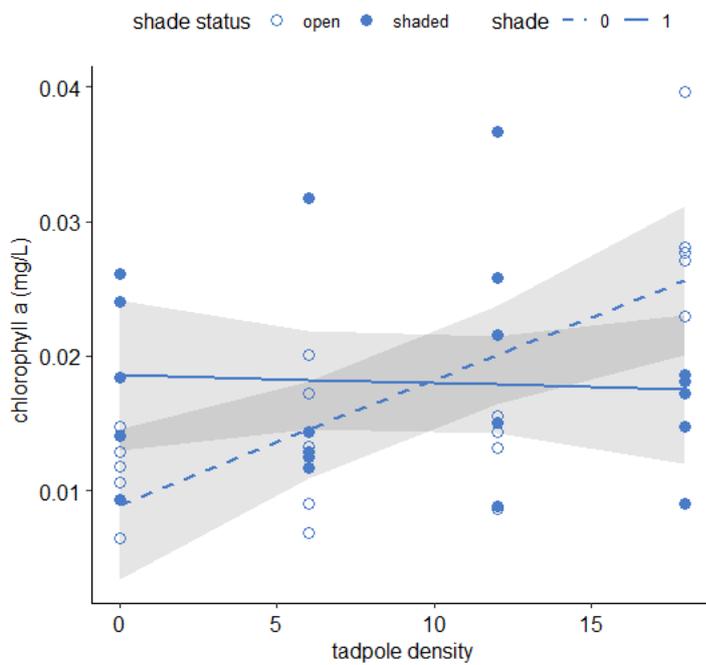


Figure 3.9. Modelled slope of phytoplankton-associated chlorophyll *a* concentration in water column in relation to tadpole density and shaded (coloured, solid line) or open (hollow, dashed line) treatments. Grey shaded areas indicate 95% confidence intervals around their corresponding lines.

To determine whether tadpoles influenced breakdown from terrestrial subsidies, I compared dry weight of terrestrial leaves added to tanks across the tadpole and predator treatments in Experiment 1. I also compared the ash free dry mass (AFDM) of pelagic organic mass as a measure of fine particulate organic matter (FPOM) across densities and in the shaded and open treatments in Experiment 2. However, neither higher tadpole density nor presence affected terrestrial leaf dry

mass or AFDM of pelagic organic mass (Table 3.3, Figure 3.10). Likewise, predators did not have an effect on terrestrial dry leaf mass, shade did not have an effect on pelagic AFDM, and there were no interaction effects in either experiment (Table 3.3, Figure 3.10).

Because tadpoles decrease primary producer mass but do not affect detrital breakdown, I investigated tadpole effects on dissolved oxygen (DO), and compared measurements made during both experiments. Neither predators nor the tadpole by predator interaction affected dissolved oxygen, however, tadpole presence significantly decreased oxygen concentration in Experiment 1 (Table 3.3, Figure 3.11a). Neither shade nor the tadpole by shade interaction in Experiment 2 affected DO, but as tadpole density increased, DO concentration decreased (Table 3.3, Figure 3.11b). Such reductions were associated with both tadpole presence and higher densities of tadpoles, therefore tadpoles must be reducing DO in the water, however the reductions were not severe, and levels were unlikely to produce hypoxic conditions.

Due to likely excretion from digestion I investigated ammonia excretion in a controlled environment, and measured ammonia excreted against the length of the tadpole. Larger tadpoles excreted more ammonia than smaller tadpoles, indicated by ANOVA ($F_{(1,10)} = 20.7$, $p < 0.01$; Figure 3.9), but all tadpole sizes (i.e. free swimming, smallest stage measured was stage 25; Gosner, 1960) excreted detectable and potentially important amounts of ammonia (Figure 3.12).

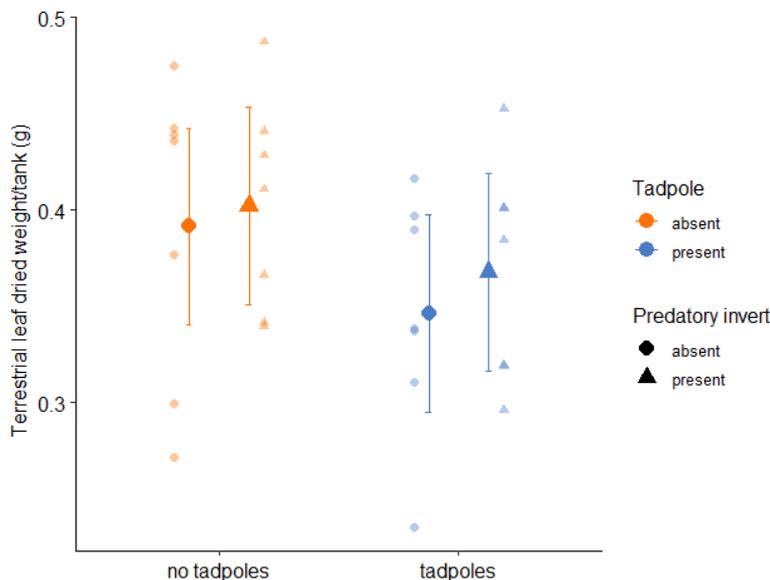


Figure 3.10. Modelled mean of dry leaf weight per tank in relation to tadpole presence (blue) or absence (orange), and predatory invertebrate presence (triangle) or absence (circle). Error bars indicate 95% confidence intervals. Smaller points indicate raw data points of invertebrate counts for each mesocosm, darker points indicate overlap.

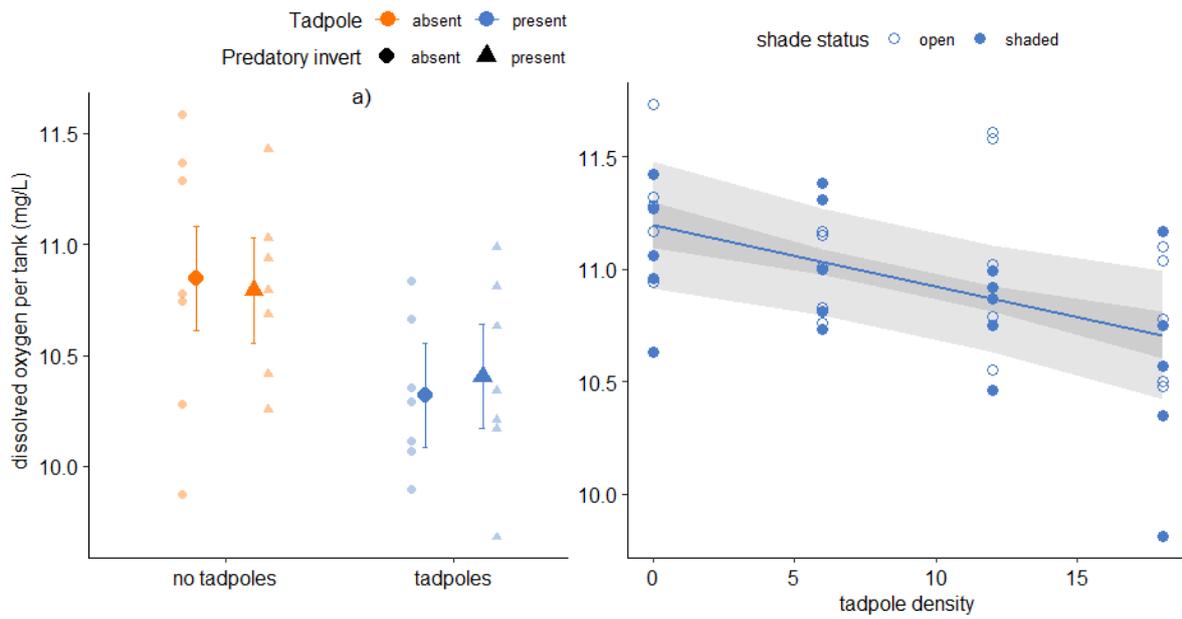


Figure 3.11. Modelled mean of dissolved oxygen per tank in relation to tadpole effect; plot a shows tadpole presence (blue) or absence (orange), and predatory invertebrate presence (triangle) or absence (circle); plot b shows tadpole density and shaded (coloured) or unshaded (open) mesocosms. Error bars indicate 95% confidence intervals. Smaller points indicate raw data points of invertebrate counts for each tank, darker points indicate overlap

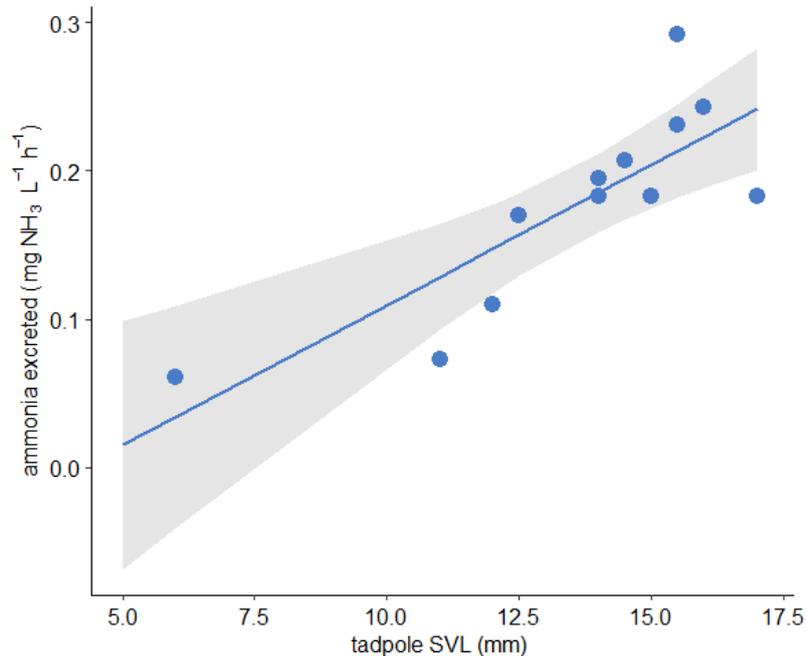


Figure 3.12. Modelled slope of ammonia excreted per tadpole (individual points) per Litre per hour according to snout-vent length (SVL) with modelled 95% confidence interval in grey.

DISCUSSION

Invasions of a particularly novel guild of organism to a system in which they were not previously present could cause large changes to trophic interactions or energy cycling within the ecosystem (Beard et al., 2009; Towns et al., 2009). Because *L. ewingii* larvae are prevalent across many potentially vulnerable ponds (Chapter Two), I investigated their ecosystem influences using mesocosm experiments, and found several tadpole-driven alterations that could result in wider ecosystem impacts. More specifically, *L. ewingii* reduced several components of pond systems: abundance of some small invertebrates, macrophyte biomass, benthic algae biomass, and dissolved oxygen levels. They also excreted ammonia and in unshaded mesocosms they increased phytoplankton growth. Therefore, *L. ewingii* tadpoles are likely to be influential generalist omnivores that may be disturbing the balance of temporary pond ecosystems, and therefore could be regarded as detrimental invaders, as I will detail below.

Consumptive effects

Litoria ewingii tadpoles may be altering pond communities directly, through predation on smaller aquatic invertebrates. There was a small shift in the overall invertebrate community composition due to tadpoles, and when investigated further this alteration was due mainly to a reduction in several small invertebrate taxa. Tadpoles primarily reduced Chironomidae and Culicidae, which are notably smaller than later stage tadpoles. Tadpole influences were assessed by comparing abundance in tadpole tanks to control tanks, so reductions could be attributed to things other than consumption (i.e. selective insect oviposition, egg predation, tadpole-driven alterations to physico-chemical aspects of the water), but direct consumption was the most likely cause due to the short time-span of the experiment. Moreover, the high trophic position of *L. ewingii* tadpoles in natural pond food-webs indicates predatory behaviour (Barclay, 2020). Very small invertebrates like zooplankton were not affected, perhaps due to limits in hunting behaviour of generalist tadpoles (Spielman & Sullivan, 1974). Snails were also not affected, likely due to their protective casing. For the two small invertebrate taxa most affected, tadpoles had a much stronger effect in my experiment than the predatory invertebrates that would usually represent their natural predators. That could have flow on effects on ecosystem function. The taxa most heavily affected, Diptera larvae, tend to play an important role in filter feeding (Berg, 1995; Kivuyo et al., 2014; Merritt, Olds, & Walker, 1992). Chironomids specifically are important and abundant primary consumers (Berg, 1995; Henriques-Oliveira, Nessimian, & Dorvillé, 2003), so large reductions in their abundance may cause an increase in phytoplankton and FPOM, potentially contributing to trophic cascades. However, observed reductions were not overly large and only affected a few taxa from the whole

community, so the threat posed to pond ecosystems will likely depend on the combination with *L. ewingii*'s other influences.

Primary production underpins a large portion of energy input for pond systems (Holgerson, Post, & Skelly, 2016), and so *L. ewingii* grazing on macrophytes could alter energy flow. In both experiments, tadpoles reduced macrophyte biomass. When *Myriophyllum* was the only macrophyte available, *L. ewingii* reduced it, but when two species were offered, only *Elodea* was significantly reduced. Therefore, *L. ewingii* tadpoles may prefer the exotic *Elodea* pond weed to *Myriophyllum* which has several native and introduced species in New Zealand. A reduction in plant mass could lead to flow-on effects such as decreases in dissolved oxygen, loss of cover for invertebrates, and reductions in surface area for algae and nitrogen processing bacteria to grow (Coci et al., 2010; Miller & Provenza, 2007). Tadpole grazing on macrophytes could also potentially increase competition for this resource, but this is unlikely because there are few native shredders in small lentic systems. An obvious exception is kekewai or freshwater crayfish (*Paranephrops spp*), but these are primarily found only in permanent waterbodies (Coffey & Clayton, 1988; Usio & Townsend, 2001). Therefore, tadpole effects on macrophytes may be especially novel in temporary pond systems, and *L. ewingii* may add a new functional role and be important in mobilising nutrients that otherwise would be largely unavailable.

Macrophyte densities vary, so tadpole grazing may be quite different in ponds with little aquatic macrophyte representation (Calero, Morellato, & Rodrigo, 2018). In cases where there is low macrophyte biomass, *L. ewingii* tadpoles may be able to switch their diet to algae. In tadpole treatments, phytoplankton actually increased, so tadpoles were not having a large consumptive effect on phytoplankton. However, benthic algae decreased due to tadpole presence, with grazing scars indicating tadpoles were directly consuming benthic algae (Figure 3.2). Because benthic algae was only measured in the experiment where shade was not a factor, I cannot draw conclusions related to shade. However, Whiles et al. (2010) found that tadpoles consume more algae in less shaded conditions due to increased algal availability, so this may be the case for *L. ewingii* as well. In warm ponds alone, benthic algae and biofilm are usually more abundant (Villanueva et al., 2011), so tadpole competition with invertebrate grazers is unlikely to be very influential to those invertebrates. However, when ponds dry down animal densities hugely increase, which likely outweighs the increased algae growth from warming, thus increasing competition for resources. As with other anuran larvae that occur at high densities and notably reduce algal biomass (Dickman, 1968; Ocock et al., 2019), *L. ewingii* appearing in high densities are likely to further increase these effects and potential for competition for these resources. Therefore, because algae is abundant,

especially in warm temporary ponds, *L. ewingii* is likely to be able to find food, and potentially large densities could be supported.

The larvae of many frog species have large effects on algae, macrophytes, and invertebrates (Altig, Whiles, & Taylor, 2007; Anderson & Kneitel, 2015; Dickman, 1968), and their success in establishing in novel systems often is related to being able to exploit a variety of resources (Measey, 2001; Sebastián et al., 2015). *Litoria ewingii* larvae are consuming biomass at multiple trophic levels of pond systems, potentially causing cumulative changes in energy pathways, and possibly with cascading effects. The reductions in macrophyte and algae biomass and reductions in abundance of some insects indicate tadpoles are acting as omnivorous generalists in New Zealand ponds. It is unlikely that the tadpoles are directly involved in decomposition pathways, however, because they did not affect terrestrial leaf breakdown. Nevertheless, even without aiding in decomposition, they are likely mobilising nutrients by grazing on macrophytes, and excreting nutrients in different forms. However, because these conclusions were reached by measuring reductions in the affected organisms rather than investigating the tadpoles themselves, future studies investigating fatty acid biomarkers or stable isotope analysis could be useful to clarify how *L. ewingii* fits in New Zealand pond food webs (Ocock et al., 2019; Whiles et al., 2010).

Litoria ewingii diet breadth will be advantageous as an invader (Cousineau, 1990), and could partially explain their success establishing widely in New Zealand (Chapter One). Additionally, invertebrates in temporary ponds may be at greater risk when high densities of *L. ewingii* tadpoles and pond drying coincide, because habitat shrinkage increases intraspecific competition, so opportunistic predation may be favoured as primary producers are depleted (Petranka & Kennedy, 1999). Understanding how invasive anurans acquire food is essential when predicting invasive consequences (Sebastián et al., 2015; Whiles et al., 2010), and in this case food resources seem to be abundant for *L. ewingii* because they consumed macrophytes, algae, and invertebrates. Thus, large densities of this invasive species may be able to be supported, exacerbating any non-consumptive effects.

Non-consumptive effects

Litoria ewingii tadpoles also impacted chemical-physical aspects of my experimental ponds, by decreasing oxygen and excreting ammonia, which perhaps facilitated phytoplankton growth. When *L. ewingii* tadpoles were present they likely altered nutrient pathways, which may have driven an increase in phytoplankton in temporary ponds. Bacteria on macrophytes can oxidise ammonia to nitrates or nitrites, meaning an increase in ammonia causes an increase in available nitrogen for producers, potentially leading to increased algal growth (Coci et al., 2010; Dai, Shang, & Qiu, 2012). The increase in phytoplankton in high density tadpole treatments could potentially be due to the

reduction in filter feeders, but this is unlikely because phytoplankton only increased in open treatments. It is more likely that ammonia excreted by a high density of tadpoles, in conjunction with high temperatures and sunlight in unshaded mesocosms is what drove the growth of phytoplankton. This increase in phytoplankton is consistent with previous studies of high-density tadpoles influences on trophic linkages (Altig et al., 2007). For example, larval plain grass frogs also facilitated phytoplankton in exposed temporary pools (Osborne & McLachlan, 1985). Phytoplankton growth and potential blooms may already be an issue for temporary ponds especially, due to their lack of flow, higher temperatures, and additional sunlight (Celewicz & Goldyn, 2021). Despite the increase in photosynthesizing phytoplankton, tadpoles also decreased dissolved oxygen, perhaps through a combination of direct respiration and their impact on other primary producers. This could also have implications for pond life: lower dissolved oxygen can be stressful to pond inhabitants, or cause die-offs if it reaches critical levels (McKee et al., 2003). Overall, the increase in phytoplankton and reduction of dissolved oxygen indicate that high densities of *L. ewingii* larvae in temporary ponds are altering these systems in through multiple trophic pathways.

Predation on tadpoles

If tadpoles are altering multiple elements of pond systems across trophic levels, it is important to understand whether their effects could be suppressed by top down control of native predatory invertebrates. I found that native predatory invertebrates did not reduce tadpole numbers, so in fishless ponds there may be no top-down control of larval *L. ewingii*. The tadpoles range in size, and at their earlier stages are similar sizes to typical prey, and yet predatory invertebrates weren't catching them in my experiment. However, the smallest sized tadpoles were not present during collection for the experiment, so these very small tadpoles may be more susceptible to predatory invertebrates. Additionally, the predatory invertebrates used in this experiment were similarly sized to the tadpoles in their later developmental stages, so tadpoles may simply become too big to catch (Formanowicz Jr, 1986). The lack of observed predation could be otherwise due to native pond invertebrates having no coevolution with tadpoles, because the larvae of the native frogs are not aquatic for the most part (Bell, 2010). Like other successful tadpole invasions, such as that of *Xenopus laevis*, *L. ewingii* may have general anti-predator behaviour which enable them to escape novel predators (Kruger et al., 2019). In their natural Australian habitats *L. ewingii* tadpoles are preyed upon by predatory insects, and have evolved behaviours such as utilising the water column to escape benthic predation (Peterson et al., 1992). Therefore, the little evidence for top-down control from native temporary-pond predators in this experiment may contribute to the widespread distribution and high densities of *L. ewingii* thus far (Chapter Two).

Conclusions & Implications

Overall, *L. ewingii* larvae likely have many direct and indirect effects on processes within pond systems, potentially altering multiple energy pathways within pond food webs. They are consumers of primary producers in the form of macrophytes and benthic algae, prey on several small insects to a higher degree than native predators, and yet predation on them by invertebrates is negligible. In total this means they are likely acting as an energy pathway out of ponds when they metamorphose and emerge. Because these tadpoles occur at high densities (Chapter Two), exodus to the terrestrial landscape may export a large quantity of nutrients and aquatic production, that go on to subsidise any terrestrial animals that prey on the frogs (van Tol, 2001). How invasive freshwater species alter the connectivity between terrestrial and aquatic systems is important to consider when assessing potential threats to ecosystems (Gergs et al., 2014), and is unknown currently about *L. ewingii*, so this research on trophic linkages is vital for determining the impact this anuran may have had in New Zealand.

Freshwater systems and especially temporary pond systems may be some of the most vulnerable systems to climate change (Celewicz & Gołdyn, 2021; Woodward et al., 2010). For temporary drying ponds which rely heavily on continual recolonization or sufficient moisture to holdover dormant species, climate irregularity and droughts can reduce diversity (Celewicz & Gołdyn, 2021). Moreover, many waterbodies are already becoming eutrophic due to human activities such as farming, and warming can increase occurrence of severe anoxic events (McKee et al., 2003), so increased pressure from introduced anurans on temporary pond communities in particular may exacerbate stresses. Without top-down control in temporary ponds, *L. ewingii* tadpoles are limited only by pond conditions and adult spread. They seem tolerant to many pond conditions (Chapter Two; Lauck et al. 2005a), and because of warming, alpine ponds may be increasingly colonized (Barclay, 2020). The ecological consequences of the introduction of *L. ewingii* have been largely ignored until now, and due to the timespan of this invasion, pond species may have already been lost, and there may be no model uninvaded ponds remaining. Thus, frog distribution should be more closely monitored, and preserving ponds unaffected by *L. ewingii* is important, and research on their effects on whole pond ecosystems is important to determine real world impacts.

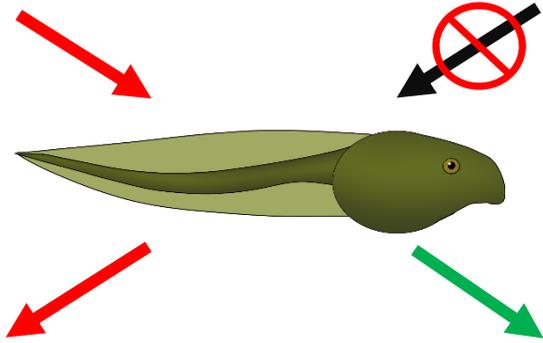
Chapter 4: Discussion

Implications of *Litoria ewingii* invasion for New Zealand

Dynamic freshwater systems, already sensitive to climate warming are also likely to be at greater risk of impact from invasion because of warming-related alterations to their dynamism. *Litoria ewingii*, a frog not native to New Zealand, is adapted for breeding in temporary ponds (Lauck et al., 2005a), and may be causing harm to vulnerable communities in dynamic, periodically drying ponds. Through surveys and mesocosm experiments, I found that *L. ewingii* tadpoles were capable of breeding across a wide range of ponds, and have the potential to alter the structure and function of those pond ecosystems. My survey in Chapter Two showed that, like their Australian counterparts, *L. ewingii* tadpoles were capable of breeding in many different pond types in New Zealand, with a seasonal peak and continual breeding throughout the year. They quickly reached high larval densities, although survival rate of the tadpoles was not measured, so total population growth from these 'booms' is not certain. Furthermore, the wide distribution of *L. ewingii* across most of New Zealand could be attributed to the tolerance of their tadpoles to multiple temperatures, acidities, pond sizes, conditions, and especially flexibility to hydroperiods. The tadpoles responded to pond drying conditions by increasing rate of development. This plastic response likely enables them to avoid strong top-down control from fish in permanent ponds by increasing survival in fishless temporary ponds. Many of *L. ewingii*'s traits like plasticity and tolerance are considered to aid in invasiveness, so *L. ewingii* should be more seriously monitored, because they reach many vulnerable pond ecosystems.

Invasiveness is usually only a concern when there are ecological or economic consequences. I investigated the former in Chapter Three with mesocosm experiments. I found that tadpole presence altered invertebrate abundance, macrophyte and algae biomass. Because the tadpoles were observed grazing on algae, and their trophic position in tarns indicates they are at least partly predatory (Barclay, 2020), reductions in the above can be attributed to consumption, so they may affect biodiversity via predation or grazing (Table 4.1). Through directly reducing small invertebrates and primary producer biomass, they could also alter trophic pathways in pond ecosystems, and through excretion of ammonia could drive increased phytoplankton growth in unshaded ponds (Table 4.1). Additionally, due to *L. ewingii*'s large clutch sizes, continual breeding, and fast larval growth rate, they can reach high densities, which may amplify any ecological consequences during pulse breeding events.

Table 4.1. Potential impacts on and by *L. ewingii* tadpoles to New Zealand pond ecosystems, as well as knowledge gaps. Red arrows indicate negative impacts, green represents a positive impact, and the cross represents no impact.

Controls on tadpoles	Unlikely to be limited by
<ul style="list-style-type: none"> • Fish presence. • Pond drying. • Very high densities 	<ul style="list-style-type: none"> • Resources shortages, because of generalist diet (except at high densities). • Moderate pond drying. • Predation by invertebrates.
	
Potential tadpole negative effects on ponds	Potential tadpole positive effects on ponds
<ul style="list-style-type: none"> • Δ species compositions (predation on small invertebrates, macrophytes and algae). • Δ abiotic conditions (↓ DO and likely ↑ available N), potentially a driver for eutrophication. • Δ pond functioning (an increase in phytoplankton along with general trophic alterations). 	<ul style="list-style-type: none"> • Increase awareness for native frogs. • Umbrella species for pond wellbeing and pond protections. • More urban ponds constructed for frogs.
Knowledge gaps on controls on <i>L. ewingii</i>	Knowledge gaps on <i>L. ewingii</i> impacts
<ul style="list-style-type: none"> • Interaction with land use (e.g. agriculture or eutrophication). • Other invertebrate predation, for example, crayfish or tadpole shrimp. • Spread of frogs terrestrially. • Altitudinal influences 	<ul style="list-style-type: none"> • Predation of tadpole eggs • Food-web effects on whole pond systems. • Pond long-term dynamics – boom and bust tadpole cycles may have food-web consequences. • Frog demographics and productivity from ponds to terrestrial systems. • Frog influences on native terrestrial animals.

These ecological consequences of *L. ewingii* presence may come in the form of eutrophication or even nitrogen toxicity. Eutrophication from nutrient addition, largely from farming practices, has been named as a big driver of freshwater ecosystem deterioration in New Zealand and the world (Schindler, 1974; White, 1983). The levels of ammonia-nitrogen excreted by a tadpole (average 0.146 mg L⁻¹ h⁻¹, Chapter Three), when extrapolated for the highest densities found (0.073 mg/m³ h⁻¹,

Chapter Two), have potential to contribute to eutrophication in ponds. According to the National Policy statement for Freshwater Management, this amount of nitrogen may slightly impact ecological communities in polymictic lakes (i.e. unstratified lakes, which applies most closely to ponds) by increasing algal and plant growth, and depending on the rate of ammonia breakdown, tadpoles may cause ammonia toxicity for the most sensitive 5% of species (see Tables 3 and 5, in New Zealand Government, 2020). The increase in phytoplankton growth related to high tadpole densities found in Chapter Three, support this, because at 0.025 mg chl-a/L, the phytoplankton growth observed indicates slight eutrophication (see Table 1, in New Zealand Government 2020). However, I did not investigate whether *L. ewingii* increased total nitrogen in ponds through egg deposition, or if the tadpoles just convert pre-existing nitrogenous sources to ammonia, and thus not ultimately causing eutrophication through nutrient addition. Moreover, in multiple frog species, high larval densities suppressed ammonia excretion (Ramamonjisoa et al., 2021), so these calculations may be overestimates for *L. ewingii*, because I measured the ammonia excretion of isolated tadpoles. Although they may not largely increase nitrogen in ponds, they may contribute to available nitrogen that drive algal blooms, and so their presence should be considered when protecting and managing vulnerable ponds and wetland ecosystems.

Density limitation through life history flexibility

In addition to potentially suppressing ammonia excretion, high larval densities may affect tadpole growth. In Chapter Two I showed that *L. ewingii* trade off size at metamorphosis with development rate to escape drying ponds, presumably by increasing rate of development. However, there may be an additional developmental trade-off for larval *L. ewingii*, implied by Chapter Three results. The primary purpose of Experiment 2 in Chapter Three was to investigate whether tadpole trophic impacts were affected by shading or larval density, so tadpole characteristics were not examined. However, due to the nature of the mesocosm experiment, I could investigate their sizes at metamorphosis after the three-week experimental duration. Their size at metamorphosis was decreased both in open tanks ($F_{1,8} = 5.355$, $p = 0.049$) and by higher larval density ($F_{1,18} = 6.047$, $p = 0.024$), but there was no interaction ($F_{1,8} = 0.283$, $p = 0.601$; Figure 4.1). Their decrease in metamorphic size in open tanks can be explained simply by the development pattern shown in Chapter Two, where their rate of development increased in more temporary ponds. Open, unshaded tanks in this experiment mimicked drying ponds, as the temperature fluctuations were large, which may be a cue *L. ewingii* uses to detect drying, like other frog species (Brady & Griffiths, 2000). Therefore, *L. ewingii* could plastically increase rate of development even when exposed to different conditions for only three weeks, highlighting how flexible they could be to rapid abiotic alterations.

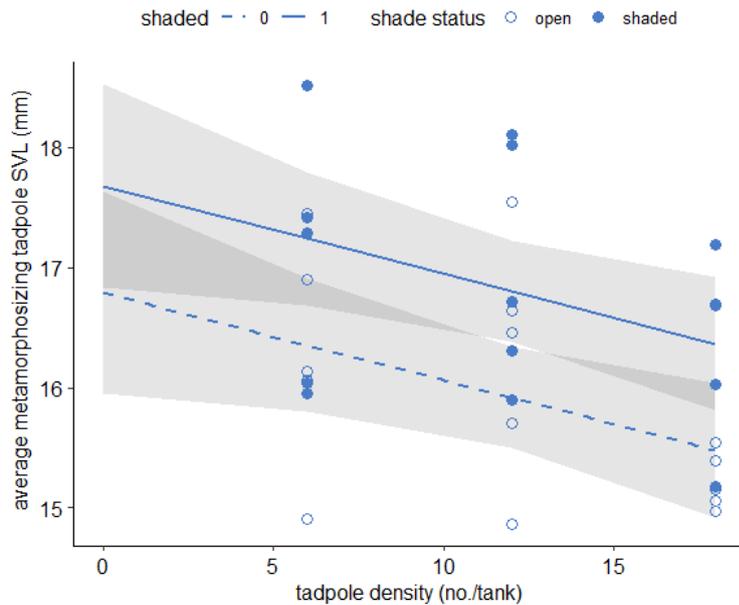


Figure 4.1. Average tadpole snout-vent-lengths (SVL, mm) at metamorphosis (between Gosner stages 32 and 43) in relation to experimental larval density (Experiment 2, Chapter Three) and shading treatment. Open circles and the dashed line represent unshaded tanks, whereas solid circles and line represent shaded tanks.

In contrast, *L. ewingii* decreased size at metamorphosis in tanks with higher larval densities may be caused by a different mechanism. Reduced size at metamorphosis may be caused by intraspecific competition for resources reducing growth rate. However, because tadpoles in all treatments were metamorphosing, development must not have been hugely delayed by reduced growth, and so was most likely a plastic developmental response. Relyea (2002) suggests that tadpoles plastically respond to different levels of competition by altering different morphological traits, consistent with this the observed response in *L. ewingii*. In *L. ewingii*'s native range, they are sympatric with other frog species that have aquatic life stages (Walker, 2002), so they may be more limited by interspecific competition. However, in New Zealand, because there are no aquatic tadpoles, they would be freed from anuran interspecific competition, and would most likely be affected by intraspecific competition. Perhaps *L. ewingii* increase rate of development when surrounded by conspecifics to escape competition. Alternately, perhaps a high density is a cue for pond drying, because shrinking habitat increases relative density, causing tadpoles to increase their rate of development to escape drying. Therefore, it is possible that *L. ewingii* uses a combination of increased temperatures and density as cues for pond drying, as seen elsewhere (Székely et al., 2017; Tejedo & Reques, 1994). However, increasing rate of development trades off size at adulthood, so the observed high densities may also cause a reduction in adult fitness. Overall, *L. ewingii* is highly adaptable, but larval populations could be regulated by density-dependent processes.

Benefits of frog introduction

Litoria ewingii tadpoles may impact pond communities, but their presence may have other benefits (Table 4.1). Ponds, especially smaller temporary ponds, are often drained during development (Grainger et al., 2018; Johnson & Rogers, 2003), effectively eliminating that aquatic habitat. Even when they are not intentionally removed, often the habitat is degraded by land use such as stock intrusion, nutrient input or habitat simplification (Johnson & Rogers, 2003). Elimination or degradation of temporary pond habitat is detrimental for native pond species, especially for the tadpole shrimp (*Lepidurus apus viridis*), and Canterbury mudfish (*Neochanna burrowsius*), both of which are often found in drying ponds and are declining (Grainger et al., 2018; Meijer, 2018). However, when people are aware of frogs inhabiting ponds on their property, they are probably more motivated to protect those ponds, some even constructing ponds for frogs (Shaw et al., 2014). While *L. ewingii* may have some negative ecological effects on pond system functioning, they may be able to serve as advocates for ponds, especially urban or farm ponds, potentially resulting in a net benefit for those pond ecosystems. Introduced salmonids in New Zealand can play a similar role – despite preying on native fish, sometimes their presence results in better protections for waterbodies they inhabit (Tadaki et al., 2022). Additionally, because native frogs are cryptic and occupy small, remote locations, many people will not encounter or be aware of them (Bishop, 2008). Encountering the much more prevalent introduced frogs can foster enthusiasm for frogs as a whole, leading to more interest in native frog conservation (Bishop, 2008). However, before declaring *L. ewingii* presence beneficial to New Zealand ecosystems, it is important to fully understand their ecological interactions, life history, and spread, and ecological impacts must not be ignored because of potential benefits. Once known, a balanced approach will be necessary to protect and manage vulnerable pond ecosystems.

Future directions

Controls on *L. ewingii* distribution and wider ecosystem impacts caused by *L. ewingii* are not well understood in New Zealand, and there are many opportunities for further research not explored by my experiments or surveys (Table 4.1). It is important to understand any negative impacts *L. ewingii* may have on real pond ecosystems rather than mesocosms, particularly whole ponds dynamics over time. Results from mesocosm experiments can often be extrapolated to larger scales (Spivak, Vanni, & Mette, 2011), but studying whole systems would be valuable, especially considering that for my experiments invertebrates and periphyton inocula (in mud) were taken from ponds with *L. ewingii* tadpoles. Consequently, my results may be obscured by a history of tadpole effects on those communities, whereas ecosystems that have not yet been invaded may be more likely to experience

large trophic disruptions upon frog colonisation. Thus, it is important to study ponds with no *L. ewingii* presence, which may be difficult to find in many parts of New Zealand.

Invasive impacts depend heavily on the timespan they take place, for example trout invasion into lakes in the USA disrupted food webs initially, but they stabilised after 50 years (Wainright et al., 2021). Because *L. ewingii* has been present since 1875, some ecosystems may have stabilised to their presence, and ponds not yet colonised may be most at risk. However, because of the 'boom and bust' life history of *L. ewingii*, New Zealand ecosystems may have had less chance to adapt, and so breeding pulses may be detrimental over time. Specifically, these 'boom and bust' cycles may destabilise ponds by severely altering energy and nutrient flow during peak densities and then frog exodus, especially for newly colonised ponds where trophic destabilisation is likely to be greater.

These pulses of frog offspring inevitably lead to an increase in *L. ewingii* frogs entering the terrestrial ecosystem, where their prey, predators, and dispersal over land have received little attention. They may spend as little as five weeks as tadpoles, but they potentially live as adults for several years, dispersing far from waterbodies when not breeding, so their ecological interactions as an adult should also be investigated. Under climate change, stress-tolerant species may experience habitat loss (Meijer et al., 2019), but temporary pond drying for *L. ewingii* will only result in wasted breeding efforts rather than local extinctions, and adults can easily recolonise upon pond filling. Ultimately, the frogs facilitate the dispersal of the tadpoles, so understanding their behaviour and spread is important, as well as understanding the adult's interactions in the terrestrial ecosystem, as they could facilitate non-native predators there.

Management suggestions

Investigating knowledge gaps such as adult frog dispersal are important, but often management should not hinge on future research, because vulnerable ecosystems may not be able to wait. Therefore, perhaps *L. ewingii* should be treated as an invasive species in New Zealand. Definitions for invasive species differ, but most agree that the first steps of invasion are introduction (mediated by humans), establishment, then spread (Lockwood, Hoopes, & Marchetti, 2013). Many definitions then require that a species have a documented negative impact on ecology or economies, but this can create delays in dealing with invasions, and so many ecologists do not require evidence of harm in their definitions (Engel et al., 2011; Lockwood et al., 2013; Young & Larson, 2011). The establishment and spread of *L. ewingii* is well documented (Shaw et al., 2014; Thomson, 1922), but ecological consequences hadn't been investigated. Thus, according to many definitions, the species is already invasive and has been for a long time. I have shown there may be negative ecological effects, and so argue they should be labelled an invasive species in New Zealand. Their status then

requires some consideration by managers and consideration of what action should be taken. New Zealand biodiversity is under strain from invasive species with large or wide impacts associated with many species (Innes et al., 2010; O'Donnell, Clapperton, & Monks, 2014), and time and resources to manage these are limited. Management of species more imminently threatening may need to be prioritised. Furthermore, frog presence may have potential benefits in specific scenarios (Table 4.1), so a balanced management approach would be required. Nevertheless, ponds often slip under the radar, and make up an important part of wetlands, which are one of New Zealand's most threatened ecosystems (Greig & Galatowitsch, 2016; Johnson & Rogers, 2003). *Litoria ewingii* may be the most important impact on some ponds, and high-altitude ponds have unique and understudied communities and so are especially vulnerable to *L. ewingii* invasion, especially due to climate warming driven range shifts (Barclay, 2020; Macinnis-Ng et al., 2021). Firstly, uninvaded ponds should be protected from *L. ewingii* colonisation, especially considering the high rate of endemism and rare species in New Zealand pond species (Greig & Galatowitsch, 2016). Uninvaded ponds are more likely to be remote, so frog distribution should be actively monitored, and more effort should go to understanding potential ecological impacts.

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