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Predator-prey interactions between mosquitofish (*Gambusia affinis holbrooki*) and whistling frog (*Litoria ewingi*) tadpoles.

A thesis presented in partial fulfilment
of the requirements for the degree of
Masters of Science in Ecology
at Massey University,
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New Zealand.

Renske van Tol

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Abstract.

Abstract.

Aspects of the impact of newly introduced predatory mosquitofish (*Gambusia affinis holbrooki*) on the resident introduced whistling frog (*Litoria ewingi*) were examined by means of experiments. Microhabitat preferences of whistling frog tadpoles and mosquitofish were investigated through a series of laboratory trials and the behaviour of both species during attacks by mosquitofish on tadpoles was also recorded. Microhabitat use of these species will overlap in natural situations, but both species changed their microhabitat use with age. In the presence of plants, small tadpoles moved towards the surface, while larger tadpoles prefered to associate with the substrate. The same pattern occurred when an alga chip (food source) was placed on the substrate. Small female mosquitofish were more attracted to plants than larger mosquitofish, and less attracted to tadpoles (potential prey).

The extensive recent literature on predation is reviewed with emphasis on existing works on predator-prey interactions involving amphibian larvae. Behaviour of both species during attacks changed with age. Small tadpoles were more likely than large tadpoles to flee from an attack. Larger female mosquitofish were more likely to attack moving tadpoles (in comparison with small female mosquitofish), and to attack the body of tadpoles (as opposed to the tail). There was no difference between the behaviour of small female and male mosquitofish. Most attacks were non-fatal. Inactive prey were more likely to be attacked in this combination of predator and prey, which is in direct contrast with previous studies. When a plant with complex three-dimensional architecture was added attacks still occurred, but overall the frequency of attacks was lower, indicating that the presence of sufficient refuge may lessen the effect of mosquitofish introductions on whistling frog tadpole populations. A field experiment was also conducted but results were not conclusive.

L. ewingi may become reduced to breeding in ephemeral waters if *G. a. holbrooki* invades permanent waters successfully and removes *L. ewingi* extensively.

Uittreksel.

Aspecten van de invloed van recent geïntroduceerde mosquitofish (*Gambusia affinis holbrooki*) op de vaste geïntroduceerde whistling frog (*Litoria ewingi*) werden onderzocht door middel van experimenten. De keuzen van mosquitofish en whistling frog kikkervissen wat betreft hun plaatsing in het water werden onderzocht met een serie proeven in het laboratorium en het gedrag van allebei de soorten gedurende aanvallen van de mosquitofish op de kikkervissen werd ook genoteerd. De plaats-voorkeur van deze twee soorten zal gedeeltelijk samenvallen in natuurlijke situaties, maar allebei de soorten veranderde hun plaats-voorkeur naar mate ze ouder werden. In de aanwezigheid van planten verplaatsten kleine kikkervissen zich naar de oppervlakte van het water, terwijl grotere kikkervissen de voorkeur gaven aan de bodem. Hetzelfde patroon kwam voor toen een alge-schijfje (voedselbron) op de bodem werd gelegd. Kleine vrouwelijke mosquitofish werden meer aangetrokken door planten dan grotere mosquitofish en ze werden minder aangetrokken door kikkervissen (potentiele prooi).

Een overzicht wordt gegeven in de uitgebreide recente literatuur over roofdieren en prooi, met de nadruk op roofdier-prooi interacties waarin amfibieën betrokken zijn. Het gedrag van beide soorten gedurende aanvallen veranderde met leeftijd. Het was waarschijnlijker dat een kleinere kikkervis zou proberen te ontsnappen na een aanval dan dat een grote kikkervis dit probeerde. Grotere vrouwelijke mosquitofish vielen vaker bewegende kikkervissen aan (in vergelijking met kleinere mosquitofish), en beten vaker in het lijf van de kikkervis (in plaats van de staart). Er was geen verschil tussen het gedrag van kleine vrouwelijke mosquitofish en dat van kleine mannelijke mosquitofish. De meeste aanvallen waren niet fataal. Al hebben eerdere werken aangegeven dat minder actieve prooi geringere kans heeft aangevallen te worden, is dit niet het geval met deze combinatie roofdier en prooi. Toen een plant met een complexe drie-dimensionale architectuur toegevoegd werd waren er nog steeds aanvallen, maar over het algemeen gebeurde aanvallen met een mindere frequentie, wat betekent dat de tegenwoordigheid van voldoende mogelijke schuilplaatsen het effect van mosquitofish introducties op whistling frog kikkervis bevolkingen mischien zal verminderen. Een experiment werd

ook gedaan in de natuur, maar de resultaten waren niet beslissend.

L. ewingi zal mischien geforceerd zijn alleen in kortstondige wateren hun eieren te leggen als *G. a. holbrooki* de permanente wateren bewoont en *L. ewingi* daaruit verdwijnt.

Chapter 1

General introduction.

Litoria ewingi.

The whistling frog (*Litoria ewingi*) is a small tree frog species (Anura: Hylidae) native to Tasmania and Victoria in Australia (Robb, 1980) where it forms part of a species complex (Watson et al., 1971). Formerly known as *Hyla ewingi* (Robb, 1980), whistling frogs were first introduced to New Zealand at Greymouth in 1875 (Robb, 1980). A further introduction to Himatangi occurred in 1948 (Robb, 1980). Today, whistling frogs are found in Southland and along the west coast of the South Island, and in the North Island throughout the Manawatu region and northern parts of the Horowhenua district (Figure 1.1) (Gill, 1978; Robb, 1980; Alderton, 1982).

Of the three *Litoria* species found in New Zealand (Robb, 1980), *L. ewingi* is the smallest. Females grow to approximately 44 mm long (from snout to vent), males reach just 34 mm (Robb, 1980). There is considerable variation in colour, with tones ranging from a uniform dark brown to light cream or grey with markings or small flecks (Robb, 1980), and individuals can vary their colour to match the background. There is almost always a dark strip leading from the nostril, across the eyes, and to the point of forelimb insertion (Robb, 1980).

L. ewingi prefer vegetated areas, but are also found in pastures near the Foxton coastline (Gill, 1970). Adults return to water only to breed (Aylward, 1978), and feed on a wide range of terrestrial invertebrates, including Amphipoda, Gastropoda, Hemiptera, Coleoptera, Diptera, Hymenoptera, Lepidoptera, Aranae, and Acarina (Kane, 1980). Tadpoles eat alga (Dickman, 1968). There is no evidence to suggest that *L. ewingi* have any negative effects on New Zealand ecosystems, but they do form a food source for some native birds including white-faced herons, kingfishers, and shags (R. A. Fordham, pers. comm.), and probably eels, rats and stoats.

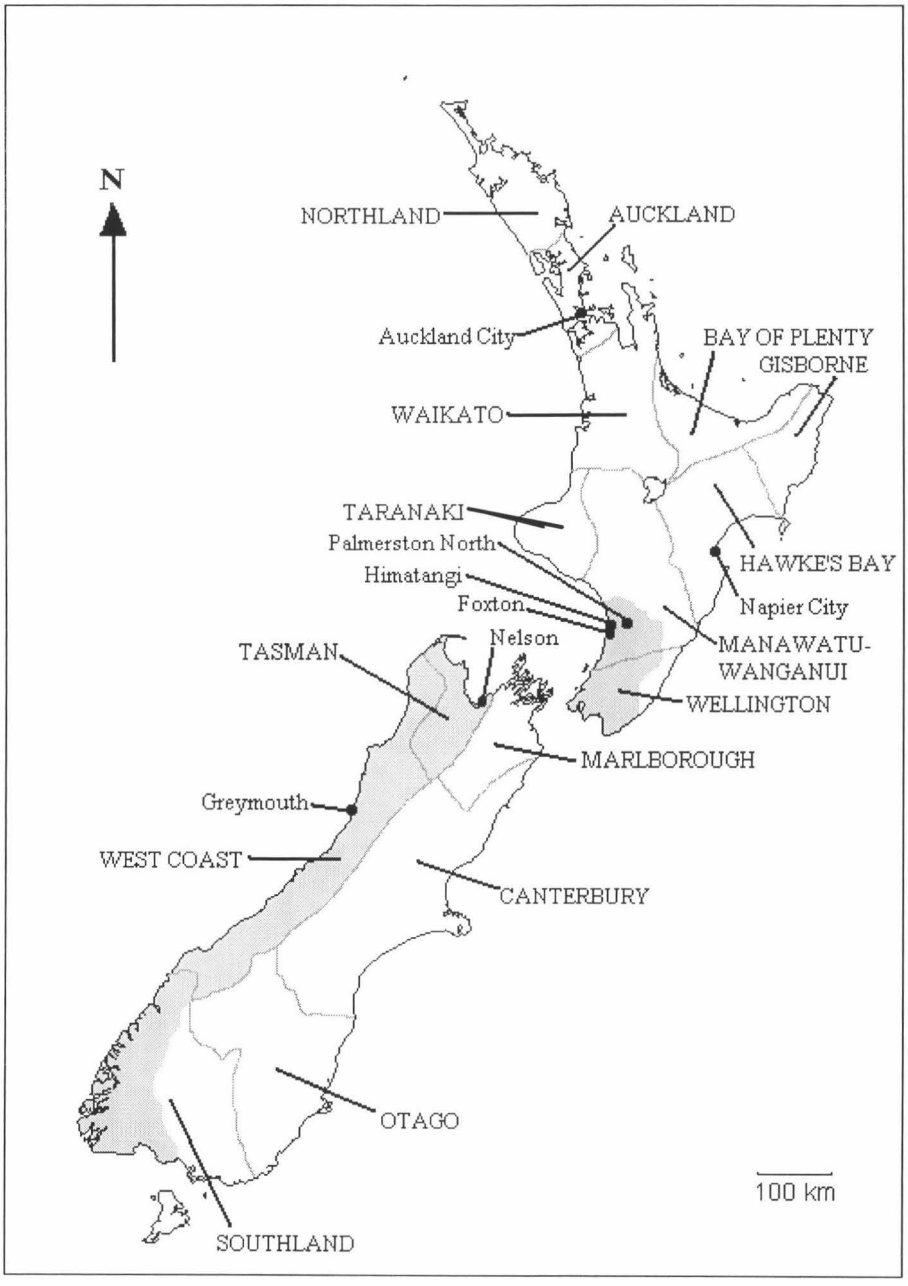

Figure 1.1. Map of New Zealand showing distribution of *L. ewingi* (shaded regions).

In New Zealand, whistling frogs breed from March through to December (Robb, 1980), with peak breeding activity occurring in July (Alderton, 1982). Female frogs lay eggs in several bundles, total clutch size usually exceeding 400 (Alderton, 1982). Metamorphs generally start to emerge from the water in November, and usually most

tadpoles have metamorphosed and disappeared by the following May (Gill, 1978), although tadpoles can overwinter in milder parts of the range (pers. ob.).

Gambusia affinis holbrooki.

Mosquitofish (*Gambusia affinis*) are native to the eastern United States (Lawler et al., 1999) where there are two subspecies, *G. a. affinis* and *G. a. holbrooki* (Komak and Crossland, 2000). Mosquitofish may be the most widespread freshwater fish species in the world because they have been introduced to Europe, Asia, Australia and New Zealand in attempts to control mosquito populations (Simberloff and Stiling, 1996; McCullough, 1998; Lawler et al., 1999). Being generalist predators, mosquitofish have preyed on native populations throughout their ever-expanding range, causing widespread destruction (Simberloff and Stiling, 1996).

Mosquitofish (*G. a. holbrooki*) were introduced from Australia to New Zealand in the 1930s, and first released in the Auckland Botanical Gardens (McCullough, 1998). Since then populations have spread throughout the Northland, Auckland, Waikato, Bay of Plenty, and Hawke's Bay regions (Department of Conservation website, 16 August 2001). Recently they have been discovered in the Manawatu (Myers, 2001) and Nelson (P. B. Studdum, pers. comm.) areas (Figure 1.2), and the Department of Conservation has conducted a survey confirming their distribution at three sites in the Manawatu (Figure 1.3; Miller, 2001).

At present, mosquitofish are not known to be in the West Coast or Southland areas (Figure 1.2), suggesting that at this time populations of whistling frogs there are still safe from predation by mosquitofish. Therefore it is important to prevent the spread of mosquitofish through these areas.

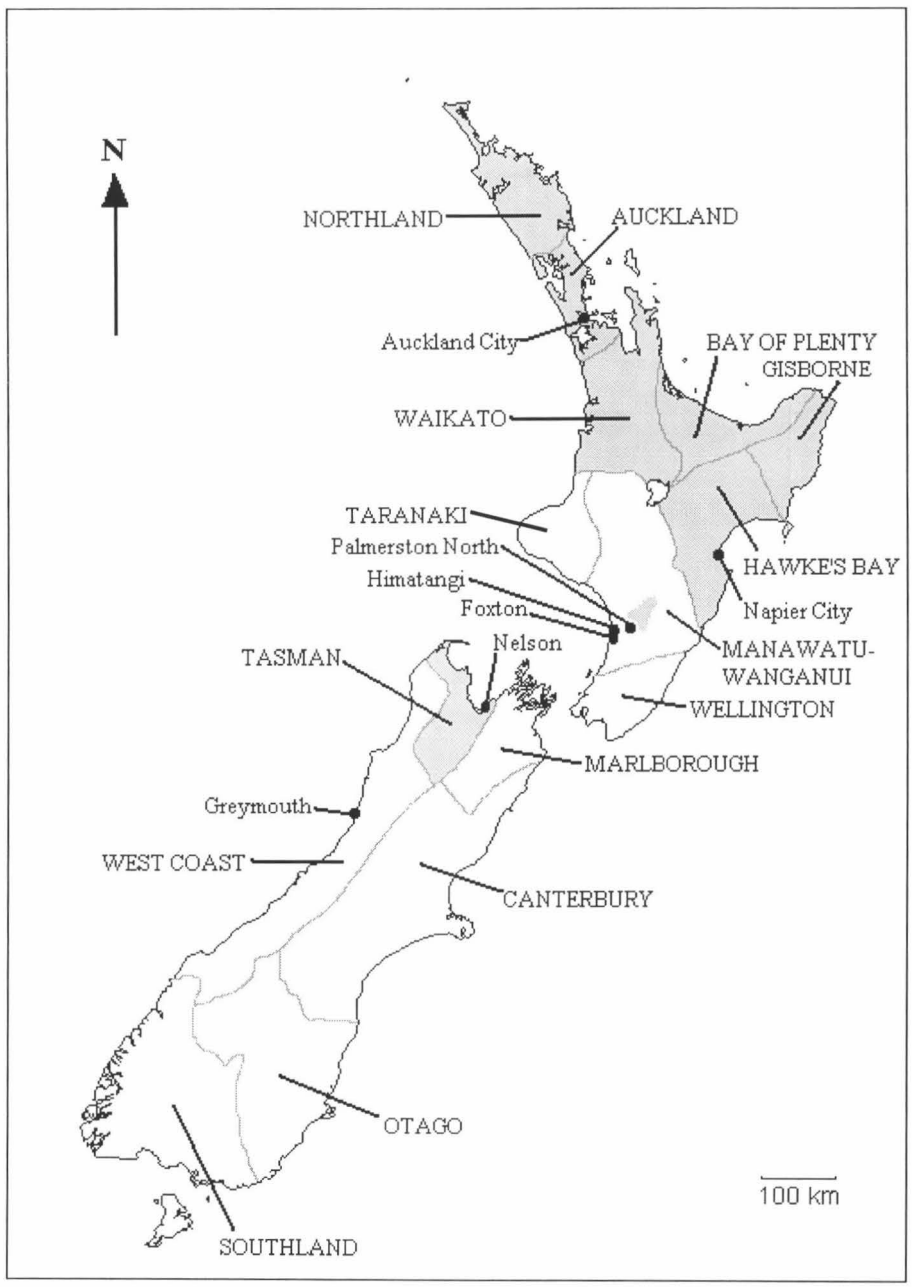

Figure 1.2. Map of New Zealand showing distribution of *G. a. holbrooki* (shaded regions). N.B. The exact status and distribution of populations in the Tasman region is currently unclear.

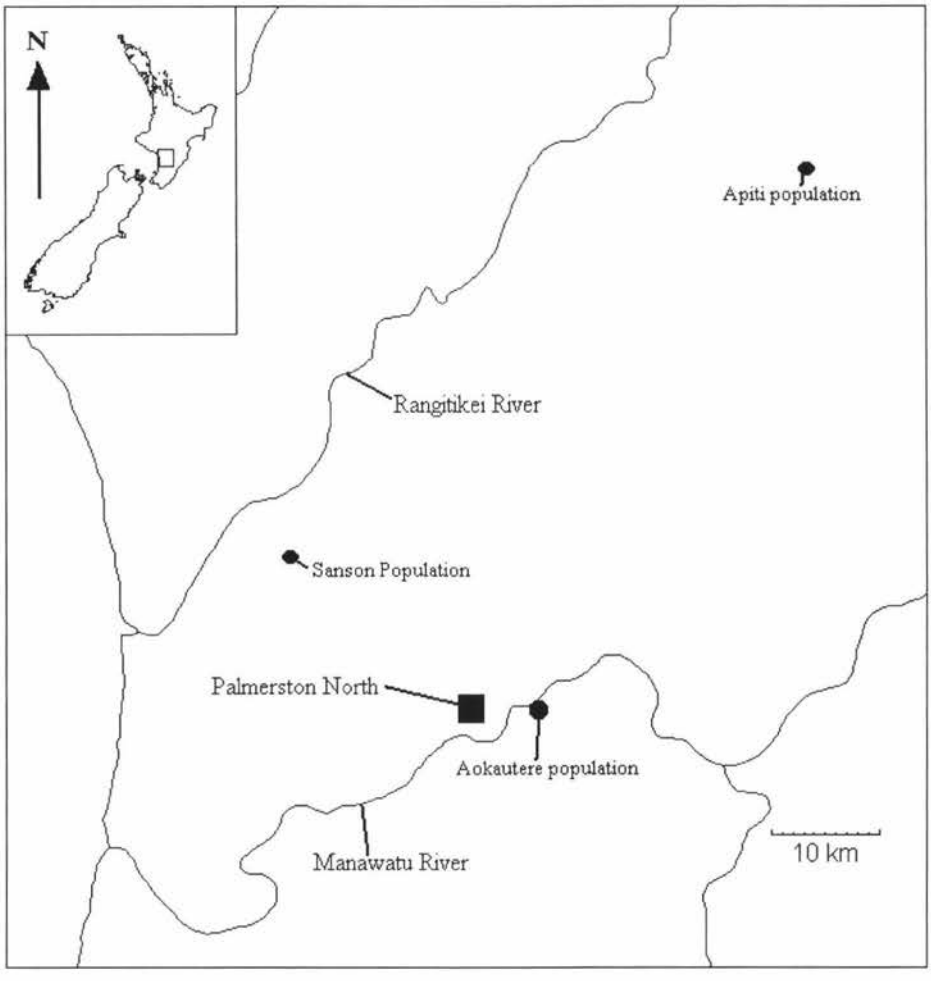

Figure 1.3. Local map showing confirmed mosquitofish populations in the Manawatu area (localities from Miller, 2001). N.B. There is no known population in Palmerston North.

Mosquitofish are small, stout, robust fish. They are grey in colour with a rounded tail and an upturned mouth that allows them to feed at the water surface (McCullough, 1998). Mature females grow up to 60 mm long (total body length), while males are much smaller at 25-35 mm. This difference in size causes males to be particularly vulnerable to cannibalism by females, and as a result the sex ratio is usually female-biased (Bisazza et al., 1989).

Like all poeciliid fish, mosquitofish bear live young (Vargas and de Sostoa, 1996). Females can store sperm in the reproductive tract for up to two months (Bisazza et al.,

1989) and they become fertile several times per year (McCullough, 1998), as a result, the entire female population is available for breeding at any time during the breeding season (Bisazza et al., 1989). Young mature rapidly (Vargas and de Sostoa, 1996; McCullough, 1998), and fecundity is age-related, with large females producing up to twenty times as many embryos as females in their first reproduction (Bisazza et al., 1989). Because of their efficient breeding system, mosquitofish populations can grow very quickly and are very difficult to eradicate (McCullough, 1998).

Impact of a new predator.

The introduction of novel fish predators can have profound effects on local amphibian populations (Heyer, 1976; Petranka et al., 1987; Sih et al., 1992; Blaustein et al., 1994*b*; Brönmark and Edenhamn, 1994; Feminella and Hawkins, 1994; Fisher and Shaffer, 1996; Bridges and Gutzke, 1997; Laurila and Aho, 1997). In New Zealand, mosquitofish invasions have already had an adverse effect on native fish populations (McCullough, 1998). Now that they have reached the Nelson and Manawatu regions it is likely that they will come into contact with populations of whistling frog tadpoles, and prey on these.

The object of this study is to investigate some possible implications of mosquitofish predation on tadpoles of *L. ewingi*, and to consider the role that habitat structure might play in this interaction. In Chapter 2 results of recent studies on predator-prey interactions are summarised, focussing on studies that involve amphibian larvae as the prey species. For the following two chapters, I conducted a series of laboratory experiments investigating habitat preferences of whistling frog tadpoles (Chapter 3) and mosquitofish (Chapter 4). I then observed attacks in the laboratory in order to collect data on the behaviour of both species during attack sequences (Chapter 5). Chapter 6 discusses a field experiment intended to support the laboratory data. Finally, Chapter 7 deals with the implications that my findings have for populations of whistling frog tadpoles in New Zealand.

In all the following chapters a summary in my native language, Dutch, is included.