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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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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 preferred 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 misschien zal verminderen. Een experiment werd

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

L. ewingi zal misschien 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.

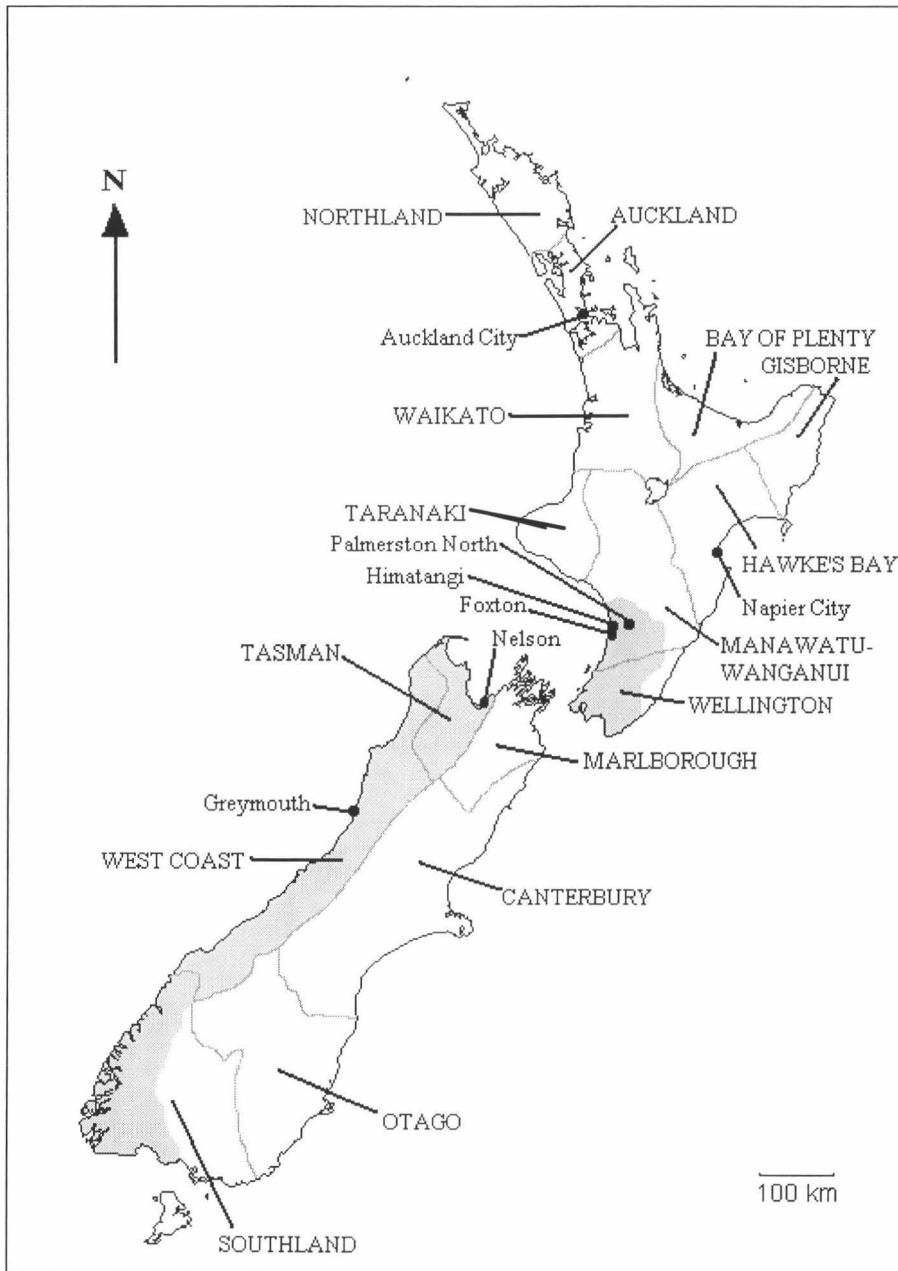


Figure 1.1. Map of New Zealand showing distribution of *L. ewingi* (shaded regions).

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.

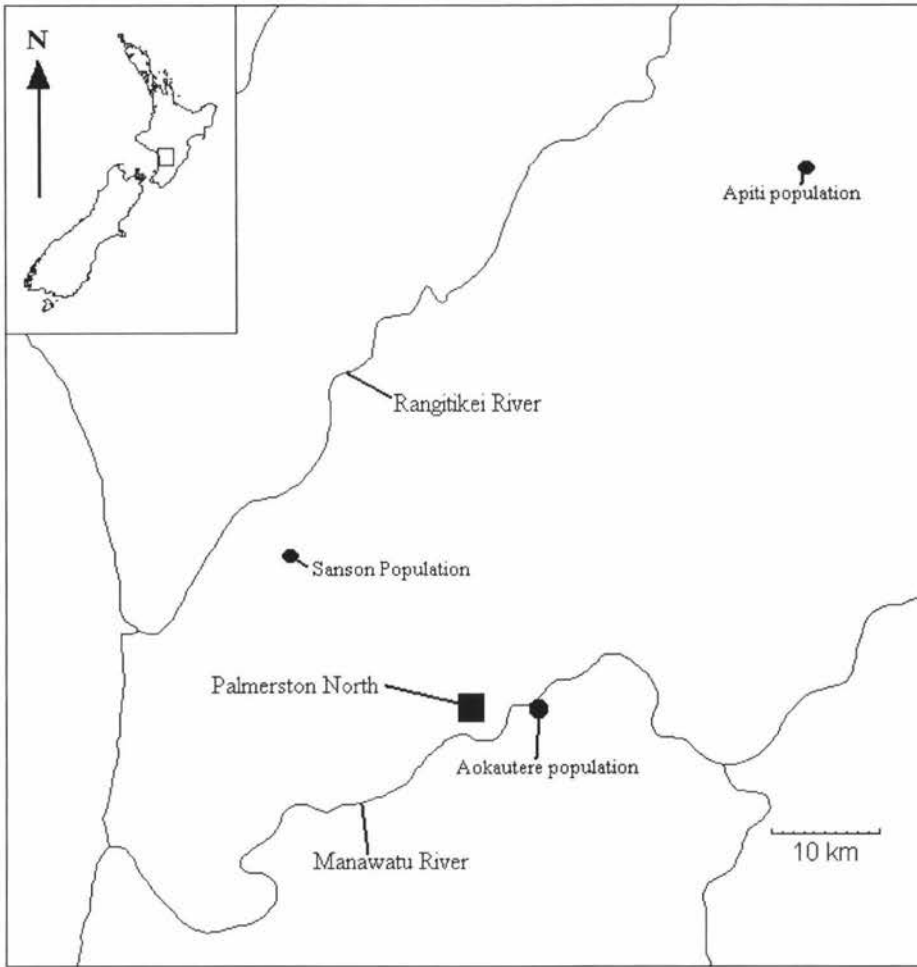


Figure 1.3. Local map showing confirmed mosquitofish populations in the Manawatu area (localities from Miller, 2001). N.B. There is no known population in Palmerston North.

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.*, 1994b; 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.

Chapter 2

**A review of literature on predator-prey interactions
involving amphibian larvae.**

Recent amphibian declines.

In recent decades scientists around the globe have been observing startling declines in a variety of amphibian groups. Amphibian communities from almost all ecosystems and regions are experiencing range restrictions and extinctions. However, these declines seem to lack a pattern or any obvious cause (Hecnar and M'Closkey, 1996). For example, although many Australian species are now under threat the introduced cane toad *Bufo marinus* is thriving (Crossland, 1998). Amphibian declines are particularly worrying because amphibians have been identified as early warning taxa for widespread global degradation. For instance they are sensitive to changes in a wide range of habitats, both land and water (Halliday, 2000) and, as top carnivores of invertebrates in the food chain, may amplify environmental changes (Phillips, 1990; Wake, 1991).

A range of recent global and regional changes have been implicated in amphibian declines. These include habitat destruction, global warming, pesticide- and other pollution, acid rain, increased exposure to UV-B radiation (as a result of habitat destruction and a break-down in the ozone layer), virulent pathogens attacking previously unexposed populations, and the influx of introduced predators (Blaustein and Wake, 1990; Phillips, 1990; Wake, 1991; Blaustein *et al.*, 1994*b*; Fisher and Shaffer, 1996; Lips, 1999; Halliday, 2000; Pounds, 2001), especially freshwater fishes such as the mosquitofish *Gambusia affinis*.

There are pronounced differences between regions of the globe in the level of declines. In the south-eastern United States of America and within 10 degrees of the equator there is little evidence for declines, except where habitat destruction has occurred (Blaustein and Wake, 1990). In temperate zones amphibians are breeding earlier each year (Halliday, 2000). Potentially this allows them a longer breeding season, which may be beneficial. However, in the tropics where the window of opportunity for breeding is already narrow, climate warming may be preventing amphibians from breeding in some years (Halliday, 2000).

Research has only recently begun on the pollution of amphibian environments by a wide array of anthropogenic chemicals (Halliday, 2000). These include the breakdown products of plastics and certain pesticides that can mimic the effects of oestrogen and other hormones (Halliday, 2000). Such products are able to cause several reproductive changes in animals, including feminisation and sex change in fishes and reptiles, and reduced sperm count in humans (Halliday, 2000). They may also interfere with development, causing morphological abnormalities, metabolic disorders, and damaged immune systems (Halliday, 2000). Because they are widespread, and effective at low levels, they are likely to affect amphibians (Halliday, 2000). Lefcort et al. (1999) have shown that the presence of heavy metals reduces tadpole survival and has an overall negative effect on tadpole development.

Amphibian embryos are also known to be sensitive to UV-B radiation (Barinaga, 1990; Smith et al., 2000; Starnes et al., 2000). UV-B radiation has been implicated in declines of a close relative of the whistling frog- *Litoria verreauxii alpina* from high altitude sites in the Snowy Mountains region of southeastern Australia (Broomhall et al., 2000). However, the eggs of some amphibian species (e.g. *Hyla regilla* (Pacific treefrog), Blaustein et al., 1994a) contain high levels of photolyase which repairs damage done by UV-B rays, which may be why they seem highly resistant to UV-B containing sunlight.

Fungi can harm amphibians, for instance *Bufo boreas* (western toad) eggs have been attacked by *Saprolegnia*, a water mold that commonly attacks fish (Blaustein et al., 1994b). This fungus is globally distributed and can kill 95% of *B. boreas* eggs (Blaustein et al., 1994b). *Saprolegnia* also infects other amphibian species, and could be responsible for other amphibian declines (Blaustein et al., 1994b). *Saprolegnia* deaths are higher in very shallow water, where tadpoles experience higher levels of UV-B compared with deeper water. Therefore with global warming *Saprolegnia* epidemics are likely to become more common (Kiesecker et al., 2001a; Pounds, 2001). *Chytrid* fungi have also been implicated in amphibian declines. A *Chytrid* fungus was identified as the cause of two declines in Panama and East Australia (Lips, 1999; Halliday, 2000) and it is present in New Zealand (Norman et al., 2000).

In montane California, predation by fishes has been implicated as a major cause of the decline of amphibian populations (Blaustein et al., 1994b). As well as preying on eggs, hatchlings, and tadpoles, fishes may bring pathogens into new areas (Blaustein et al., 1994b). In California's Great Central Valley amphibian populations are gradually being restricted to higher elevations, apparently because of the influx of exotic predators into low-elevation sites (Fisher and Shaffer, 1996). Declines are more likely to be caused by predation than by habitat degradation in this case because there are still successful amphibian populations in predator-free, but highly modified, pools in this area (Fisher and Shaffer, 1996). Despite extremely rich amphibian communities in the tropics, amphibian predator-prey studies have mainly focussed on temperate communities. Gascon (1992) studied tropical predator-prey interactions involving amphibians and found the results were qualitatively similar to those previously found in temperate systems. This suggests that predators introduced to the tropics may be causing widespread declines there. However, Blaustein et al. (1994c) warned that amphibian declines may be exaggerated, due to the short term or anecdotal nature of most studies. Many amphibians are seen only at breeding sites. Therefore, if the animals experience a breeding failure one year it may look like the population has suffered a vast decline (Pechmann et al., 1991).

Effects of predation on amphibian populations.

A wide variety of field and laboratory research has documented the effects of predators on amphibian populations (Morin, 1983; Wilbur et al., 1983; Travis et al., 1985a; Petranka et al., 1987; Van Buskirk, 1988; Semlitsch and Reichling, 1989; Ludwig and Rowe, 1990; Fauth and Resetarits, 1991; Brönmark and Edenhamn, 1994; Feminella and Hawkins, 1994; Bridges and Gutzke, 1997; Laurila and Aho, 1997; Crossland, 1998). Most of these studies have focussed on the predation of odonate (dragonfly) larvae, fishes and other amphibians on tadpoles. Aside from being consumed by a predator, a tadpole can also be injured, experience a lower growth rate, and a longer larval period. *Hyla chrysoscelis* (treefrog) tadpoles with 75% of their tails surgically removed were twice as likely to be preyed on by dragonfly larvae than those with their tails left intact (Semlitsch, 1990). As well as the likely increase in predation risk for an injured individual, there is also a cost involved if energy is diverted from

somatic growth to tissue repair (Semlitsch and Reichling, 1989; McCollum and Leimberger, 1997).

Because many predators preferentially consume prey individuals of a particular size, the size structure of the prey population can be significantly altered by predation. As well as lowering survival, adding *Ambystoma opacum* (salamanders) to communities of *Hyla gratiosa* tadpoles significantly altered the size of the tadpoles because the salamanders cropped out the larger individuals (Travis et al., 1985a). However, this happened only at intermediate densities of *H. gratiosa* (Travis et al., 1985a). In an artificial pond study, using larvae of the dragonflies *Anax junius* and *Tramea caroline* as predators, both species significantly affected the composition of an anuran guild and lengthened the larval period of some species (Van Buskirk, 1988). In other species the survivors experienced increased growth rates, presumably due to competitive release (Van Buskirk, 1988).

Crossland (1998) found that native Australian anuran tadpoles were preyed on more by other native species than by exotic *Bufo marinus* (cane toad) tadpoles. However *B. marinus* is often found in very high densities in natural waterbodies and may, therefore, still have significant impacts (Crossland, 1998). In North America the densities of *Notophthalmus viridescens dorsalis* (broken-striped newt) and *Siren intermedia intermedia* (lesser siren) both significantly affected the number of anuran tadpoles that survived to metamorphosis. *N. v. dorsalis* alone reduced anuran tadpole survival by about 60%, even at low density (Fauth and Resetarits, 1991). Aside from eliminating some insect and amphibian species from temporary ponds, *N. v. dorsalis* reverses the outcome of competition among anuran tadpoles, acting as a keystone predator (Fauth and Resetarits, 1991).

Strong predatory effects also occur in captive communities. Wilbur et al. (1983) attempted to separate out the effects of competition and predation using *N. v. dorsalis* as a predator. He found that predation accounted for 65.5% of the variation in growth rate between treatments, and that predation had a strong impact on the survival of all anuran populations. Morin (1983) noted that predation by larval *Ambystoma tigrinum* (tiger salamander) essentially deleted the entire anuran guild from a tank community,

and differences in *N. viridescens* density produced striking differences in the final composition of the tadpole community.

Fishes are particularly voracious tadpole predators, and can effectively eliminate tadpoles from ponds (Petranka *et al.*, 1987; Brönmark and Edenhamn, 1994; Bridges and Gutzke, 1997). Laurila and Aho (1997) introduced *Gasterosteus aculeatus* (threespine sticklebacks) to some pools, leaving other pools fish-free, to measure the effects of the introduction on the survival of *Rana temporaria* (common frog) tadpoles. They found that female common frogs did not adjust their breeding habitat choice in order to avoid fish preying on their offspring, and that fish predation drastically affected the survival of *R. temporaria* tadpoles in these habitats. Heyer (1976) found that out of ninety-seven ponds fish and tadpoles only co-occurred in one pond. In one pond there were fish one year, but not the next. Tadpoles were present only in the year that the fish were absent, but nests were seen both years, suggesting that the absence of tadpoles was due to predation rather than female choice of breeding habitat (Heyer, 1976).

After studying the rate at which *Dicamptodon* spp. (giant salamanders), *Cottus confusus* (shorthead sculpins), *Salmo clarki* (cutthroat trout) and *Salvelinus fontinalis* (brook trout) consumed tadpoles in the laboratory, Feminella and Hawkins (1994) concluded that these species could eliminate all tadpoles from a stream. Similarly, when *Lepomis cyanellus* (green sunfish) were added to some pools containing *Ambystoma barbouri* (small-mouthed salamander) larvae predation was the major cause of mortality in pools containing fish, while in pools without fish flooding was the most common cause of mortality (Sih *et al.*, 1992). However, increasing predation risk in a habitat may not necessarily lead to higher mortality from predation. For instance if the tadpoles have some behavioural or morphological means of avoiding predation, mortality may remain at the same level, although some other cost, eg. smaller tadpole body size is likely (Ludwig and Rowe, 1990).

The evidence summarised above clearly demonstrates that the addition of a novel predator to a system can have severe effects on existing amphibian populations (Morin, 1983; Wilbur *et al.*, 1983; Travis *et al.*, 1985a; Van Buskirk, 1988; Fauth and

Resetarits, 1991; Sih et al., 1992; Feminella and Hawkins, 1994; Laurila and Aho, 1997). However, some studies produce unexpected results (Ludwig and Rowe, 1990; Crossland, 1998) suggesting that knowledge about the dynamics of predator-prey interactions is not sufficient to confidently predict the effects a new predator will have on a community.

Ways in which amphibian larvae sense predation.

Cues from predators.

Failing to respond to a predator can be very costly for prey species. On the other hand, constantly reacting to predators that are not there can be a waste of energy, and feeding opportunities may be lost. Therefore, there should be strong selection for the development of some kind of predator-detection system (Kiesecker et al., 1999; Chivers et al., 2001). Early detection of a predator allows prey to decrease the chance that an attack will eventuate (Kiesecker et al., 1999).

When a predator is present tadpoles may sense chemical compounds excreted by the predator or by an individual that has been attacked (Spieler and Linsenmair, 1999). The ability to detect predators through chemical cues is particularly well developed in amphibians. *Ascaphus truei* (tailed frog) tadpoles, for example, are sensitive to cues from a wide range of fish species, suggesting that they may be responding to a generalist cue. However, they do not react to shorthead sculpins (Feminella and Hawkins, 1994). Captive *Rana temporaria* (common frog) tadpoles react to chemical cues from fish by abruptly decreasing their activity and avoiding the central section of a test tray (Niecieza, 1999). In a study combining chemical, visual and tactile cues from predators, Stauffer and Semlitsch (1993) showed that tadpoles reacted most strongly to chemical cues regardless of what other cues were present.

Laurila et al. (1998) measured the responses of *R. temporaria* to late-instar *Aeshna juncea* (dragonfly) larvae that had been fed either chironomid larvae, *R. temporaria* tadpoles or *Bufo bufo* (common toad) tadpoles. The tadpoles of both species reacted to predator presence, regardless of predator diet, by avoiding the predator side of the test

container. *B. bufo* tadpoles avoided the predator side significantly more when the predator had been fed *B. bufo* tadpoles compared with insect- or *R. temporaria*-fed predators. *R. temporaria* tadpoles also reduced their activity in response to predator presence. The work of Mathis and Vincent (2000) further illustrates the complexity of prey reactions to predators. *Notophthalmus viridescens louisianensis* (central newt) larvae reduced their activity in response to visual cues from both predatory *Ambystoma tigrinum tigrinum* (tiger salamander) larvae and non-predatory *Hyla chrysochloris/versicolor* (grey treefrog) complex tadpoles. However, when they were given chemical cues they responded only to *A. t. tigrinum*, not to *H. chrysochloris/versicolor*. Lack of visual acuity in amphibian larvae may explain a reliance on chemical cues to fine-tune antipredator responses (Mathis and Vincent, 2000). A different anti-predator response is described for *H. chrysochloris* (McCollum and Leimberger, 1997). In the laboratory *H. chrysochloris* tadpoles developed a predator-resistant morph in response to *Aeshna umbrosa* (dragonfly) larvae only when these had been fed on *H. chrysochloris*. McCollum and Leimberger (1997) suggest the tadpoles may have been responding to a metabolite of the conspecifics, or an alarm substance emitted by them before they died.

Cues from other prey.

Some species of amphibians have been found to respond to alarm substances emitted by other prey individuals (Pfeiffer, 1966; Caldwell, 1982; Hews, 1988; Rödel and Linsenmair, 1997; Adams and Claeson, 1998; Kiesecker *et al.*, 1999). Such responses, in the form of antipredator behaviours, can affect the survival of prey in the presence of predators (Semlitsch and Reyer, 1992). Bridges and Gutzke (1997) point out that for an alarm response system to be efficient, the substance should be detectable in minute concentrations.

B. bufo tadpoles exhibit an alarm reaction when conspecifics are attacked. Even though newly hatched *B. bufo* produce this response it reaches a high point just prior to metamorphosis (Pfeiffer, 1966). An extract can be produced from young metamorphs which also elicits this response (Pfeiffer, 1966). *Bufo calamita* also exhibit an alarm response when conspecifics are attacked, however, a wide variety of

other genera tested do not (Pfeiffer, 1966). In most cases *B. bufo* produces an alarm response when exposed to *B. calamita* skin extracts but not to extracts from non-bufoanids (Pfeiffer, 1966). The skin of *B. bufo* and *B. calamita* tadpoles contains large cells not found in other anuran taxa that are a likely origin for an alarm substance (Pfeiffer, 1966).

Alarm responses have been found in some non-bufoanid species including *Plethodon vehiculum* (western redback salamander) (Chivers et al., 1997), *Phrynomantis microps* (African savanna frog) (Rödel and Linsenmair, 1997), *Hyla regilla* (Pacific treefrogs)(Adams and Claeson, 1998), and *Rana aurora* (red-legged frogs)(Kiesecker et al., 1999). Wilson and Lefcort (1993) found that *R. aurora* tadpoles responded with reduced activity to predators feeding on conspecifics, and to the spilling of tadpole cell contents regardless of whether predators were involved. However, although *Rana cascadae* (Cascades frog) tadpoles became less active in the presence of newt predators, there was no evidence of an alarm response in addition to the observed antipredator response (Hokit and Blaustein, 1995).

Amphibians appear, therefore, to rely mostly on chemosensors to detect predators (Stauffer and Semlitsch, 1993; Wilson and Lefcort, 1993; Mathis and Vincent, 2000). The chemical cues picked up can come either from the predator itself (Stauffer and Semlitsch, 1993; Feminella and Hawkins, 1994; Laurila et al., 1998; Niecieza, 1999; Mathis and Vincent, 2000) or from alarmed or injured prey individuals (Pfeiffer, 1966; Caldwell, 1982; Hews, 1988; Wilson and Lefcort, 1993; Chivers et al., 1997; Rödel and Linsenmair, 1997; Adams and Claeson, 1998; Kiesecker et al., 1999). Tadpoles commonly react to chemical cues by reducing activity (Wilson and Lefcort, 1993; Niecieza, 1999; Mathis and Vincent, 2000), spatially avoiding the area where the cue (and presumably the predator) is found (Adams and Claeson, 1998; Laurila et al., 1998; Niecieza, 1999; Laurila, 2000), swarming (Rödel and Linsenmair, 1997; Spieler and Linsenmair, 1999), and developing predator-resistant morphs (McCollum and Leimberger, 1997).

Methods commonly employed by tadpoles to avoid predation.

Breeding habitat selection.

When laying their eggs, female frogs have a choice of ponds or water bodies supporting different communities, and this can affect the competition tadpoles later face, the quality of food available to them (Wilbur and Alford, 1985), and the predators they may encounter. There are various ways in which female frogs might choose breeding habitats to avoid fish (Heyer, 1976). One option is to breed in ponds that do not tend to have fish in them. Another option is to spread egg bundles out over a wide range of ponds, so that it is likely that some will be in fishless ponds (Heyer, 1976).

In a survey of ponds where all fish were electro-fished out Brönmark and Edenhamn (1994) found that *Hyla arborea* (tree frogs) were significantly more likely to breed in the absence of fish than where fish were present. Furthermore, they described two ponds where reproduction of *H. arborea* began only after fish were removed, and one where reproduction ceased following the introduction of trout and carp. Although it is not certain that *H. arborea* stopped breeding in ponds with fish, (all eggs and hatchlings may have been consumed by fish), males did not call much, suggesting at least lowered reproduction (Brönmark and Edenhamn, 1994).

Many amphibians choose to breed in ephemeral water. Presumably predation pressure is reduced in these sites because predator populations are usually less well-established (Morin, 1987), although predatory salamanders and insects abound in ephemeral pools (Morin, 1983). Woodward (1983) found that in 17 out of 20 choice experiments predators preferred temporary pond species over those from permanent ponds. He also found that permanent ponds tended to contain more individual aquatic predator species. This suggests that temporary pond species lack the defences to withstand predation in permanent ponds.

Breeding late in ephemeral water may not always be disadvantageous because the sites can dry and refill several times in one breeding season (Morin et al., 1990). This may

be why some frog species have long breeding seasons but tend to breed only during rainy periods (Morin et al., 1990).

Spatial avoidance of predators by tadpoles.

One way in which tadpoles avoid predation is by leaving the area. This can include swimming away from predators when they enter the area, or generally avoiding areas where predators tend to be or where chemical cues from predators linger.

In the presence of *Aeshna juncea* (dragonfly larvae) chemicals, *Rana temporaria* (common frog) tadpoles spent less time at the water surface and maintained a greater distance from the inflow pipe bringing the chemicals into the enclosure (Laurila, 2000). However, when the same experiment was repeated using *Perca fluviatilis* (European perch) chemicals tadpoles spent more time at the water surface just above the inflow pipe. Perch are likely to produce a greater volume of chemical cues than *A. juncea*, so a concentration gradient may have formed. If this were the case, the area above the inflow pipe may have actually had the lowest concentration of perch chemicals (Laurila, 2000). According to Relyea and Werner (1999) *Rana catesbeiana* (bullfrog) tadpoles and *Rana clamitans* (green frog) tadpoles also exhibit spatial avoidance responses to *Umbra limi* (mudminnows) and *Anax junius* (dragonfly) larvae.

In a study of a complex system of waterbodies, Sih et al. (1992) found that when fish were added to a pool the proportion of *Ambystoma barbouri* (small-mouthed salamander larvae) in the centre area of the pools decreased. Also, migration out of these pools increased.

Relyea (2001a) studied a number of tadpole species, all of which spatially avoided some predator species. However, none of the tadpoles avoided all four predators tested.

Size refuges.

Some predators are gape-limited, meaning that they can consume prey items only up to a certain size. This affords an opportunity for tadpoles to avoid predation by quickly reaching a large size, thus significantly increasing survival rates (Calef, 1973; McCollum and Leimberger, 1997; Anholt and Werner, 1998). This is usually referred to as a “size refuge” (Travis *et al.*, 1985*a*; Sih and Moore, 1993; Gotthard and Nylin, 1995; Persson *et al.*, 1996; Babbit and Tanner, 1998). The rate at which an individual reaches a size refuge is related to its growth rate which in turn depends on predator density (Persson *et al.*, 1996). The trait of rapid growth can lead to a “growth race” where tadpoles grow quickly to reach a size refuge before predators such as dragonfly larvae are big enough to prey on them (Babbit and Tanner, 1998). Dragonfly larvae are less gape-limited than many other predators because they tend to eat their prey one bite at a time, rather than swallowing them whole (McCollum and Van Buskirk, 1996). However, dragonflies feed to satiation, so a smaller dragonfly will eat less, thus exerting less predation pressure on a population of tadpoles (Travis *et al.*, 1985*b*). Further, in a study using *Rana utricularia* (southern leopard frog) tadpoles Babbit and Tanner (1998) found that small predators were largely ineffective, and there was no significant difference in tadpole survival between treatments with small predators and the controls.

When *Hyla chrysoscelis* (treefrog) tadpoles are exposed to dragonfly larvae they grow to a larger size than would be expected from their activity and food intake (McCollum and Van Buskirk, 1996). These authors believe that the tadpoles may achieve this by increasing metabolic efficiency, changing resource-allocation patterns, or getting extra food by filtering suspended phytoplankton rather than scraping periphyton, and this increased body size may result in an early size refuge.

Size refuges also occur in other groups. For instance *Carassius carassius* (crucian carp) in lakes with fish-eating predators tend to develop deeper bodies than those in ponds without predators. This means they can reach a size refuge at a smaller total body size (Brönmark and Miner, 1992; Brönmark and Pettersson, 1994; both cited in Gotthard and Nylin, 1995). In a similar way *Ambystoma barbouri* (small-mouthed

salamander) eggs tend to hatch later at a larger size in the presence of *Phagocotus gracilis*, a predatory flatworm, giving them a type of size refuge (Sih and Moore, 1993). However, Laurila et al. (2001) found that the presence of *Dytiscus marginalis* (diving beetle), which preys on *Rana temporaria* (common frog) tadpoles, had no effect on hatching times.

Once tadpoles have reached a size refuge, they may, according to Puttlitz et al. (1999), reduce the effort expended in other means of predator avoidance. This is a form of threat-sensitive predator avoidance, where the intensity of a prey animal's antipredator responses is related to its vulnerability to predators (Puttlitz et al., 1999). Niecieza (1999) found that older *Rana temporaria* (common frog) tadpoles were less susceptible to gape-limited predators and could swim faster. They were also less responsive to predator chemicals compared to their younger smaller conspecifics. *Hyla regilla* (Pacific treefrog) tadpoles also reduced their antipredator response as they grew bigger and less vulnerable (Puttlitz et al., 1999).

Differences between sibling cohorts in growth rate thus allow some groups to reach a size refuge before others, resulting in differential survival among groups of related individuals (Travis et al., 1985a). This differential survival may drive further evolution. Predators can also change the structure of prey communities if some prey species outgrow the predator before others do (Travis et al., 1985b).

Changes in life history.

Changes in life history patterns are often referred to as phenotypic plasticity (Van Buskirk, 1988; Skelly and Werner, 1990; Sih and Moore, 1993; Warkentin, 1995; Laurila and Kujasalo, 1999; Van Buskirk and Schmidt, 2000). There are two possible responses, slowing development, thus lengthening the larval period (eg. Skelly and Werner, 1990; Laurila and Kujasalo, 1999; Van Buskirk and Schmidt, 2000; Relyea, 2001b), or increasing development rate so as to metamorphose earlier (eg. Noland and Ultsch, 1981; Van Buskirk, 1988; Warkentin, 1995; Laurila and Kujasalo, 1999). The effect of changing the larval period can carry on into adulthood. For example, *Rana sylvatica* (wood frog) tadpoles that have been previously exposed to predators have

longer larval periods. When they finally do achieve metamorphosis, froglets have longer limbs and narrower bodies (Relyea, 2001*b*).

Unpalatability.

Bufoiid larvae secrete unpalatable compounds as a mean of deterring predators (Pfeiffer, 1966; Kruse and Stone, 1984; Crossland and Alford, 1998), possibly allowing them to relax other defense mechanisms like reduced activity (Laurila et al., 1998). The general unpalatability of *Bufo* tadpoles may also be partly responsible for the success of *Bufo marinus* (cane toads) in Australia (Lawler and Hero, 1997). Although *B. bufo* is eaten by both chewing and sucking predators, more often it is released, indicating that it might be unpalatable to both (Henrikson, 1990). However, in general, predators that swallow tadpoles whole or invertebrate predators that suck body fluids should be less affected by bufotoxin than predators that masticate, bite or somehow taste the tadpoles (Kiesecker et al., 1996). *Bufo boreas* (western toad) tadpoles graded their antipredator responses according to the sensitivity of the predator to bufotoxin (Kiesecker et al., 1996), and Relyea and Werner (1999) obtained similar results for *Rana catesbeiana* (bullfrogs) and *R. clamitans* (green frogs). However, *Bufo woodhousei* (Fowler's toad) tadpoles reduced activity in the presence of both *Notophthalmus viridescens* (newts) and *Enneacanthus obesus* (black-banded sunfish) even though they are palatable only to newts (Lawler, 1989). Unpalatability is exhibited by several other animal groups including aquatic beetles *Dineutes hornii* (Eisner and Aneshansley, 2000) and larval *N. viridescens* (Mathis and Vincent, 2000).

Unpalatable compounds are thought to be produced in the granular glands of the skin. Because these glands develop with age, older individuals tend to become increasingly unpalatable (Mathis and Vincent, 2000). Unpalatable species may also take advantage of predator learning by swarming; a predator is likely to avoid the rest of the swarm after consuming one distasteful individual (Kruse and Stone, 1984).

The literature summarised above demonstrates that unpalatable compounds are produced in several genera of amphibians (Relyea and Werner, 1999; Mathis and Vincent, 2000), although they are most common in bufoinids (Pfeiffer, 1966; Lawler,

1989; Henrikson, 1990; Kiesecker et al., 1996; Lawler and Hero, 1997; Crossland and Alford, 1998; Laurila et al., 1998). The production of unpalatable compounds is thought to increase with age (Mathis and Vincent, 2000), and they may reduce the need for other antipredator defences (Kiesecker et al., 1996; Relyea and Werner, 1999).

Morphological defenses.

In some cases, various elements of morphology develop differently in tadpoles exposed to predators. Most commonly, the size, depth and width of the tail muscle increases relative to total body length (McCollum and Van Buskirk, 1996; Van Buskirk et al., 1997; Van Buskirk and Relyea, 1998; Van Buskirk and McCollum, 1999; Van Buskirk, 2000; Van Buskirk and McCollum, 2000; Laurila et al., 2001; Relyea, 2001*a*), although some of the species tested in Relyea's (2001*a*) experiment developed shallower tails in the presence of predators. Tail size affects the transient propulsion of the caudal fin, which is important for rapid turns and acceleration (McCollum and Van Buskirk, 1996; Lardner, 1998; Van Buskirk and Schmidt, 2000). Colour can also be modified, with some species developing spots on the tail, or even completely changing tail colour (Caldwell, 1982; McCollum and Van Buskirk, 1996; McCollum and Leimberger, 1997; Van Buskirk and McCollum, 1999; Van Buskirk and McCollum, 2000) in order to distract predator attention away from the more vulnerable body (Caldwell, 1982; McCollum and Van Buskirk, 1996; McCollum and Leimberger, 1997). Tadpoles caught by the tail may also be more likely to wriggle free (Van Buskirk and McCollum, 2000). Van Buskirk (2000) suggests that morphological defenses should be the most costly, and therefore be reduced in conditions where food is scarce. Morphological modifications can be seen very soon after hatching, indicating that either the responses were already present at hatching, or they develop very rapidly (Laurila et al., 2001).

Overall it appears that predator morphs enjoys better survival in the presence of predators (Caldwell, 1982; McCollum and Van Buskirk, 1996; Van Buskirk et al., 1997; Van Buskirk, 2000; Van Buskirk and McCollum, 2000), but may be at a disadvantage when predators are absent, compared to non-predator morphs (Van

Buskirk et al., 1997). In contrast, *Rana clamitans* (green frog) development slows in the presence of predators, so that stage, but not total length is affected (Thiemann and Wassersug, 2000). Tadpoles developing in the presence of predators had significantly wider bodies and shallower tails than those not exposed to predators. Perhaps in these tadpoles resources are allocated to accelerated growth, allowing them to escape predators earlier (Thiemann and Wassersug, 2000). In general, when variation in predator presence is temporal rather than spatial polyphenisms will be favoured (Moran, 1992).

In summary, tadpoles can alter their morphology in the presence of predators. The two most common changes are an increase in tail size (McCollum and Van Buskirk, 1996; Van Buskirk et al., 1997; Van Buskirk and Relyea, 1998; Van Buskirk and McCollum, 1999; Van Buskirk, 2000; Van Buskirk and McCollum, 2000; Laurila et al., 2001), and a change in tail colour (Caldwell, 1982; McCollum and Van Buskirk, 1996; McCollum and Leimberger, 1997; Van Buskirk and McCollum, 1999; Van Buskirk and McCollum, 2000). These tend to be plastic changes expressed only when predators are present because in the absence of predators, predator-resistant morphs tend to experience lower survival (Van Buskirk et al., 1997).

Kin selection.

Tadpoles of various species aggregate in order to lessen individual probability of predation (Milinski, 1979; Kruse and Stone, 1984; Semlitsch and Reyer, 1992; Hokit and Blaustein, 1997; Rödel and Linsenmair, 1997; Watt et al., 1997; Spieler and Linsenmair, 1999; Laurila, 2000). In some species aggregations appear to be kin-biased (Blaustein and O'Hara, 1987; Blaustein et al., 1993; Pfennig et al., 1993; Hokit and Blaustein, 1997; Niecieza, 1999). Blaustein et al., 1993 noted that *Rana aurora* (red-legged frog) tadpoles discriminate between kin and non-kin in early developmental stages, but cease to do so later on in their development. Even in early larval stages, *R. aurora* exposed to non-kin lose their preference to associate with kin (Blaustein et al., 1993). Possibly the recognition signal is lost later in life because the tadpoles vulnerability to predation diminishes, and therefore aggregations are of less use. *Rana cascadae* (Cascades frog) tadpoles also prefer to aggregate with kin

(Blaustein and O’Hara, 1987). One theory on how aggregations might benefit the individual is that predators are less successful when faced with aggregations (Hokit and Blaustein, 1997). However, the presence of predators reduced aggregations in *R. cascadae*, which contradicts this theory (Hokit and Blaustein, 1997). Possibly the recognition of kin could help related individuals reform groups after disturbances such as predation (Blaustein and O’Hara, 1987).

The literature cited gives various examples of kin recognition in tadpoles (Blaustein and O’Hara, 1987; Blaustein et al., 1993; Pfennig et al., 1993; Hokit and Blaustein, 1997; Niecieza, 1999), and suggests that the most likely benefit of kin recognition is helping individuals form aggregations which can lessen individual predation risk (Milinski, 1979; Kruse and Stone, 1984; Semlitsch and Reyer, 1992; Hokit and Blaustein, 1997; Rödel and Linsenmair, 1997; Watt et al., 1997; Spieler and Linsenmair, 1999; Laurila, 2000).

Aggregations.

Aggregations in tadpoles are thought to have a number of functions, including increased feeding efficiency and reduced risk to individuals of predation (eg. Hokit and Blaustein, 1997). One of the ways in which aggregations can reduce predator success is through the “confusion effect” described by Miller (1922; cited in Milinski, 1979): “when attacking a swarm (school), the predator’s attention is diverted by a great number of simultaneously visible targets. The predator hesitates before attacking because it has difficulty in taking aim at one of the many similar prey targets before it is quickly masked by another individual”. Several species of amphibians form aggregations in response to predation cues, including *Phrynomantis microps* (African savanna frog) (Rödel and Linsenmair, 1997), *Bufo maculatus* (Spieler and Linsenmair, 1999), and *Bufo bufo* (common toad) (Watt et al., 1997). However, in *R. cascadae*, aggregation frequency is significantly higher when predators are few suggesting that these aggregations do not serve an antipredator function (Hokit and Blaustein, 1997).

As mentioned above, in distasteful tadpoles such as bufonids aggregations may serve an antipredator function by taking advantage of predator learning. A predator may

attack one tadpole from the aggregation, find it distasteful and leave without approaching any other tadpoles. This might increase an individual's chance of survival (Kruse and Stone, 1984). Coming to the surface for air may increase the risk of predation as an individual must move away from refuge and travel up through the open water column. Synchronous breathing as observed in *Xenopus laevis* may reduce predation risk at such times in a similar way to other types of aggregation (Baird, 1983; cited in Lima and Dill, 1990).

Aggregations therefore form in a variety of frog species in response to predator cues (Kruse and Stone, 1984; Lima and Dill, 1990; Rödel and Linsenmair, 1997; Watt et al., 1997; Spieler and Linsenmair, 1999), and can therefore be assumed to have some antipredator function.

Use of refuges.

Many animals flee into a refuge when predators appear. Refuges can consist of crevices, plants and other geographical habitat features. Residence in refuges acts as a primary defence before detection or attack by a predator. Withdrawing into a crevice is a secondary behavioural defence which acts during an encounter with a predator (Cooper et al., 1999). Increased shelter use and decreased movement may be particularly important defence behaviours from predators that catch prey by detecting movement (Kiesecker et al., 1999). For example when *Rana utricularia* (southern leopard frog) tadpoles are exposed to predators, survival is better at higher cover levels (Babbitt and Tanner, 1998). In caged predator trials using *Triturus alpestris* (alpine newt) and *T. helveticus* (palmate newt) larvae both species avoided the open (Van Buskirk and Schmidt, 2000).

In laboratory experiments (Kiesecker et al., 1999) *Rana aurora* (red-legged frog) tadpoles decreased activity, increased shelter use and spatially avoided conspecifics that had been disturbed, compared to controls. However, when exposed to non-disturbed conspecifics, they increased activity and did not change shelter use or distance between individuals (Kiesecker et al., 1999). Lefcort et al. (1999) found that *Rana luteiventris* (Columbia spotted frog) tadpoles exposed to odour from fish fed

with conspecifics spent more time in refuge and moved less than tadpoles exposed to odour from fish fed crickets or worms.

In nature tadpoles may react promptly to a predator. Kats et al. (1988) showed that within 2.5 hours of the addition of predatory fish palatable tadpoles from permanent ponds shifted to a refuge. There were differences between congeneric tadpoles, and conspecific tadpoles from habitats with different predator loads, suggesting that this refuge behaviour is genetic. Sih et al. (1992) also found that *Ambystoma barbouri* (small-mouthed salamander) larvae generally spent less time in the centre section of waterbodies, and more time under rocks, particularly after fish were added. The benthic area thus acts as an important refuge from visually foraging predators because light decreases with depth, tadpoles are more cryptic against an irregular, similarly coloured background than in the water column, and motion near the bottom, may stir up silt, further hiding them (Lawler, 1989).

In more complex habitats *Anax junius* (dragonfly larvae) predation efficiency was reduced (Folsom and Collins, 1984) suggesting that plants were associated with the biggest drop in predation success, followed by gravel substrate, sand substrate and soda straws. When the actual quantity of water plants present was increased, *A. junius* predation efficiency was lower still.

Sih et al. (1988) warned that snapshot observations of how many prey are in refuge at any one time provides limited information about prey refuge use, because there are no indications of prey emergence rates and probable predator attack rates. They found that when *Lepomis macrochirus* (bluegill sunfish) were added to an enclosure containing *Ambystoma texanum* (small-mouthed salamanders) larvae, the larvae showed a significant decrease in emergence rates and a significant increase in re-entry rate. Emergence from refuge can also be affected by other prey needs, eg. mating, foraging (there is usually less food in the refuge), and aggression. In this study, hungry larvae showed a significantly higher emergence rate from refuge.

The literature demonstrates that refuge use is a very important aspect of antipredator behaviour for many species, with plants and the benthos providing the most useful

refuges (Folsom and Collins, 1984; Lawler, 1989). Tadpoles may retreat into refuges in response to chemical cues as well as predators (Kiesecker et al., 1999; Lefcort et al., 1999).

Decreasing activity.

In the presence of predators, prey often decrease their activity to avoid detection (Lawler, 1989; Skelly, 1992; Feminella and Hawkins, 1994; Hokit and Blaustein, 1995; McCollum and Van Buskirk, 1996; Van Buskirk and McCollum, 2000; Relyea, 2001a; Richardson, 2001). This method of defence is particularly effective against visually foraging predators (eg. many fishes) or predators that sense their prey through tactile means, such as dragonfly larvae (Kiesecker et al., 1999). Consumption of prey by *Anax* (dragonfly) larvae increased steadily with prey activity until a plateau was reached and prey capture became independent of activity (Folsom and Collins, 1984). Similarly, Pritchard (1965) found that dragonfly larvae struck at stationary objects that had been moving recently, but prey movement during the preparation for the strike appeared necessary. However, although *Pseudacris triseriata* (chorus frog) tadpoles reduce activity in the presence of *Anax* larvae, relatively inactive tadpoles do not survive better in the presence of *Anax* than the more active groups (Van Buskirk et al., 1997), which suggests that changes to levels of activity are not successful in all species. Van Buskirk and McCollum (2000) found that while overall changes in activity level did not affect susceptibility of *Hyla versicolor* (treefrog) tadpoles to predation by *Ambystoma tigrinum* (tiger salamanders) and *Anax longipes* (dragonfly) larvae, time spent swimming did. Thiemann and Wassersug (2000) found that the reduction in activity in response to predators is stronger when trematode parasites are also present. The effect of trematodes on tadpole life history may be more pronounced in temporary ponds (Kiesecker and Skelly, 2001). At high food levels tadpoles also show a stronger decrease in activity (Laurila et al., 1998; Van Buskirk and McCollum, 2000), which may lead to a decrease in predation in those situations (Anholt and Werner, 1995). The magnitude of the reduction in activity shown may be dependent on how serious the threat is (Relyea, 2001a).

As demonstrated above, reducing activity is a widely exhibited response to predation (Lawler, 1989; Skelly, 1992; Feminella and Hawkins, 1994; Hokit and Blaustein, 1995; McCollum and Van Buskirk, 1996; Laurila *et al.*, 1998; Thiemann and Wassersug, 2000; Relyea, 2001*a*), but the magnitude at which the response is expressed depends on other environmental variables such as parasite presence (Thiemann and Wassersug, 2000) and food availability (Laurila *et al.*, 1998).

The reduction of food intake as a consequence of reduced activity.

Resource-rich patches tend to have the most prey and the most predators (Feminella and Hawkins, 1994). Therefore, by spatially avoiding predators, tadpoles can experience a reduction in food intake (eg. Relyea and Werner, 1999). Reducing activity also decreases food intake, because tadpoles tend to feed by moving and scraping periphyton off surfaces. Thus, tadpoles are faced with a conflict (McNamara and Houston, 1987; Anholt and Werner, 1998); they must avoid being preyed on whilst still garnering enough resources to successfully metamorphose. This conflict is particularly important for tadpoles inhabiting ephemeral waterbodies, because there is a limited time before they must metamorphose or die from desiccation. Even for tadpoles in permanent water bodies, there is a cost to lengthening the larval period because the longer they remain in the water, the greater their cumulative chance of predation (Lawler, 1989; Niecieza, 1999). In general, tadpoles living in habitats with fewer predators tend to be more active (Laurila, 2000).

Two components of activity influence the risk of predation; speed while foraging, and the proportion of time spent foraging (Werner and Anholt, 1993). The effect of an increase in tadpole speed depends on the relative speed of tadpole and predator (Werner and Anholt, 1993). If the tadpole is much slower than the predator an increase in tadpole speed may have little effect on the encounter rate. If, however, the tadpole can move quite fast in relation to the predator, any increase in speed can have a dramatic effect on encounter rate (Werner and Anholt, 1993).

Many models have been developed to try and predict optimal tadpole behaviour in the face of various levels of predation and resource supply (eg. Ludwig and Rowe, 1990;

Abrams, 1992; Werner and Anholt, 1993). In a study comparing the responses of *Pseudacris crucifer* (spring peeper) and *P. triseriata* (chorus frog) tadpoles to predation risk and food availability, Skelly (1995) found that food availability affected the reduction in activity for *P. triseriata*, with a weaker response when food was short. However, food supply had no effect on the reduction in activity for *P. crucifer*. *P. crucifer* breeds in permanent ponds with many predators, thus they may have a fixed response to predators. *P. triseriata*, on the other hand, breeds in temporary ponds where there are fewer predators, which may explain the maintenance of plasticity in their antipredator responses (Skelly, 1995).

In the presence of caged *Anax junius* (dragonfly) larvae *Rana sphenoccephala* (southern leopard frog) tadpoles reduced their activity to the same low level regardless of the amount of food they were receiving (Babbit, 2001). Tadpoles that were on a high-food treatment had reduced growth rates and metamorphosed at a smaller size when *A. junius* was present. In the low food treatment, although tadpoles did reduce their activity their growth rate was not reduced. They were slightly larger at metamorphosis, and had a significantly longer larval period (Babbit, 2001).

Overall, reduced activity in the presence of predators commonly leads to reduced growth rates (eg. Ball and Baker, 1996; Relyea and Werner, 1999; Van Buskirk and Schmidt, 2000)

The quality of food available, as well as the amount, is important to tadpoles. For example in the presence of predators, mortality of *Rana temporaria* (common frog) tadpoles is higher when only phytoplankton is available for feeding than when only benthic food is available, or both sources of food (Eklöv and Halvarsson, 2000). This is because phytoplankton is a lower quality food resource. Tadpole activity decreased in response to predators in the benthic treatment, and benthic combined with phytoplankton treatment, but not in the phytoplankton only treatment. Structural complexity in the phytoplankton treatment was lower, which may partly account for the heightened mortality rate in that situation (Eklöv and Halvarsson, 2000).

General costs of antipredator defenses.

As mentioned above, certain antipredator behaviours such as reduction of activity have an associated cost. Commonly this cost is reduced growth and slower development, which in turn can lead to a longer larval period, reduced size at metamorphosis, and reduced adult breeding success (Smith, 1987; Skelly and Werner, 1990; Wilson and Lefcort, 1993; Beck and Congdon, 1999). While these costs are not severe when compared to the alternative, death, they can still present a considerable disadvantage to an individual which incurs them unnecessarily. In the case of group antipredator behaviour, the cost of the behaviour is paid by all the prey individuals, unlike the individual cost of predation which is paid only by those animals that are eaten (Ives and Dobson, 1987).

Antipredator mechanisms likely to come with an associated cost include aggregations (Spieler and Linsenmair, 1999), physical features such as shells or toxicity (Werner and Anholt, 1993), shortened larval periods to escape aquatic predators sooner (Berven, 1990), deeper tail fins and other morphological plasticity (Smith and Van Buskirk, 1995; McCollum and Van Buskirk, 1996; Van Buskirk and Relyea, 1998).

Genetic origins of antipredator defenses.

Little is known about the genetic origins of antipredator behaviour (Semlitsch and Reyer, 1992), but much behaviour is plastic and is maintained in many populations and species experiencing different predator regimes. This may prevent populations from evolving and diverging (Laurila, 2000).

In some cases phylogenetic patterns are mirrored by antipredator behaviours. For example, Richardson (2001) found that phylogenetic relationships of a number of anuran groups were correlated with the magnitude of the decrease in activity that they exhibited in the presence of predators.

Indirect effects of predation on prey communities.

Many studies document the indirect effects of predators on prey communities (eg. Holomuzki, 1986; McNamara and Houston, 1987; Fauth, 1990; Henrikson, 1990; Fauth and Resetarits, 1991; Werner, 1991; Sih, 1992; Skelly, 1992; Werner, 1992; Soluk, 1993; Wissinger and McGrady, 1993; Holt and Lawton, 1994; Werner and McPeck, 1994; Anholt and Werner, 1998; Babbit and Tanner, 1998; Relyea and Werner, 1999; Van Buskirk, 2000). Indirect effects are those which cannot be anticipated from combining simple pair-wise interactions. For example, the decimation of one prey population might cause an increase in another population through competitive release or the removal of its predator (eg. Van Buskirk and Yurewicz, 1998). As antipredator behaviour becomes more efficient, the amplitude of fluctuations typical of predator-prey systems are damped (Ives and Dobson, 1987).

If two prey species experience different predation pressures from two predators, predator community structure may impact on prey competitive interactions. For example, *Rana catesbeiana* (bullfrog) tadpoles are more vulnerable to predation from *Anax* (dragonfly) larvae than are *R. clamitans* (green frog) tadpoles, but *R. clamitans* are more vulnerable to fish. Fish also prey on *Anax* (Werner, 1992). *Notophthalmus viridescens* (newts) are keystone predators, whose addition to a system can change competitive interactions. Because *N. viridescens* prey preferentially on dominant tadpole species, subordinate species experience competitive release and flourish (Fauth and Resetarits, 1991).

Effects of environmental factors on antipredator defenses.

Bufo boreas (western toad) metamorphs that had been exposed to UV-B radiation exhibited less avoidance of conspecific alarm cues than metamorphs who had not been exposed to UV-B (Kats et al., 2000). The same trend was seen in *Taricha torosa* (newt) larvae, but the results were not significant (Kats et al., 2000). *Rana aurora* (red-legged frog) tadpoles exposed to UV-B also showed a non-significant reduction in avoidance of the predator side of the tank, and a significant increase in activity in the presence of predators compared with non-exposed conspecifics (Kats et al., 2000).

Pollutants (Lefcort *et al.*, 1999) and physical tissue damage (Semlitsch, 1990; Figiel and Semlitsch, 1991) can also affect the strength and efficacy of antipredator mechanisms.

The chapters that follow describe experiments on the use of space by whistling frog (*Litoria ewingi*) tadpoles and mosquitofish (*Gambusia affinis holbrooki*) and the predator-prey interaction between these two species. The reason for exploring microhabitat use by these two species was to see whether they would be likely to encounter each other in a natural setting. In predation experiments the focus was on the behaviour of both species and the effect of habitat complexity on predation events. This work relates to many of the topics discussed above.

Summary.

1. Amphibians employ a wide variety of antipredator defenses including: selecting breeding habitats without predators (eg. Brönmark and Edenhamn, 1994), spatially avoiding predators (eg. Laurila, 2000), accelerating growth to reach a size refuge quickly (eg. Anholt and Werner, 1998), producing unpalatable compounds (eg. Laurila *et al.*, 1998), modifying morphology to be less susceptible to predation (eg. McCollum and Van Buskirk, 1996), aggregating (eg. Spieler and Linsenmair, 1999), entering refuges (eg. Lefcort *et al.*, 1999), and reducing their general activity (eg. Relyea, 2001a).
2. However, these defenses can cost individuals dearly if they are employed in the absence of predators (Berven, 1990; Werner and Anholt, 1993; Smith and Van Buskirk, 1995; McCollum and Van Buskirk, 1996; Van Buskirk and Relyea, 1998; Beck and Congdon, 1999; Relyea and Werner, 1999; Spieler and Linsenmair, 1999).

Samenvatting.

1. Amfibieën gebruiken verscheidene anti-roofdier verdedigingsmethodes waaronder: de keus hun eieren leggen in water met minder roofdieren (bv. Brönmark en Edenhamn, 1994), het ruimtelijke vermijden van roofdieren

(bv. Laurila, 2000), vlug groeien om eerder een veilige maat te bereiken (bv. Anholt en Werner, 1998), de productie van onsmakbare stoffen (bv. Laurila *e. a.*, 1998), het modificeren van de morfologie om minder blootgesteld te zijn aan plundering (bv. McCollum en Van Buskirk, 1996), aggregatie (Spieler en Linsenmair, 1999), het binnentreden van schuilplaatsen (bv. Lefcort *e. a.*, 1999), en het verminderen van hun algemene bedrijvigheid (bv. Relyea, 2001*a*).

2. Hoe dan ook, deze verdedigingsmethodes kunnen kostelijk zijn voor de individueel als ze worden gebruikt in de afwezigheid van roofdieren (Berven, 1990; Werner en Anholt, 1993; Smith en Van Buskirk, 1995; McCollum en Van Buskirk, 1996; Van Buskirk en Relyea, 1998; Beck en Congdon, 1999; Relyea en Werner, 1999; Spieler en Linsenmair, 1999).

Chapter 3

**Use of microhabitat by whistling frog (*Litoria ewingi*)
tadpoles.**

Introduction.

Litoria ewingi (whistling frog or brown tree frog) was introduced to New Zealand from Australia (Robb, 1980). It is not known to be in decline in New Zealand, but other *Litoria* species are declining in Australia (White and Pyke, 1996), and localised breeding failures do occur in New Zealand (Van Tol, unpublished data). In New Zealand, whistling frogs are found in the Manawatu and Wellington regions, down the west coast of the South Island, and in Southland (Gill, 1978; Robb, 1980).

Whistling frogs have an extended breeding season from March to December (Robb, 1980), with peak breeding activity in July (Alderton, 1982). Eggs are laid in several bundles with a total clutch size of usually more than 400 (Alderton, 1982). Newly metamorphosed frogs are first seen in November, and in general most tadpoles have metamorphosed and dispersed by the following May (Gill, 1978). Metamorphs then go through a period of growth before reaching maturity, they can more than double in length before they are full grown (Aylward, 1978).

When female frogs are selecting breeding sites, submerged vegetation and alga content appear to be most important (Alderton, 1982). The tadpoles feed on surface algae (Dickman, 1968; Alderton, 1982; pers. ob.).

As detailed in the previous chapter, predation can have large-scale effects on amphibian populations (Morin, 1983; Wilbur et al., 1983; Travis et al., 1985a; Petranka et al., 1987; Van Buskirk, 1988; Semlitsch and Reichling, 1989; Ludwig and Rowe, 1990; Fauth and Resetarits, 1991; Brönmark and Edenhamn, 1994; Feminella and Hawkins, 1994; Bridges and Gutzke, 1997; Laurila and Aho, 1997; Crossland, 1998). Encounter rates have a major effect on the predation rate (Werner and Anholt, 1993). Anuran larvae exhibit various mechanisms for decreasing their encounter rates with predators, including reduction of activity (Lawler, 1989; Skelly, 1992; Feminella and Hawkins, 1994; Anholt and Werner, 1995; Hokit and Blaustein, 1995; McCollum and Van Buskirk, 1996; Laurila et al., 1998; Thiemann and Wassersug, 2000), retreat into refuge (Folsom and Collins, 1984; Kats et al., 1988; Sih et al., 1988; Lawler, 1989; Sih et al., 1992; Babbit and Tanner, 1998; Kiesecker et al., 1999; Lefcort et al.,

1999; Van Buskirk and Schmidt, 2000), and spatial avoidance of predators (Sih et al., 1992; Relyea and Werner, 1999; Laurila, 2000). However, differences between predators and prey in microhabitat use may also lead to decreased encounter rates. A predator that preys heavily on one species may not have as large an effect on another due to differences in use of the microhabitat. Thus, in order to estimate the effects of a newly introduced predator species on an existing potential prey species, we cannot rely solely on laboratory palatability experiments. We must also evaluate microhabitat use by the two species in order to ascertain whether they are likely to encounter each other in field situations.

This chapter investigates the use of microhabitat by whistling frog tadpoles under laboratory conditions. Preferences for regions and for various habitat features added to the aquarium were assessed by recording the position of tadpoles in the water column. The role of ontogeny in determining tadpole microhabitat preferences was also considered through the use of two distinct size groups. In Chapter 4, the results of similar experiments with predacious *Gambusia affinis holbrooki* (mosquitofish) are presented.

Materials and methods.

L. ewingi tadpoles used in these experiments were obtained from two locations. The main site was a large trough in the Esplanade, Palmerston North, where *L. ewingi* have been breeding for eight years. In this trough tadpole density is high during the peak breeding months (pers. ob.), and tadpoles frequently over-winter. No other vertebrate species live in the trough, although predators may visit it from time to time (eg ducks). The trough holds an invertebrate community consisting mainly of insect larvae, plus alga-covered branches that provide grazing for the tadpoles. Egg bundles were collected from the trough and reared in the laboratory to small tadpoles. These tadpoles were used in experiments on the use of space by small tadpoles and the laboratory and field experiments on direct attack by mosquitofish (covered in Chapter 5). Tadpoles raised from egg bundles collected in the Nelson area by Bruce Thomas (Landcare, CRI) were reared in the laboratory and used in experiments on the use of

space by large tadpoles. It was necessary to have two different sources of tadpoles because local frogs were late in breeding and therefore unavailable.

All the tadpoles were reared in an aquarium identical to the test aquarium, measuring inside 40 x 30 x 30 cm, and made from 5 mm thick window glass. In the rearing tank, tadpoles were given a gravel substrate in which a small plant (*Lindernia rotundifolia*) was fixed, and were fed ad lib. on a mixture of *Spirulina* chips and fish food (Wardley spirulina discs and Masterpet Multimix gold fish food). The tank was held in a controlled temperature (15-17°C) room with a light: dark cycle of 11:13 hours. Water in both the rearing tank and the experimental tank was tap water aged using Masterpet Water Ager.

Animals used for the small tadpole experiments were less than 10 mm long snout to vent (Gosner stage 24-31, Gosner, 1960). Animals used for the large tadpole experiments were more than 10 mm long (Gosner stage 37-40). The tadpoles were measured in a wet petrie dish over a sheet of paper marked in mm and those fitting the size requirement were housed in a separate tank.

Fifty tadpoles of each size class were used for each treatment of the experiment on the use of space. Individuals were re-used when possible. The fifty tadpoles (ten individuals times five replicates) were kept together and all replicates of any treatment were run on the same day. The ten tadpoles for each replicate were drawn from the experimental tank. After the experiment they were held in a separate tank. Therefore the composition of replicate groups changed between treatments. Tadpoles that died or outgrew their class were replaced from the rearing tank. Small tadpoles were replaced if they grew to more than 10 mm snout to vent, and large tadpoles if the front limbs emerged. Large tadpoles could not be used after forelimb emergence because their behaviour changes significantly at this point. Tadpoles with emerged forelimbs were observed “sitting” in the plants, a behaviour unknown in the other large tadpoles. Forelimb emergence is also widely viewed to be the mark of metamorphic climax among anurans and tadpoles usually stop feeding at this point (Skelly and Werner, 1990).

The experiments on large tadpoles were carried out from 26 October 1999 to 10 December 1999, while those on small tadpoles experiments took place between 15 February 2000 and 8 March 2000.

The experimental tank was marked on the outside in the following way (Figure 3.1):

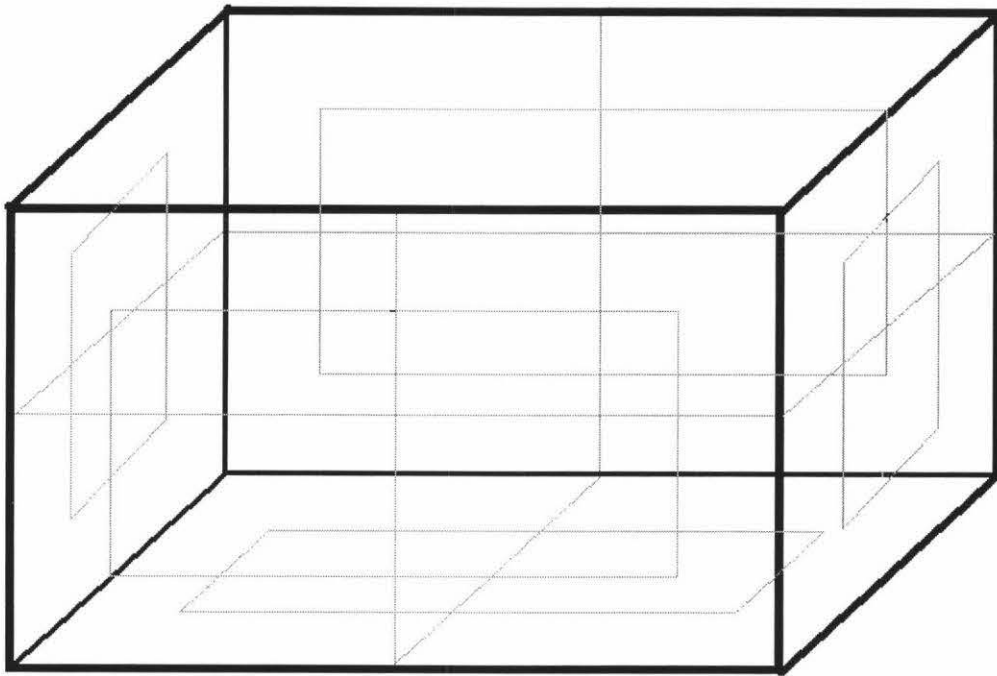


Figure 3.1: Diagram to show water body and lines used to demarcate the different areas.

- A vertical line separated the left from the right (20.5 cm from the outside left edge).
- A horizontal line separated the top half from the bottom half (12.5 cm from the bottom for control treatment, 15 cm from the bottom for all other treatments to allow for the 5 cm layer of gravel on the bottom).
- Boxes were marked on each surface to mark out the half edge and half centre habitats. For the control treatment, the edges of these boxes were 3.1 cm from each edge, giving an edge area of volume c. 14,900 cm³ and a centre volume of c. 15,100 cm³. For the treatments containing a gravel substrate, the edges of the boxes were 2.8 cm from each edge, to give an edge area of volume 11,900 cm³, and a centre volume of 12,000 cm³.

Nine treatments were tested in the following order:

1. Control: Nothing in the tank but water.
2. Gravel: A five-cm layer of gravel was placed at the bottom of the tank.
3. Gravel versus sand: The left hand side of the tank had a 5 cm layer of gravel (mean diameter c. 5 mm) on the bottom, the right hand side had a 5 cm layer of coarse stream sand (mean diameter c. 0.5mm). The sand was sieved and baked to remove possible diseases and other living organisms.
4. Shade: The left-hand side of the tank was shaded by placing black paper over the side, top, and back of the tank, and speckled overhead projector sheets (black paper photocopied onto OHP sheets) over the front to allow viewing.
5. Simple plant: A clump of architecturally simple plants (*Acorus gramineus*) occupied the left two thirds of the left-hand half of the tank (one third of the total tank volume). These plants provided a habitat feature with relatively little complexity compared to the architecturally complex plant used in the “complex plant” treatment.
6. Complex plant: A clump of architecturally complex plants (*Lindernia rotundifolia*) occupied the left two thirds of the left-hand half of the tank. This species was also present in their rearing tank so this may have meant that the tadpoles were more familiar with the “complex” plant than the “simple” plant. However, a different individual plant was used, to avoid the possibility of tadpole odours being associated with the plant.
7. Simple versus complex plant: A clump of “simple” plants on the left hand side (occupying two thirds of this volume), and a clump of “complex” plants on the right hand side (occupying two thirds of this volume).
8. Alga chip: An alga chip (of the sort regularly fed to the tadpoles) was placed along the left-hand wall of the tank, and allowed to soften for 15 minutes before the tadpoles were introduced.
9. Mosquitofish: Three mosquitofish that had been starved for 48 hours were placed in a mesh cage along the left-hand wall of the tank.

Each experiment was started by placing 10 tadpoles in the experimental tank, and leaving the room to allow them to acclimatise. The tadpoles were left for 15 minutes,

after which I entered the room and sat directly in front of the tank. I sat still for a further 5 minutes, after which observations were made every minute for 10 minutes, to give ten observations of habitat choice (left versus right, top versus bottom, and edge versus centre). A similar experimental design was used by Petranka *et al.* (1987) when they investigated tadpole responses to chemical cues from prey, competitors and predators. For some trials (simple plant, complex plant, simple versus complex plant, alga chip and mosquitofish) the edge versus centre observations were not taken. Instead, I recorded every minute how many tadpoles were in contact with the stimulus object. Tadpoles were considered to be in contact with an object if any body part was directly touching the object. For the mosquitofish treatment, touching the cage was considered contact with the stimulus object. Throughout the observation time, I sat still so as to distract the tadpoles as little as possible.

Analysis.

The response of tadpoles to habitat structures was recorded as the proportion occupying the half of the tank containing the structure. Proportions were arc-sin transformed before performing ANOVA.

Initially, a two-way ANOVA was carried out using tadpole size and treatment to investigate use of the left half of the tank, the top half, and the edge. In cases where the ANOVA showed a significant interaction term, pairwise comparisons of treatments against the control were done separately for each size class. Pairwise comparisons were also used to compare the large and small tadpoles' contact with the alga chip, mosquitofish, simple, and complex plants. The Bonferroni method was used for pairwise comparisons (SYSTAT Version 8; SPSS, 1998). P values referred to are corrected probabilities.

A large bias towards use of the right side of the tank was exhibited by tadpoles, particularly in the empty tank ("control") treatment. There was a significant difference between the "gravel" (gravel on both sides of the tank) and the "control" (empty tank) treatments, with the "gravel" treatment being less skewed to the right. Therefore, I

used the “gravel” treatment as a control for all pairwise comparisons concerning the use of the left half of the tank.

Results.

Comparisons with the control (movements to the left, top or edge).

In the two way ANOVA, there was a significant effect of stimulus type (Table 3.1), meaning that tadpoles in general responded differently depending what environmental stimulus they were being exposed to. More important, however, is the significant interaction term (Table 3.1), which indicates that different sized tadpoles respond differently to the stimuli.

| Dependent variable | Tadpole type | Stimulus type | Interaction term |
|------------------------------|---------------------|----------------------|-------------------------|
| Left | NS | 0.001673 | 0.000001 |
| Top | NS | 0.000015 | <0.000001 |
| Edge | NS | NS | NS |
| Contact with stimulus | 0.000725 | 0.000007 | 0.003938 |

Table 3.1. ANOVA probabilities for use of microhabitat by tadpoles.

Pairwise comparisons with the control (gravel treatment for the use of the left) were done to see which stimuli the tadpoles were responding differently to. Only those pairwise comparisons that gave significant results are presented here.

In the presence of simple plants small tadpoles moved to the upper half of the water column ($P=0.026240$). They did the same in the presence of both complex and simple plants together ($P=0.004896$), although there was no significant attraction to the top half of the tank in the presence of complex plants alone. Conversely, large tadpoles moved to the bottom half of the tank in the presence of simple plants alone ($P=0.000080$) and in the presence of simple plants and complex plants together ($P=0.000016$). Large tadpoles also moved to the right, though not significantly ($P=0.006871$) in the presence of simple plants. When an alga chip was added to the

bottom left side of the tank small tadpoles moved to the upper half of the water ($P=0.024512$), while large tadpoles moved towards the left ($P=0.020146$) and bottom ($P=0.000631$). Although they were not significantly attracted to mosquitofish ($P=0.074690$), small tadpoles certainly did not avoid them. Large tadpoles spent more time in the lower part of the water column in the presence of mosquitofish ($P=0.000592$).

Both small and large tadpoles showed a very large bias towards the edge in all treatments, which remained regardless of which stimulus the tadpoles were exposed to.

Comparisons between tadpole sizes for contact with stimulus.

There was a significant difference between the tadpole size classes for contact with stimulus (Table 3.1). Stimulus type also had a significant effect. Again, there was also a significant interaction term (Table 3.1), indicating that the treatment affected the difference between tadpoles with respect to the amount of contact with the stimulus. In this case there was no control treatment to compare results to, therefore I did pairwise comparisons of small and large tadpoles. The only significant result was that large tadpoles were more likely than small tadpoles to be in contact with the alga chip ($P=0.000132$).

Discussion.

Ontogenic changes in tadpole behaviour.

There was a difference between small and large tadpoles in the amount of contact they had with the stimuli. Overall, large tadpoles were more likely to be in contact with stimuli.

There was no other consistent pattern of change in the behaviour of tadpoles during their development. This was somewhat surprising because recent studies have noted shifts in behaviour during development as metabolic and behavioural trends (eg.

resource allocation, predator avoidance, growth rates) change (Ludwig and Rowe, 1990; Blaustein *et al.*, 1993; Werner and Anholt, 1993; Babbit and Tanner, 1998; Niecieza, 1999). Microhabitat preferences of *Rana catesbeiana* (bullfrog) (Smith, 1999), *Limnaoedus ocularis*, *Bufo terrestris*, and *Rana utricularia* (Alford, 1986) tadpoles change over the course of their development, although tadpoles of *Pseudacris ornata* do not appear to show such a change in behaviour over ontogeny (Alford, 1986).

There was a significant interaction effect in all cases except the use of edge, which suggests that behaviour under various treatment regimes was in some way influenced by the tadpole size class in question.

Use of the edge of the tank.

Tadpoles showed a very high attraction to the edge of the tank under all treatments. This is probably because their food tends to accumulate on surfaces (Dickman, 1968; Kupferberg, 1997), to which they are more attracted than open water areas. There was no significant difference in tadpole use of edge between any of the treatments and the control.

Control treatment.

In the control treatment, tadpoles appeared to prefer aggregating in the right hand half of the tank. There are several possible reasons for this unexpected bias. There may have been some subtle undetected habitat difference between the two halves of the tank, or light intensity may have varied in different positions. The room for these experiments was lit from a diffuse ceiling source, which cast no shadows. No subtle differences in habitat, light intensity, or position of the whole tank could be identified to explain this bias, which remains unclear. It is also possible that there was some observer bias in the observations. For most of the treatments, the tadpoles in the left hand half were counted first, then the tadpoles on the right side as a counter checking measure, however there was still a potential for error in this method.

Substrate treatments.

Large tadpoles showed a non-significant trend towards the bottom of the tank when gravel was added ($P=0.093136$). Although the gravel was clean, we may have expected tadpoles to be attracted to it because they perceived it to be a possible source of food. In the wild, benthic alga communities can be quite rich (Buelker and Gunkel, 1996; Robson and Barmuta, 1998) and alga is the major food source for tadpoles (Dickman, 1968; Kupferberg, 1997). Also, gravel can provide some refuge from predators (Folsom and Collins, 1984) by camouflaging the tadpoles (Lawler, 1989; Fuller and Rand, 1990) or providing crevices as refuge (Gotceitas and Brown, 1993; Gotceitas et al., 1995; Valdimarsson and Metcalfe, 1998). In general, *L. ewingi* tadpoles prefer the surface of the water (Peterson et al., 1992). This preference is significantly stronger when the tadpoles are alone, compared to when they are in groups of five (Peterson et al., 1992). Alford and Crump (1982) found a similar pattern for *Rana utricularia* (southern leopard frog) tadpoles. Possibly the stress of reduced inter-individual distances causes tadpoles to seek protection near the substrate (Alford and Crump, 1982). Odonate larvae tend to forage on the substrate (Pritchard, 1965; Richards and Bull, 1990; Peterson et al., 1992), and Richards and Bull (1990) found that in the field *L. ewingi* tadpoles were less susceptible to predation from *Hemicordulia tau* (dragonfly) larvae than tadpoles of *Ranidella signifera*, a more benthic species. However, Peterson et al. (1992) found that in the laboratory *L. ewingi* was preyed on more than *R. signifera*. They attribute this to a difference in swimming speed of *L. ewingi* and *R. signifera*, *R. signifera* is a faster swimmer (Peterson et al., 1992). Heyer (1976) found that different species of tadpoles prefer different regions of the water column, and within species these preferences may vary ontogenetically, and within years.

When tadpoles were offered the choice between a gravel or a sand substrate they were expected to prefer the former for better camouflage (Lawler, 1989; Fuller and Rand, 1990) but they showed no significant preference either way. This may have been because the sand was closer in colour to the tadpoles than the gravel was. It is possible

that the addition of a predator might cause a sudden increase in the use of one habitat. In the presence of a predator, the sand may provide better refuge as it stirs up and conceals animals (Lawler, 1989), and tadpoles may be able to burrow into it. On the other hand, gravel would have provided crevices large enough for at least small tadpoles to hide in (Gotceitas and Brown, 1993; Gotceitas *et al.*, 1995; Valdimarsson and Metcalfe, 1998). Small *Rana catesbeiana* (bullfrog) tadpoles prefer gravel to rock substrates and are often seen buried in the interstitial spaces between pieces of gravel, even in the absence of predators (Smith, 1999). Also, as mentioned above, gravel may be perceived as a possible food source by tadpoles (Buelker and Gunkel, 1996; Robson and Barmuta, 1998). However, the sand may also have contained small particles of organic matter. *R. utricularia* tadpoles of less than 10 mm snout to vent avoid sand, possibly because it supports less periphyton. Older *R. utricularia* tadpoles show no significant preference (Alford and Crump, 1982).

Shade treatment.

Shade had no effect on tadpole spacing or on their vertical position in the water column. Shade might provide a refuge for tadpoles in the presence of predators, and in the wild tadpoles may frequent shaded areas to avoid excessive exposure to UV-B radiation (Barinaga, 1990; Blaustein *et al.*, 1994a; Halliday, 2000). However, food is likely to be more abundant in sunlit areas, although a number of studies have reported a lowering in phytoplankton biomass productivity under intense UV-light regimes (Hermann *et al.*, 1996; Häder, 1996; Kasai and Arts, 1998; Marwood *et al.*, 1999; Marwood *et al.*, 2000). Peterson *et al.* (1992) found that *L. ewingi* were actually more susceptible to predation by odonate larvae in the dark.

Plant treatments.

When simple plants were added, large tadpoles responded by moving away from the plants (to the open right hand side), although this result was not significant ($P=0.006871$). Young *Rana temporaria* (common frog) tadpoles exhibit a preference for green light which is replaced by a preference for blue light by the time metamorphosis is reached (Jaeger and Hailman, 1976). The authors speculate that this

preference might enable tadpoles to find food quickly. At metamorphosis the diet becomes carnivorous, and tadpoles leave the water. A preference for blue might help tadpoles find the water surface (Jaeger and Hailman, 1976). Simple plants might provide a refuge for tadpoles (Folsom and Collins, 1984; Bridges and Gutzke, 1997; Babbit and Tanner, 1998; Lefcort et al., 1999), but it is possible that they also provide an ambush site for sit-and-wait predators like dragonfly larvae (Pritchard, 1965). Thus tadpoles might be best off staying out of the plants when there are no obvious predators around, and retreating to refuge only when predators appear (Kats et al., 1988; Sih et al., 1992; Sih and Kats, 1994; Bridges and Gutzke, 1997; Babbit and Tanner, 1998; Kiesecker et al., 1999; Lefcort et al., 1999; Van Buskirk and Schmidt, 2000). Smith (1999) found that small *Rana catesbeiana* (bullfrog) tadpoles preferred non-vegetated areas, while larger tadpoles showed no preference. In *Limnaoedus ocellularis* tadpoles, smaller individuals prefer areas with more extensive ground cover. This preference weakens with development (Alford, 1986). Lower food stocks in refuge areas (Sih et al., 1988; Ludwig and Rowe, 1990; Feminella and Hawkins, 1994) would not encourage tadpoles to remain there.

In the presence of simple plants on the left hand side, small tadpoles moved towards the top of the tank, and large tadpoles towards the bottom. Peterson et al. (1992) found that when alone *L. ewingi* tadpoles spend most of their time at the surface. When in groups of five, tadpoles spend significantly more time nearer the substrate, but still spend most of their time at the surface. The tadpoles they used were 12-15 mm total length, which means they would have been put into my small size class (of less than 10 mm snout to vent). Perhaps in the presence of simple plants larger tadpoles are more able to feed off the benthos, while smaller tadpoles are more able to feed on smaller algae found on the plant leaves and in the water column (Van Buskirk and Schmidt, 2000).

Hyla arborea arborea (treefrog) tadpoles prefer the surface of the water to the bottom and middle layers (Waringer-Löschenkohl, 1988). *Rana dalmatina* (spring frog) tadpoles generally prefer the bottom water layer to the middle or the top, but in the presence of plants a higher percentage of tadpoles are found at the surface. However, although *Bufo bufo bufo* (common toad) and *Pelobates fuscus* (spadefoot) tadpoles

also prefer the lower reaches of the water column, they show no shift to the top in the presence of plants (Waringer-Löschenkohl, 1988). Finally, *Rana ridibunda* (lakefrog) tadpoles preferred the bottom of the tank in the absence of vegetation, but when plants were added smaller tadpoles moved to the surface, while larger tadpoles moved to the bottom (Waringer-Löschenkohl, 1988). Waringer-Löschenkohl (1988) speculates that perhaps in the absence of plants tadpoles feed off the benthos but when plants are present they prefer to feed off phytoplankton. Löschenkohl (1986) reports that most species of tadpoles tested spent more time at the surface in the presence of vegetation. In one case larger tadpoles shifted their distribution towards the bottom, the author speculates that this might be due to the development of forelimbs in the individuals used (Löschenkohl, 1986). Because I stopped using tadpoles after forelimb emergence, this is unlikely to have been a factor in my experiments.

Neither tadpole size class showed a significant attraction to complex plants when these were added to the left hand side. In the absence of predators, 70.8% of *Phrynomantis microps* (African savanna frog) tadpoles are found in areas without vegetation (Rödel and Linsenmair, 1997). In contrast, Formanowicz and Bobka (1989) found that in the absence of predaceous diving beetles (*Dytiscus verticalis*) both *Rana sylvatica* and *Hyla versicolor* tadpoles prefer to be in a more complex environment. However, when put together both predator and prey changed microhabitat use so that they were both uniformly distributed through the habitat (Formanowicz and Bobka, 1989). Possibly plants can harbour predators, which could dissuade tadpoles from using plant refuges when no predators are present outside the refuge. Unlike the simple plant treatment, there was no significant tendency towards or away from the bottom in either tadpole size class in the presence of complex plants.

No bias was shown towards either simple or complex plants when both were placed in the tank. This indicates that either tadpoles preferred to stay out of refuge, i.e. in the centre third of the tank, or they did not discriminate between the two types of refuge. During these trials tadpoles did appear to be aggregating in the central section of the tank but these data were not formally recorded. When both simple and complex plants were present in the tank, small tadpoles moved to the top of the water column while large tadpoles sank towards the bottom. This is probably due to the presence of the

simple plants, and unrelated to the presence of the complex plants, because complex plants alone did not elicit this response, whereas simple plants did.

Alga chip treatment.

Small tadpoles were not significantly attracted to the alga chip, but large tadpoles used the left hand side of the tank more when the alga chip was present. This is what would be expected. Possibly the small tadpoles did not respond to the addition of the food because they were unaware of its presence, although this is unlikely as they had 15 minutes to acclimatise to the tank with the alga chip present before observations started, and tadpoles are known to have well-developed chemosensory abilities (Stauffer and Semlitsch, 1993; Wilson and Lefcort, 1993; Feminella and Hawkins, 1994; McCollum and Van Buskirk, 1996; McCollum and Leimberger, 1997; Laurila *et al.*, 1998; Mathis and Vincent, 2000). It is also possible that the small tadpoles did not go near to the algae chip for fear of being ousted by larger tadpoles (Alford and Crump, 1982; Flores-Nava and Vera-Munoz, 1999). Alford (1986) speculates that presence of more efficient tadpoles might cause less efficient types to move to different areas. *Rana aurora* (red-legged frog) tadpoles avoid feeding in the same areas as *Rana catesbeiana* (bullfrog) tadpoles (Kiesecker *et al.*, 2001*b*). However, as small tadpoles were raised together in one cohort and were never exposed to larger tadpoles after hatching, this is also unlikely. Also, Heyer (1976) concluded that competition among tadpoles should not occur normally in nature as resources are super-abundant.

Mosquitofish treatment.

Small tadpoles spent more time in the left side of the tank ($P=0.074690$) when caged mosquitofish were present, although this result was not quite significant, and they were also observed sucking on the mesh sides of the cage. Large tadpoles did not spend more time in the left side of the tank, but did not actively avoid the mosquitofish, which would be the expected optimal behaviour, although Wildy and Blaustein (2001) found that larval *Ambystoma macrodactylum* (long-toed salamanders) decrease activity in the presence of predators but do not change their

spatial distribution. When several species were investigated for spatial responses to predators, some species, but not all, avoided *Umbra limi* (mudminnows) (Relyea, 2001a).

Possibly neither size class of tadpoles recognised the mosquitofish as potential predators. Large tadpoles may also have perceived themselves to be in a size refuge (Travis *et al.*, 1985a; Sih and Moore, 1993; Gotthard and Nylin, 1995; Persson *et al.*, 1996; Babbit and Tanner, 1998) as the mosquitofish were only slightly larger than they were. In *Cottus cognatus* (slimy sculpins) being preyed on by *Salvelinus fontinalis* (brook trout) the strength of antipredator responses depends on the ratio of trout size to sculpin size (Chivers *et al.*, 2001). Mosquitofish do attack animals larger than themselves (McCullough, 1998; Lawler *et al.*, 1999; Willis and Ling, 2000), but because they have reached populations of whistling frog tadpoles in Manawatu only recently (Myers, 2001), it is unlikely that these tadpoles would have evolved an antipredator response to them. Once the populations meet whistling frogs may learn to respond fairly quickly. Previous work has shown that naïve animals may not respond to some predators at first, but may develop antipredator behaviour after they are exposed to chemical cues from injured or consumed conspecifics (see Wildy and Blaustein, 2001 for examples).

If whistling frog tadpoles do not display a response to mosquitofish, they may not have an evolved response to fish in general. Some amphibian species show genetically inherited antipredator behaviours, the naïve larvae respond to predators even though they have never encountered them before e.g. *Rana lessonae* and *R. esculenta* (Semlitsch and Reyer, 1992) and *Ambystoma barbouri* (streamside salamander) hatchlings (Sih and Kats, 1994). Kats *et al.* (1988) found that *Hyla chrysoscelis* (grey treefrog) larvae native to predator-rich sites showed stronger antipredator behaviour and were less palatable than tadpoles from predator-poor sites, although neither groups had ever encountered predators before, indicating that those from predator-rich sites had evolved antipredator responses. However, Bridges and Gutzke (1997) found no such difference between *H. chrysoscelis* sibships from predator-rich and predator-poor sites. Tadpoles that do not have a genetically programmed antipredator response

can develop one if reared in the presence of conspecifics (Semlitsch and Reyer, 1992; Bridges and Gutzke, 1997).

In the presence of caged mosquitofish, the large tadpoles spent more time at the bottom of the tank. This may be one way to avoid predators as the substrate may provide camouflage (Lawler, 1989; Fuller and Rand, 1990) or tadpoles may be able to burrow into the gravel (Gotceitas and Brown, 1993; Gotceitas *et al.*, 1995; Valdimarsson and Metcalfe, 1998). However, other predators (e.g. dragonfly larvae) are concentrated on the substrate (Richards and Bull, 1990; Peterson *et al.*, 1992), so this kind of behaviour could increase the risk of predation from these other predators. Possibly this is why only larger tadpoles showed an increased association with the substrate in the presence of mosquitofish, in natural situations the substrate might be dangerous for small tadpoles but larger individuals may have some protection from predation by dragonfly larvae through a size refuge (Richards and Bull, 1990).

Summary.

1. Tadpoles were found to be highly attracted to surfaces.
2. The addition of simple plants, either alone or together with complex plants, caused large tadpoles to move towards the bottom of the tank while small tadpoles preferred the top half of the water column.
3. Large tadpoles were attracted to an alga chip placed in the bottom left hand side of the tank, but small tadpoles showed no attraction to the food.
4. Neither large nor small tadpoles showed any significant attraction or aversion to mosquitofish.

Samenvatting.

1. Kikkervissen vonden de zijanten, bodem en oppervlakte van het water zeer aantrekkelijk.
2. De toevoeging van architectureel simpele planten, zelfstandig of in combinatie met complexe planten, maakte dat grote kikkervissen zich in de

richting van de bodem verplaatsten terwijl kleinere kikkervissen voorkeur gaven aan de oppervlakte van het water.

3. Grote kikkervissen werden aangetrokken door een alga-schijfje dat op de bodem was geplaatst, maar kleine kikkervissen werden niet aangetrokken door deze voedselbron.
4. Grote noch kleine kikkervissen lieten attractie of afkeer merken in reactie tot mosquitofish.

Chapter 4

Use of microhabitat by mosquitofish (*Gambusia affinis holbrooki*).

Introduction.

Gambusia affinis holbrooki (mosquitofish) are generalist predators, consequently their widespread introductions have had disastrous effects (Hurlbert et al., 1972; Gamradt and Kats, 1996; Simberloff and Stiling, 1996; Howe et al., 1997; McCullough, 1998; Goodsell and Kats, 1999; Lawler et al., 1999; Malakoff, 1999; Komak and Crossland, 2000; Willis and Ling, 2000). Aside from preying on other fish species (Howe et al., 1997; McCullough, 1998; Malakoff, 1999), amphibian larvae (Gamradt and Kats, 1996; McCullough, 1998; Goodsell and Kats, 1999; Lawler et al., 1999; Malakoff, 1999; Komak and Crossland, 2000), and aquatic invertebrates (Hurlbert et al., 1972; Bence, 1988), they are also cannibalistic (Winkelman and Aho, 1993).

Because they are able to withstand a wide range of salinities and temperature, mosquitofish can colonise an extensive variety of habitats (Vargas and De Sostoa, 1996; Willis and Ling, 2000), although water must be permanent (Willis and Ling, 2000). In densely vegetated areas they tend to be found only in open water (Willis and Ling, 2000). In New Zealand these fish are found throughout the Northland, Auckland, Waikato, Bay of Plenty, and Hawkes Bay regions (Department of Conservation website, 16/08/2001), and populations have recently been found in the Manawatu (Myers, 2001) and Nelson areas (P. B. Studdum, pers. comm.).

In order to assess the likely extent to which these fish might prey on whistling frogs we need to be able to estimate how often individuals of the two species will encounter each other. This requires some knowledge of the microhabitat use of both species (see also Chapter 3 introduction). Chapter 3 gives details about whistling frog tadpoles' use of microhabitat. This chapter does the same for mosquitofish, through a series of laboratory experiments exploring preferences for certain microhabitat features.

Materials and methods.

The mosquitofish used in these experiments were obtained from Massey University stocks, originally captured from a ditch in Anderson park, Napier. This ditch runs between two lakes in the park, and usually houses a very large population of mosquitofish. The University's captive mosquitofish population is fed on dried commercial fish flakes, similar to Bisazza *et al.* (1989).

The mosquitofish were divided into three size/sex categories: small male, small female, and large female. There was no "large male" class because males do not grow to a large size. Small fish were 10 to 30 mm long (total body length), large fish were more than 40 mm long, and never longer than 60 mm. The fish were measured by placing them in a water-filled petrie dish over a sheet of paper with millimetre gradations. Fish that fitted the size requirement were housed in a separate tank.

Twenty-five mosquitofish of each size class were used for each treatment in experiments on the use of space. Fish were reused for each treatment when possible. The twenty-five mosquitofish (five individuals times five replicates) were kept together, and all replicates of any one treatment were carried out on the same day. For each replicate five fish were drawn from the experimental tank and afterwards were placed in a separate tank. Therefore, while the composition of the replicate groups changed between treatments, they were made up of the same twenty-five fish. If an experimental mosquitofish died it was replaced with a new animal from the rearing tank. No fish ever outgrew its size class during the experiments.

The experiments with large female mosquitofish were carried out from 15 March 2000 to 26 March 2000, and with small females between 28 March 2000 and 10 April 2000. The small male mosquitofish experiments were carried out from 13 April 2000 to 25 April 2000.

During the experiments the mosquitofish were placed in the same tank used for the experiments on tadpoles in Chapter 3 (Chapter 3, Figure 3.1).

The following eight treatments were tested in the sequence described in Chapter 3, running through a series of control, gravel, gravel vs. sand, shade, simple plant, complex plant, simple versus complex plant, and tadpoles. In the tadpole treatment, five tadpoles (Gosner stages 24-31, Gosner 1960) were placed in a mesh cage along the left-hand wall of the tank.

The experimental procedure was as described in Chapter 3, with observations taken every minute. Again, the amount of fish in contact with the stimulus object was measured for the last four treatments. Fish were considered to be in contact with an object if any body part was directly touching the object. In the "tadpoles" treatment, contact with the cage was considered to be in contact with the stimulus object. For the shade treatment, only the left versus right observation was recorded, because the light colouration of the fish made them impossible to see through the speckled plastic.

Analysis.

Statistical analysis follows the methods described in chapter three. However, when comparing size classes against each other with pairwise comparisons only the large female and small female mosquitofish were compared, because the results for small male mosquitofish were very similar to those for small female mosquitofish. For the pairwise comparisons the Bonferroni method was used. P values presented here are corrected probabilities.

Results.

Comparisons with the control (movements to the left, top or edge).

Stimulus type had a significant effect on use of the left hand side of the tank (Table 4.1), suggesting that mosquitofish use of the left hand side differed among treatments. There was also a significant interaction term (Table 4.1), indicating that mosquitofish responses to stimuli varied with the size and/or gender of the mosquitofish.

| Dependent variable | Mosquitofish type | Stimulus type | Interaction term |
|------------------------------|--------------------------|----------------------|-------------------------|
| Left | NS | <0.000001 | 0.003997 |
| Top | NS | NS | 0.037396 |
| Edge | NS | NS | NS |
| Contact with stimulus | 0.000006 | <0.000001 | <0.000001 |

Table 4.1. ANOVA probabilities for the use of microhabitat by mosquitofish.

Pairwise comparisons with the control treatment were done to see which treatments accounted for this variation, and significant results are presented below.

Small female mosquitofish were significantly attracted to the left hand side in the complex plants treatment ($P=0.006984$). In the gravel versus sand treatment they showed a significant preference for the right (sand) side of the tank ($P=0.000609$).

Although there was also a significant interaction term for use of the top half of the tank (Table 4.1), pairwise comparisons with the control did not yield any significant results. Mosquitofish showed no bias in association with the edge, regardless of the size or gender of the mosquitofish, or the habitat features present.

Comparisons between mosquitofish sizes for contact with stimulus.

A two-way ANOVA showed that contact with the stimulus provided depended not only on the type of stimulus, but also on the size and/or gender of the mosquitofish (Table 4.1). More importantly, there was a highly significant interaction term (Table 4.1), indicating that contact with the stimulus was not consistent among mosquitofish sizes/genders. In general, small female and male mosquitofish appeared to behave very similarly. Therefore, pairwise comparisons were done between small and large female mosquitofish, and males were omitted from this part of the analysis.

Compared to large female fish, small females showed a significantly greater attraction to simple plants ($P=0.000009$) and complex plants ($P<0.000003$). Large females were significantly more attracted to tadpoles ($P=0.010962$).

Discussion.

Ontogenic changes in mosquitofish behaviour.

The only significant overall difference noted in mosquitofish behaviour over the course of development was in the level of contact with the stimulus. We might have expected large and small mosquitofish to behave differently because smaller fish are prone to predation from their larger counterparts (Winkelman and Aho, 1993; Benoit *et al.*, 2000). In general males are smaller than females and are therefore also vulnerable to predation (Busack and Gall, 1983; Bisazza *et al.*, 1989; McCullough, 1998).

Small male mosquitofish behaviour was very similar to that of small female mosquitofish. It may be beneficial for small males to associate with small females rather than larger females because larger females are more likely to prey on them (Winkelman and Aho, 1993). However, larger females have a higher fecundity (Bisazza *et al.*, 1989), so the potential benefit of mating with a larger female is far greater in terms of reproductive success.

Use of the edge of the tank.

Mosquitofish showed no bias towards or away from the edge of the tank.

Substrate treatments.

Small female mosquitofish showed a significant attraction to sand in the treatment where gravel was the alternative substrate to sand, but other size/sex classes did not. Small female mosquitofish are vulnerable to predation from larger individuals (Winkelman and Aho, 1993; Benoit *et al.*, 2000), and the sand may have provided

them with some refuge (Folsom and Collins, 1984; Holomuzki and Stevenson, 1992; Closs, 1996), although Usio and Townsend (2000) found that coarse substrates afford better protection to *Paranephrops zealandicus* (freshwater crayfish) from predation. In contrast, predation on another mobile crustacean, *Metapenaeus ensis* (burying shrimp), is not affected by sediment size (Primavera, 1997).

When offered a choice between sand, gravel-pebble and cobble, juvenile *Gadus morhua* (Atlantic cod) preferred sand or gravel-pebble in the absence of predators. In the presence of predators, juveniles hid in the interstitial spaces of the cobble (Gotceitas and Brown, 1993). Two and a half hours after exposure to a predator larger juveniles again showed a preference for sand or gravel-pebble, but smaller individuals continued to associate with cobble (Gotceitas and Brown, 1993).

The gravel was closer in colour to the mosquitofish than the sand, therefore we would have expected the mosquitofish to prefer gravel over sand for camouflage reasons. Male mosquitofish are just as vulnerable to predation as small female mosquitofish because of their small size (Busack and Gall, 1983; Bisazza et al., 1989; McCullough, 1998). By hiding from larger female fish, however, males may lose out on breeding opportunities. Therefore they may risk predation in order to increase their chances of reproducing.

One other possible reason why small female mosquitofish might prefer a sand substrate to gravel is that more prey might be found on sand substrates. On the one hand, sand might hide some prey species better because it is easily shifted to conceal prey (Lawler, 1989) and prey might be able to bury themselves in it. However, it is also likely that some prey would be better hidden in the interstitial spaces of gravel substrates (Gotceitas and Brown, 1993; Gotceitas et al., 1995; Valdimarsson and Metcalfe, 1998). On balance, therefore, if prey is better hidden in gravel than in sand, then predators might be advantaged by congregating over sand.

Plant treatments.

Small female mosquitofish were significantly attracted to complex plants, although they showed no significant attraction to simple plants compared to the control. Complex plants might supply a refuge for smaller individuals seeking to hide from larger individuals (Gotceitas and Colgan, 1989; Turner, 1996; Primavera, 1997; Swisher et al., 1998). However, Savino and Stein (1989) found that at higher vegetation densities *Micropterus salmoides* (largemouth bass) change foraging tactics from searching to ambushing. This suggests that increasing habitat complexity may not necessarily provide extra refuge for prey because predators may change their behaviour, leaving prey still vulnerable to predation. When compared to large female fish, small female mosquitofish did show a significantly higher attraction to both simple and complex plants. Again, both types of plants may provide refuge for smaller individuals (Gotceitas and Colgan, 1989; Turner, 1996; Primavera, 1997; Swisher et al., 1998). It is likely that complex plants provide a better refuge because of their more elaborate architecture (Folsom and Collins, 1984; Gotceitas and Colgan, 1989; Pennings, 1990; Mattingley and Butler, 1994; Persson and Eklöv, 1995; Moksnes et al., 1997; Swisher et al., 1998; Corona et al., 2000; Manatunge et al., 2000). However, prey individuals using more complex refuges may have reduced foraging efficiency (Gotceitas, 1990)

Tadpole treatment.

Compared to small female fish, large female mosquitofish were significantly more attracted to tadpoles. Although the tadpoles used in this experiment were less than 10 mm in length (snout to vent), small mosquitofish may have been less eager to attack them because of their size. Small mosquitofish can attack tadpoles larger than these, but usually just bite small amounts out of the tail fin rather than attempting to consume the whole tadpole (Lawler et al., 1999; Komak and Crossland, 2000), as detailed in the next chapter. Since the tadpoles were inside a mesh cage, the mosquitofish may not have perceived them to be suitable prey items.

Summary.

1. Small female mosquitofish were attracted to sand and complex plants, they moved to the right in the gravel versus sand treatment and to the left in the complex plant treatment.
2. This may reduce the efficacy of sand and complex plants as refuge habitats for tadpoles.
3. Small male mosquitofish behaved very similarly to small female mosquitofish.
4. Compared to large female mosquitofish, small female fish were more attracted to simple plants and complex plants, and less attracted to tadpoles.

Samenvatting.

1. Kleine vrouwelijke mosquitofish werden aangetrokken door zand en complexe planten, zij bewogen naar rechts in de grint versus zand behandeling, en naar links in de complexe plant behandeling.
2. Dit kan mogelijk de werkzaamheid van zand en complexe planten als schuilplaatsen voor kikkervissen verminderen.
3. Kleine mannelijke mosquitofish gedroegen zich op dezelfde manier als kleine vrouwelijke mosquitofish.
4. In vergelijking met grote vrouwelijke mosquitofish, werden kleine vrouwelijke mosquitofish meer aangetrokken door simpele planten en complexe planten, en minder aangetrokken door kikkervissen.

Chapter 5

**Behaviour of whistling frog (*Litoria ewingi*) tadpoles and
mosquitofish (*Gambusia affinis holbrooki*) during attack
sequences.**

Introduction.

Mosquitofish (*Gambusia affinis holbrooki*) were introduced to New Zealand in the 1930s (McCullough, 1998). Since their original release in Auckland they have spread through both natural and artificial introductions, and are now found throughout the Auckland, Waikato, and Hawkes Bay regions (McCullough, 1998). They have recently been discovered in the Manawatu (Myers, 2001) and Nelson areas (P. B. Studdum, pers. comm.).

Whistling frogs (*Litoria ewingi*) were introduced to the South Island in 1875 (Robb, 1980), and a further introduction was made to Himatangi in the North Island in 1948 (Robb, 1980). They are now found around the top and down the west coast of the South Island, and throughout the Manawatu and Wellington regions in the North Island (Gill, 1978; Robb, 1980).

With the discovery of mosquitofish in the Manawatu and in Nelson, it seems likely that populations of whistling frog tadpoles will soon be exposed to them, if they are not already. Mosquitofish are voracious predators of aquatic invertebrates, fish, and tadpoles (Bence, 1988; McCullough, 1998). They have been blamed for declines in a wide range of species overseas, including amphibians (Hurlbert *et al.*, 1972; Bence, 1988; Gamradt and Kats, 1996; Simberloff and Stiling, 1996; Howe *et al.*, 1997; McCullough, 1998; Goodsell and Kats, 1999; Lawler *et al.*, 1999; Malakoff, 1999; Komak and Crossland, 2000; Willis and Ling, 2000).

In Chapters 3 and 4, I presented results from laboratory experiments on the microhabitat preferences of both species. These were conducted to see whether tadpoles and fish would be likely to encounter each other in natural situations. The results suggested that mosquitofish and whistling frog habitat preferences overlap, and they are therefore likely to be found in the same areas of water. This means that it is highly likely that mosquitofish will prey on whistling frog tadpoles. The series of laboratory experiments presented here investigate behaviour of both species during attack sequences. Habitats with and without available refuge are compared to see whether refuge availability has any effect on attack frequency and the behaviour exhibited by either species.

Materials and Methods.

In these experiments, 10 whistling frog tadpoles and five mosquitofish were placed together in a tank. The experiments were run in a two x three x two factorial design, with two size classes of tadpoles (small and large), three size/sex classes of mosquitofish (small male, small female, large female), and two environments (no refuge and with refuge). Small tadpoles were less than 10 mm snout to vent (Gosner stages 24-31, Gosner, 1960) and large tadpoles were more than 10 mm (Gosner stages 37-40). Small mosquitofish were 10-30 mm long and large mosquitofish 40-60 mm long.

All treatments were tested in a 30 cm x 30 cm x 40 cm tank (inside dimensions) made out of 5 mm thick window glass. There was a 5 cm layer of gravel on the bottom of the tank, and the water was filled to 5 cm from the top, giving a water volume of 24 litres. The refuge used in the refuge trials was a single plant (*Lindernia rotundifolia*) in the middle of the left hand half of the tank. This plant was quite well grown and occupied approximately half the tank. Water used was tap water aged with Masterpet Water Ager.

During a trial the tadpoles were added first and allowed to acclimatise for 15 minutes, with no one in the room. Five fish were then added at time zero and every attack that followed was recorded. Features of each attack that were noted were:

1. Time (of attack).
2. Tadpole behaviour prior to the attack (swimming, feeding, or resting).
3. Direction of the attack relative to the tadpole (from above, below, front, behind, or from the side).
4. Tadpole body part that was attacked (body or tail).
5. Tadpole reaction (flight, reorientation, immobility, death, or, in the refuge trials, hiding in the refuge).
6. Location of the attack (inside or outside the refuge; measured only in the refuge trials).

Each trial was continued until ten attacks had occurred or one hour had elapsed. Any lunge towards a tadpole was considered to be an attack. Before trials, mosquitofish were starved for 48 hours. Immediately prior to the trial they were placed in a small plastic container, fed a small amount of fish flakes (Wardley Total Tropical) to ensure they were feeding (similar to Komak and Crosland, 2000), then released straight into the experimental tank. Animals were moved from tank to tank using a standard fish net. After the trial, the fish were removed first, as quickly as possible. Tadpoles were then moved back to their rearing tank and fed. Tadpoles were never used more than once in a week but the fish had to be re-used owing to shortages of large female fish. At least 15 individuals of each size class were used.

At the start, trials were video taped, but because of poor visibility this was discontinued.

Analysis.

Pearson's two way Chi Square tests were used to make comparisons between animal groups (small versus large tadpoles; small female mosquitofish versus large female mosquitofish; female mosquitofish versus male mosquitofish), and refuge treatments (no refuge or refuge present) for the variables recorded (location of the attack etc.).

Attacks were considered to be independent events. While it is possible that two attacks in one treatment were made by the same fish on the same tadpole, the probability of this happening is low. There was no way to distinguish between individual animals, but where a mosquitofish was seen to lunge at the same tadpole several times in rapid succession only the first attack was recorded.

There were so few attacks made from above, below and the front that for the two way tests on direction of attack these three options were grouped together, and compared with the frequency of attacks from behind, or from the side. Although direction of the attack was affected by tadpole size (Table 5.1) it was not significantly affected by the size or gender of mosquitofish ($P=0.0733$) or by the presence or absence of refuge ($P=0.4286$). The data from the two refuge treatments were, therefore, amalgamated, and a one-way chi square test comparing all five possible directions with expected

probability was calculated (Table 5.1). The two refuge treatments were amalgamated to raise the expected values above 5. These probabilities were one sixth for each direction, except for “side” which accounted for the remaining two sixths as there were two possible sides the fish could have attacked from.

Tadpoles did not often respond to an attack with complete immobility, so responses were divided up into “active” responses (flight, and also hiding in the refuge treatment) and “passive” responses (reorientation, immobility or death). This also eliminated the problem that tadpoles could not “hide” in the no refuge treatment.

The time between consecutive attacks was calculated, then analysed by ANOVA with tadpole size, fish size, and presence/absence of refuge as factors. I also did two-sample t-tests using separate variances on each tadpole/fish size class combination (6 in total) to see whether there was a significant difference in time between attacks when refuge was present compared to when it was absent.

Two way Chi Square tests, two-sample t-tests, and ANOVA were done using SYSTAT version 6.0 for Windows (SPSS, 1996). One way chi square tests were done by hand.

Results.

Overall patterns.

Some general trends emerged from the results. Almost all attacks made from behind or the side were on the tail. In total 85.2% (n=271) of attacks recorded were made on the tail (Figure 5.1).

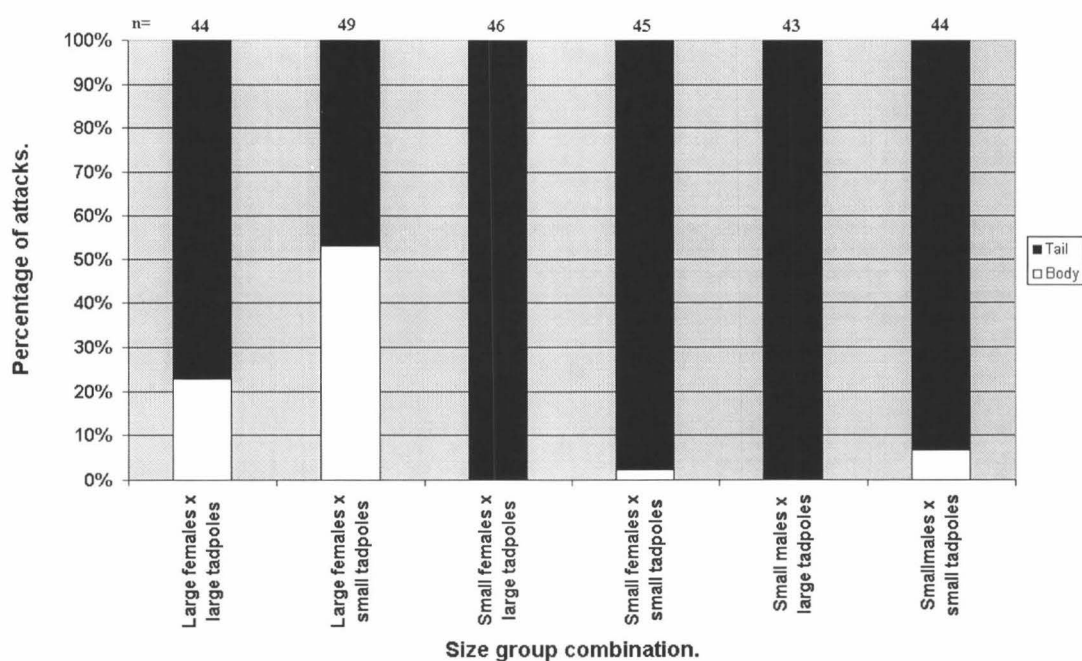


Figure 5.1. Tadpole body part attacked by female or male mosquitofish in each of the six combinations of mosquitofish and tadpole size classes.

The same percentage of attacks were made from behind and the side combined as were made on the tail (Figure 5.2). As expected, most attacks made on the tail came from the side or from behind, but there were a few attacks made on the body that came from the side, and a few attacks made on the tail from above.

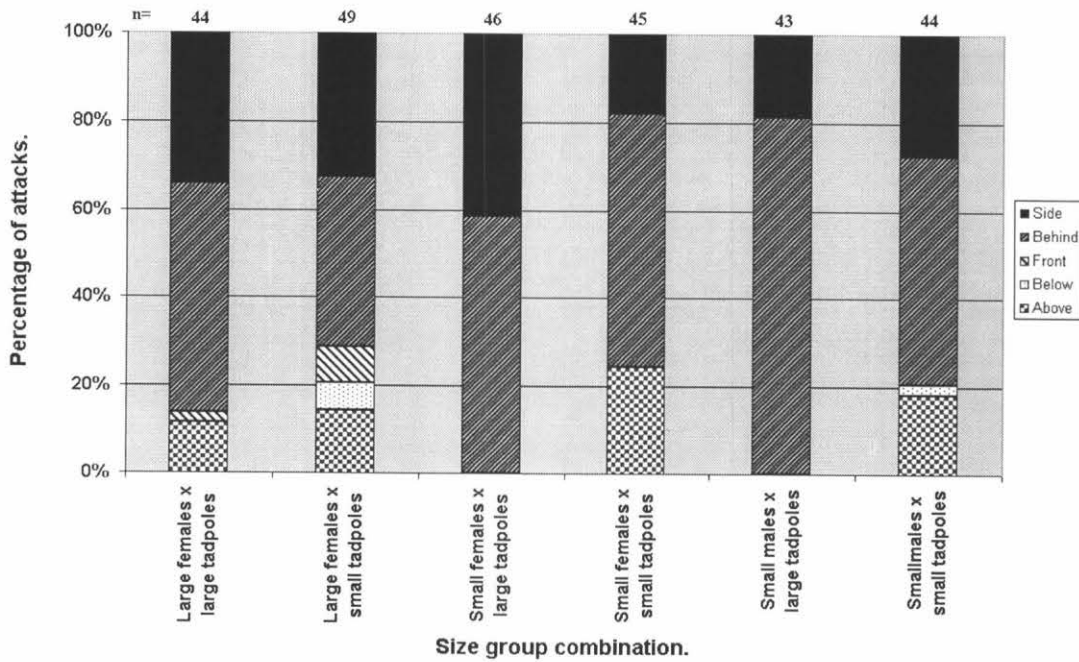


Figure 5.2. Direction of attacks on tadpoles for each of the six combinations of female or male mosquitofish and tadpole size classes.

When attacks were separated into two groups, comprising (a) those that came from behind or the side and (b) those that came from the front, above or below, in a one way chi square test on direction of attack, all tests were significant (Table 5.1). This result was influenced by a disproportionately high number of attacks coming from behind.

| Fish/tadpole combination | P value |
|---------------------------------------|---------|
| Large female fish with large tadpoles | <0.0001 |
| Large female fish with small tadpoles | 0.0005 |
| Small female fish with large tadpoles | <0.0001 |
| Small female fish with small tadpoles | <0.0001 |
| Small male fish with large tadpoles | <0.0001 |
| Small male fish with small tadpoles | <0.0001 |

Table 5.1. Probabilities from one-way chi square tests on direction of attack, combining the no refuge and refuge treatments, for each of the six combinations of female or male mosquitofish.

Attacks were most commonly made on resting tadpoles (57.9%, n=271; Figure 5.3).

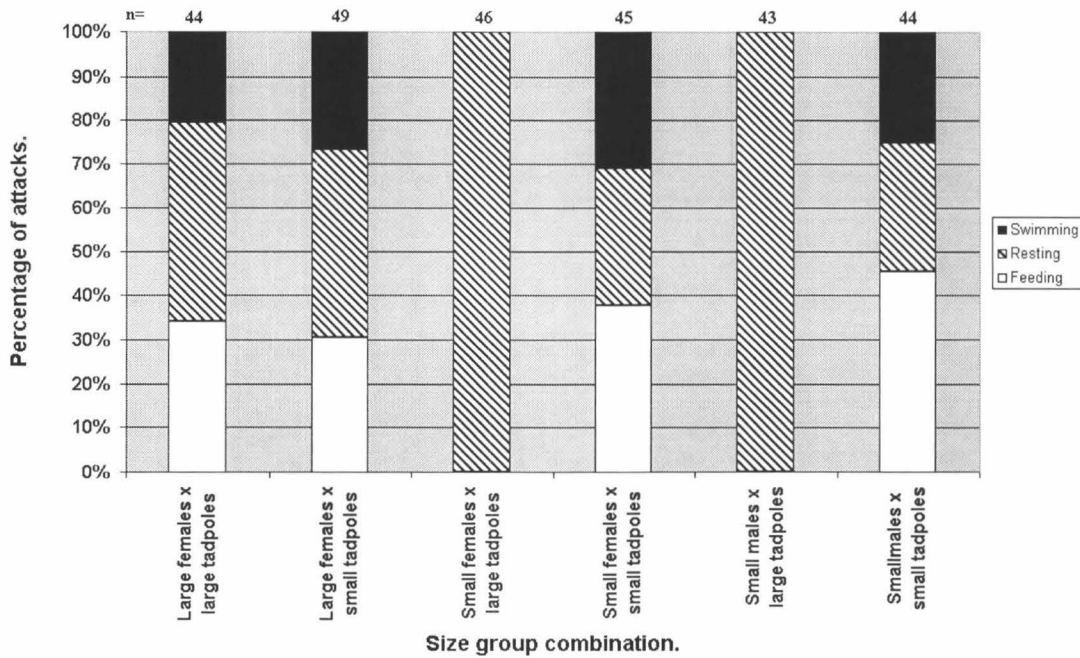


Figure 5.3. Tadpole state prior to the attack for each of the six combinations of female or male mosquitofish and tadpole size classes.

Focal animal sampling showed that tadpoles spent 8.4% of their time swimming, 71.0% feeding and 20.6% resting (Figure 5.4). I expected the number of tadpoles attacked while resting to be proportional to the time tadpoles spent resting, however, a one-way chi square test proved that this was not so ($P < 0.0001$). Indeed a far higher proportion of attacks than expected occurred on resting tadpoles, but both tadpole size and mosquitofish size/gender group affected these trends. While large female fish attacked large tadpoles whether they were resting, swimming or feeding, small male and small female fish only ever attacked large tadpoles that were resting. Small male and small female fish did attack some small tadpoles while they were feeding or swimming.

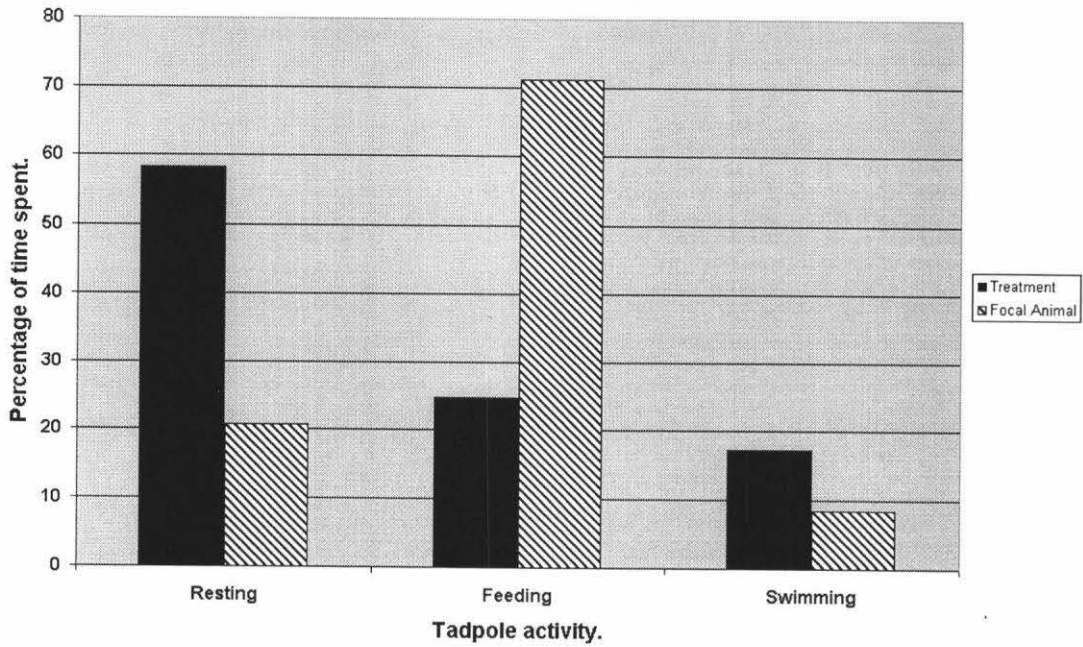


Figure 5.4. Time tadpoles spent in each activity during focal animal sampling versus percentage of attacks made on tadpoles in each of those three states.

The tadpoles usually responded to attack with an active response (flight or hiding 81.6%, n=271) rather than a passive response (reorientation, immobility or death; Figure 5.5).

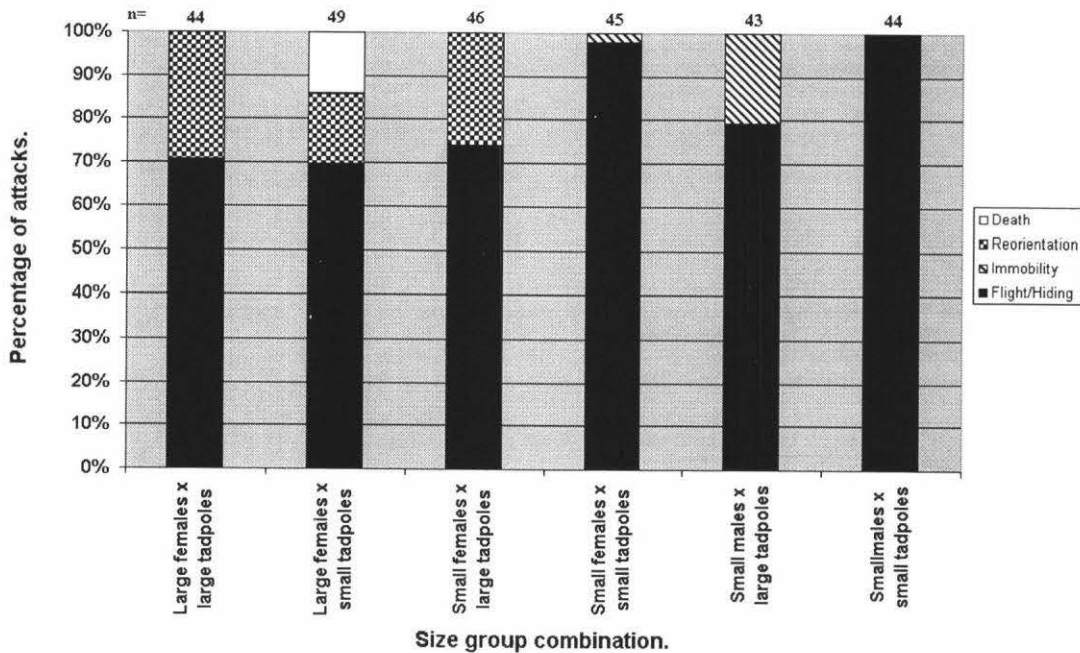


Figure 5.5. Tadpole reaction to being attacked in each of the six combinations of female or male mosquitofish and tadpole size classes.

When refuge habitat was provided, attacks occurred both inside and outside the refuge in approximately equal numbers (Figure 5.6).

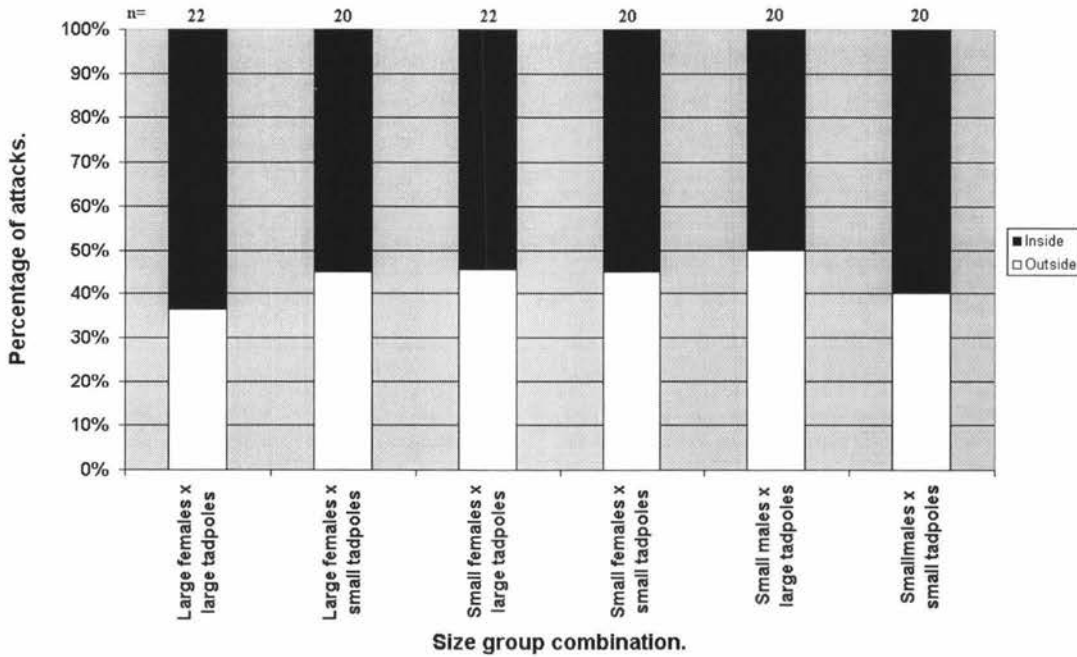


Figure 5.6. Location of attacks (refuge treatment only) in each of the six combinations of female or male mosquitofish and tadpole size classes.

Effect of tadpole size.

Small tadpoles were more likely to receive an attack on the body (rather than the tail) than large tadpoles ($P=0.0010$), and were also more likely to be attacked from above, below, or the front ($P<0.0001$). Small tadpoles were more likely to be attacked while swimming or feeding than large tadpoles ($P<0.0001$). Large tadpoles showed a passive response more often than small tadpoles ($P=0.0030$).

Effect of mosquitofish fish size/gender.

Large female mosquitofish made more attacks on swimming and feeding tadpoles than either of the smaller mosquitofish groups ($P=0.0194$). They were also more likely to attack the tadpole body rather than the tail ($P<0.0001$). Tadpoles attacked by these large female mosquitofish were more likely to show a passive response than tadpoles attacked by smaller mosquitofish groups ($P=0.0013$).

Effect of refuge habitat.

Presence or absence of refuge habitat had no effect on any of the variables measured except for time of the attacks (Table 5.2). The size of the fish, the size of the tadpole being attacked, and the presence or absence of refuge all affected the amount of time between consecutive attacks ($P < 0.0001$, $P < 0.0001$, and $P = 0.0003$ respectively), which is related to attack frequency. There was a significant interaction effect between tadpole and fish size/gender group ($P < 0.0001$), tadpole size and presence/absence of refuge ($P = 0.0088$), and also between fish size/gender, tadpole size and refuge presence or absence ($P = 0.0004$).

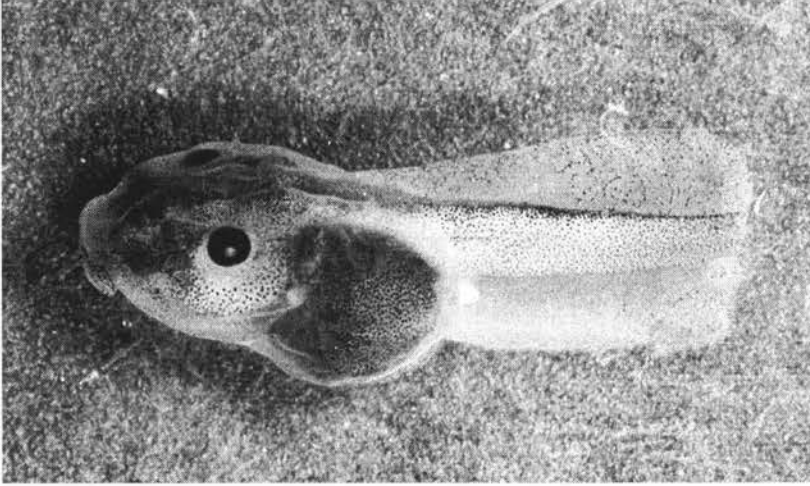
| Source | P |
|---|----------|
| Fish size/gender | <0.0001 |
| Tadpole size | <0.0001 |
| Refuge treatment | 0.0003 |
| Fish size/gender x tadpole size | <0.0001 |
| Fish size/gender x refuge treatment | 0.6028 |
| Tadpole size x refuge treatment | 0.0088 |
| Fish size/gender x tadpole size x refuge treatment | 0.0004 |

Table 5.2. Results of ANOVA on time between consecutive attacks.

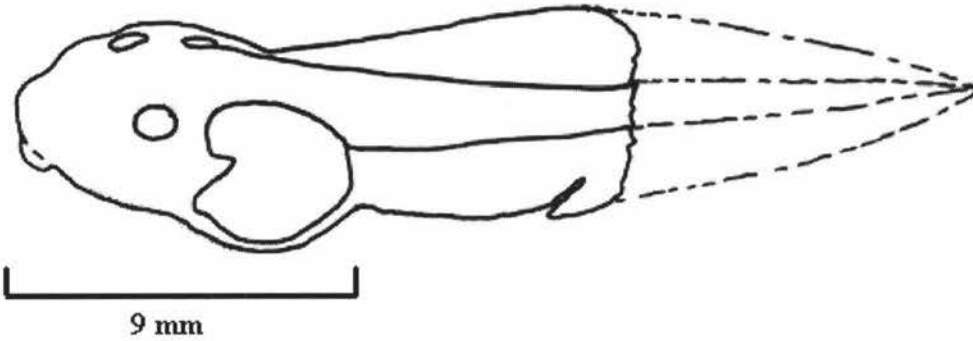
Separate two-sample t-tests revealed that attack frequency was significantly lower in refuge habitat when small tadpoles were attacked by large female mosquitofish ($P = 0.0087$), or by small female mosquitofish ($P < 0.0001$), and when large tadpoles were being attacked by small male mosquitofish ($P = 0.0138$). Within the refuge treatment, the location of the attack (inside or outside of the refuge) had no effect on the prior state of the tadpoles (swimming, resting, or feeding) or the reaction of the tadpoles (active escape or a passive reaction), and neither the size of the tadpoles attacked nor the size/gender of the fish affected the eventual location of the attack. Although there was a significant effect of mosquitofish size/gender on time between consecutive attacks in the ANOVA, when tadpole sizes and refuge treatments were lumped, the average time between attacks was between 11.4 seconds and 15.7 seconds

for all three fish groups, indicating that overall attack frequency does not differ much in relation to fish size or gender.

Injuries sustained during attacks.



a.



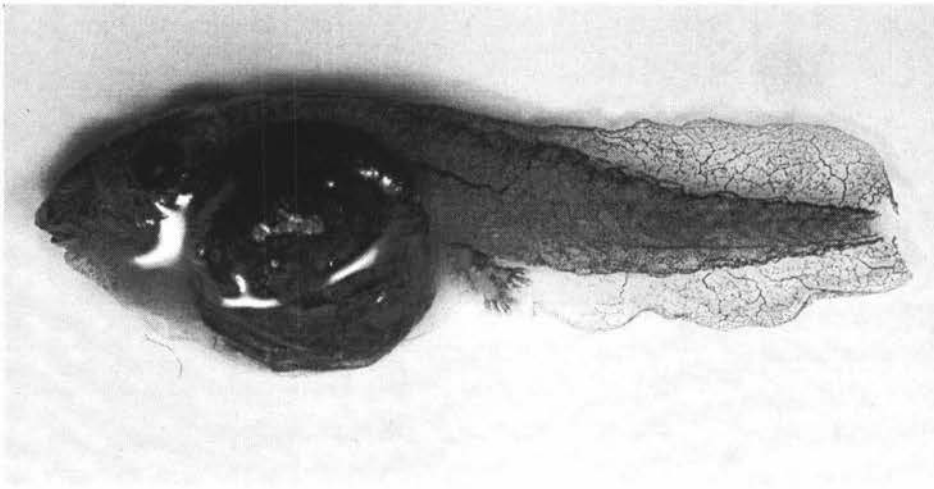
b.

Figure 5.7. Photo of small tadpole injured by mosquitofish (a) and line drawing of the same tadpole to show extent of tissue loss (b).

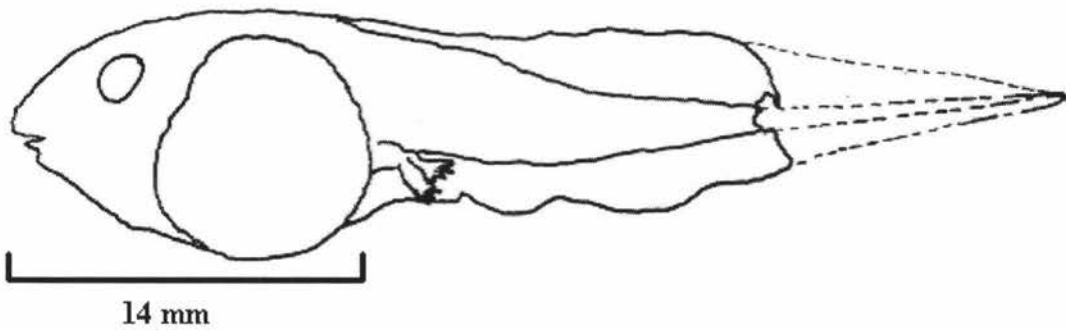
The tadpole in Figure 5.7a was swallowed whole during a trial using small tadpoles, large female mosquitofish, and no refuge habitat. As this attack was the tenth of that trial, the fish were removed immediately after, and the individual that had swallowed this tadpole regurgitated it just after being transferred to the mosquitofish housing tank, presumably due to shock from being moved. The tadpole was still alive on exiting the fish's mouth, but died soon after. Figure 5.7b shows the extent of tissue

loss that the tadpole had suffered (approximately 26% total body mass). There were also lacerations round the eye and oral disc.

The tadpole shown in Figure 5.8a was a large tadpole which received extensive injuries from several attacks during a pilot study. Again, Figure 5.8b shows the amount of tissue loss (approximately 12% total body mass). This tadpole was still alive when removed from the aquarium, but it was sluggish and losing a lot of blood, and was therefore euthanased.



a.



b.

Figure 5.8. Photo of large tadpole injured by mosquitofish (a) and line drawing of the same tadpole to show extent of tissue loss (b).

Discussion.

General observations.

During each trial the mosquitofish roamed the entire tank space. Attacks on tadpoles appeared to be the result of chance encounters, rather than active searching or hunting. This means that as tadpoles increase activity, the encounter rate with a potential predator and therefore attack frequency should increase. However, in the case of a fast moving predator (such as mosquitofish), an increase in prey speed should have little effect on encounter rates, especially if prey speed is low to begin with (Werner and Anholt, 1993), as is the case with whistling frog tadpoles.

Mosquitofish attacked most, but not every, tadpole they encountered, and occasionally attacked each other. This suggests that in the presence of alternative prey whistling frog tadpoles may experience lower predation pressure. The amount of alternative prey available at any time will depend on the life history of resident invertebrate populations. In natural situations mosquitofish reach extremely high densities (pers. ob.) which will result in increased encounter rates.

Attacks on the tail.

Although the same number of attacks were made on the tail as those that came from behind or the side, these were not exactly the same subset of attacks, ie. there were a few occasions where an attack was made on the tail from above, or on the body from the side. However, in general, most attacks made from behind or the side were made on the tail.

One possible advantage to the mosquitofish of attacking the tail rather than the body is that during the attack the fish might go unnoticed longer by the tadpole, thus increasing its chances of a successful attack. The fact that large female fish were more likely than smaller fish to attack the body of a tadpole may be related to their increased swimming speed. Larger fish tend to be faster swimmers (Nashimoto, 1980; Meng, 1993; Drucker and Jensen, 1996; Lightfoot and Jones, 1996; Booth *et al.*,

1997; McDonald et al., 1998), and a faster mosquitofish can afford to be detected earlier. Smaller tadpoles were also more likely than larger tadpoles to be attacked in the body. This could also be related to swimming speeds, smaller tadpoles being slower swimmers (Jung and Jagoe, 1995; Van Buskirk and McCollum, 2000; Watkins, 2000), thus even if they detect approaching predators earlier, small tadpoles may still be too slow to evade attack. Tadpoles of *Pseudophryne bibroni*, *Ranidella signifera*, and *L. ewingi* are all more susceptible to predation by *Hemicordulia tau* (dragonfly) larvae when they are smaller (Richards and Bull, 1990). This is probably related to swimming speed as all sizes encounter the predator equally frequently, and the dragonfly larvae seem to strike at all tadpoles regardless of size (Richards and Bull, 1990).

Alternatively, mosquitofish bias towards attacking the tail may be related to their innate cannibalism. When attacking their own kind, it is probably safer to attack the tail rather than the head region which is likely to bite back. In *Sinea diadema* (spined assassin bugs) first-instar larvae prefer to feed on other, non-predatory species rather than cannibalise conspecifics. This may be because conspecifics have effective self-defence mechanisms (Taylor and Schmidt, 1996). *Heterobranhus longifilis* (vundus) larvae aged 4-17 days cannibalise their siblings by attacking the tail. Larvae older than 17 days switch to an opportunistic ambushing mode of foraging (Baras, 1999). Alternatively, it is possible that tail attacks are a more successful means of catching the tadpoles. Komak and Crossland (2000) noted that when attacking free-swimming *Limnodynastes ornatus* tadpoles mosquitofish ate the tails first and moved on to the body only when the tadpoles were immobilised. In larval *Stizostedion vitreum* (walleyes) trunk attacks are far more common than tail attacks. However, trunk attacks almost always result in the escape of the victim, whereas tail attacks almost always result in the ingestion of the victim (Loadman et al., 1986). Thus, the fact that mosquitofish tend to direct attacks towards tadpole tails may be associated with behaviours learned whilst attacking their own species.

Lastly, as mosquitofish are somewhat gape-limited (they cannot usually consume the entire tadpole in one gulp) the tail might be an easier body part to attack successfully. The tail fin is the thinnest part of the tadpole body, so the fish may have the least trouble getting it's jaws around this section. This could explain why large female fish

made more attacks on the body than smaller fish. Larger fish have larger mouths (Nilsson and Brönmark, 2000), and may be more able to deliver a significant bite to a tadpole body. More body attacks were also made on small tadpoles, possibly because mosquitofish were more able to successfully get their jaws around smaller bodies. As well as receiving more attacks to the body, more attacks on small tadpoles came from above, below or the front. This is not surprising as attacks on the tail would be more likely to come from behind.

The delivery of most attacks to the tail rather than the body is beneficial to the tadpoles. In tadpoles injuries to the tail fin have a good chance of healing, whereas injuries to the body are much more likely to be fatal (Caldwell, 1982; McCollum and Van Buskirk, 1996; McCollum and Leimberger, 1997). However, if the tail fin is attacked repeatedly the tadpole's chance of surviving diminishes. Even in the case of just one attack on the tail, if the centre part of the tail is damaged the tadpole may die from loss of blood (pers. ob.). Additionally, although the mid-portion of the tadpole tail generates most of the thrust, the end is important for reducing turbulence and drag by damping the oscillations of the tail anterior to it (Wassersug and Hoff, 1985). Therefore, a tadpole with a damaged tail end may still be more vulnerable to predation as a result of reduced maximum swimming speed (Wassersug and Hoff, 1985; Figiel and Semlitsch, 1991).

De Santi *et al.* (2001) show that mosquitofish use their left and right eyes differently when inspecting other animals. Therefore, if this research were to be repeated, it may be beneficial to distinguish between attacks made from the left side, and those made from the right side of the tadpole.

Attacks on resting tadpoles.

Although tadpoles spent only about 20% of the time resting (completely immobile), 60% of attacks overall were made on resting tadpoles (Figure 5.4). This is in direct contrast with previous literature, which tends to suggest that immobility is a form of defence for tadpoles because predators are less likely to notice an unmoving prey item (Lawler, 1989; Skelly, 1992; Feminella and Hawkins, 1994; Hokit and Blaustein, 1995; McCollum and Van Buskirk, 1996). This unexpected result may be connected

to the relative sizes of prey (tadpoles) and predators (mosquitofish) in these experiments. As shown in Figure 5.9, feeding and swimming tadpoles quiver their tails. The amplitude of this tail movement may exceed the head width of a mosquitofish. This could make it very difficult for a mosquitofish to accurately direct a strike towards the tail, which is a constantly moving target. During my observations I repeatedly saw mosquitofish miss attempted strikes at the tails of feeding or swimming tadpoles. Wassersug and Hoff (1985) found that the maximum amplitude of a tadpole tail beat increases with swimming speed, finally approaching an asymptote at about 25% of total body length. However, in *Hyla versicolor* (treefrogs), large active tadpoles and small inactive tadpoles are more susceptible to predation than large inactive tadpoles and small active tadpoles (Van Buskirk and McCollum, 2000).

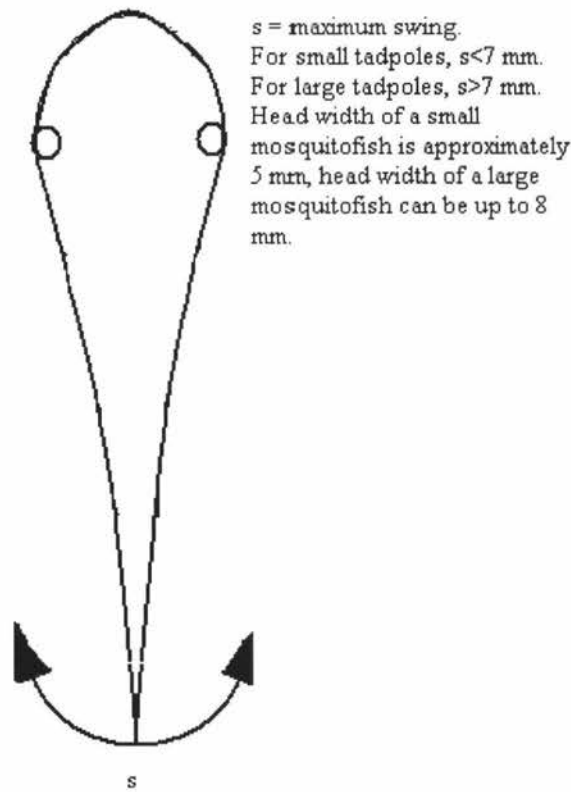


Figure 5.9. Amplitude of tadpole tail movement relative to the head width of mosquitofish. Mosquitofish head widths were measured on a petrie dish with 1 mm gradations.

Small tadpoles were more likely to be attacked while swimming or feeding than large tadpoles. The tail movement of a small tadpole is less wide (Figure 5.9) than that of a large tadpole, so mosquitofish are more likely to successfully attack a smaller moving tadpole than a large moving tadpole. Similarly, larger mosquitofish have wider heads, and therefore are more likely to succeed in any attack on a moving tadpole tail (Figure 5.9).

Tadpole flight response.

Tadpoles commonly reacted to attacks with an active flight response (fleeing or hiding in the refuge where available) rather than a passive response (reorienting, immobility or death). This in itself is no surprise. Large tadpoles passively responded to attack more often than smaller tadpoles by reorientation and immobility (no large tadpoles died during these experiments). There are two possible reasons for this difference. Firstly, in larger tadpoles the mosquitofish is likely to bite off only the very outer rim of the tail fin. This outer edge may have less nerve tissue than more proximal parts of the fin. In general, tadpole tails may have less nerve tissue than the body (Nishikawa and Wassersug, 1989). Alternatively, larger tadpoles lose a smaller percentage of their body mass from each mosquitofish bite, therefore they may have less to lose from receiving a second injury. This seems unlikely though as any injury, no matter how minor, will still cost the tadpole resources to repair.

Tadpoles were more likely to show a passive response if the attack came from a large female mosquitofish. As larger fish are faster swimmers (Nashimoto, 1980; Meng, 1993; Drucker and Jensen, 1996; Lightfoot and Jones, 1996; Booth *et al.*, 1997; McDonald *et al.*, 1998), there may be no point in trying to escape them, although this seems unlikely. Alternatively, perhaps a bite from a larger fish so badly damaged the tail fin that the tadpole was unable to move. However, this is also unlikely because, apart from those tadpoles that died during these experiments (7 in total, from 271 attacks), all tadpoles were able to swim at the end of each trial. All tadpoles that died during these experiments were swallowed whole by the individual fish that attacked them (in other words there was no food sharing).

Other species have been known to vary their response to predators according to the size of the individual attacking them. For example, small *Cottus cognatus* (slimy sculpins) avoid large *Salvelinus fontinalis* (brook trout) but not smaller brook trout individuals. Smaller sculpins also show a greater reduction of activity in the presence of brook trout compared with large sculpins (Chivers *et al.*, 2001).

Time between attacks.

In general, there was more time between consecutive attacks in the presence of refuge (Figure 5.10). More time between attacks would result in a lower attack frequency. This would mean a tadpole would experience fewer attacks over the course of its larval period and, all other things being equal, would have a greater probability of surviving to metamorphosis. There was a significant interaction effect between tadpole size and fish size/gender, and between tadpole size and presence/absence of refuge. This was because refuge presence only significantly decreased attack frequency for some fish/tadpole combinations, small tadpoles in combination with large or small female mosquitofish, and large tadpoles in combination with small male mosquitofish. Small tadpoles may be able to make better use of plant refuges as they are more able to conceal themselves among the foliage.

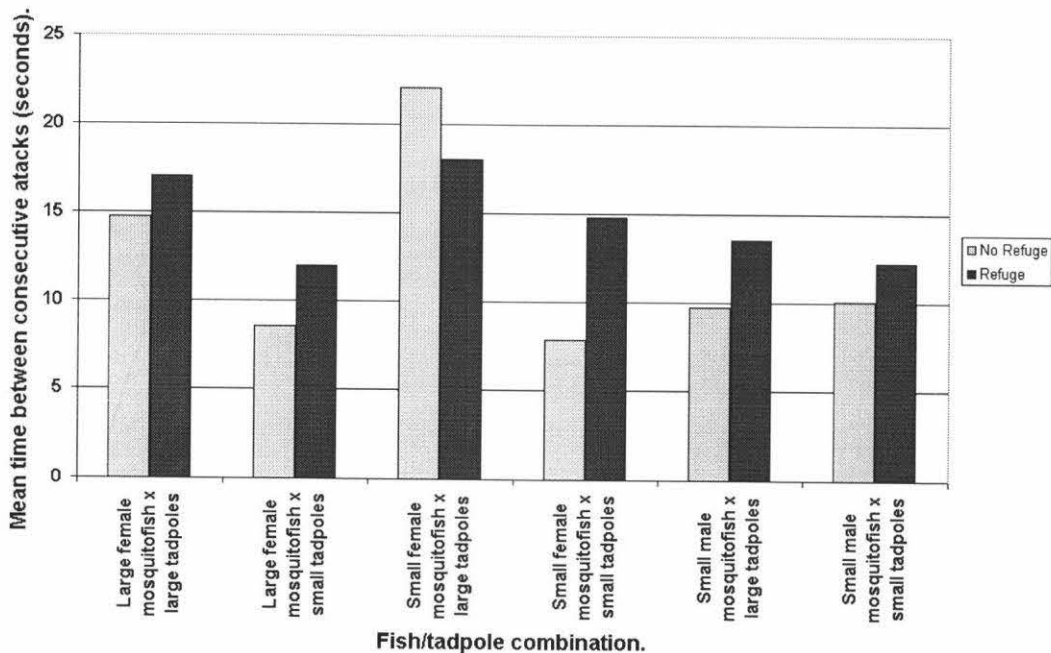


Figure 5.10. Mean time between consecutive attacks for each possible combination of tadpole and mosquitofish size classes, showing no refuge and refuge treatments.

When large tadpoles were attacked by small female mosquitofish, the presence of refuge actually increased attack frequency (Figure 5.10), although the result was not quite significant ($P=0.0728$). Small female mosquitofish prefer to associate with plants (Chapter 4) so perhaps the large tadpoles encountered more fish in the refuge habitat. However, it must be remembered that these experiments were carried out in a small aquarium where the mosquitofish had only a limited amount of places to go. In the wild, they may not spend as much time in the refuge, which would mean that the refuge would be more effective. Also, different types of refuge might have different impacts, for example, a denser plant species might have been a more effective refuge (Folsom and Collins, 1984; Gotceitas and Colgan, 1989; Gotceitas, 1990), although it would have made it more difficult to make observations on attacks.

Injuries sustained during attacks.

Small tadpoles were swallowed whole by the larger fish, suggesting that hatchlings and small tadpoles will suffer fatal attacks from mosquitofish. Large tadpoles tended to receive injuries to the tail fin. In these experiments injuries to the tail fins of large tadpoles were never fatal, but the tadpole in Figure 5.8a which was attacked during a pilot study, was severely damaged and probably would have died from tail injuries if it had not been euthanased. If an individual suffers repeated attacks it may become immobile (Komak and Crossland, 2000) and vulnerable to more serious trauma (Caldwell, 1982; McCollum and Van Buskirk, 1996; McCollum and Leimberger, 1997). At the high densities of mosquitofish found in natural waterbodies (pers. ob) tadpoles may experience frequent attacks. This suggests that mortality will occur, even among the largest tadpoles, and mosquitofish invasions will have a significant effect on whistling frog tadpole populations.

Tadpoles with fatal wounds became sluggish. They tended to float towards the water surface and remain just below it, where they did not invest any effort in maintaining their position. Unlike healthy tadpoles, those with injuries showed little or no response to direct physical stimulus and did not attempt to escape the net when they were removed from the water. These tadpoles were euthanased.

Summary.

1. Attacks appeared to occur as a result of random encounters.
2. Mosquitofish attacked each other as well as the tadpoles, suggesting that in the presence of alternative prey predation pressure on tadpoles will be lessened.
3. Mosquitofish did not attack at each opportunity, but often swam past without lunging at a tadpole.
4. Large fish could swallow small tadpoles whole, but small fish never swallowed whole tadpoles, and large tadpoles were never consumed whole.
5. Most attacks were made on the tail region rather than the body, although small tadpoles received more body injuries than large tadpoles, and large fish were more likely to attack the body than smaller fish.
6. Most attacks were made on resting tadpoles, possibly because fish found it difficult to attack a moving tail on a feeding or swimming tadpole.
7. In most cases tadpoles reacted to attacks by fleeing or hiding in refuge. However, large tadpoles were more likely to respond passively to an attack (by reorienting or being immobile) than small tadpoles, and tadpoles attacked by large mosquitofish were more likely to respond passively than those attacked by small fish.
8. The presence or absence of refuge had no effect on any of the aspects of tadpole and mosquitofish behaviour that were measured, but did affect the overall attack frequency.
9. Bites from mosquitofish can remove a significant proportion of the tadpole tail. Repeat attacks on the same tadpole were observed.
10. Tadpoles with fatal tail wounds became sluggish and remained just below the water surface.

Samenvatting.

1. Aanvallen schenen voor te komen als gevolg van toevallige ontmoetingen.
2. Mosquitofish vielen elkaar aan net als kikkervissen, wat suggereert dat in de aanwezigheid van alternatieve prooi kikkervissen minder zullen worden aangevallen.

3. Mosquitofish vielen niet op elke gelegenheid aan, maar vaak zwommen ze langs zonder op de kikkervis toe te schieten.
4. Grote vissen konden kikkervissen in een bijt opeten, maar kleine vissen aten nooit hele kikkervissen, en grote kikkervissen werden nooit geheel opgegeten.
5. De meeste aanvallen werden gemaakt op de staart (niet het lichaam), al ontvingen kleine kikkervissen meer wonden op hun lichaam dan grote kikkervissen. Grote mosquitofish maakten meer aanvallen op het lichaam van de kikkervissen.
6. De meeste aanvallen werden gemaakt op niet-bewegende (rustende) kikkervissen, mogelijk omdat mosquitofish het moeilijk vonden de bewegende staart van een etende of zwemmende kikkervis aan te vallen.
7. In de meeste gevallen reageerden kikkervissen op aanvallen door te vluchten of door zich te verschuilen in de schuilplaats. Daarentegen reageerden grote kikkervissen vaker met een passieve reactie (door te heroriënteren of door bewegingloos te zijn) dan kleine kikkervissen, en kikkervissen die aangevallen werden door grote mosquitofish reageerden vaker passief dan die die aangevallen werden door kleine mosquitofish.
8. De aan- of afwezigheid van schuilplaatsen had geen effect op enig van de gemeten aandachtspunten van kikkervis of mosquitofish gedrag, maar had wel een effect op de frequentie van aanvallen.
9. Beten van mosquitofish kunnen een gewichtig deel van de kikkervisstaart verwijderen. Herhaalde aanvallen op dezelfde kikkervis werden waargenomen.
10. Kikkervissen met fatale wonden aan hun staart werden traag en bleven net onder de oppervlakte van het water.

Chapter 6

**Field experiment on predator-prey interactions between
mosquitofish (*Gambusia affinis holbrooki*) and whistling frog
(*Litoria ewingi*) tadpoles.**

Introduction.

Laboratory studies are most commonly used to investigate predator-prey interactions (Skelly, 1995; see also Chapter 2). Because conditions in the laboratory can be controlled data collection is usually simplified and results easier to interpret with fewer confounding factors (Martin and Bateson, 1993). However, events recorded in the laboratory may not be observed in the wild, so laboratory studies can give misleading results (Travis *et al.*, 1985*a*; Martin and Bateson, 1993). It is, therefore, useful to repeat indoor experiments in the field to compare the relationships in captive and wild settings.

The preceding three chapters deal with laboratory experiments on the use of microhabitat in whistling frog tadpoles (Chapter 3) and mosquitofish (Chapter 4), and the behaviour of both species in the event of an attack on a tadpole by a mosquitofish (Chapter 5). In addition to these laboratory experiments I conducted a simple field experiment to support what I observed in the laboratory. The purpose of this experiment was to measure the effect that mosquitofish presence had on tadpole biomass, by loss of mass through bites from the fish, and the loss of weight through reduced activity (Ball and Baker, 1996; Relyea and Werner, 1999; Van Buskirk and Schmidt, 2000). I expected the tadpoles exposed to mosquitofish to lose more weight during trials compared to those in the control treatment.

Materials and Methods.

The field experiment was conducted in Anderson Park, Napier. At this site there are two artificial lakes connected by a ditch. This ditch usually contains a large population of mosquitofish. There are no whistling frog tadpoles at this location, so study individuals were transported from laboratory populations at Massey University, Palmerston North.

The trials were conducted using a large mesh enclosure measuring 1 x 1 x 1.5 m. The mesh bag was made of coarse whitebait net (hole diameter c. 2 mm), and supported by a metal frame. The corners of the bag were tied to this frame to keep the shape.

At the start of each trial 10 tadpoles measuring 5-10 mm snout to vent (Gosner stages 24-31, Gosner, 1960) were weighed collectively on an electronic balance in grams to two decimal places. They were weighed by taring the scale with a beaker of water on it, and then adding the tadpoles one by one by hand. This may have produced some error as a film of water would have been present on the surface of the tadpoles, but this error should have been fairly consistent among tadpole groups. As there was no power supply at the field site in Anderson Park, weighing was done at a nearby cabin (approximately ten minutes drive away). The tadpoles were then added to the enclosure, which was placed in the middle of the ditch in water approximately 1 m deep. There were two treatments, a control (“control treatment”) in which the tadpoles were left alone, and a predator treatment (“fish treatment”) where 19.43 g of mosquitofish (approximately 200 individuals) were added. It is not unusual to see this density of fish in the ditch (pers. ob.). There were three replicates each of the control and fish treatments and the same mosquitofish were used for the three fish treatment trials, but different tadpoles were used in each of the six trials.

After all animals (tadpoles in the control; tadpoles and fish in the fish treatment) had been added, they were left undisturbed in the enclosure for 1.5 hours, at which time tadpoles were removed and re-weighed, and fish were placed in a bucket, or, in the case of consecutive fish treatment trials, left in the mesh enclosure to await the next group of tadpoles. No behavioural observations could be taken while the animals were in the enclosure because the mosquitofish reacted strongly to observer presence by diving towards the substrate and the water was extremely turbid. The trials were done in pairs to lessen the effect of time of day. In the first pair the control treatment had to be done first as we needed time to collect the fish. The order of the other two pairs was randomised using a coin toss, thus the order of the treatments was: control, fish, control, fish, fish, control. All trials were conducted on the same day (3 April, 2000). Afterwards the tadpoles were taken back to the laboratory and eventually released to their natal trough. The fish were returned to the laboratory population at Massey University.

Analysis.

Data were analysed with a repeated measures ANOVA. Analysis was done using SYSTAT 6.0 for Windows (SPSS, 1996).

Results.

In the repeated measures ANOVA none of the results was significant (Table 6.1).

| | P |
|--|----------|
| Control versus treatment | 0.4616 |
| Weight before trial versus weight after | 0.6324 |
| Interaction effect | 0.6375 |

Table 6.1. ANOVA probabilities for weights of tadpoles in the field experiment.

When combining weights of tadpoles before and after the trials, there was no significant difference between the control tadpoles and those used in the fish treatment ($P=0.4616$). Combining both the control treatment and the fish treatment, there was no significant difference between the weights of tadpoles before the trials and the weights at the conclusion of the trial ($P=0.6324$). The treatment did not have an effect on the weight change of the tadpoles during the trials ($P=0.6375$).

Discussion.

None of the results of the trial was significant (Table 6.1). Predictably there was no difference overall between the tadpoles used in the control and the fish treatment because the tadpoles were selected from the same experimental pool. For a similar reason there was no overall difference in the weights of tadpoles before and after trials when both treatments were combined. Under predator-free conditions tadpoles tend to feed almost continuously, either by scraping periphyton off surfaces or filtering suspended alga (Dickman, 1968). However, in the presence of predators, tadpoles tend to reduce their activity and therefore their food intake (McNamara and Houston,

1987; Anholt and Werner, 1998). If tadpoles in the control treatment were free to feed on the available alga and therefore gained weight, but tadpoles in the fish treatment lost weight because they were inhibited by the presence of predators, these two opposing effects on weight change could have balanced each other out over the course of trials.

There was, however, expected to be a greater loss of tadpole biomass in the fish treatments than in the control treatments as a result of predacious attacks by the fish. If the fish were taking bites from the tadpoles this would result in overall weight loss, and we expected the tadpoles to decrease their activity and therefore feeding efficiency in the presence of predators, as noted by McNamara and Houston (1987) and Anholt and Werner (1998). With separate pairs, tadpoles in the first control lost 8.2% of their original collective mass, whereas in the fish treatment they lost 33.9%. After this tadpoles gained 2.3% (control), 25.2% (fish), 21.2% (fish), and 61.5% (control) in the other four treatments ie. there was no consistent pattern.

In the field enclosure there were no alga-covered surfaces for the tadpoles to scrape for food, so filter feeding was probably their only option. Tadpoles can still filter feed when inactive, although generally their intake rate is higher while on the move (Werner and Anholt, 1993). It is, therefore, unlikely that tadpoles could decrease their activity in response to predators yet still maintain the same rate of food intake (Eklöv and Halvarsson, 2000).

The tadpoles were transported from Palmerston North to Napier, a journey of approximately 2.5 hours, in a bucket containing fresh water, on the evening before the trials. Although the trials were all conducted over the course of one day, it is possible that as time wore on, the tadpoles defecated their gut contents and started metabolising reserves. This theory is supported by the start weights of the last four tadpole groups which get progressively lower through time. Although all the tadpoles were roughly the same size, the first group weighed 2.19 g at the start of their trial, and the last group weighed just 0.78 g. Tadpoles feed by grazing on alga (Dickman, 1968). Grazing animals characteristically have a fairly constant intake of food, although their food is generally of low nutritional value. It is likely that food deprivation even for just a few hours might cause the tadpoles to start metabolising

their fat reserves (Hervant *et al.*, 1999; Hervant *et al.*, 2001), accounting for the hefty decrease in weight noticed over the course of the day. The water in the field enclosure contained periphyton and other sources of food. After being starved some time the tadpoles' metabolism may have been geared to assimilate food at a very fast rate in order to replenish energy stores, accounting for the increasing weight gains over the course of the trials. Hervant *et al.* (2001) found that salamanders adapted to habitats with a variable food source can recover from food deprivation very quickly. Tadpoles may also have failed to respond to predators because the urgent need to replenish lost energy reserves took priority over normal antipredator responses (Laurila *et al.*, 1998; Van Buskirk and McCollum, 2000).

In conducting these experiments, it was not possible to transport the tadpoles' home tank to Napier complete with their normal alga food source. This problem is inherent in field studies using two animals whose ranges do not overlap, as one species must always be transported, and it is not always possible to provide the same conditions as in the laboratory. This can impact on the results of the field experiments. It may be useful to repeat these experiments or similar trials when mosquitofish populations reach whistling frog breeding grounds. Alternatively, trials could be run simultaneously to eliminate the problem of different groups having different hunger levels. It may also be possible to even out hunger levels between groups by feeding each one a set period of time before the start of their trial.

Summary.

1. Mosquitofish presence had no significant effect on the net weight gain of the tadpoles over the course of the 1.5 hour field trials.
2. Tadpoles had been out of their normal housing aquarium and may therefore have been hungry. This could have caused them to forego normal antipredator responses in order to replenish lost energy stores by feeding on available alga resources in the field enclosure.

Samenvatting.

1. De aanwezigheid van mosquitofish had geen significant effect op de netto vermeerdering van het gewicht van de kikkervissen gedurende de anderhalfuur lange veldproeven.
2. Kikkervissen waren niet in hun normale aquarium en hadden daarom misschien honger. Dit had kunnen veroorzaken dat ze hun normale anti-roofdier reacties voorafgingen om hun verloren voorraad energie bij te vullen door beschikbare alge te eten in de veldkooi.

Chapter 7

General discussion.

This thesis has reviewed previous work on predator-prey interactions, focussing on those involving amphibian larvae as the prey species (Chapter 2); the use of microhabitat by both whistling frog tadpoles (Chapter 3) and mosquitofish (Chapter 4); the behaviour of both species during attack sequences (Chapter 5); and attempts to duplicate some of the laboratory experiments in the field (Chapter 6). Here all the results are pulled together in a synthesising discussion.

Microhabitat preferences and encounter rates.

In the laboratory tadpoles were very strongly attracted to the edge of the tank whereas mosquitofish showed no preference for the edge. This difference in behaviour could reduce the encounter rate of the two species in the wild if tadpoles tend to aggregate at the edges of waterbodies in which mosquitofish occupy more open water.

Large tadpoles were attracted to an alga chip (food source), but small tadpoles avoided such food, although they readily consumed it in the rearing tank. It is possible that, for innate reasons, they were avoiding competition with larger tadpoles by not approaching the food (Alford and Crump, 1982; Flores-Nava and Vera-Munoz, 1999). Since the small tadpoles had never been exposed to larger individuals a learned response to the larger individuals is very unlikely.

Small female mosquitofish exhibited a significant preference for complex plants over open water, and for sand substrates over gravel, but large female mosquitofish, small male mosquitofish and tadpoles showed no preference for either of these features. This suggests that, in the absence of other constraints, in a heterogeneous habitat large female mosquitofish, small male mosquitofish and tadpoles will distribute themselves equally over open water and densely planted habitats, and over gravel and sand substrates. This indicates, therefore, that sooner or later tadpoles and mosquitofish would be likely to encounter each other in natural habitats. Tadpoles have previously been proven to spatially avoid predators and predator signals (Sih *et al.*, 1992; Relyea and Werner, 1999; Laurila, 2000; Relyea, 2001*a*). In these experiments, however, tadpoles did not appear to spatially avoid mosquitofish.

Small male and small female mosquitofish responded to stimuli in a very similar way. Associating with smaller females may be beneficial for males as it allows them to breed with less risk of being cannibalised (Winkelman and Aho, 1993; Benoit et al., 2000), although smaller female mosquitofish do have a lower fecundity (Bisazza et al., 1989). In terms of inclusive fitness, therefore, breeding with smaller rather than larger females carries a cost for the male.

Small mosquitofish show a higher preference for plant habitats than their larger counterparts. Because mosquitofish are cannibalistic, with larger individuals commonly attacking the smaller fish (Winkelman and Aho, 1993; Benoit et al., 2000), this association with a refuge habitat may be a form of predator avoidance on the part of small mosquitofish.

Larger mosquitofish showed a greater attraction than did small fish to tadpoles, but there was no overall difference in attack frequency between the three mosquitofish size/gender groups. Possibly, although larger fish spend more time around tadpoles they do not attack them as often. Certainly during my observations mosquitofish frequently came very close (c. 5-10 mm) to tadpoles without making any move to attack.

Refuge and attack rates.

Sometimes prey modify the strength of their antipredator behaviour in relation to the amount of risk posed by a predator. For instance *Cottus cognatus* (slimy sculpins) avoid areas containing large *Salvelinus fontinalis* (brook trout) but show no such aversion to smaller brook trout individuals (Chivers et al., 2001). However, whistling frog tadpoles did not spatially avoid mosquitofish - a novel predator - when simultaneously exposed to both visual and chemical signals from them, which suggests that they will not change their microhabitat preferences in response to the presence of mosquitofish in the wild. However, large tadpoles did spend more time at the substrate in the presence of the caged mosquitofish. Sand is readily stirred up and can conceal prey items (Lawler, 1989). Therefore, large tadpoles in sand habitats might be less vulnerable to predation, especially from the small female mosquitofish that aggregate there. Somewhat surprisingly, the presence of plants as a potential

refuge increased the attack frequency when small female mosquitofish were exposed to large tadpoles. This may be because the small female mosquitofish prefer the refuge surroundings provided by the plants. Attacks were less frequent, however, when refuge (provided by a complex plant) was available for some combinations of mosquitofish and tadpole size classes, indicating that tadpoles may still gain some protection from such a refuge.

Activity and attack rates.

In the presence of mosquitofish large tadpoles spent more time on the substrate, and reduced their activity. Lowered activity can make prey less conspicuous and therefore less vulnerable from predation by both visual and tactile predators (Lawler, 1989; Skelly, 1992; Feminella and Hawkins, 1994; Hokit and Blaustein, 1995; McCollum and Van Buskirk, 1996; Van Buskirk and McCollum, 2000; Relyea, 2001a; Richardson, 2001). However, these experiments demonstrated that in the case of mosquitofish and whistling frog tadpoles, resting tadpoles are actually more likely to be attacked, possibly because their immobile tails provide a less difficult target for mosquitofish. Van Buskirk *et al.* (1997) found that although *Pseudacris triseriata* (chorus frog) tadpoles reduce their activity in response to *Anax* (dragonfly) larvae the more inactive individuals do not survive any better. Therefore, when exposed to predatory mosquitofish, it is non-adaptive for large tadpoles to decrease their activity. This may be the case for other predatory fish of a similar size relative to the tadpoles.

In the presence of simple plants in isolation, and simple and complex plants combined, large tadpoles drop down towards the substrate, whereas small tadpoles move up to the surface and quiver their tails constantly. The tadpoles are more vulnerable to predation when their tails are still which suggests that in the presence of plants smaller tadpoles experience lower predation rates than their larger counterparts. Once attacked, larger tadpoles may be more likely to survive because they are faster swimmers (Wassersug and Hoff, 1985) and therefore better able to escape. Large tadpoles also have wider tail fins, thus a bite into one fin is not as likely to damage the central muscle part of the tail, which bleeds readily.

Body versus tail wounds.

In general, when mosquitofish attacked tadpoles they made more attacks on the tail, coming from behind or the side of the prey. This may allow the mosquitofish to go unnoticed by its prey for longer. It is also possible that mosquitofish attack all things from behind because, when attacking each other, this is least likely to result in a counter-attack from the prey (Taylor and Schmidt, 1996; Baras, 1999). Attacks on the tail may be more likely to result in successful capture than attacks on the body because the tadpole may be immobilised, allowing the mosquitofish to come back for further pieces of food (Komak and Crossland, 2000).

If attacks to the body are more likely to be fatal (Caldwell, 1982; McCollum and Van Buskirk, 1996; McCollum and Leimberger, 1997; Van Buskirk and McCollum, 2000), and smaller tadpoles receive a greater proportion of attacks on the body it might be advantageous for tadpoles to divert some of their nutrients from development into growth. Other tadpole species have been observed to do this in order to reach a size refuge more quickly (Calef, 1973; McCollum and Leimberger, 1997; Anholt and Werner, 1998). Slowing development can lengthen the larval period (Skelly and Werner, 1990; Laurila and Kujasalo, 1999; Van Buskirk and Schmidt, 2000; Relyea, 2001a). This means in turn that the tadpoles spend longer in water, and therefore are more likely to be preyed on by mosquitofish sometime during the course of their development. However, Relyea (2001b) found that *Rana sylvatica* (wood frog) tadpoles that had been previously exposed to predacious *Anax* (dragonfly) larvae did lengthen their larval period in order to modify their morphology to a more predator-resistant form.

Implications for adult populations.

Recruitment of adult populations of whistling frogs is largely dependent on tadpole survival. At present in New Zealand there are no known populations of whistling frog tadpoles in contact with mosquitofish. A large part of the whistling frog range (Figure 1.1), along the West Coast and in Southland, has no reported mosquitofish populations, so these frogs are currently safe. However, mosquitofish spread very easily, and are difficult to eradicate (McCullough, 1998), and now that they have

invaded the Manawatu region (Myers, 2001) and Nelson area (P. B. Studdum, pers. comm.) it is very likely that whistling frog tadpoles will soon be exposed to mosquitofish in the wild. The effect of mosquitofish on exposed tadpole populations will carry over into the adult populations, and ultimately have a roll-on influence in community food webs, some of which include native fish and birds.

Many prey species gain partial protection from predators through aggregating in refuge areas (Folsom and Collins, 1984; Kats *et al.*, 1988; Lawler, 1989; Sih *et al.*, 1992; Cooper *et al.*, 1999; Kiesecker *et al.*, 1999; Lefcort *et al.*, 1999). However, in these trials presence of complex plants as a refuge had a limited effect on attack frequency. This indicates that although augmenting natural plant stocks to provide additional refuge may lessen the effect of mosquitofish invasions on whistling frog populations, there will still be considerable predation and therefore considerable loss to adult recruitment. The frequency of attacks in the laboratory suggests that mosquitofish are capable of eliminating whistling frog tadpoles from water bodies even when refuges are present. Therefore it would be much better to halt the spread of mosquitofish before they reach populations of whistling frog tadpoles, or other prey species who might be susceptible to predation by these generalist predators (Simberloff and Stiling, 1996). Public education is an important step in halting the spread of this species, as it is still viewed by many to be the ideal mosquito control agent and therefore frequent introductions occur.

Summary.

1. When mosquitofish arrive as novel predators in whistling frog breeding habitats, they are likely to prey on whistling frog tadpoles.
2. Microhabitat preferences of both species may affect the efficacy of mosquitofish predation on tadpoles.
3. Although it may protect them from other aquatic predators, reduction of activity is not a sufficient defence against mosquitofish predation.
4. The existence of a refuge reduced attack frequency, but not very much. Therefore, augmenting refuge habitats in natural water bodies will have limited effect on mosquitofish predation rates.

5. Tadpoles can recover from injuries to the tail, but it presumably requires new resources to repair these and continue growing.
6. Injuries to the tail can be fatal.
7. Young tadpoles (up to at least two weeks after hatching) can be swallowed whole.
8. At densities of mosquitofish observed in the field, predation rates could be very high, and whistling frog tadpoles may be excluded from certain water bodies due to predation from mosquitofish.
9. Once mosquitofish and whistling frogs are sympatric whistling frog adults may be reduced to breeding in temporary water where their offspring are less likely to be exposed to predation from mosquitofish.
10. The impact of mosquitofish on whistling frog populations could have roll-on effects through community food webs on some native fish and birds.

Samenvatting.

1. Wanneer mosquitofish als nieuwe roofdieren aankomen in whistling frog kikkervis woonplaatsen is het waarschijnlijk dat ze whistling frog kikkervissen zullen plunderen.
2. Keuzen van allebei de soorten wat betreft hun plaatsing in het water zal misschien een effect hebben op de uitwerking van mosquitofish plundering van deze kikkervissen.
3. Al beschermt het ze misschien van andere aquatische roofdieren, de vermindering van bedrijvigheid is niet een geschikte bescherming tegen plundering door mosquitofish.
4. De aanwezigheid van een schuilplaats verminderde de frequentie van aanvallen, maar niet bepaald veel. Daarom zal de vermeerdering van schuilplaatsen in natuurlijke wateren een beperkt effect hebben op de veelvuldigheid van aanvallen.
5. Kikkervissen kunnen genezen van wonden tot de staart, maar het kost vermoedelijk nieuwe middelen om deze te repareren.
6. Wonden tot de staart kunnen fataal zijn.
7. Jonge kikkervissen (tot minstens twee weken nadat ze uit het ei komen) kunnen geheel opgegeten worden.

8. Met de dichtheid van mosquitofish die in natuur bestaat kunnen aanvallen zeer veelvuldig voorkomen, en whistling frog kikkervissen zullen mogelijk van sommige wateren buitengesloten worden als gevolg van plundering door mosquitofish.
9. Eens mosquitofish en whistling frogs dezelfde regio's bewonen zijn whistling frogs mogelijk verplicht om hun eieren in tijdelijke wateren te leggen waar kikkervissen minder kans hebben geplundert te worden door mosquitofish.
10. De invloed van mosquitofish op whistling frog bevolkingen zal mogelijk voortrollende effecten hebben op sommige inheemse vissen en vogels omdat deze ook kikkervissen en volwassene kikkers eten.

Chapter 8

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