IMPACT OF EGGS, HATCHLINGS AND TADPOLES OF THE INTRODUCED CANE TOAD *Bufo marinus* (ANURA: BUFONIDAE) ON NATIVE AQUATIC FAUNA IN NORTHERN QUEENSLAND, AUSTRALIA

Thesis submitted by

Michael Richard CROSSLAND BSc(Hons) *JCUNQ*

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in the Department of Zoology and Tropical Ecology
James Cook University of North Queensland

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ABSTRACT

The impact of the eggs, hatchlings and tadpoles of the introduced cane toad, *Bufo marinus*, on native aquatic fauna in northern Queensland, Australia was investigated using a series of replicated laboratory and artificial pond experiments. Specifically, the project investigated: (1) the toxic effects of *Bufo* on native aquatic species, (2) predation by *B. marinus* tadpoles on native aquatic species, (3) competition between *B. marinus* tadpoles and native aquatic species, and (4) higher order effects produced by *Bufo* on other trophic interactions within native aquatic animal assemblages.

The toxic effects of *Bufo* on native aquatic fauna were always associated with the consumption of *Bufo*; there was no evidence that toxins are released from *Bufo* into solution. Native aquatic species exhibited considerable inter- and intraspecific variation in their susceptibility to *B. marinus* toxins. *Bufo* were highly toxic to certain species but were non-toxic to others. Interspecific variation in toxic effects was not related to the number of *Bufo* ingested, and there was no clear pattern of distribution of vulnerability among species within higher taxa. Intraspecific variation in responses to toxins may result from (1) differences in the number of *Bufo* ingested by individuals, (2) individual variation in resistance to *B. marinus* toxins, or (3) individual variation in toxicity of *Bufo*.

Two "susceptible" native aquatic taxa (fish and anuran larvae) were chosen for detailed studies. Native fish (barramundi: *Lates calcarifer*; sooty grunter: *Hephaestus fuliginosus*) usually learned with minimal trauma to avoid *B. marinus* tadpoles. Populations of these species are therefore unlikely to experience significant declines in water bodies where they co-occur with *Bufo*. Anuran larvae, however, exhibited considerable interspecific variation in their ability to detect and avoid *B. marinus* toxins. Artificial pond experiments demonstrated that populations of species which have limited ability to detect and avoid *B. marinus* toxins (*Litoria bicolor*, *L. nigrofrenata*, *Limnodynastes ornatus*) experienced significant increases in mortality when exposed to *Bufo*. However, the toxic effects of *Bufo* on *L. ornatus* tadpoles indirectly facilitated the survival of eggs and hatchlings of later breeding native anurans by reducing the intensity of predation on these early life history stages by *L. ornatus* tadpoles.

Bufo tadpoles were not significant predators of native anuran eggs, hatchlings or tadpoles, but did compete with native tadpoles (*L. ornatus*). The outcome of competition between *B. marinus* tadpoles and *L. ornatus* tadpoles was determined by their order of introduction into ponds. Generally, each species performed better when added to ponds before the other species, and performed worse when added to ponds after the other species, as compared to when both species were added to ponds simultaneously. However, the toxic effects of *B. marinus* eggs

and hatchlings on *L. ornatus* tadpoles reversed these competitive priority effects and allowed late breeding *Bufo* to perform as well as, or better than, conspecifics which were added to ponds prior to *L. ornatus*.

The results demonstrate that *B. marinus* eggs, hatchlings and tadpoles may have a significant impact on the composition and dynamics of native aquatic communities, and in particular, on native larval anuran communities.

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any University or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

30/4/97

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This thesis is dedicated to my family, without whom, none of this would have been possible.

CHAPTER 1. GENERAL INTRODUCTION: ECOLOGICAL IMPACTS OF INTRODUCED SPECIES

1.1 Introduction

Human activities have resulted in the accidental or deliberate introduction of many plant and animal species into areas outside their natural ranges. Some species have been introduced from foreign countries, while others have been translocated from one region to another within the same country. Large-scale examples of species introductions include the construction of the Suez Canal that connected the Red and Mediterranean Seas, and the Welland Canal that connected the Atlantic Ocean to most of the North American Great Lakes (Aron and Smith 1971). Smaller scale introductions also occur frequently (Lodge 1993). In a recent review, Lodge (1993) reported that the number of introduced species in most countries ranges between 100 to 10 000. However, these are likely to be underestimates as many species introductions probably go undetected (Lodge 1993).

Once a species is introduced into a community, its successful establishment is contingent upon a variety of abiotic and biotic factors including appropriate climatic conditions, the availability of suitable habitat, its competitive ability relative to native species, and its vulnerability to local predators and disease (Crawley 1986; Diamond and Case 1986; Brown 1989). Introduced species which become established may subsequently affect native biota via a variety of direct

and indirect mechanisms. Direct interactions include competition, herbivory, predation, parasitism and hybridisation. Indirect effects may occur via changes in habitat characteristics, cascading trophic effects, and alteration of ecosystem processes (e.g. fire regimes, hydrological cycles, nutrient cycles).

The impacts of introduced species on native biota range from little or no detectable impact, through to large-scale effects which alter the composition and dynamics of native communities and, in some instances, change properties of entire ecosystems (Elton 1958; Drake *et al*. 1989). The extent to which a particular introduced species will affect native biota depends on a number of factors. Pimm (1987) suggested that introduced species are likely to have severe impacts when (1) species are introduced into predator-free areas, (2) polyphagous species are introduced, or (3) species are introduced into relatively simple communities where the removal of a few species may alter entire food chains. In addition, introduced species have the potential to change ecosystems when they (1) differ substantially from native species in resource acquisition or utilisation, (2) alter the trophic structure of native communities, or (3) alter the frequency and/or intensity of disturbance (Vitousek 1990).

1.2 Impacts of Introduced Flora

1.2.1 Effects on Native Flora

Many studies have documented changes in native plant communities resulting from competition between introduced plants and native flora. Brockie *et al.* (1988) reported the replacement of native plants in the Galápagos Islands by the competitively superior introduced guava (*Psidium guajava*). In Australia, the introduced shrub *Chrysanthemoides monilifera* outcompetes and displaces native *Acacia longifolia* from coastal dunes due a more effective arrangement of photosynthetic tissue (Weiss and Noble 1984a, b; Noble 1989). Prickly pear shrub (*Mimosa pigra*) also outcompetes and replaces a variety of native Australian plant communities (Braithwaite *et al.* 1989), while introduced pine trees (*Pinus radiata*) restrict the recruitment of native eucalypt trees by outcompeting them (Burdon and Chilvers 1994). In the Mediterranean, the introduced seaweed *Caulerpa taxifolia* replaces native marine flora due to its competitive superiority (Boudouresque *et al.* 1992), while in Chesapeake Bay, the introduced aquatic plant *Hydrilla verticillata* outcompetes and displaces native aquatic plants (Posey *et al.* 1993).

Introduced plants may also inhibit the growth and/or recruitment of native plants by altering light regimes or by producing dense layers of leaf litter. In Ireland, introduced *Rhododendron ponticum* inhibits recruitment of native oak (*Quercus petraea*) and holly (*Ilex aquifolium*) by creating a dense shade and by forming an impenetrable litter layer (Usher

1986). Similarly, *Casuarina litorea* prevents the establishment of native plants in the Bahamas by creating dense shade (Heywood 1989). In Australia, the alteration of light regimes by *Mimosa pigra* reduces the density and diversity of many native herbs and tree seedlings (Braithwaite *et al.* 1989). However, in some instances, increased shading by introduced plants may favour native species. In Hawaii, shading by the introduced tree *Myrica faya* enhances the survival and growth of native trees (*Metrosideros polymorpha*) in areas where *M. polymorpha* seedlings are established prior to colonisation by *M. faya* (Vitousek and Walker 1989). However, the dense leaf litter layer produced by *M. faya* prevents the germination of *M. polymorpha* seeds (Vitousek and Walker 1989).

Introduced plants may also have significant impacts on native flora via alteration of ecosystem processes. These effects are discussed in Section 1.2.3.

1.2.2 Effects on Native Fauna

The introduction of non-native plants into a community may adversely affect native fauna by reducing the quantity or quality of food resources which are available to them. Areas of northern Australia which have been colonised by *Mimosa pigra* and *Tamarix aphylla* have reduced populations of native birds and reptiles (Braithwaite *et al.* 1989; Griffin *et al.* 1989). One of the main reasons for this is a reduction in food

resources for these species in *Mimosa* and *Tamarix* dominated communities (Braithwaite *et al.* 1989; Griffin *et al.* 1989). Similarly, colonisation of intertidal mudflats of south-eastern Australia by the introduced sea grass *Spartina* spp. has resulted in a reduction in benthic microfauna which are an important component of the diet of native water birds (Humphries 1991). The domination of coastal areas of the Mediterranean by the introduced seaweed *Caulerpa taxifolia* poses a major risk to native sea urchins (*Paracentrotus lividus*). This seaweed possesses toxins which produce lethal or sublethal effects when consumed by *P. lividus* (Lemée *et al.* 1993; Boudereseque *et al.* 1996). Since *C. taxifolia* has become the only potential food source for sea urchins in many areas, populations of *P. lividus* are likely to be reduced in the near future (Boudereseque *et al.* 1996).

Introduced plants may also adversely affect native fauna by inhibiting their recruitment. On the Galápagos island of Floreana, dense thickets of lantana (*Lantana camara*) prevent petrels (*Pterodroma phaeopygia*) from accessing their nest burrows (Macdonald *et al.* 1989). Replacement of native vegetation by *Tamarix aphylla* in northern Australia is also likely to affect native avifauna by reducing the number of available nesting sites for native birds which require tree-hollows for nesting (Griffin *et al.* 1989). However, in some instances, introduced plants may have positive effects on native fauna. The dominance of aquatic plant communities by *Hydrilla verticillata* in Chesapeake Bay has

resulted in an increase in eight of the thirteen common macrofaunal taxa, probably due to increased refugia from predators and/or increased nutrients (Posey *et al.* 1993). In northern Australia, invasion by *Mimosa pigra* may benefit small native mammals by increasing shelter from avian predators (Braithwaite *et al.* 1989). However, this advantage is likely to diminish as *M. pigra* spreads and eventually displaces the native vegetation on which these mammals feed (Braithwaite *et al.* 1989).

1.2.3 Effects on Ecosystem Processes

Several studies have demonstrated that introduced plants may alter various ecosystem processes and thereby affect native biota. Many introduced plants alter fire regimes by increasing the probability, extent and intensity of fires, which in turn may alter native communities. In South Africa, the introduced climbing plant (*Chromolaena odorata*) has colonised savanna-forest ecotones which normally act as natural fire breaks. Due to the high flammability of this species, fires now burn from the savanna into the forest margins and kill the fire-sensitive forest trees. As a result, the size of native forest patches has been reduced (Macdonald *et al.* 1989). In western North America, introduced cheatgrass (*Bromus tectorum*) has replaced native perennial grasses by increasing the fire intensity experienced by native species (Macdonald *et al.* 1989). Similarly, in north-western Australia, introduced buffel grass (*Cenchrus ciliaris*) displaces native grasses and sedges by altering the intensity and extent of the fire regime (Humphries 1991), while in north-

eastern Australia, the proliferation of lantana (*Lantana camara*) increases fire intensity, causing severe damage to native vegetation (Fensham *et al.* 1994).

Introduced plants may also impact upon native biota by altering biogeochemical cycles. In Australia and California, the introduced ice-plant (*Mesembryanthemum crystallinum*) accumulates salt from the rooting zone and consequently increases the salt content in the soil to levels above the osmotic tolerance of many native species. As a result, *M. crystallinum* is able to dominate native grassland communities where it has been introduced (Vivrette and Muller 1977; Kloot 1983; Macdonald *et al.* 1989; Ramakrishnan and Vitousek 1989). Similarly, *Tamarix aphylla* alters soil salinity in northern Australia by secreting large quantities of salt from its foliage which subsequently leach into the soil when the leaves drop. Consequently, few native plants are able to persist in areas colonised by *T. aphylla* (Griffin *et al.* 1989). In Papua New Guinea, the water fern *Salvinia molesta* alters benthic faunal communities by reducing the amounts of dissolved oxygen, phosphate-phosphorus and nitrate nitrogen present in aquatic ecosystems (Mitchell *et al.* 1980; review by Thomas and Room 1986), while in Hawaii, the introduced nitrogen-fixing tree *Myrica faya* alters the nature of ecosystem development by quadrupling the amount of nitrogen present in the soil (Vitousek *et al.* 1987b; Vitousek and Walker 1989; Aplet 1990; Vitousek 1990; Walker and Vitousek 1991).

Several studies have demonstrated that introduced plants may also affect native communities by altering hydrological cycles. The introduction of *Tamarix* spp. into south-western North America has resulted in marshes and swamps being converted into dry areas because *Tamarix* has higher transpiration rates than native plants (Ramakrishnan and Vitousek 1989). Similarly, the increased transpiration rates of introduced trees and shrubs such as *Hakea sericea*, *Pinus pinaster*, *Acacia longifolia* and *Acacia mearnsii* in South Africa has reduced stream flow from mountain catchments where these species have become established (Macdonald *et al.* 1989). In contrast, the introduced grass *Andropogon virginicus* has transformed areas of native forest in Oahu, Hawaii into permanent swamps (Mueller-Dombois 1973). During the wet season, most of this grass dries up and forms a relatively impermeable layer on the soil surface which prevents effective transpiration and soil evaporation. The increased water runoff in areas covered with *A. virginicus* also results in increased erosion and silt deposition in nearby bays (Mueller-Dombois 1973).

Finally, introduced plants may also alter the structure and development of native communities by altering geomorphological processes. In Australia, the introduced sand dune grasses *Elymus farctus* and *Atriplex arenaria* are more efficient than native grass species at trapping sand. Consequently, these introduced species enhance the development and size of sand dunes in areas where they occur (Heyligers 1985).

Similarly, the introduction of *Casuarina equisetifolia* into coastal areas of Florida has produced steeper shorelines due to increased sand accumulation. One of the consequences of this is that the altered shorelines cannot be surmounted by sea turtles (*Caretta caretta*) attempting to lay eggs during the nesting season (Macdonald *et al.* 1989).

1.3 Impacts of Introduced Fauna

1.3.1 Effects on Native Flora

Introduced animals may directly affect native flora via their feeding activities and/or habitat destruction. Grazing and/or trampling by introduced herbivores (e.g. sheep, rabbits, goats, cattle, pigs) has altered native plant communities in many countries throughout the world (Scowcroft 1983; Usher 1986, 1989; Vitousek *et al.* 1987a; Brockie *et al.* 1988; Kruger *et al.* 1989; Macdonald *et al.* 1989; Humphries 1991). Such impacts have often been particularly severe on islands whose flora has evolved in the absence of native browsers (Diamond and Case 1986; Mack 1989; Simberloff 1995).

In addition to consuming or trampling native flora, introduced animals may also inhibit the recruitment of native plants via indirect mechanisms. In South Africa, the seeds of many native plants, including *Mimetes cucullatus*, are dispersed by native ants. The displacement of native ants by Argentine ants (*Iridomyrmex humilis*) has disrupted this

coevolved dispersal system and consequently reduced the establishment of native seedlings. Bond and Slingsby (1984) reported that Argentine ants differ from native ants in being slower to discover *M. cucullatus* seeds, in moving them shorter distances, and in failing to store them in nests below the soil. Seeds which are left on the soil surface by *I. humilis* are subsequently eaten by vertebrate and invertebrate predators, resulting in minimal seedling establishment in areas dominated by *I. humilis*. Bond and Slingsby (1984) speculated that continued invasion by Argentine ants may eventually lead to the extinction of native plants which rely on native ants for seed dispersal. The Argentine ant also poses a similar threat to native flora in Hawaii as it has reduced populations of moths (*Agrotis* sp.) and bees (*Hylaeus volcanica*) which pollinate many native plants (Cole *et al.* 1992).

Introduced animals may also indirectly affect native flora by facilitating the establishment and/or dispersal of introduced plants which, once established, may subsequently affect native flora. The decimation of native plants in the Salvage Islands, Portugal, by rabbits has allowed introduced plants (*Nicotiana glauca, N. tabacum*) to dominate native communities (Brockie *et al.* 1988). In Australia, grazing by sheep has aided the establishment of introduced grasses (*Hordeum* spp.) (Humphries 1991), while rooting by pigs kills native trees and thereby produces favourable light regimes for lantana (*Lantana camara*) (Fensham *et al.* 1994). Trampling and digging by introduced goats and

pigs also creates suitable sites for the germination of introduced plants in Hawaii (Vitousek *et al.* 1987a). Many introduced animals are also important dispersal agents for the seeds of introduced plants. The success of introduced guavas (*Psidium guajava*, *Psidium cattleianum*) and vines (*Passiflora mollisima*) on many oceanic islands is closely linked to seed dispersal by pigs and cattle (Brockie *et al.* 1988; Ramakrishnan and Vitousek 1989). Similarly, an important factor in the success of the introduced tree *Myrica faya* in Hawaii is its dispersal by exotic birds (LaRosa *et al.* 1985; Vitousek and Walker 1989).

1.3.2 Effects on Native Fauna

The impact of introduced animals on native vegetation may in turn affect native fauna which rely on that vegetation for food, shelter or breeding habitat. The destruction of native flora in Hawaii by rabbits, sheep, and deer has resulted in range reductions or extinctions of several native bird species (Greenway 1958; Mills and Mark 1977; Scowcroft 1983). Similarly, the aldabran brush warbler (*Nesillas aldabranus*) is now only found in areas of the Seychelle Islands where native vegetation has not been destroyed by goats (Prys-Jones 1979). The black-naped hare (*Lepus nigricollis nigricollis*) is also affecting native avifauna in the Seychelle Islands: these hares prevent the regeneration of *Casuarina equisetifolia* trees which are used as foraging and nesting sites by many bird species (Kirk and Racey 1992). In areas of the Great Smoky Mountains of North America, pigs have almost eliminated voles

(*Clethrionomys gapperi*) and shrews (*Blarina brevicauda*) by destroying native vegetation and leaf litter (Singer *et al.* 1984). However, in a recent study, Corbett *et al.* (1996) found that buffalo (*Bubalus bubalis*) had no significant effect on the abundance or distribution of vegetation types important to native magpie geese (*Aneranas semipalmata*) in northern Australia.

Introduced animals may also indirectly affect native fauna by increasing their exposure to disease, parasites or predators. The importation of avian malaria with introduced birds was a major factor contributing to the decline of native avifauna in Hawaii (Van Riper *et al.* 1986). In the Great Lakes of North America, heavy infestations of introduced zebra mussels (*Dreissena polymorpha*) increase the susceptibility of native bivalves to parasites and predators by reducing their ability to close their valves and by increasing stress levels (Mackie 1991; Haag *et al.* 1993; Gillis and Mackie 1994).

In addition to indirect effects, introduced fauna may also adversely affect native species via direct interactions including competition, predation, parasitism and reproductive interference/hybridisation. Changes in the distribution or abundance of native species following the introduction of a non-native species have often been attributed to interspecific competition (e.g. Lachner *et al.* 1970; Kinzie 1968; Moyle 1973, 1976; Capelli and Munjal 1982; Holway 1995). However, in

many instances evidence supporting the competition hypothesis is weak and other potential mechanisms have not been investigated (Butler and Stein 1985). Nonetheless, several studies have provided evidence that introduced species may competitively exclude native species. When planktivorous fish (alewife: *Alosa pseudoharengus* and rainbow smelt: *Osmerus mordax*) were introduced into Lake Michigan, three native fish having negligible diet overlap with the introduced species remained common, while seven native species that had feeding habits similar to those of the introduced species declined drastically (Crowder *et al.* 1981). Similarly, the introduction of inland silversides (*Menidia beryllina*) into Lake Texoma, Oklahoma resulted in the complete elimination of borrk silverside (*Labidesthes sicculus*). Laboratory studies suggested that this was probably due to the competitive superiority of *M. beryllina* in capturing copepod prey (McComas and Drenner 1982).

In San Francisco Bay, the intertidal snail *Cerithidea californica* is now restricted to only a portion of its normal habitat range following the introduction of another intertidal snail (*Hyanassa obseleta*). *Cerithidea californica* normally inhabits marsh pans, tidal creeks and mudflats in estuaries. However, it is now confined to pans for most of the year, while *H. obseleta* inhabits the creeks and mudflats. Race (1982) demonstrated that the range reduction of *C. californica* was primarily due to interference competition with *H. obseleta*. Despite this competitive exclusion, *C. californica* has not become extinct because of

the existence of a refuge in pan habitats which are beyond the physiological tolerance limits of *H. obseleta*. Interestingly, this competitive displacement occurs repeatedly due to seasonal migrations by both species and changes in abiotic factors along the habitat gradient (Race 1992). Losos *et al.* (1993) observed that native anole lizards (*Anolis conspersus*) on Grand Cayman altered their habitat use following the introduction of *A. sagrei*, probably as a result of competitive interactions. Competitive exclusion is also probably a major factor facilitating the replacement of native crayfish (*Astacus astacus*) by introduced crayfish (*Pacifastacus leniusculus*) in Sweden (Söderbäck 1995). Recently, Barr *et al.* (1996) demonstrated that the introduced wasp *Vespula vulgaris* is likely to compete with native birds for food in New Zealand. In natural forests, wasps caused high removal rates of an artificial food (mealworms: *Tenebrio molitor*). This suggests that *V. vulgaris* may be having a significant impact on native invertebrates which in turn is likely to adversely affect native insectivorous birds (Barr *et al.* 1996).

Introduced predators have also often had dramatic impacts on native fauna. Egg predation by pigs has decimated bird populations on many islands throughout the world (Greenway 1958). Similarly, the introduction of rats and/or cats is believed to have caused major population declines of native birds in Hawaii (Atkinson 1977) and New Zealand (Atkinson 1973; Brockie *et al.* 1988). Savidge (1987) and

Rodda and Fritts (1992) documented range reductions and extinctions in several species of native birds and lizards in Guam following the introduction of the brown tree snake (*Boiga irregularis*). Predation by introduced fish (*Poecilia mexicana*) has reduced populations of native fish (*Moapa coriacea*) in North America (Scoppettone 1993), while the introduction of predatory nile perch (*Lates niloticus*) has resulted in a dramatic decrease in the species diversity and biomass of native fishes in Lake Victoria (Ogutu-Ohwayo 1990).

Introduced predators may also indirectly affect native fauna via cascading trophic effects. Zaret and Paine (1973) documented the impact of introduced cichlid fish (*Cichla ocellaris*) in Gutan Lake, Panama. Predation by *C. ocellaris* eliminated most of the native fish species including *Melaniris chagresi*. A major food item for *M. chagresi* was the cladoceran *Ceriodaphnia cornuta* which has horned and unhorned morphs. Since *M. chagresi* preyed differentially on unhorned *C. cornuta*, the proportion of horned *C. cornuta* in zooplankton populations decreased following the introduction of *C. ocellaris* due to reduced predation on unhorned forms by fewer *M. chagresi*. In addition, the reduction in *M. chagresi* populations coincided with reductions in populations of native fish and birds whose diet normally incorporates this species. Finally, the introduction of *C. ocellaris* coincided with a dramatic increase in mosquito populations, probably because many of the native fish eliminated by *C. ocellaris* normally feed upon mosquito

larvae (Zaret and Paine 1973). Spencer *et al.* (1991) reported similar cascading trophic effects when oppossum shrimp (*Mysis relicta*) were released into Flathead Lake, Montana. Predation by *M. relicta* dramatically reduced zooplankton populations. As a result, populations of planktivorous salmon were reduced, which in turn led to decreases in the populations of bald eagles and grizzly bears whose diet consisted primarily of salmon (Spencer *et al.* 1991).

Introduced predators may also cause cascading trophic effects without reducing populations of native fauna. In New Zealand streams, native mayfly nymphs have altered their behaviour following the introduction of brown trout (*Salmo trutta*). Mayfly nymphs are normally diurnal. However, in the presence of *S. trutta* they become nocturnal and reduce their grazing activity to minimise the risk of predation by trout. As a result, algal biomass in streams containing trout increases, which in turn is likely to impact upon other native aquatic biota (McIntosh and Townsend 1994, 1995 and references cited therein).

While introduced predators often cause dramatic reductions in populations of native fauna, the impact of introduced parasites may also be severe. Zimmerman (1970) reported that endemic moth populations in Hawaii have been decimated by the introduction of several species of parasitic wasps. Similarly, the introduction of the tachinid fly *Bessa*

remota into Fiji probably caused the extinction of the native moth *Heteropan dolens* (Howarth 1991).

Finally, introduced animals may also directly affect native fauna via reproductive interference (inappropriate mate choice) or hybridisation. One of the primary mechanisms driving the replacement of native crayfish (*Oronectes sanborni*) by introduced crayfish (*O. rusticus*) in North America is reproductive interference (Butler and Stein 1985). Similarly, reproductive interference contributes to the replacement of native crayfish (*Astacus astacus*) by introduced crayfish (*Pacifastacus leniusculus*) in Sweden (Söderbäck 1995). In North America, the mohave chub (*Gila mohavensis*) has become endangered due to hybridisation with the introduced minnow (*G. orcutti*) (Lachner *et al.* 1970). Hybridisation has created similar problems between the lahontan tui chub (*G. obesa*) and the endangered Owens tui chub (*G. bicolor synderi*), and between armoured (*Gasterosteus aculeatus*) and unarmoured (*G. aculeatus williamsoni*) three-spine sticklebacks (Moyle 1976).

1.3.3 Effects on Ecosystem Processes

Numerous studies have demonstrated that grazing and trampling by introduced herbivores often results in accelerated soil erosion rates. Destruction of native vegetation by European rabbits (*Oryctolagus cuniculus*) has resulted in considerable loss of topsoil via erosion in

California (Macdonald *et al.* 1989). Similarly, browsing and trampling by the introduced Himalayan tahr (*Hemitragus jemlahicus*) has accelerated soil erosion in South Africa (Macdonald *et al.* 1989), while goats (*Capra hircus*) and sheep (*Ovis aries*) have increased soil erosion rates in many countries including Hawaii, New Zealand and Australia (Vitousek *et al.* 1987a; Brockie *et al.* 1988; Parkes *et al.* 1996).

In addition to altering soil erosion rates, introduced animals may also alter biogeochemical cycles and energy flow within ecosystems. Rooting by introduced pigs (*Sus scrofa*) in the deciduous forests of North America accelerates the leaching of Ca, P, Zn, Cu and Mg from leaf litter and soil, and also alters nitrogen transformation processes (Singer *et al.* 1984). In the Great Lakes of North America, large colonies of the introduced zebra mussel (*Dreissena polymorpha*) remove vast amounts of seston and algae from the water column and biodeposit them on the substrate as pseudofaeces. These nutrients thus become available to the benthic community. Consequently, an ecosystem once driven from the pelagic zone is now likely to become driven from energy from the benthic zone. Ultimately, many native aquatic species may be adversely affected (Mackie 1991; Mellina *et al.* 1995).

1.4 Conclusions

Introduced species may adversely affect native biota via a variety of direct and indirect mechanisms. These impacts range from subtle

changes which are not easily detected, through to large scale alterations in community structure and dynamics. However, caution must be exercised when attributing any observed change in the abundance or distribution of native fauna or flora to an introduced species. In many instances it is difficult or impossible to separate the effects of the introduced species from other factors such as habitat alteration (e.g. Moyle and Nichols 1974; Moyle 1976; Butler and Stein 1985; Vitousek *et al.* 1987a; Macdonald *et al.* 1988; Ng *et al.* 1993; Light *et al.* 1995). Furthermore, changes in native biota which coincide with the introduction of a non-native species may in fact be unrelated to the introduced species. For example, population declines of European otters (*Lutra lutra*) in Great Britain coincided with the introduction of North American mink (*Mustela vison*), but were probably caused by pesticide pollution (Chanin and Jeffries 1978).

At present, much of our knowledge of the ecological impacts of introduced species is anecdotal (Simberloff 1985; Vitousek *et al.* 1987a) and the mechanisms controlling these effects are often poorly understood (e.g. Clark *et al.* 1982; Nichols *et al.* 1990). Consequently, controlled experimental studies of the impact of introduced species on native biota are both highly desirable and recommended (Simberloff 1985; Vitousek *et al.* 1987a).

CHAPTER 2. RESEARCH BACKGROUND, STUDY AIMS, SITES AND METHODOLOGY

2.1 History of *Bufo marinus* in Australia

The toad *Bufo marinus* is native to Mexico, Central and tropical South America, with a natural range extending from approximately 27°N latitude in southern Texas and north-western Mexico to 10°S in central Brazil (Zug and Zug 1979). Throughout this region, *B. marinus* inhabits lowland forest margins and grassland savanna habitats (Zug and Zug 1979). The species was extensively introduced into many tropical regions during the 1900's, primarily as a biological control agent for agricultural pests. Consequently, the range of *B. marinus* has now extended to include islands throughout the Caribbean and the Pacific, as well as continental Australia and Florida USA (Sabath *et al.* 1981).

Bufo marinus was introduced into Australia in 1935 in an attempt to control larvae of the greyback beetle, *Dermolepida albohirtum*, and the frenchi beetle, *Lepidiota frenchi*, which were causing extensive damage to sugar cane crops in north eastern Queensland (Mungomery 1935; Covacevich and Archer 1976). It has since become locally known as the "cane toad". However, *B. marinus* has failed to control these agricultural pests and is now considered a pest species itself (Tyler 1989; Cogger 1992). The range of *B. marinus* in Australia has expanded greatly since its introduction to northern Queensland. This has been achieved by human actions (both intentional and accidental

introductions), as well as natural dispersal by the toad. *Bufo marinus* is now found over more than 50% of Queensland, and populations have also become established in the Northern Territory and northern New South Wales (Van Beurden and Grigg 1980; Sabath *et al.* 1981; Van Beurden 1981; Easteal *et al.* 1985; Freeland and Martin 1985; Seabrook 1991; Sutherst *et al.* 1996; Figure 2.1). Estimates of the rate of expansion of *B. marinus* vary from 1km/year in northern New South Wales (Van Beurden and Grigg 1980) to 27km/year in north-western Queensland (Freeland and Martin 1985). *Bufo marinus* occupies a wide variety of habitats within its range in Australia (Covacevich and Archer 1975), and is physiologically capable of eventually colonising areas in at least four of the mainland Australian States or Territories (Van Beurden 1981; Sutherst *et al.* 1996; Figure 2.1).

2.2 Impact of Introduced *Bufo marinus* on Native Fauna

Although *B. marinus* has been introduced into many localities throughout the world (>90 distinct places: Easteal 1981), very few studies have investigated the impact of introduced *B. marinus* on native fauna in these localities. This lack of data is somewhat surprising, given that the introduction history of *B. marinus* is probably the best documented of any species (Easteal 1981).

2.2.1 Overseas Studies

Studies conducted overseas which have investigated the impact of introduced *B. marinus* on native species have concentrated primarily on competitive interactions between adult *B. marinus* and native herpetofauna. These studies have been mainly qualitative, and none has found irrefutable evidence of a negative impact of introduced *B. marinus* on native fauna. Where changes in native faunal assemblages have coincided with the arrival of *B. marinus*, alternate explanations may often be proposed to account for these changes.

Rabor (1952) observed declines in the populations of four native anuran species (*Rana erythraea*, *R. cancrivora*, *Oxyglossus laevis* and *Kaloula conjuncta*) in Dumaguete City, Philippines, following the introduction of *B. marinus*. As these species retained their former abundance in areas not colonised by *B. marinus*, it was concluded that these population declines resulted from competition with *B. marinus* (Rabor 1952). However, Alcala (1957) observed that many native anurans were abundant in their proper habitats (i.e. not in the city) in the Philippines, despite the presence of *B. marinus*. Alcala (1957) concluded that *B. marinus* does not compete with native frogs of the Philippines, and suggested that Rabor's (1952) observations were "an illusory phenomenon".

Schultze-Westrum (1970) observed that increases in *B. marinus* populations in savanna habitat in Papua New Guinea coincided with declines in native reptile populations, possibly as a result for competition for shelter sites. In contrast, Zug *et al.* (1975) found no evidence that *B. marinus* was displacing native savanna frog species in Papua New Guinea. They noted that the native species are either arboreal (*Litoria congenita, L. caerulea, L. infrafrenata, L. bicolor, L. impura*) or closely associated with water (*Rana papua, Litoria nasuta*). Consequently, they concluded that adult *B. marinus* are unlikely to compete with these native frogs due to differences in the "habits and habitats" of *B. marinus* and native frogs. Similarly, Pernetta and Goldman (1976) concluded that *B. marinus* did not compete with either of the two endemic frog species of Fiji (*Platymantis vitianus* and *P. vitiensis*) because (1) *B. marinus* mainly occurs in open areas while native frogs are found in forest habitat, (2) *B. marinus* never climbs and therefore feeds at a different level in the habitat, and (3) *B. marinus* is larger than the native frogs and would therefore eat larger insects. Ryan (1988) also concluded that *B. marinus* does not compete with *P. vitiensis* in Fiji. However, Ryan (1988) believed that the range of *P. vitianus* in Fiji was more widespread prior to the introduction of *B. marinus*. He attributed this range reduction to competition between adult *P. vitianus* and *B. marinus*, but conceded that the introduction of the mongoose into Fiji may also have affected the range of *P. vitianus*.

2.2.2 Australian Studies

The introduction of *B. marinus* into Australia was protested by naturalists who were concerned that native species would be adversely affected (Froggatt 1936; Kinghorn 1938). This concern has continued to the present day, and *B. marinus* is popularly believed to be having a catastrophic impact on native wildlife. However, quantitative data either supporting or refuting this notion are lacking (Easteal and Floyd 1986; Freeland 1987).

As with overseas studies, many previous Australian studies have focussed on the competitive impact of adult *B. marinus* on native anuran fauna. Cassels (1966) warned that *B. marinus* was "ousting" native Australian frogs but did not provide any data to support this claim. Covacevich and Archer (1975) attributed reductions in populations of some native frogs to competition for, and dominance of, breeding grounds by *B. marinus*. However, they did concede that habitat alteration also may have adversely affected native frog populations. Sabath *et al.* (1981) reiterated the assumption that *B. marinus* is "believed to compete with native anurans". However, in the first rigorous scientific study of competitive interactions between *B. marinus* and native fauna, Freeland and Kerin (1988) found that *B. marinus* had no observable impact on the patterns of habitat and food use by native frog species in the Gulf of Carpentaria. *Bufo* did not affect the species compositions, equitabilities or population sizes of the native frog

communities active during the dry season, nor did they affect the recovery of native frog communities following experimental perturbation (Freeland and Kerin 1988). However, this study only examined the short-term responses of native anurans to *Bufo*; longer-term effects remain to be determined.

In addition to competing with native fauna, *B. marinus* may also adversely affect native predators due to their highly toxic skin secretions (Low 1972). Adult *B. marinus* are consumed by some native predators without ill effect (water rats: Cassels 1966; crayfish: Hutchings 1979; turtles: Hamley and Georges 1985). However, other native predators (varanid lizards, elapid snakes, marsupial cats) are highly susceptible to *B. marinus* toxins and die after "mouthing" or eating adult *B. marinus* (Covacevich and Archer 1975). Anecdotal reports suggest that populations of these "susceptible" species decline following the arrival of *B. marinus* (Covacevich and Archer 1975; Easteal *et al.* 1985), but recover within several years (Freeland 1987). Covacevich (1974) and Shine and Covacevich (1983) used museum records to determine the possible impact of *B. marinus* on native elapid snakes. While the data were suggestive of a negative impact of toads on these species, they could not distinguish between the possible effects of *B. marinus* and the possible effects of habitat alteration. Thus, to date no study has unequivocally demonstrated that the introduction of *B. marinus* into

Australia has had a detrimental impact on the population of any native species (Freeland 1990).

From the above discussion, it can be seen that previous research in Australia has concentrated primarily on the effects of adult *B. marinus* on native terrestrial fauna. Few published data exist regarding the impact of *B. marinus* eggs, hatchlings or tadpoles on native aquatic fauna in Australia, or in any other country where *B. marinus* has been introduced. These early life-history stages also possess toxins (Flier *et al.* 1980; Akizawa *et al.* 1994) which may adversely affect native aquatic predators (Covacevich and Archer 1975; Pearse 1980; Hearnden 1991). In addition, *B. marinus* tadpoles may prey upon native aquatic fauna, and may also compete with native aquatic herbivores.

2.3 The Aquatic Life History Stages of *Bufo marinus*

Breeding ponds available to anurans represent a gradient of pond types, from ephemeral to permanent (Wilbur 1980). Most anurans breed in seasonal or temporary water bodies (Alford in press). *Bufo marinus*, however, are catholic in their choice of breeding sites, reproducing in both temporary and permanent water bodies (Stuart 1951; Alcala 1957; Straughan 1966; Covacevich and Archer 1975; Zug and Zug 1979; Hearnden 1991). In addition, *B. marinus* are also capable of successfully developing and metamorphosing in up to 15% sea water (Ely 1944).

Bufonids are typically opportunistic breeders, with reproductive activities generally co-ordinated with patterns of rainfall (Blair 1972). Overseas studies have reported that *B. marinus* breed throughout the year (Philippines: Alcala 1957; Papua New Guinea: Zug *et al.* 1975; Costa Rica: Crump 1989). In northern Australia, *B. marinus* reproduce throughout the year, with reproductive activity peaking during the wet season (December-April: Hearnden 1991). Female toads lay up to 35000 eggs each time they breed (Straughan 1966; Zug and Zug 1979; Crump 1989; Hearnden 1991), and each female may breed several times during a year (Hearnden 1991).

Bufo marinus eggs are laid in shallow water in long gelatinous strings which are usually entwined in vegetation or rocks if present (Easteal and Floyd 1986). Hatchlings (Gosner 1960 stages 17-18) emerge from the egg string within 2-3 days after egg deposition, the time of development depending on water temperature (Straughan 1966; Van Beurden 1979; Floyd 1983). The hatchlings are initially immobile, but become increasingly mobile as they develop. Hatchlings do not feed on external food sources but obtain their nutrition from a yolk sac. When the external gills have been absorbed and operculum development is completed, the hatchlings become free swimming tadpoles (Gosner 1960 stage 25) and commence feeding (Straughan 1966). Development time from egg deposition to the free swimming tadpole stage is from 70-91 hours (Hearnden 1991).

Initially, *B. marinus* tadpoles feed on the remains of the gelatinous egg string from which they have emerged (Easteal and Floyd 1986). Thereafter, their diet consists of detritus and suspended organic material (Crump 1989), but may also include conspecific eggs and hatchlings (Hearnden 1991). *Bufo marinus* tadpoles are diurnal in habit, and often form large dense aggregations (Zug and Zug 1979). Tadpole growth and survival rates are highly variable and depend upon water temperature (Zug and Zug 1979), and the presence of predators and competitors (Hearnden 1991). The thermal tolerance limits of *B. marinus* tadpoles have been determined as ranging from 8°C to 45°C (Heatwole *et al.* 1968; Krakauer 1970; Floyd 1983, 1984), but vary according to developmental stage, photoperiod and starvation condition of the tadpoles (Floyd 1983, 1984, 1985).

As *B. marinus* tadpoles approach metamorphosis (Gosner 1960 stages 41-44) they cease feeding and commence breathing air (Floyd 1984). At this time their swimming abilities are greatly reduced, and the tadpoles aggregate in the shallow margins of the water body until they metamorphose and commence the terrestrial phase of their life cycle (Floyd 1984; Easteal and Floyd 1986). Development from the egg stage to metamorphosis is usually completed in 1-2 months (Alcala 1957; Krakauer 1970; Zug and Zug 1979; Hearnden 1991), but may be completed in as little as 16 days if densities of conspecifics are low and food is abundant (Hearnden 1991).

2.4 Study Aims

The aim of this study was to investigate the impact of *B. marinus* eggs, hatchlings and tadpoles on native aquatic fauna in northern Queensland, Australia. It will also provide the first quantitative data on the impact of the early life history stages of *B. marinus* on native aquatic fauna in any country where *B. marinus* has been introduced.

Specifically, the project investigated:

(1) the toxic effects of *B.marinus* eggs, hatchlings and tadpoles on native aquatic fauna,

(2) *B. marinus* tadpoles as predators of native aquatic fauna,

(3) competition between *B. marinus* tadpoles and native aquatic herbivores, and

(4) higher-order effects produced by *B. marinus* on other trophic interactions within native aquatic communities.

2.5 Study Sites

The research was conducted at James Cook University, Townsville (19°20'S 146°46'E), and at Heathlands Departmental and Official Purposes Reserve, Cape York Peninsula (11°45'S 142°35'E), Queensland, Australia (Figure 2.2) between June 1991 and April 1995. These sites support well established and newly established *B. marinus* populations respectively. *Bufo marinus* have been present in Townsville since 1944 (Easteal 1986), but first colonised Heathlands during the

1989/90 wet season (T. McLeod pers. comm.). Habitat in the Townsville region is predominantly eucalypt woodland, while habitat at Heathlands includes grassland, heath, eucalypt woodland and vine forest. Permanent water bodies (creeks and rivers) exist at both localities, while temporary water bodies form at both locations during the summer wet season (December-April).

2.6 Methodology

The project consisted of field observations and replicated laboratory and artificial pond experiments. Laboratory experiments were used to identify which native aquatic species were likely to be adversely affected by *B. marinus* eggs, hatchlings and/or tadpoles. When such species were identified, artificial pond experiments were employed to quantify the impact of *B. marinus* on populations of "susceptible" species.

The use of artificial ponds in community ecology studies has been criticised by Jaeger and Walls (1989), but defended by Hairston (1989), Morin (1989) and Wilbur (1989). Artificial ponds are analogs of natural ponds and function as discrete mesocosms (Morin 1983a, 1989). The value of artificial ponds is that they can be controlled, manipulated and censused better than natural ponds, and allow rigorous experimental investigation of the processes structuring aquatic communities (Morin 1983a, 1989). The realism of these reconstituted communities is confirmed by the successful development, persistence, and reproduction

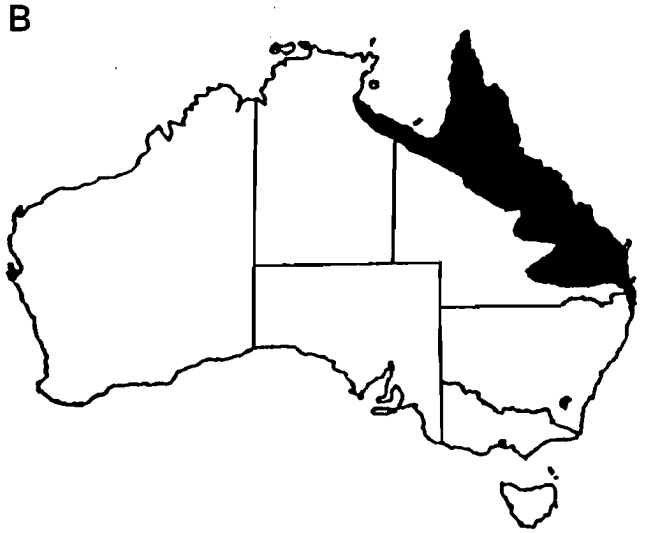
of component species (e.g. Morin 1983b). Consequently, artificial ponds have been extensively used by ecologists to investigate species interactions within aquatic communities (e.g. Morin 1983a, b; Morin *et al.* 1983; Alford and Wilbur 1985; Wilbur and Alford 1985; Morin 1986; Semlitsch 1987; Wilbur 1987; Morin *et al.* 1988; Rahel and Stein 1988; Semlitsch and Gibbons 1988; Alford 1989a, b; Wissinger 1989; Figiel and Semlitsch 1990; Wilbur and Fauth 1990; Griffiths 1991; Lawler and Morin 1993; Semlitsch and Walls 1993; Warner *et al.* 1993; Blaustein and Margalit 1994; Werner and Anholt 1996).

All research reported in this thesis was conducted within the guidelines of "The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes" and "The NHMRC Statement on Human Experimentation and Supplementary Notes", and received ethical clearance from the James Cook University Experimentation Ethics Review Committee (Approval Number A117).

Figure 2.1 Distribution of *B. marinus* in Australia: A. 1935; B. current distribution; C. predicted ultimate distribution (after Easteal *et al.* 1985; Sutherst *et al.* 1996). Darkened areas indicate range of *B. marinus*. Arrow indicates site of initial introduction.

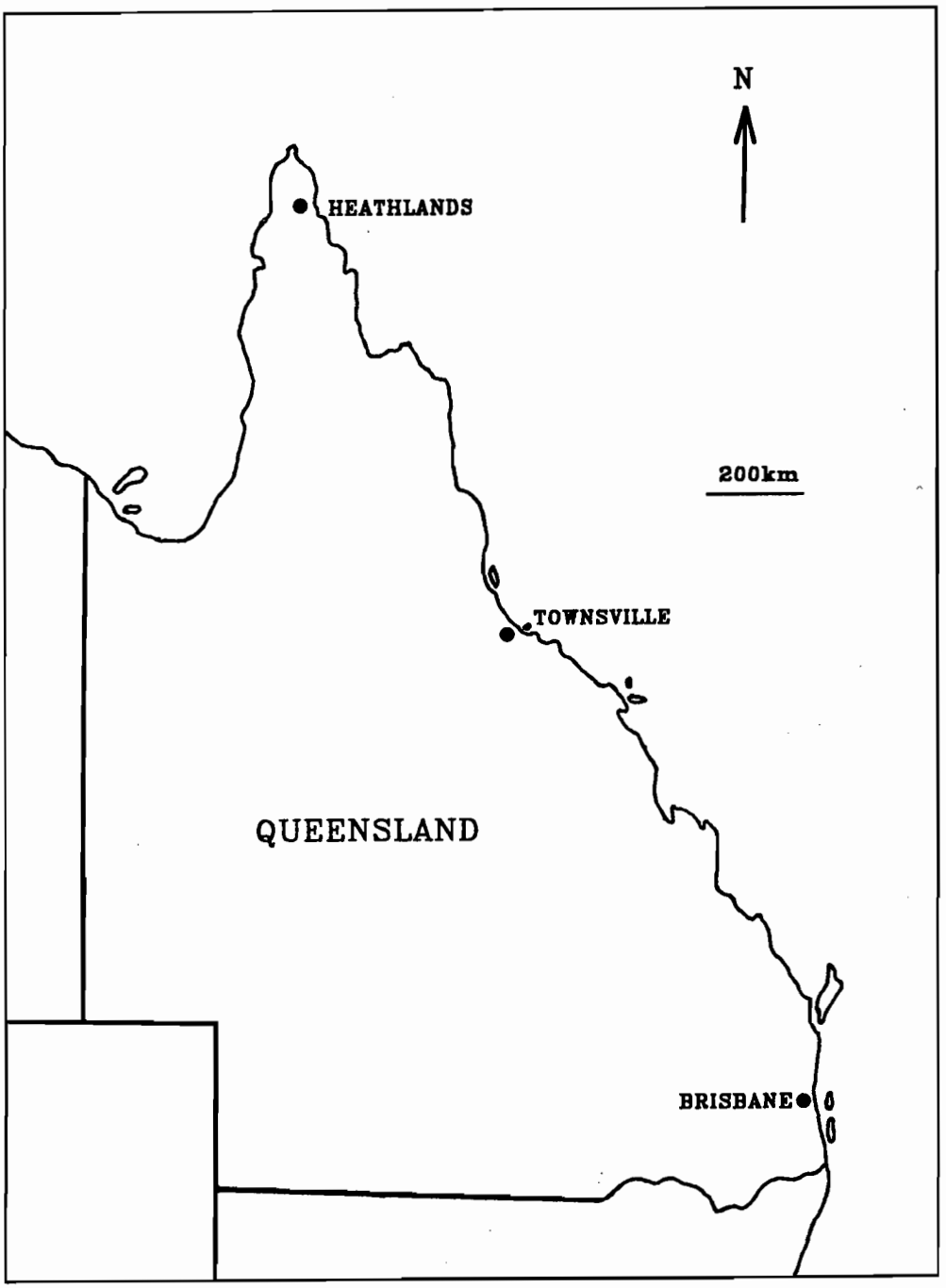
A

B



C

Figure 2.2 Map of Queensland showing the locations of the study sites.


N
HEATHLANDS
200km
TOWNSVILLE
QUEENSLAND
BRISBANE

CHAPTER 3. TOXIC EFFECTS OF *BUFO* ON NATIVE AQUATIC FAUNA: AN OVERVIEW

3.1 Introduction

Numerous plants and animals possess noxious or toxic chemicals in their tissues (Fraenkel 1959; Mosher *et al.* 1964; Brodie 1968a, b; Freeland and Janzen 1974; Garton and Mushinsky 1979; Rhoades 1979; Pawlik *et al.* 1986; Harvell *et al.* 1988; Hough-Goldstein *et al.* 1993; Vicari and Bazely 1993; Madsen and Shine 1994; Tullrot 1994; Van Alstyne *et al.* 1994; Rowell-Rahier *et al.* 1995). Species which consume such organisms may be adversely affected by these chemicals. Some consumers die after ingesting toxic food items (Brodie 1968a; Blau *et al.* 1978; White *et al.* 1989; Madsen and Shine 1994), while others experience sublethal effects such as reduced activity (Blau *et al.* 1978) or growth rates (Feeny 1970; Reese and Beck 1976a, b, c; Bernays 1978; Hough-Goldstein *et al.* 1993; Leather and Walsh 1993). However, some species consume chemically defended taxa without any apparent ill effect (Brodie 1968a; Licht and Low 1968; Pawlik *et al.* 1986; Robineau *et al.* 1991). In addition, "non-susceptible" species may sequester chemicals from food items and subsequently become noxious or toxic themselves (Brower and van Zandt Brower 1964; Aplin *et al.* 1968; Brower *et al.* 1968; Reichstein *et al.* 1968; Brower 1969; Price *et al.* 1980; White *et al.* 1989; Cronin *et al.* 1995; Rowell-Rahier *et al.* 1995).

One factor which is likely to influence the behavioural and physiological responses of an organism to the toxins present in food items is its evolutionary history of exposure to them. Species which regularly incorporate toxic food items in their diet may evolve effective detoxification mechanisms (Ehrlich and Raven 1964; Licht and Low 1968; Krieger *et al.* 1971; Whittaker and Feeny 1971; Blau *et al.* 1978; Scriber 1978). In contrast, species which are naive to such chemicals may be unable to detect (Speiser *et al.* 1992) or detoxify them (Blau *et al.* 1978; Ryan and Byrne 1988; Gilbert 1994).

The eggs (Licht 1967a, 1968, 1969; Walters 1975; Wells 1979; Henrikson 1990; Denton and Beebee 1991), hatchlings (Brodie and Formanowicz 1987; Denton and Beebee 1991) and tadpoles (Voris and Bacon 1966; Wassersug 1971; Kruse and Stone 1984; Kats *et al.* 1988; Henrikson 1990) of anurans belonging to the genus *Bufo* are unpalatable and/or toxic to many predators. However, some species readily consume these stages without ill effect (Brockelman 1969; Heusser 1970; Grubb 1972; Cooke 1974; Heyer *et al.* 1975; Beebee 1977; Brodie *et al.* 1978; Wells 1979; Wilbur *et al.* 1983; Banks and Beebee 1987; Van Buskirk 1988; Henrikson 1990; Kehr and Schnack 1991; Tejedo 1991; Blaustein and Margalit 1994; Petranka *et al.* 1994; Babbitt and Jordan 1996). Few data exist regarding the toxicity of *Bufo marinus* eggs, hatchlings and larvae to native Australian aquatic predators. Chemical analyses have verified the presence of

bufodienolides in *B. marinus* eggs and larvae (Flier *et al.* 1980; Akizawa *et al.* 1994), and previous studies indicate that these early life-history stages are toxic to some native Australian predators but not others (Covacevich and Archer 1975; Hutchings 1979; Pearse 1980; Hamley and Georges 1985; Hearnden 1991). Since no species of *Bufo* are native to Australia, and no Australian frog is known to possess chemical defences based on steroidal bufogenins and bufotoxins (Tyler 1987), native aquatic species which ingest *B. marinus* eggs, hatchlings and/or tadpoles may be particularly susceptible to the toxins present in these early life history stages.

In this chapter I investigate the toxic effects of *B. marinus* eggs, hatchlings and larvae on native aquatic fauna. *Bufo marinus* breeds in both temporary and permanent water bodies in northern Queensland, Australia, so a wide variety of invertebrate and vertebrate aquatic species are exposed to their eggs and larvae. There are several mechanisms by which native aquatic species may potentially be adversely affected by *B. marinus*. Species which consume *B. marinus* eggs, hatchlings or tadpoles may be adversely affected by the ingestion of the toxins present in these stages. Alternately, species which are unaffected by the consumption of *B. marinus* may sequester toxins and adversely affect other native aquatic taxa at higher trophic levels. In addition, *B. marinus* toxins may be released from *Bufo* into solution, either passively from eggs or larvae, or as a result from predators which

tear and shred *Bufo*. The aim of these initial experiments was to identify which native aquatic species are susceptible to *B. marinus* toxins, and to determine the mechanism(s) by which they are adversely affected. Once these have been identified, more detailed experiments investigating the impact of *B. marinus* on "susceptible" native aquatic species will be performed.

3.2 Methods

The following experiments were conducted in the laboratory at either James Cook University, Townsville (22-26°C air temperature; approximately 12HD:12HL photoperiod) or Heathlands Reserve, Cape York Peninsula (19-33°C air temperature; approximately 14HD:10HL photoperiod). Each experiment was a randomised block design. Experimental containers were randomly positioned on a benchtop in an array whose size was predetermined by the number of treatments included in the experiment, and the number of replicates per treatment. Containers were filled with either aged tap water (Townsville experiments) or local pond water (Heathlands experiments). Treatments were randomly allocated to containers within blocks with the constraint that no adjacent containers had the same treatment. All native species and *Bufo* were randomly allocated to containers within treatments. All random allocations were made using a random number table.

Except for crayfish (*Cherax quadricarinatus*: Sections 3.2.1, 3.2.2, 3.2.3) and purple spotted gudgeon (*Mogurnda adspersa*: Section 3.2.7.2), all native aquatic species used in the experiments were collected from local water bodies using dipnets and baited traps. Crayfish were obtained from breeding stock maintained at the James Cook University aquaculture facility. Purple spotted gudgeon were obtained from a local fish collector.

All *B. marinus* eggs (fertilised) and tadpoles used in the experiments were collected from local temporary and permanent water bodies. The *B. marinus* hatchlings used in the experiments were initially collected as eggs from local water bodies and were reared to hatchling stage in the laboratory. *Bufo* eggs were used in experiments on the day of collection (= day of deposition). *Bufo* tadpoles were maintained in 20 l buckets filled with 10 l pond water for up to five days before being included in an experiment. The tadpoles were not offered an artificial diet (e.g. frozen lettuce) while being maintained in the laboratory in case this might affect their toxicity. However, since the diet of *B. marinus* tadpoles usually consists of detritus and suspended organic material (Crump 1989), it is likely that the pond water and detritus present in the holding buckets provided adequate nutrition for the tadpoles. Individuals of all species tested were only used once in the experiments. Native species which consumed *Bufo* without apparent ill effect were kept for

three days after the experiment was completed to monitor their condition.

3.2.1 Toxicity of *Bufo marinus* Eggs, Hatchlings and Tadpoles to Predators

To determine which native aquatic predator species are susceptible to the toxins present in *B. marinus* eggs, hatchlings and tadpoles, I conducted a series of experiments at James Cook University and at Heathlands Reserve between January 1992 and June 1994. Experiments were conducted in either 440 ml plastic containers (containing 350 ml water) or 10 l buckets (containing 6 l water). The size of the container depended on the size of the predator being tested. The predator species tested are listed in Tables 3.1-3.3. Containers in the fish, dytiscid, belastomatid, nepid, snail and crustacean predation experiments were covered with lids to prevent predator escape. Containers in the crustacean, snail, leech and fish predation experiments were also constantly aerated to ensure a sufficient supply of oxygen to the predators.

The containers were positioned on a benchtop in a 3 X *N* array (*N*=1-10 replicates). Each experiment consisted of three treatments:

(1) 1 predator per container (predator control),

(2) 1 predator per container plus *B. marinus* eggs, hatchlings, or tadpoles (predator exposure), and

(3) *B. marinus* eggs, hatchlings, or tadpoles with no predator (*B. marinus* control).

Treatments were replicated up to ten times depending on the availability of predators. Predators in *Bufo* treatment containers (treatment 2) were offered either 50 *B. marinus* eggs, 10 *B. marinus* hatchlings (3.2-4.4 mm snout-vent length (SVL), Gosner 1960 stages 21-24) or 10 *B. marinus* tadpoles (4.0-8.0 mm SVL, Gosner 1960 stages 25-28).

Potential predators of hatchlings and tadpoles were starved for 24 hours prior to the experiments. Potential egg predators were not starved because it was difficult to predict when I would obtain eggs, and because of the short duration of the egg stage. All predators were measured using vernier calipers prior to each experiment. Predator sizes are given as total lengths, except for tadpoles (SVL), crabs (carapace width) and leeches (wet weight). Prior to each hatchling or tadpole predation experiment, one container in the predator exposure treatment was randomly chosen and all *B. marinus* hatchlings or tadpoles in it were measured using vernier calipers (SVL) and staged (Gosner 1960).

Predator condition and the number of *Bufo* present in each experimental container were monitored at 12-hour intervals. Eggs were exposed to predators until hatching commenced (36-48 hours). Similarly, hatchlings were exposed to predators until they reached Gosner (1960) stage 25 (24-36 hours). Tadpoles were exposed to predators for 72 hours.

Differences between the survival of predators in the presence and absence of *Bufo* were analysed in the following manner. If predators did not experience any mortality following consumption of *Bufo*, I did not statistically compare the mortality rates of predators in control and *Bufo* treatments. When all individuals of a predator species consumed *Bufo* and there was predator mortality, I compared the numbers of living and dead predators in control and *Bufo* treatments using 2 X 2 Fisher's Exact Tests. When only some individuals of a predator species consumed *Bufo* and there was predator mortality, I compared the numbers of living and dead predators in the following categories: (1) control animals, (2) animals exposed to *Bufo* that consumed at least one *Bufo*, and (3) animals exposed to *Bufo* that did not consume any *Bufo*, using 2 X 3 Fisher's Exact Tests (SAS Institute Inc. 1988).

3.2.2 Ontogenetic Variation in Toxicity of *Bufo marinus* Tadpoles to Predators

To determine whether there is any ontogenetic variation in the toxicity of *B. marinus* tadpoles to native aquatic predators, I tested three predator species (crayfish: *C. quadricarinatus*, dytiscid beetles: *Hydaticus vittatus*, and belastomatids: *Lethocerus insulanus*) with early (Gosner 1960 stage 25), mid (Gosner 1960 stages 30-35), and late (Gosner 1960 stages 38-41) developmental stage *B. marinus* tadpoles. Each developmental stage of *Bufo* was tested in a separate experiment. The experiments were conducted at James Cook University (crayfish

experiment) and Heathlands Reserve (dytiscid beetle and belastomatid experiments) between December 1992 and March 1993 using 10 l plastic buckets filled with 6 l water.

Experimental buckets were positioned on a benchtop in a 2 X 10 array. Each experiment consisted of two treatments:

(1) 1 predator per bucket (control treatment), and

(2) 1 predator per bucket plus 10 *B. marinus* tadpoles (*Bufo* treatment).

Each treatment was replicated ten times.

The buckets were covered with lids to prevent predator escape. Two sticks (5-10 cm length) were added to each bucket in the dytiscid and belastomatid experiments to serve as perching sites for the predators. Buckets in the crayfish experiment were constantly aerated to ensure a sufficient oxygen supply.

Predators were collected the day prior to an experiment and were starved for 24 hours before being tested. Prior to each experiment, all predators were measured (total length) using vernier calipers. One bucket in the predator plus *Bufo* treatment was randomly chosen and all *B. marinus* tadpoles in it were measured using vernier calipers (SVL) and staged (Gosner 1960).

Predator condition and the number of live *Bufo* tadpoles present in each bucket were monitored at 12 hour intervals for 72 hours. Differences between the survival of predators in the presence and absence of *Bufo* were analysed as per Section 3.2.1.

3.2.3 Toxicity of Dead *Bufo marinus* Tadpoles to Detritivores

During the experiments conducted in Sections 3.2.1 and 3.2.2, several native predators killed *B. marinus* tadpoles but did not entirely consume them (see Sections 3.3.1.3 and 3.3.2). In nature, the carcasses of such *B. marinus* tadpoles become available as a food source for aquatic detritivores. To determine the toxicity of dead *B. marinus* tadpoles to native aquatic detritivores, I performed a series of experiments at James Cook University and at Heathlands Reserve between June 1994 and March 1995. The experimental containers used were as per Section 3.2.1.

The containers were positioned on a benchtop in a 2 X *N* array ($N = 6$-10 replicates). Each experiment consisted of two treatments:

(1) 1 detritivore per container (control), and

(2) 1 detritivore per container plus 10 dead *B. marinus* tadpoles (*Bufo* treatment).

Treatments were replicated up to ten times depending on the availability of detritivores. *Bufo* tadpoles (4.2-8.2 mm SVL, Gosner 1960 stages 25-28) were sacrificed by placing them in a 440 ml container with a

small quantity of water, and then placing the container in a freezer for one hour. This technique avoided the use of chemicals which might have altered the toxicity of the tadpoles.

All detritivores were measured prior to each experiment as per Section 3.2.1. One container in the detritivore plus *Bufo* treatment was randomly chosen and all tadpoles in it were measured using vernier calipers (SVL) and staged (Gosner 1960). All detritivores were starved for 24 hours prior to each experiment.

Detritivore condition and the number of dead tadpoles present in each container were monitored at 12-hour intervals for 72 hours. Differences between the survival of detritivores in the presence and absence of *Bufo* were analysed as per Section 3.2.1.

3.2.4 Detoxification of Dead *Bufo marinus* Tadpoles with Time

Experiments conducted in Section 3.2.3 demonstrated that dead *B. marinus* tadpoles are highly toxic to some native aquatic detritivores (see Section 3.3.3). The following experiment was designed to determine the length of time it takes for dead *B. marinus* tadpoles to detoxify so that they may be consumed by "susceptible" native aquatic detritivores without ill effect. Native aquatic snails (*Austropeplea lessoni*) are susceptible to *B. marinus* toxins (Sections 3.3.1.1 and 3.3.3) and were

therefore used as a bioassay for the toxicity of dead *B. marinus* tadpoles in this experiment.

The experiment was conducted at James Cook University using 440 ml plastic containers filled with 350 ml water on 24 June 1994. The containers were positioned on a benchtop in a 6 X 10 array. Each container was covered with a lid and constantly aerated.

A single snail (12.5-18.9 mm; N=60) was allocated to each container and offered one of six diet treatments:

(1) frozen lettuce (control),

(2) 5 dead *B. marinus* tadpoles (tadpoles killed 1 hour ago),

(3) 5 dead *B. marinus* tadpoles (tadpoles killed 24 hours ago),

(4) 5 dead *B. marinus* tadpoles (tadpoles killed 48 hours ago),

(5) 5 dead *B. marinus* tadpoles (tadpoles killed 72 hours ago), and

(6) 5 dead *B. marinus* tadpoles (tadpoles killed 96 hours ago).

Each treatment was replicated ten times.

Bufo marinus tadpoles (4.8-7.4 mm SVL; Gosner 1960 stages 25-31) were sacrificed as per Section 3.2.3. All of the *Bufo* tadpoles (N=250) used in the experiment were sacrificed at the same time. After being sacrificed, the dead *B. marinus* tadpoles were allocated to 5 ml plastic dishes containing 3 ml water (1 tadpole per dish). Groups of five dishes were then randomly chosen and combined into 440 ml containers (i.e.

each 440 ml container held five dead *Bufo* tadpoles plus their associated water). The 440 ml containers were then left on a benchtop in the same room as used for the experiment until they were added to treatments. Prior to the start of the experiment, one container from each treatment was randomly chosen and all tadpoles in it were measured (SVL) using vernier calipers and staged (Gosner 1960).

Snails in the control treatment were fed frozen lettuce *ad libitum* throughout the experiment. Snails in *Bufo* treatments were initially fed frozen lettuce, and then starved for 24 hours prior to being offered dead *B. marinus* tadpoles. Snails which consumed all of the *B. marinus* tadpoles available without ill effect were then maintained on a diet of frozen lettuce until the completion of the experiment. The condition of snails and the number of *Bufo* consumed were monitored at 12 hour intervals after *Bufo* were added to containers for a period of 72 hours.

All of the snails offered dead *B. marinus* tadpoles fed on *Bufo* during the experiment. Therefore, differences in the numbers of live and dead snails among treatments were tested for using a 2 X 6 Fisher's Exact Test.

3.2.5 Possible Leaching of Toxins from *Bufo marinus* Eggs

Experiments conducted in Sections 3.2.1 and 3.2.2 demonstrated that *B. marinus* eggs, hatchlings and tadpoles are toxic to some native

aquatic predators, while experiments conducted in Sections 3.2.3 and 3.2.4 demonstrated that dead *B. marinus* tadpoles are toxic to some native aquatic detritivores (see Sections 3.3.1-3.3.4). In all of these experiments, mortality of native species occurred following the ingestion of *Bufo*. It is also possible that the toxins present in *Bufo* may leach into the surrounding water. If this occurs, then native aquatic predators which are susceptible to *B. marinus* toxins need not necessarily consume *Bufo* to be adversely affected by them.

The following experiments were designed to investigate whether the toxins present in *B. marinus* eggs leach into the surrounding water. Previous experiments had shown that native tadpoles are highly susceptible to *B. marinus* egg toxins (see Section 3.3.1.1). Therefore, native tadpoles (*Limnodynastes ornatus*: 8.2-13.2 mm SVL, Gosner 1960 stages 28-34; and *Litoria rubella*: 10.6-13.4 mm SVL, Gosner 1960 stages 33-38) were used as a bioassay for the presence of *B. marinus* toxins in solution. Each species was tested in a separate experiment. Native tadpoles were maintained on a diet of frozen lettuce (fed *ad libitum*) for up to two weeks before being included in an experiment.

The experiments were conducted at James Cook University using 680 ml plastic containers filled with 500 ml water on 22 December 1992. Each container was partitioned into two halves by a piece of fibreglass

flyscreen (2 mm mesh size) which allowed water exchange between the two halves of the container. The containers were positioned on a benchtop in a 3 X 10 array. Each experiment consisted of three treatments:

(1) 1 native tadpole per container (control),

(2) 1 native tadpole plus 50 *B. marinus* eggs per container (tadpole and *Bufo* eggs on opposite sides of flyscreen partition), and

(3) 1 native tadpole plus 50 *B. marinus* eggs per container (tadpole and *Bufo* eggs on same side of flyscreen partition)

Native tadpoles were thus either not exposed to *Bufo*, exposed to *Bufo* but unable to consume *Bufo*, or exposed to *Bufo* and able to consume *Bufo*. All treatments were replicated ten times. Allocation of native tadpoles to one side of each container was made by tossing a coin.

All tadpoles were measured (SVL) using vernier calipers and staged (Gosner 1960) prior to each experiment. Tadpole condition in each container was monitored at 12 hour intervals until hatching commenced (48 hours).

3.2.6 Possible Release of Toxins by Predators of *Bufo marinus*

In addition to possibly leaching from eggs, *B. marinus* toxins may also be released into solution by predators which tear and shred *Bufo* (e.g. crayfish). If this occurs, then native species which are susceptible to *B.*

marinus toxins may either absorb or inadvertently ingest these released toxins, and thus be adversely affected without consuming *Bufo*. The following experiment was designed to test this possibility. As in Section 3.2.5, native tadpoles (*L. ornatus*: 9.0-12.9 mm SVL; Gosner 1960 stages 32-40) were used as a bioassay for the presence of *B. marinus* toxins in solution.

The experiment was conducted at James Cook University using 10 l buckets filled with 3 l water on 20 April 1994. The buckets were positioned on a benchtop in a 3 X 10 array. A single 440 ml plastic container was randomly allocated to each bucket. The bottom of each container (80 mm diameter) had been removed and replaced with fibreglass flyscreen (2 mm mesh size). Two pieces of styrofoam (20 mm x 20 mm) attached to the outside of each container allowed the containers to remain partially submerged at the water surface within each bucket. A single *L. ornatus* tadpole was allocated to each container within each bucket. Thus, *L. ornatus* tadpoles were restricted to the containers within buckets, but remained exposed to the water present in each bucket.

The experiment consisted of three treatments:

(1) no dead *B. marinus* tadpoles added to buckets (control treatment),

(2) remains of 2 dead *B. marinus* tadpoles added to each bucket (low density *Bufo* treatment), and

(3) remains of 10 dead *B. marinus* tadpoles added to each bucket (high density *Bufo* treatment).

Each treatment was replicated ten times.

I simulated predation on *B. marinus* tadpoles in the following manner. *Bufo* tadpoles (7.8-11.0 mm SVL; Gosner 1960 stages 30-38) were sacrificed as per Section 3.2.3. Dead tadpoles were allocated to 5 ml plastic dishes containing 3 ml water (1 tadpole per dish). Each dead tadpole was then cut into 20 pieces with a pair of scissors. The remains of dead tadpoles and their associated water were randomly combined into 440 ml plastic containers until there were ten 440 ml containers each with the remains of two dead *B. marinus* tadpoles, and ten 440 ml containers each with the remains of ten dead *B. marinus* tadpoles. The remains of dead *B. marinus* tadpoles were then added to buckets, but not to containers within buckets. As soon as dead *B. marinus* tadpoles were added they sank to the bottom of the bucket. Thus, live *L. ornatus* tadpoles in floating containers had no direct contact with the remains of dead *B. marinus* tadpoles.

The condition of live *L. ornatus* tadpoles in floating containers was monitored at 12 hour intervals for a period of 72 hours.

3.2.7 Possible Accumulation of Bufotoxins by Native Fauna

3.2.7.1 Anuran Larvae

Previous experiments demonstrated that native tadpoles die after eating *B. marinus* eggs (see Sections 3.3.1.1, 3.3.5). The following experiment was designed to investigate whether *L. ornatus* tadpoles which die after consuming *B. marinus* eggs become toxic to conspecifics which feed upon their carcasses.

The experiment was conducted at James Cook University on 6 March 1994. Twenty 440 ml plastic containers were positioned on a benchtop in a 2 X 10 array and filled with 350 ml water. A single *L. ornatus* tadpole (10.1-12.9 mm SVL; Gosner 1960 stages 28-38; N = 20) was allocated to each container and exposed to one of two diet treatments:

(1) 1 cold-killed *L. ornatus* tadpole (8.7-9.7 mm SVL; Gosner 1960 stages 26-29; N = 10) (non-*Bufo* treatment), or

(2) 1 *L. ornatus* tadpole (9.2-10.1 mm SVL; Gosner 1960 stages 27-31; N = 10) which had recently (< 1 hour) died after eating *B. marinus* eggs (*Bufo* treatment).

Each treatment was replicated ten times.

The tadpoles to be sacrificed were selected haphazardly from a holding bucket. Cold-killed *L. ornatus* tadpoles were sacrificed as per Section 3.2.3. *Bufo*-killed *L. ornatus* tadpoles were sacrificed by placing a single tadpole in a 440 ml plastic container with a small quantity of water plus

20 *B. marinus* eggs. The containers were monitored at hourly intervals. As soon as *L. ornatus* tadpoles had consumed *B. marinus* eggs and died they were added to treatment 2 containers.

The condition of live *L. ornatus* tadpoles and the number of dead tadpoles consumed were monitored at 12 hour intervals for a period of 96 hours.

3.2.7.2 Odonate Larvae

Experiments conducted in Sections 3.2.1 and 3.2.2 demonstrated that some native aquatic predators can consume *Bufo* without ill effects (see Sections 3.3.1, 3.3.2). The following experiment was designed to determine whether such "non-susceptible" native predators accumulate *B. marinus* toxins and thus become toxic to other native predators at higher trophic levels. Odonate larvae (*Pantala flavescens*) were maintained on a diet of either *B. marinus* tadpoles or native tadpoles (*L. rubella*) before being offered as prey to purple-spotted gudgeon (*Mogurnda adspersa*). Purple spotted gudgeon were used as a bioassay for the presence of *B. marinus* toxins in *P. flavescens* larvae as these fish are highly susceptible to *B. marinus* toxins (Pearse 1980). In contrast to *B. marinus* tadpoles, *L. rubella* tadpoles are palatable and non-toxic to a variety of invertebrate and vertebrate native aquatic predators (pers. obs.). Larvae of *P. flavescens* readily consume tadpoles of both species without ill effect (Heyer *et al.* 1975; pers. obs.).

The experiment was conducted at James Cook University on 30 March 1995. Ten 440 ml plastic containers were positioned on a benchtop in a 2 X 5 array. Containers were filled with 350 ml water, covered with lids and constantly aerated. A single *P. flavescens* larva (20.5-23.7 mm; N = 10) was added to each container and offered one of two diet treatments:

(1) 5 *B. marinus* tadpoles (6.0-10.1 mm SVL; Gosner 1960 stages 28-40) per day, or

(2) 5 *L. rubella* tadpoles (5.8-8.5 mm SVL; Gosner 1960 stages 25-28) per day.

Each treatment was replicated five times. After three days, odonate larvae were removed from containers and offered to fish as described below.

Purple-spotted gudgeon (7.5-12.1 cm total length; N = 10) were maintained in 60 l plastic tanks filled with 30 l water (one fish per tank). The tanks were positioned on a benchtop in a 2 X 5 array, covered with lids and constantly aerated. Fish were maintained on a diet of Tetramin™ Tropical Fish Flakes (fed *ad libitum*) until used in the experiment. All fish were starved for 24 hours before being offered odonate larvae.

Each fish was offered one of two diet treatments:

(1) 1 *P. flavescens* larva which had previously consumed *B. marinus* tadpoles, or

(2) 1 *P. flavescens* larva which had previously consumed *L. rubella* tadpoles.

Each treatment was replicated five times. Responses of fish were recorded at 12 hour intervals for a period of 48 hours.

3.3 Results

Data analyses were performed using SAS PROC FREQ (SAS Institute Inc. 1988). All hypothesis tests were performed at $\alpha = 0.05$.

3.3.1 Toxicity of *Bufo marinus* Eggs, Hatchlings and Tadpoles to Predators

Results from the *B. marinus* egg, hatchling and tadpole predation experiments are presented in Tables 3.1-3.3 respectively. Mortality of *B. marinus* eggs, hatchlings and tadpoles in control treatments during all experiments was minimal. Egg mortality in controls was apparently due to fungal infection. The causes of hatchling and tadpole mortality in control treatments are unknown. Only one predator control (a fish, *Craterocephalus stercusmuscarum*) died during the experiments. Mortality of predators exposed to *Bufo* was always associated with consumption of eggs, hatchlings or tadpoles. None of the predators that

successfully consumed *Bufo* during the experiments died during the three day monitoring period following the completion of the experiments.

3.3.1.1 Predation on Eggs

Bufo marinus eggs were eaten by nepids (*Laccotrephes* sp.), larval (*Cybister* sp.) and adult (*C. godeffroyi, H. vittatus, Sandracottus bakewelli*) dytiscid beetles, belastomatids (*L. insulanus*) and crustaceans (*Holthuisana* sp., *C. quadricarinatus, Macrobrachium* sp.) without any apparent ill effect. However, eggs were toxic to tadpoles, snails and fish. All of the tadpoles (*Litoria alboguttata, L. bicolor, L. infrafrenata, L. nigrofrenata, L. rubella, L. ornatus*), snails (*A. lessoni*) and fish (*C. stercusmuscarum*) that consumed eggs died within 12 hours of egg consumption. Snails and tadpoles of all of the anuran species experienced significantly reduced survival when exposed to *B. marinus* eggs (Table 3.1). Relative survival of fish (*C. stercusmuscarum*) was not significantly reduced by exposure to eggs because only one individual consumed eggs (Table 3.1).

3.3.1.2 Predation on Hatchlings

Bufo marinus hatchlings were consumed by nepids (*Laccotrephes* sp.), dragonfly larvae (*Trapezostigma* sp., *Hemianax papuensis*), adult dytiscids (*H. vittatus, C. godeffroyi, S. bakewelli*) and crustaceans (*Holthuisana* sp., *C. quadricarinatus*) without any apparent ill effect.

Some of these predators (*Trapezostigma* sp., *H. papuensis*) also killed hatchlings but did not eat them.

Bufo marinus hatchlings were toxic to tadpoles (*L. alboguttata*, *L. infrafrenata*, *L. ornatus*), dytiscid larvae (*Cybister* sp.) and notonectids (*Anisops* sp.). Native tadpoles that ate hatchlings always died within 12 hours of hatchling consumption. Dytiscid larvae (*Cybister* sp.) and notonectids (*Anisops* sp.) experienced differential mortality after eating hatchlings. Four *Cybister* sp. larvae ate 1-3 hatchlings without ill effect, while one individual ate 2 hatchlings and died within 12 hours. Three *Anisops* sp. ate 1-2 hatchlings without any apparent ill effect, while another *Anisops* sp. ate 8 hatchlings and died within 12 hours. The only predators to experience significantly reduced survival when exposed to *B. marinus* hatchlings were tadpoles of *L. alboguttata*, *L. infrafrenata* and *L. ornatus* (Table 3.2).

3.3.1.3 Predation on Tadpoles

Early developmental stage *B. marinus* tadpoles were eaten by nepids (*Laccotrephes* sp.), dragonfly larvae (*Trapezostigma* sp., *H. papuensis*), adult dytiscids (*H. vittatus*, *C. godeffroyi*, *S. bakewelli*), crayfish (*C. quadricarinatus*) and turtles (*Elseya latisternum*, *Emydura krefftii*) without any apparent ill effect (Table 3.3). Several of these predators (*Trapezostigma*, *H. papuensis*, *E. krefftii*) also killed tadpoles without

eating them. Belastomatids (*L. insulanus*) also killed *B. marinus* tadpoles but only consumed a small portion of them.

Dead *B. marinus* tadpoles were also found in experimental containers with some fish (*C. stercusmuscarum*, *Hypseleotris compressa*). Single dead *B. marinus* tadpoles were found in two *C. stercusmuscarum* containers and one *H. compressa* container. Single *B. marinus* tadpoles disappeared from one *Ambassis agrammus* container and one *Neosilurus hyrtlii* container. These tadpoles may have been consumed by the fish, or killed by the fish and later eaten by the remaining *B. marinus* tadpoles.

Bufo marinus tadpoles were toxic to dytiscid larvae (*Hydaticus* sp.) and leeches (*Goddardobdella elegans*). Both species experienced differential mortality after eating tadpoles. Two of the five *Hydaticus* larvae each ate 10 tadpoles and showed no obvious ill effects. The remaining three *Hydaticus* larvae ate 5-8 tadpoles and died within 24 hours. Six of the ten leeches partially consumed 1-4 tadpoles and died within 12 hours. Another three leeches each partially consumed 3 tadpoles but did not die. Two of these leeches were observed lying on the bottom of their experimental containers, writhing and contorting their bodies. This behaviour ceased within 12 hours, after which the leeches apparently recovered fully. The third leech which preyed upon 3 *B. marinus* tadpoles showed no obvious ill effects. Control leeches did not display any writhing or body contortion behaviour. Leeches were the only

predator to experience significantly reduced survival when exposed to *B. marinus* tadpoles (Table 3.3).

3.3.2 Ontogenetic Variation in Toxicity of *Bufo marinus* Tadpoles

The responses of crayfish (*C. quadricarinatus*), dytiscid beetles (*H. vittatus*) and belastomatids (*L. insulanus*) to early, mid and late developmental stage *B. marinus* tadpoles are listed in Table 3.4. Only one of the control predators (*L. insulanus* vs. stage 30-35 *Bufo* experiment) died during the experiments. The cause of mortality in this instance is unknown.

Differences in predator feeding technique resulted in differences in the amounts of *B. marinus* tadpoles consumed by each predator species. The crayfish and dytiscid beetles fed on tadpole tissues, while the belastomatids fed on the internal fluids of tadpoles (pers. obs.). Consequently, crayfish and dytiscid beetles consumed all of the *B. marinus* tadpoles which they killed (i.e. no dead *B. marinus* tadpoles were found lying on the bottom of buckets containing crayfish and dytiscids). In contrast, belastomatids only partially consumed the *B. marinus* tadpoles they killed.

Crayfish and dytiscid beetles readily consumed early, mid, and late developmental stage *B. marinus* tadpoles without any apparent ill effects. Belastomatids also fed on early developmental stage *B. marinus*

without any apparent ill effects. However, belastomatids which fed on mid and late developmental stage *B. marinus* tadpoles experienced significant mortality (Table 3.4). In such instances, death of the predator occurred within 12 hours after the consumption of *Bufo*. There was intraspecific variation in the toxic effects of mid and late developmental stage *B. marinus* tadpoles on belastomatids. Three *L. insulanus* fed on 8-10 mid developmental stage *B. marinus* tadpoles without any ill effect, while seven *L. insulanus* died after feeding on 1-6 mid developmental stage *Bufo*. Similarly, four *L. insulanus* fed on 10 late developmental stage *B. marinus* tadpoles without any ill effect, while six *L. insulanus* died after feeding on 5-9 late developmental stage *Bufo*.

3.3.3 Toxicity of Dead *Bufo marinus* Tadpoles to Detritivores

Toxic effects of dead *B. marinus* tadpoles on native aquatic detritivores are listed in Table 3.5. No detritivores died in the control treatment during any of the experiments.

Chironomids (*Chironomus tepperi*), crustaceans (*Macrobrachium* sp., *Holthuisana* sp., *C. quadricarinatus*) and eel-tailed catfish (*N. hyrtlii*) consumed dead *B. marinus* tadpoles without any apparent ill effects. However, snails (*A. lessoni*) and tadpoles (*L. bicolor*, *L. nigrofrenata*, *L. alboguttata*, *L. gracilenta*, *L. ornatus*, *C. brevipes*) died within 12 hours after partially or completely consuming dead *B. marinus* tadpoles. All

snails and tadpoles that consumed dead *B. marinus* tadpoles experienced significant mortality (Table 3.5).

Consumption of dead *B. marinus* tadpoles was always fatal to tadpoles of *L. bicolor*, *L. nigrofrenata*, *L. gracilenta* and *L. ornatus*. However, other tadpoles (*L. alboguttata*, *C. brevipes*) and snails (*A. lessoni*) experienced differential mortality after consuming dead *B. marinus* tadpoles. Three *L. alboguttata* tadpoles each consumed 1 *B. marinus* tadpole and died, and another three *L. alboguttata* tadpoles each grazed the dorsal surface of a *B. marinus* tadpole and died. However, two *L. alboguttata* tadpoles each consumed 1 *B. marinus* tadpole without any apparent ill effect. Similarly, three *C. brevipes* tadpoles ate 1 *B. marinus* tadpole each and died, while two *C. brevipes* tadpoles each grazed the dorsal surface of a *B. marinus* tadpole and died. Another three *C. brevipes* tadpoles, however, ate 1 *B. marinus* tadpole each without ill effect. Eight *A. lessoni* died after consuming 0.5-2 *B. marinus* tadpoles, while two *A. lessoni* each ate 1 *B. marinus* tadpole without ill effect.

3.3.4 Detoxification of Dead *Bufo marinus* Tadpoles with Time

Survival of snails (*A. lessoni*) which fed on frozen lettuce (control treatment) or *B. marinus* tadpoles which had died 1-96 hours previously is plotted in Figure 3.1. Dead *B. marinus* tadpoles were always consumed within 24 hours after being offered to snails.

There was a significant difference in the survival of snails among diet treatments (Fisher's Exact Test $P<0.001$). None of the snails in the control treatment died during the experiment. However, all (N=10) snails that fed on *B. marinus* tadpoles which had been killed 1 hour previously died within 12 hours (1-2 tadpoles eaten). Similarly, most (N=8) of the snails that fed on *B. marinus* tadpoles which had been killed 24 hours previously died within 12 hours (1-3 tadpoles eaten), although 2 snails consumed "24-hour dead" tadpoles (1-4 tadpoles eaten) without ill effect.

In contrast, most *B. marinus* tadpoles which had been dead for more than 24 hours were non-toxic to snails. Eight snails consumed *B. marinus* tadpoles which had been killed 48 hours previously without ill effect (2-5 tadpoles eaten), while two snails died after eating "48-hour dead" tadpoles (1-2 tadpoles eaten). Similarly, seven snails consumed *B. marinus* tadpoles which had been killed 72 hours previously without ill effect (5 tadpoles eaten), while three snails died after eating "72-hour dead" tadpoles (3-5 tadpoles eaten). All (N=10) snails consumed *B. marinus* tadpoles which had been killed 96 hours previously without ill effect (5 tadpoles eaten).

3.3.5 Possible Leaching of Toxins from *Bufo marinus* Eggs

Survival of *L. rubella* and *L. ornatus* tadpoles in the presence and absence of *B. marinus* eggs is plotted in Figure 3.2. No statistical

analyses were performed due to obvious trends in the data. None of the *L. rubella* or *L. ornatus* tadpoles died in the control treatment, or in the treatment where tadpoles were exposed to *B. marinus* eggs but were unable to consume eggs. In addition, none of the native tadpoles exposed to *B. marinus* eggs but unable to consume eggs exhibited any behaviour (e.g. lethargy, erratic swimming) which differed from the behaviour of tadpoles in the control treatment.

The only *L. rubella* and *L. ornatus* tadpoles which died during the experiments were those which consumed *B. marinus* eggs. Three *L. rubella* tadpoles died after eating 1 *B. marinus* egg each, while nine *L. ornatus* tadpoles died after eating 0.5-3 eggs. Death occurred within 12 hours after the consumption of *Bufo*. Native tadpoles which were able to consume *B. marinus* eggs but failed to do so experienced no apparent ill effects.

3.3.6 Possible Release of Toxins by Predators of *Bufo marinus*

There was no evidence that simulated predation on *B. marinus* tadpoles released *B. marinus* toxins into solution. None of the *L. ornatus* tadpoles experienced any ill effects in the control treatment, or in treatments where they were exposed to the remains of dead *B. marinus* tadpoles but were unable to consume *Bufo*.

3.3.7 Possible Accumulation of Bufotoxins by Native Fauna

3.3.7.1 Anuran Larvae

There were no apparent toxic effects associated with the consumption of *L. ornatus* tadpoles which had previously died after consuming *B. marinus* eggs. All (N = 10) of the cold-killed *L. ornatus* tadpoles were consumed by conspecifics without ill effect. Similarly, eight of the ten *Bufo*-killed *L. ornatus* tadpoles were consumed by conspecifics without any apparent ill effect. The remaining two *L. ornatus* tadpoles each partially consumed a *Bufo*-killed conspecific without ill effect. The *Bufo*-killed *L. ornatus* tadpoles offered as food to conspecifics had consumed 0.5-2 *B. marinus* eggs before dying.

3.3.7.2 Odonate Larvae

Odonate larvae (*P. flavescens*) consumed 11-13 *B. marinus* tadpoles (mean consumption rate 4.1 ± 0.8 s.d. tadpoles per day) and 8-15 *L. rubella* tadpoles (mean consumption rate 4.2 ± 1.7 s.d. tadpoles per day) over a three day period before being offered as prey to purple spotted gudgeon (*M. adspersa*). All fish consumed *P. flavescens* larvae within 24 hours. None of the fish experienced any apparent ill effects after consuming odonate larvae which had previously fed on either *B. marinus* or *L. rubella* tadpoles.

Table 3.1 Predators tested with *B. marinus* eggs. N = number of replicates; H = Heathlands; T = Townsville; numbers in parentheses indicate the number of predators which ate *B. marinus* eggs; *P* values are results of Fisher's Exact Tests as explained in the text.

Predator	N	Mean Predator Size(mm) ± SD	Gosner Stage	Number Eggs Eaten	Predator Mortality (%)	*P*
NEPIDAE						
Ranatra sp.[H]	10(0)	44.0 ± 16.1		0	0	-
Laccotrephes sp.[H]	1(1)	66.1		7	0	-
DYTISCIDAE						
Cybister sp.[H]	10(6)	28.0 ± 8.1		0-25	0	-
Hydaticus sp.[H]	5(0)	20.8 ± 4.5		0	0	-
Cybister godeffroyi[H]	4(4)	31.8 ± 5.4		3-26	0	-
Hydaticus vittatus[H]	10(10)	14.1 ± 0.6		23-50	0	-
Sandracottus bakewelli[H]	4(4)	11.5 ± 2.4		16-25	0	-
BELASTOMATIDAE						
Lethocerus insulanus[H]	10(10)	66.3 ± 3.2		1-16	0	-
ODONATA						
Trapezostigma sp.[H]	10(0)	23.5 ± 3.0		0	0	-
CRUSTACEA						
Macrobrachium sp.[H]	5(4)	33.1 ± 11.6		0-4	0	-
Holthuisana sp.[T]	10(10)	28.2 ± 10.7		10-50	0	-
Cherax quadricarinatus[T]	10(10)	85.1 ± 3.9		48-50	0	-
GASTROPODA						
Austropeplea lessoni[T]	10(10)	23.8 ± 2.6		1-2	100	<0.0001
ANURA						
Litoria bicolor[H]	10(10)	10.5 ± 0.7	31-40	0.5-2	100	<0.0001
Litoria rubella[T]	10(3)	5.7 ± 0.6	25	0-2	30	0.0009
Litoria infrafrenata[H]	10(10)	12.5 ± 1.2	26-31	1-3	100	<0.0001
Litoria nigrofrenata[H]	10(6)	18.2 ± 1.5	26-35	0-2	60	<0.0001
Litoria alboguttata[T]	10(10)	19.0 ± 1.9	25-32	1-7	100	<0.0001
Limnodynastes ornatus[T]	10(9)	11.2 ± 1.4	30-38	0-4	90	<0.0001
PISCES						
Craterocephalus stercusmuscarum[T]	10(1)	40.4 ± 5.0		0-1	10	0.1000

Table 3.2 Predators tested with *B. marinus* hatchlings. N = number of replicates; H = Heathlands; T = Townsville; numbers in parentheses indicate the number of predators which ate *B. marinus* hatchlings; *P* values are results of Fisher's Exact Tests as explained in the text.

Predator	N	Mean Predator Size(mm) ± SD	Gosner Stage	Number Hatchlings Eaten	Predator Mortality (%)	*P*
NEPIDAE						
Ranatra sp.[H]	10(0)	44.3 ± 15.9		0	0	-
Laccotrephes sp.[H]	6(6)	46.6 ± 3.5		4-10	0	-
DYTISCIDAE						
Cybister sp.[H]	5(5)	34.2 ± 13.3		0-3	20	1.0000
Cybister godeffroyi[H]	2(2)	36.1 ± 3.9		6-8	0	-
Hydaticus vittatus[H]	10(10)	14.2 ± 0.4		10	0	-
Sandracottus bakewelli[H]	2(2)	12.9 ± 2.4		10	0	-
ODONATA						
Trapezostigma sp.[H]	5(5)	20.5 ± 3.5		8-10	0	-
Hemianax papuensis[H]	5(4)	42.4 ± 13.4		0-10	0	-
NOTONECTIDAE						
Anisops sp.[H]	5(4)	7.9 ± 1.1		0-8	20	0.500
CRUSTACEA						
Cherax quadricarinatus[T]	10(10)	88.2 ± 8.7		10	0	-
Holthuisana sp.[T]	8(8)	23.9 ± 4.3		3-10	0	-
ANURA						
Litoria rubella[T]	10(0)	12.3 ± 1.0	33-37	0	0	-
Litoria infrafrenata[H]	5(4)	13.9 ± 0.9	29-32	0-1	80	0.0048
Litoria nigrofrenata[H]	5(0)	17.4 ± 0.6	26-31	0	0	-
Litoria alboguttata[T]	10(2)	22.7 ± 2.4	26-37	0-1	20	0.0053
Limnodynastes ornatus[T]	10(1)	9.4 ± 0.8	28-32	0-1	10	0.0500

Table 3.3 Predators tested with *B. marinus* tadpoles. N = number of replicates; H = Heathlands; T = Townsville; numbers in parentheses indicate the number of predators which ate *B. marinus* tadpoles; *P* values are results of Fisher's Exact Tests as explained in the text; *weight (grams); †consumption of tadpoles not certain.

Predator	N	Mean Predator Size(mm) ±SD	Gosner Stage	Number Tadpoles Eaten	Predator Mortality (%)	*P*
NEPIDAE						
Ranatra sp.[H]	10(0)	32.4±2.3		0	0	-
Laccotrephes sp.[H]	4(4)	42.3±2.9		8-10	0	-
DYTISCIDAE						
Hydaticus sp.[H]	5(5)	24.2±9.8		5-10	60	0.1667
Cybister godeffroyi[H]	5(5)	29.8±4.2		5-10	0	-
Hydaticus vittatus[H]	10(10)	14.0±0.5		10	0	-
Sandracottus bakewelli[H]	4(4)	13.5±2.5		10	0	-
BELASTOMATIDAE						
Lethocerus insulanus[H]	10(10)	60.3±4.5		5-10	0	-
ODONATA						
Trapezostigma sp.[H]	5(4)	21.2±3.4		0-7	0	-
Hemianax papuensis[H]	5(4)	41.1±5.7		0-8	0	-
NOTONECTIDAE						
Anisops sp.[H]	5(0)	7.1±1.8		0	0	-
CRUSTACEA						
Cherax quadricarinatus[T]	10(10)	87.4±6.8		7-10	0	-
Holthuisana sp.[T]	2(0)	46.9±4.5		0	0	-
HIRUDINEA						
Goddardobdella elegens[T]	10(9)	1.2±6.7*		0-4	60	0.0022
ANURA						
Litoria rubella[T]	10(0)	11.9±0.9	33-39	0	0	-
Litoria infrafrenata[H]	10(0)	13.1±0.5	28-31	0	0	-
Litoria nigrofrenata[H]	10(0)	17.1±1.1	27-34	0	0	-
Litoria alboguttata[T]	10(0)	23.9±2.5	28-37	0	0	-
Limnodynastes ornatus[T]	10(0)	10.5±0.7	28-34	0	0	-
PISCES						
Hypseleotris compressa[T]	3(0)	66.3±3.6		0	0	-
Neosilurus hyrtlii[T]	10(1)†	77.7±9.9		0-1	0	-
Ambassis agrammus[T]	10(1)†	45.1±2.0		0-1	0	-
Craterocephalus stercusmuscarum[T]	5(0)	50.4±5.1		0	0	-
Melanotaenia splendida australis[T]	5(0)	56.0±2.2		0	0	-
CHELIDAE						
Elseya latisternum[H]	1(1)	100.2		8	0	-
Emydura krefftii[T]	4(4)	132±32.2		3-10	0	-

Table 3.4 Ontogenetic variation in the toxicity of *B. marinus* tadpoles to native aquatic predators. *Tadpoles completely consumed; **tadpoles partially consumed; *P* values are results of Fisher Exact Tests as explained in the text.

Predator Species	Mean Predator Size ± SD(mm)	*B. marinus* Tadpoles		Number Tadpoles Eaten	Predator Mortality (%)	*P*
		Mean SVL ± SD(mm)	Gosner Stage			
Cherax quadricarinatus	86.8 ± 7.8	5.0 ± 0.4	25	7-10*	0	-
" "	85.0 ± 5.8	8.7 ± 1.1	31-35	6-10*	0	-
" "	90.5 ± 6.7	10.7 ± 0.4	38-41	9-10*	0	-
Hydaticus vittatus	14.0 ± 0.5	4.7 ± 0.8	25	10*	0	-
" "	12.1 ± 1.5	9.8 ± 0.9	30-35	9-10*	0	-
" "	13.0 ± 0.9	12.2 ± 0.8	38-41	5-10*	0	-
Lethocerus insulanus	63.5 ± 4.9	4.9 ± 0.5	25	9-10**	0	-
" "	63.2 ± 5.9	9.5 ± 0.8	30-35	1-10**	70	0.0198
" "	68.2 ± 2.8	11.8 ± 0.9	38-41	5-10**	60	0.0108

Table 3.5 Detritivores tested with dead *B. marinus* tadpoles. N = number of replicates; numbers in parentheses indicate the number of detritivores which consumed dead *B. marinus* tadpoles; H = Heathlands; T = Townsville; *P* values are results of Fisher's Exact Tests as explained in the text.

Species	N	Mean Size (mm) ± SD	Gosner Stage	Number Tadpoles Eaten	Detritivore Mortality (%)	*P*
CHIRONOMIDAE						
Chironomus tepperi[H]	10(10)	9.8 ± 1.0		2-5	0	-
CRUSTACEA						
Macrobrachium sp.[H]	7(7)	41.0 ± 8.1		8-10	0	-
Holthuisana sp.[T]	6(6)	26.0 ± 2.5		10	0	-
Cherax quadricarinatus[T]	10(10)	89.2 ± 4.2		10	0	-
GASTROPODA						
Austropeplea lessoni[T]	10(10)	17.4 ± 3.1		0.5-2	80	0.0007
ANURA						
Litoria bicolor[H]	10(8)	6.7 ± 0.4	27-31	0-1	80	<0.0001
Litoria rubella[T]	10(0)	12.2 ± 0.5	32-38	0	0	-
Litoria nigrofrenata[H]	10(10)	6.9 ± 0.9	26-30	1	100	<0.0001
Litoria alboguttata[T]	10(8)	28.2 ± 2.5	33-38	0-1	60	0.0007
Litoria gracilenta[T]	10(2)	10.5 ± 1.0	25-33	0-1	20	0.0053
Limnodynastes ornatus[T]	10(9)	6.5 ± 1.1	25-29	0-1	90	<0.0001
Cyclorana brevipes[T]	10(8)	15.7 ± 4.0	26-35	0-1	50	0.0072
PISCES						
Neosilurus hyrtlii[T]	10(10)	78.7 ± 16.0		1-10	0	-

Figure 3.1 Survival of snails (*A. lessoni*) which fed on dead *B. marinus* tadpoles. Numbers indicate the number of tadpoles consumed.

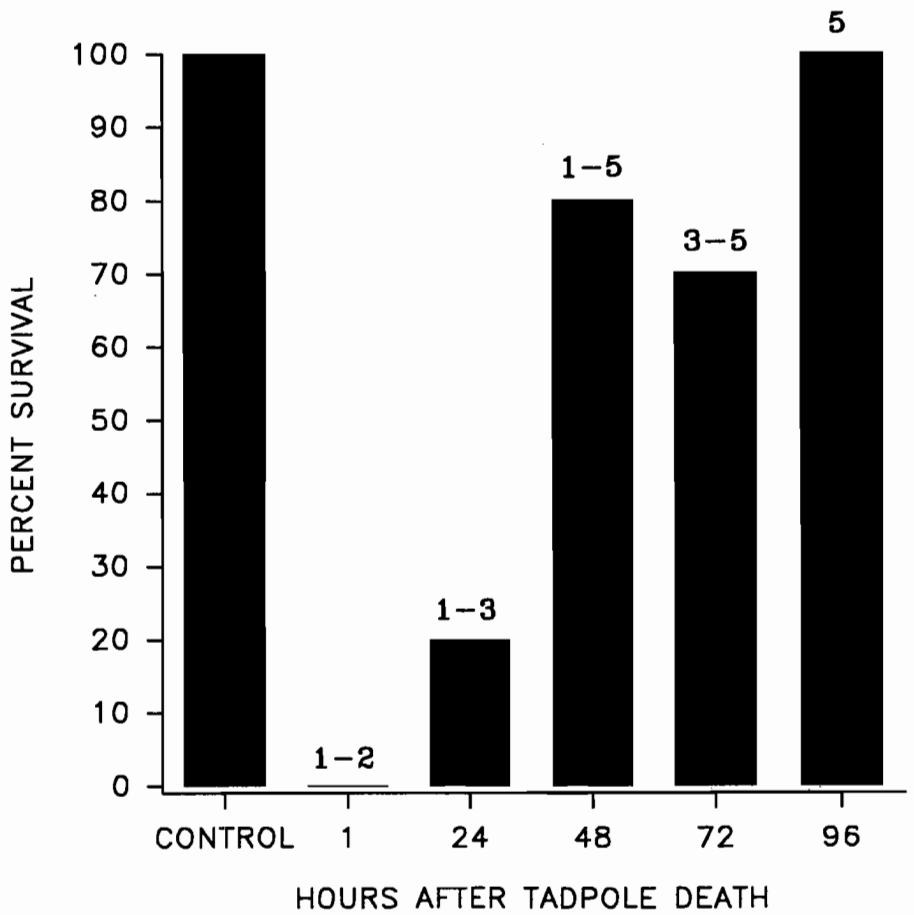
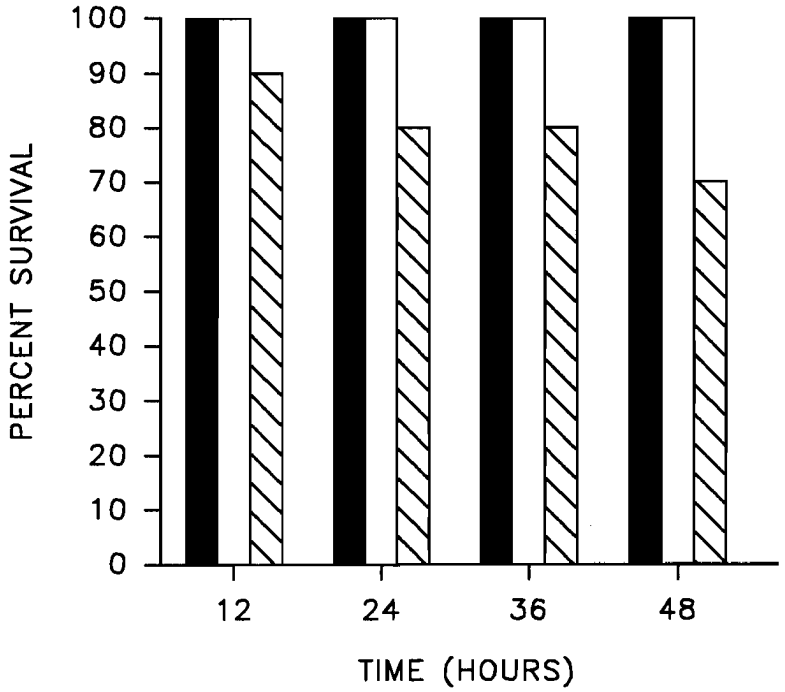
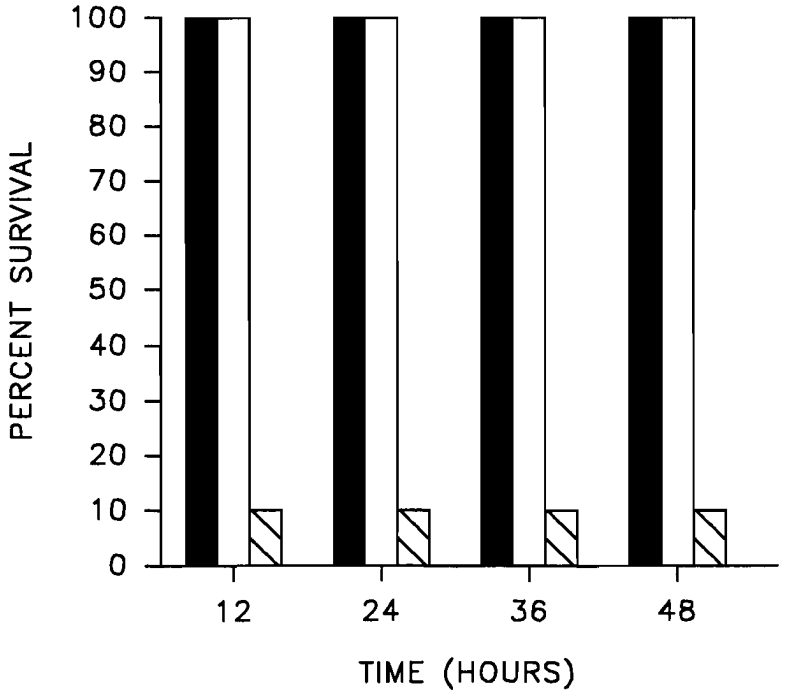

PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
1–2
1–3
1–5
3–5
5
CONTROL
1
24
48
72
96
HOURS AFTER TADPOLE DEATH

Figure 3.2 Survival of *L. rubella* and *L. ornatus* tadpoles in the absence of *B. marinus* eggs (solid bar), exposed to *B. marinus* eggs but unable to consume *B. marinus* eggs (open bar), and exposed to *B. marinus* eggs and able to consume *B. marinus* eggs (hatched bar).

L. rubella



L. ornatus



3.4 Discussion

The toxic effects of *B. marinus* eggs, hatchlings and tadpoles on native aquatic fauna were always associated with their ingestion. There was no evidence that toxins leach from *Bufo* into solution, or that predation on *Bufo* releases toxins into solution. Native tadpoles which were exposed to *B. marinus* eggs, hatchlings and tadpoles in a small volume of water but which failed to consume *Bufo* did not experience any mortality or aberrant behaviour (Tables 3.1-3.3; Figure 3.2). Similarly, native tadpoles exposed to water containing the remains of *B. marinus* tadpoles which had been "preyed upon" also experienced no apparent ill effects (Section 3.3.6). Anuran larvae are primarily filter feeders (Kenny 1969; Wassersug 1974, 1975; Wilbur 1980) and would therefore be likely to ingest at least some *B. marinus* toxins if they were present in solution. Since native tadpoles are highly susceptible to *B. marinus* toxins (Tables 3.1, 3.2, 3.5), these results suggest that toxins are not released from *Bufo* into solution, or that the quantities released are too small, under any realistic scenario, to have major negative effects. There was also no evidence that native species which prey upon *Bufo* accumulate *B. marinus* toxins. Odonate larvae (*P. flavescens*) which fed on *B. marinus* tadpoles were non-toxic to native fish (*M. adspersa*) which are known to be highly susceptible to *B. marinus* toxins (Section 3.3.7; Pearse 1980). Whether other native predator taxa which were not tested accumulate *B. marinus* toxins remains to be determined. These results indicate that the native aquatic species which are at risk

from *B. marinus* eggs, hatchlings and tadpoles are predators which consume these early life history stages, and detritivores which consume *B. marinus* eggs, immobile hatchlings or dead tadpoles.

There was considerable interspecific variation in the toxicity of *B. marinus* eggs, hatchlings and tadpoles to native aquatic predators and detritivores. *Bufo* were highly toxic to some native species, while other species either consumed them without ill effect or avoided consuming them at all. None of the "susceptible" native species are adversely affected by the consumption of the equivalent life history stages of native anurans (Chapter 6; pers. obs.). The ability of many native aquatic predators to consume *Bufo* without ill effect was surprising as it has generally been presumed that few native Australian predators are able to consume *B. marinus* eggs and larvae due to their high toxicity (Covacevich and Archer 1975). However, the large numbers of *Bufo* consumed by some predators (e.g. crustaceans, dytiscid beetles) suggest that these species may be major predators of *B. marinus* in nature.

The reasons why certain native aquatic taxa are susceptible to *B. marinus* toxins, while others remain unaffected, are unknown. Most of the "non-susceptible" taxa are invertebrates (Tables 3.1-3.5). However, "susceptible" taxa appear in most of the taxonomic groups containing "non-susceptible" taxa. Similarly, the extent of toxic effects of *B.*

marinus on different native taxa did not depend on the number of *Bufo* consumed. Some species (e.g. crayfish, dytiscid beetles) consumed large numbers of *Bufo* without ill effect, while others (e.g. tadpoles, snails) died after consuming very few *Bufo* (Tables 3.1-3.5). The mechanisms which allow some native aquatic species to consume *B. marinus* without ill effect remain to be determined.

There was no difference in the toxicity of *B. marinus* eggs, hatchlings and early developmental stage (Gosner 1960 stage 25) tadpoles to particular native taxa. These stages were either always toxic to certain taxa (e.g. anuran larvae) or were always non-toxic to other taxa (e.g. crustaceans, dytiscid beetles). However, the toxicity of *B. marinus* tadpoles varied ontogenetically, with toxicity increasing as the tadpoles developed. Belastomatids (*L. insulanus*) preyed upon early developmental stage (Gosner 1960 stage 25) *B. marinus* tadpoles without ill effect, but experienced significant mortality after preying upon mid (Gosner 1960 stages 30-35) and late (Gosner 1960 stages 38-41) *B. marinus* tadpoles. This increase in toxicity may be correlated with the development of the poison glands in *Bufo*. Previous studies have found that bufonid (*B. americanus*: Brodie *et al.* 1978; *B. bufo*: Delfino *et al.* 1995a, b) and non-bufonid (*Gastrophryne carolinensis*: Garton and Mushinsky 1979; *Rana sylvatica*: Formanowicz and Brodie 1982) tadpoles become toxic immediately prior to or just after metamorphosis when the granular or poison glands mature.

Interestingly, not all native aquatic predators were affected by the ontogenetic increase in toxicity of *B. marinus* tadpoles. Crayfish (*C. quadricarinatus*) and dytiscid beetles (*H. vittatus*) consumed early, mid and late developmental stages of *B. marinus* tadpoles without ill effects (Sections 3.3.3 and 3.3.4). This interspecific variation did not result from differences in the number of *Bufo* consumed as crayfish and dytiscids preyed upon similar numbers of mid and late developmental stage *B. marinus* tadpoles as did belastomatids (Table 3.4). In addition, *C. quadricarinatus* and *H. vittatus* consumed all of the *B. marinus* tadpoles they killed, while *L. insulanus*, which feeds on internal fluids, only partially consumed *B. marinus* tadpoles. Since the toxins are believed to be located in the skin tissue of *B. marinus* tadpoles (Wassersug 1971; Flier *et al.* 1980), it is likely that belastomatids ingested less toxin than either crayfish or dytiscid beetles. The interspecific variation in the toxicity of mid and late developmental stage *B. marinus* tadpoles to these predator species probably reflects interspecific variation in their susceptibility to *B. marinus* toxins (e.g. Wassersug 1973; Peterson and Blaustein 1992).

There are few published data regarding the toxic effects of *B. marinus* eggs, hatchlings and tadpoles on aquatic fauna in native *B. marinus* habitat. In a series of experiments conducted in Costa Rica, Heyer *et al.* (1975) found that *B. marinus* hatchlings and tadpoles were non-toxic to anuran larvae (*Leptodactylus pentadactylus*), while *B. marinus* tadpoles

were also non-toxic to dragonfly larvae (*Pantala flavescens*). During the present study, *B. marinus* tadpoles were non-toxic to *P. flavescens* larvae in Australia, as well as to other native Australian odonate larvae. However, *B. marinus* hatchlings and tadpoles of similar size and developmental stage to those used by Heyer *et al.* (1975) were highly toxic to native Australian tadpoles. The ability of native Australian odonate larvae to consume *B. marinus* tadpoles without ill effect may indicate that they have adapted to detoxify *B. marinus* toxins, or that they have always been unaffected by these toxins. Differences in the responses of anuran larvae in Australia and Costa Rica to *B. marinus* toxins may result from differences in their evolutionary histories of exposure to these toxins (e.g. Ehrlich and Raven 1964; Krieger *et al.* 1971; Whittaker and Feeny 1971; Blau *et al.* 1978; Scriber 1978; Ryan and Byrne 1988; Gilbert 1994). Alternately, these differences may reflect geographic variation in the toxicity of different populations of *Bufo* as previous studies have shown that the toxicity of adult anurans may vary among populations (Myers *et al.* 1978; Myers and Daly 1980; Daly *et al.* 1986).

Interestingly, some "susceptible" native species (notonectids, dytiscid larvae, leeches, belastomatids, snails, tadpoles) exhibited intraspecific variation in their susceptibility to *B. marinus* toxins: some individuals died after consuming *Bufo* while others survived (Tables 3.2-3.5). In the case of notonectids, this intraspecific variation in toxicity may result

from differences in the number of *Bufo* consumed. Three notonectids each consumed a single *B. marinus* hatchling without ill effect, while one notonectid consumed eight hatchlings and died. For the remaining species, however, intraspecific variation in toxicity did not relate to the number of *Bufo* consumed. While a number of dytiscid larvae, leeches and belastomatids died after preying upon *B. marinus* hatchlings or tadpoles, other individuals consumed an equal or greater number of *Bufo* and survived. Similarly, some snails and tadpoles of *L. alboguttata* and *C. brevipes* died after grazing on dead *B. marinus* tadpoles, while other individuals of these species consumed an equal or greater number of *Bufo* without ill effect. These results may indicate that there is intraspecific variation in the ability of these species to cope with *B. marinus* toxins, or that there is intraspecific variation in the toxicity of *B. marinus* hatchlings and tadpoles. Previous studies have investigated intraspecific variation in the palatability or toxicity of anuran larvae of different developmental stages (Brodie *et al.* 1978; Garton and Mushinsky 1979; Formanowicz and Brodie 1982; Brodie and Formanowicz 1987; Peterson and Blaustein 1992; Delfino *et al.* 1995a, b). However, I know of no published data regarding intraspecific variation in the toxicity of early developmental stage anuran larvae, or in the ability of aquatic predators to detoxify them.

Despite the high toxicity of *B. marinus* to several native aquatic taxa, not all of these taxa experienced significantly reduced survival when

exposed to *Bufo*. It seems likely that this lack of statistical significance is due, in most cases, to small sample sizes; no experiment produced results suggesting that predator mortality occurred at random. However, these results do indicate that variation in the propensity to consume and be poisoned by *B. marinus* exists in some taxa. This variation may reflect differences in the abilities of "susceptible" native taxa to detect and avoid *B. marinus* toxins. For example, *B. marinus* eggs were highly toxic to native fish. However, native fish did not experience significantly reduced survival in the presence of *Bufo* eggs because few fish ate *Bufo* eggs (Table 3.1). This avoidance of *Bufo* eggs is probably due to the ability of fish to detect their noxiousness (Licht 1968, 1969; Wells 1979; Hearnden 1991). In contrast, other "susceptible" native taxa (anuran larvae, gastropods, dytiscid larvae, leeches, belastomatids) did not avoid eating *Bufo* and consequently experienced significantly reduced survival when exposed to *Bufo* (Tables 3.1-3.5). This lack of avoidance may indicate that these species have limited ability to detect *B. marinus* toxins, although the absence of alternate food may have forced some predators to attempt to feed on unpalatable *Bufo*. The behavioural responses of most native tadpoles and snails to *Bufo*, however, suggest that these species may be unable to detect the toxicity of *Bufo*. Native tadpoles and snails showed no aversive response when they came into contact with *B. marinus* eggs. Rather, they persisted in grazing on egg strings until they had penetrated the gelatinous string and consumed the fertilised eggs within, after which

they always died. Similarly, most native tadpoles and snails readily consumed dead *B. marinus* tadpoles and subsequently died. Interestingly, very few *L. rubella* tadpoles ate *B. marinus* eggs, hatchlings or tadpoles. This may indicate that *L. rubella* tadpoles have a greater ability to detect the noxiousness of *Bufo* than do other native tadpoles. Alternately, *L. rubella* tadpoles may not normally incorporate anuran eggs, hatchlings or tadpoles in their diet.

From an evolutionary viewpoint, feeding deterrence is not necessarily expected for novel compounds (Speiser *et al.* 1992). Previous studies have found that gastropods (Speiser *et al.* 1992), dytiscid larvae (Brodie and Formanowicz 1981) and leeches (Licht 1969, Pough 1971) are able to detect and avoid noxious food items. However, Australian species of these taxa may have limited ability to detect and avoid *B. marinus* toxins because they have no evolutionary experience with them. Tadpoles also may have limited ability to taste their food (Heyer *et al.* 1975). For example, Wells (1979) observed that eggs of *Bufo typhonius* were unpalatable to fish, but were readily consumed by *L. pentadactylus* tadpoles. This may indicate that tadpoles have less ability to assess the palatability of their food items than do fish. However, it may also indicate that there are species-specific differences in the palatability of *B. typhonius* eggs to different predators. Such differences may not necessarily reflect the abilities of different predators to taste food items *per se*. However, if anuran larvae do have limited ability to taste their

food, then native tadpoles may continue to be highly susceptible to the toxins present in *B. marinus* eggs because selection to avoid their taste cannot lead to the evolution of aversion if native tadpoles cannot detect that taste.

Native predators at Heathlands and Townsville differ in their history of evolutionary exposure to *B. marinus*. *Bufo* have been present in Townsville since 1944 (Easteal 1986), but first colonised Heathlands during the 1989/90 wet season (T. McLeod pers. comm.). I did not compare the responses of individual native predator species to *Bufo* at Heathlands and Townsville. However, several higher taxa (anuran larvae, crustaceans, turtles) were tested at both sites, allowing some speculation as to the possible evolutionary responses of native predators to *Bufo*. There was no evidence of any difference in the susceptibility of native predator taxa to *Bufo* between Heathlands and Townsville. *Bufo* eggs, hatchlings and dead tadpoles were toxic to native tadpoles at both sites. Similarly, *Bufo* eggs and dead tadpoles were non-toxic to crustaceans at Heathlands and Townsville, and live *Bufo* tadpoles were non-toxic to turtles at both sites.

The extent to which "susceptible" native aquatic species are affected by *Bufo* in natural water bodies will depend on (1) their ability to detect and avoid *Bufo* toxins, (2) which stage of *Bufo* they prey upon, and (3) their ability to tolerate or detoxify *Bufo* if they consume *Bufo*. Native species

which can detect and avoid *B. marinus* toxins are likely to be unaffected by *Bufo*, while species that are unable to detect and avoid *B. marinus* toxins may be significantly affected. Since the tadpole stage of *B. marinus* lasts longer than the egg and hatchling stages, species that prey on *Bufo* tadpoles (e.g. dytiscid larvae, leeches) are exposed to risk for longer periods and may be more adversely affected than species that only prey upon *Bufo* eggs or hatchlings (e.g. anuran larvae, snails). In addition, some developmental stages of *B. marinus* tadpoles may be more toxic to certain predators (e.g. belastomatids) than other developmental stages. The toxic effects of dead *B. marinus* tadpoles on native detritivores will also depend on how long *Bufo* tadpoles have been dead prior to being ingested by the detritivore. Under laboratory conditions, dead *B. marinus* tadpoles became non-toxic to "susceptible native detritivores (snails) within 2-4 days after the death of the tadpole. These toxins may possibly break down even faster under natural conditions where they would be exposed to ultraviolet light.

Predators may play an important role in structuring freshwater communities by regulating the abundance and composition of prey species (e.g. Brooks and Dodson 1965; Hutchinson 1971; Hurlbert *et al.* 1972; Zaret and Paine 1973; Dodson 1974; Macan 1977; Zaret 1980; Wilbur 1987), and altering the intensity and outcome of ecological interactions among prey species (e.g. Zaret 1972; Morin 1981, 1983, 1987; Wilbur *et al.* 1983; Wilbur 1987; Alford 1989a; Wilbur and Fauth

1990; Werner 1991; Skelly 1992). Therefore, any adverse effect of *B. marinus* eggs or larvae on native aquatic predators may in turn affect the structure and dynamics of native freshwater communities. In Chapters 4 and 5, I will investigate the ability of two taxa of "susceptible" native predators (fish and anuran larvae) to learn to avoid *Bufo*. Where these species are unable to learn to avoid *Bufo*, the impact of *Bufo* on their populations will be quantified.

CHAPTER 4. ABILITY OF NATIVE FISH TO LEARN TO AVOID *BUFO* TADPOLES

4.1 Introduction

Numerous studies have demonstrated that fish are capable of learning a variety of complex behaviours (see Hughes *et al.* 1992 for recent review). This ability to learn plays an important role in the foraging ecology of many fish. By learning from previous encounters with prey, fish may alter their behaviour during subsequent predation events to increase foraging efficiency. For example, bluegill sunfish (*Lepomis macrochirus*) learn to adjust their foraging behaviour depending on the physical structure of the habitat, and consequently improve foraging success (Ehlinger 1989). Fifteen-spined stickleback (*Spinachia spinachia*) learn about specific characteristics of their prey, and alter their foraging behaviour accordingly to increase foraging efficiency (Croy and Hughes 1991). Similarly, larval white seabass (*Atractoscion nobilis*) learn to prey more effectively on zooplankton with experience (Dutton 1992).

Fish may also learn to differentiate and selectively choose between prey species (e.g. Coates 1980). This discriminatory ability is particularly advantageous when food items contain noxious or toxic chemicals (e.g. dinoflagellates: Robineau *et al.* 1991; ascidians: Stoecker 1980; sponges: Pawlik *et al.* 1995; corals: Harvell *et al.* 1988, Van Allstyne *et al.* 1994). Several previous studies have investigated the ability of fish

to learn to avoid noxious prey. Kerfoot *et al.* (1980) found that guppies (*Lebistes reticulatus*) are able learn to avoid unpalatable water mites. McClintock and Janssen (1990) demonstrated that planktivorous fish (*Pagothenia borchgrevinki*) learn to avoid amphipods (*Hyperiella dilatata*) which carry unpalatable pteropods (*Clione limacina*). Similarly, cod (*Gadus morhua*) can learn to avoid unpalatable marine invertebrates (Tullrot and Sunberg 1991; Tullrot 1994).

While many *Bufo* tadpoles are unpalatable to fish (Voris and Bacon 1966; Kruse and Stone 1984; Duellman and Trueb 1986; for exceptions see Holomuzki 1995), few studies have investigated the ability of fish to learn to avoid *Bufo* tadpoles. Voris and Bacon (1966) observed that small bluegills (*Lepomis macrochirus*) learned to avoid unpalatable *B. americanus* tadpoles. Similarly, Kruse and Stone (1984) demonstrated that largemouth bass (*Micropterus salmoides*) are able to learn to avoid unpalatable *B. woodhousei* tadpoles. In Australia, *B. marinus* tadpoles are unpalatable to many native fish, and may be highly toxic if ingested (Pearse 1980; Hearnden 1991). Consequently, the ability to learn to avoid *B. marinus* tadpoles should be an important factor determining the survival of native fish in water bodies where they co-occur with *B. marinus* tadpoles.

In this chapter I investigate the ability of two species of predatory native fish (sooty grunter: *Hephaestus fuliginosus*, and barramundi: *Lates*

calcarifer) to learn to avoid *B. marinus* tadpoles. Both fish species occur in freshwater creeks and rivers in northern Queensland (Allen 1989) which are also often used by *B. marinus* as breeding sites (pers. obs.). Thus, both sooty grunter and barramundi are likely to encounter *B. marinus* tadpoles in nature. Previous studies have recorded that the diet of sooty grunter and barramundi includes aquatic insects, crustaceans, frogs and fish (Davis 1985; Allen 1989). However, as both species are opportunistic predators, they are also likely to consume tadpoles when they encounter them.

4.2 Methods

Hatchery reared barramundi and sooty grunter were obtained from the Department of Primary Industries Research Station, Walkamin, Queensland. All fish were naive to *B. marinus* tadpoles prior to being tested (A. Hogan pers. comm.; N. Milward pers. comm.). The fish were maintained in 500 l holding tanks (separate holding tank for each species) at the James Cook University aquaculture facility until used in the trials.

The trials were performed in the laboratory at James Cook University between January and May 1993. For each fish species tested, ten aquaria (35 cm x 30 cm x 23 cm) were positioned contiguously on a benchtop in an isolated room (23-25°C; 10HL:14HD photoperiod). The tanks were filled with 30 l aged tap water and aerated constantly from a

common source. Fish (barramundi 10-12 cm total length, N = 10; sooty grunter 10-16 cm total length, N = 10) were haphazardly netted from the holding tanks and randomly allocated to aquaria (one fish per tank) using a random number table. Three sides of each tank were covered with white paper to ensure that the fish were visually isolated from each other. One side remained uncovered to allow recording of fish-tadpole interactions by video-camera.

The fish were maintained in the aquaria for 14 days prior to the trials to allow them to acclimatise to the tanks, and to ensure that they were feeding before being tested. Fish were offered two food pellets (Tetramin^{tm} Tropical Fish Pellets) each day to determine whether they had commenced feeding. All fish commenced feeding within seven days after being added to the aquaria. Once feeding, each fish was offered 5 food pellets daily between 0800-0900 hours. All fish were starved for 24 hours prior to being offered tadpoles.

Bufo marinus tadpoles were collected from a local creek using a dipnet on the day prior to the commencement of the trials. Tadpoles were maintained in the laboratory in a 20 l bucket which was filled with 15 l creek water and constantly aerated. The tadpoles were not fed while kept in the laboratory in case an artificial diet might affect their palatability or toxicity. However, the creek water and detritus present in the holding bucket probably provided adequate nutrition for the tadpoles

(Crump 1989). *Bufo* tadpoles used in the sooty grunter trials were collected in January 1993, while *Bufo* tadpoles used in the barramundi trials were collected in April 1993. This was necessary as it was not possible to conduct the sooty grunter and barramundi trials simultaneously.

4.2.1 Exposure to *Bufo marinus* Tadpoles (Day 1)

Sooty grunter were first offered *B. marinus* tadpoles on 3 January 1993, while barramundi were first offered *B. marinus* tadpoles on 24 April 1993. Each fish was offered one *B. marinus* tadpole during a trial. The *B. marinus* tadpoles were initially netted haphazardly from the holding bucket, and then randomly assigned to aquaria. The order of fish to be tested was also chosen randomly. All random allocations made using a random number table. Each tadpole was measured (SVL) using vernier calipers and staged (Gosner 1960) prior to being offered to a fish. The tadpoles offered to sooty grunter were 7.8-9.4 mm SVL, stages 29-33. The tadpoles offered to barramundi were 7.6-9.9 mm SVL, stages 30-35. Each tadpole was only used once during the experiments.

The responses of each fish to the *B. marinus* tadpole were recorded using a video-camera for a period of 20 minutes. Tapes were later viewed and analysed in the laboratory. At the completion of each trial, the tadpole (if not eaten) was removed and the fish was offered 5 food pellets to determine whether it had ceased feeding. Tadpoles which

were not consumed were placed individually in 440 ml plastic containers filled with 350 ml aged tap water for a period of 24 hours to monitor their condition.

4.2.2 Exposure to *Bufo marinus* Tadpoles (Day 2)

To investigate the short term memory of sooty grunter and barramundi, the trials were repeated one day after the fish were initially offered *B. marinus* tadpoles (methodology as per Section 4.2.1). Thus, sooty grunter were retested on 4 January 1993, while barramundi were retested on 25 April 1993. The sizes and developmental stages of the *B. marinus* tadpoles offered to fish on day 2 were comparable to those offered on day 1 (sooty grunter trials: *Bufo* 7.8-8.9 mm SVL, stages 30-34; barramundi trials: *Bufo* 8.1-10.1 mm SVL, stages 29-35).

4.2.3 Exposure to *Limnodynastes ornatus* Tadpoles

After the *Bufo* trials were completed, each fish was offered a single native tadpole (*Limnodynastes ornatus*: 9.1-10.5 mm SVL, stages 30-34) which was of comparable size and developmental stage to the *Bufo* tadpoles it had previously been offered. *Limnodynastes ornatus* tadpoles are palatable and non-toxic to a variety of invertebrate and vertebrate native aquatic predators (pers. obs.). Consequently, these trials were to ensure that tadpole attributes other than unpalatability (e.g. size, shape) did not prevent sooty grunter and barramundi from consuming *B. marinus* tadpoles. The *L. ornatus* tadpoles were collected from a local

temporary pond early on the day of each trial. Sooty grunter were tested on 21 January 1993, while barramundi were tested on 11 May 1993. The methodology employed was as per Section 4.2.1. In the period between the *B. marinus* and *L. ornatus* trials, fish were maintained in the test aquaria and were each offered five food pellets daily.

4.2.4 Behavioural Definitions

The number of approaches and attacks made by each fish during a trial was determined in the following manner. An approach was defined as any movement of the fish directly towards the tadpole. This usually occurred at speed. An attack refers to the engulfing of the tadpole by the fish (i.e. the tadpole was taken into the fish mouth).

4.2.5 Statistical Analyses

Significant trends in (1) the number of fish to approach and attack *B. marinus* tadpoles, and (2) the number of approaches and attacks made by fish during the day 1 trial period were tested for using Spearman rank correlations. Since the same sooty grunter and barramundi tested on day 1 were retested on day 2, the data for days 1 and 2 for each fish species cannot be considered to be independent. Therefore, differences in the responses of fish to *B. marinus* tadpoles on days 1 and 2 were tested for using Sign Tests and McNemar's Tests (Zar 1984). All hypothesis tests were performed at $\alpha = 0.05$.

4.3 Results

4.3.1 Response to *Bufo marinus* Tadpoles (Day 1)

4.3.1.1 Sooty Grunter

The responses of sooty grunter to *B. marinus* tadpoles during the day 1 trials are plotted in Figures 4.1 and 4.2. There was no significant trend in the number of sooty grunter that approached (r_s=-0.3966, $0.10>P>0.05$) or attacked (r_s=-0.3381, $0.20>P>0.10$) *B. marinus* tadpoles over time (Figure 4.1). However, there was a significant negative correlation between the mean number of approaches made by sooty grunter and time since the start of the trial period (r_s=-0.6352, $P<0.005$, Figure 4.2). There was no significant correlation between the mean number of attacks made by sooty grunter, and time since the start of the trial period (r_s=-0.3794, $0.20>P>0.10$, Figure 4.2). The number of attacks declined markedly during the first four minutes of the trials. Thereafter, *B. marinus* tadpoles were attacked by sooty grunter at low rates for the remainder of the trial period (Figure 4.2).

All of the sooty grunter attacked *B. marinus* tadpoles within 2 minutes 13 seconds after the start of the trials on day 1. Tadpoles were rejected (i.e. spat out) by all fish within 1 second after being attacked. After rejecting the tadpole, sooty grunter vigorously opened and closed their mouth for up to 35 seconds. In addition, many fish also shook their head from side to side for up to 5 seconds. *Bufo marinus* tadpoles were reattacked by sooty grunter between 1-56 times during the remainder of

the trial period. In all instances, tadpoles were rejected within 2 seconds after being attacked. Vigorous "mouthing" and/or "head shaking" behaviour was observed following most attacks.

While all sooty grunter continued to approach *B. marinus* tadpoles during the trial period, not all approaches were followed by attacks. In many instances, fish would approach to within 2 cm of the *B. marinus* tadpole before halting, viewing the tadpole for up to 7 seconds, and then turning away. This behaviour was exhibited throughout the trial period, although many fish did attack *B. marinus* tadpoles on subsequent approaches. On several occasions during the trials, *B. marinus* tadpoles swam towards sooty grunter. In such instances, the fish often retreated from the advancing tadpole by swimming backwards while still facing the tadpole.

No sooty grunter consumed *B. marinus* tadpoles during the day 1 trials, and none of the tadpoles showed any ill effects 24 hours after being attacked by fish. All sooty grunter immediately consumed food pellets at the completion of each trial.

4.3.1.2 Barramundi

The responses of barramundi to *B. marinus* tadpoles during the day 1 trials are plotted in Figures 4.3 and 4.4. There was a significant negative correlation between the number of barramundi that approached

B. marinus tadpoles and time since the start of the trial (r_s=-0.8615, P<0.001, Figure 4.3). There was also a significant negative correlation between the mean number of approaches made by barramundi and time since the start of the trial (r_s=-0.8784, P<0.001, Figure 4.4). The number of attacks made by barramundi was not analysed statistically due to the obvious trend in the data: all attacks on *B. marinus* tadpoles ceased after the first minute of the trial.

All of the barramundi attacked *B. marinus* tadpoles within 6 seconds after the start of the trials on day 1. One barramundi consumed the *B. marinus* tadpole and was observed to open and close its mouth vigorously for 53 seconds following the attack. This tadpole was not later regurgitated and the fish showed no obvious ill effects. The remaining nine barramundi rejected the *B. marinus* tadpole less than 1 second after attacking it, and vigorously opened and closed their mouths for up to 10 seconds. Two of these fish did not attack the tadpole again. The other seven fish reattacked the *B. marinus* tadpole 1-3 times in the first minute of the trial before ceasing attacks.

All of the barramundi which did not eat the *B. marinus* tadpole reapproached the tadpole during the remainder of the trial period. Barramundi would typically approach to within 2 cm of the tadpole before halting, viewing the tadpole for up to 10 seconds, and then turning away from the tadpole. Five fish also swam slowly up to the

tadpole and gently nudged it 1-2 times with their mouth closed before turning away. As in the sooty grunter trials, barramundi retreated when approached by *B. marinus* tadpoles by swimming backwards while still facing the tadpole.

None of the *B. marinus* tadpoles which were not eaten showed any ill effects 24 hours after being attacked by barramundi. All barramundi immediately consumed food pellets at the completion of each trial.

4.3.2 Response to *Bufo marinus* Tadpoles (Day 2)

4.3.2.1 Sooty Grunter

There was no difference between the number of sooty grunter which approached and attacked *B. marinus* tadpoles on days 1 and 2; all fish approached and attacked *B. marinus* tadpoles on both days. There was also no significant difference between the number of approaches made by sooty grunter to *B. marinus* tadpoles on days 1 and 2 (Sign Test $P=0.1719$; Figure 4.5). However, there was a significant difference between the number of attacks on *B. marinus* tadpoles by sooty grunter on days 1 and 2 (Sign Test $P=0.0195$), with tadpoles being attacked fewer times on day 2 than day 1 (Figure 4.5).

There was also a significant difference between the number of approaches made by sooty grunter prior to first attacking *B. marinus* tadpoles at the start of the trials on days 1 and 2 (Sign Test $P=0.0312$;

Figure 4.6). On day 1, all fish attacked *B. marinus* tadpoles on their first approach to the tadpole. On day 2, five fish attacked the *B. marinus* tadpole on their first approach, while the other five fish approached and turned away from the *B. marinus* tadpole 1-4 times prior to their first attack. The difference in the time that *B. marinus* tadpoles were first attacked by sooty grunter on days 1 and 2 is marginally non-significant (Sign Test $P=0.0547$; Figure 4.7).

No sooty grunter consumed *B. marinus* tadpoles during the day 2 trials. One *B. marinus* tadpole died after being attacked and rejected by a sooty grunter 24 times. The remaining *B. marinus* tadpoles showed no ill effects after being attacked and rejected by sooty grunter between 1-36 times. All sooty grunter immediately fed on food pellets at the completion of each trial.

4.3.2.2 Barramundi

There was no difference between the number of barramundi that approached *B. marinus* tadpoles on days 1 and 2; all fish approached *B. marinus* tadpoles on both days. However, there was a significant difference between the number of barramundi which attacked *B. marinus* tadpoles on days 1 and 2 (McNemar's Test $P=0.0082$). While all ($N=10$) barramundi attacked *B. marinus* tadpoles on day 1, only seven of these fish attacked *B. marinus* tadpoles on day 2. One barramundi consumed the *B. marinus* tadpole it was offered during the day 2 trials

without any apparent ill effect, although it did open and close its mouth vigorously for 30 seconds after ingesting the tadpole. This was the same fish which had consumed a *B. marinus* tadpole during the day 1 trials. The three barramundi which did not attack *B. marinus* tadpoles on day 2 approached to within 3 cm of the tadpole soon (<10 seconds) after the tadpole was added to the tank, viewed the tadpole for up to 5 seconds, and then turned away. These fish repeated this behaviour throughout the trial period.

There was no significant difference between the number of approaches to *B. marinus* tadpoles by barramundi during the day 1 and 2 trials (Sign Test $P=0.5000$; Figure 4.8). However, there was a significant difference between the number of attacks by barramundi on *B. marinus* tadpoles during the day 1 and 2 trials (Sign Test $P=0.0195$), with tadpoles being attacked fewer times on day 2 than day 1 (Figure 4.8). All barramundi which attacked *B. marinus* tadpoles on days 1 and 2 did so on their first approach to the tadpole. For barramundi which did attack *B. marinus* tadpoles on days 1 (N=10 fish) and 2 (N=7 fish), there was no significant difference between the time that tadpoles were first attacked at the start of the trial period (Sign Test $P=0.3125$; Figure 4.9).

None of the *B. marinus* tadpoles which were attacked and rejected by barramundi showed any ill effects 24 hours after the trials were

completed (each tadpole was only attacked once). All barramundi immediately consumed food pellets at the completion of each trial.

4.3.3 Response to *Limnodynastes ornatus* Tadpoles

All sooty grunter and barramundi attacked and consumed *L. ornatus* tadpoles within 30 seconds after the tadpole was added to the tank. Tadpoles were consumed within 1-3 attempts. *Limnodynastes ornatus* tadpoles which escaped from an attack were immediately reattacked. Neither sooty grunter or barramundi displayed any vigorous "mouthing" or "head shaking" behaviour after attacking *L. ornatus* tadpoles.

Figure 4.1 Number of sooty grunter that approached (solid bar) and attacked (open bar) *B. marinus* tadpoles during the trial period on day 1.

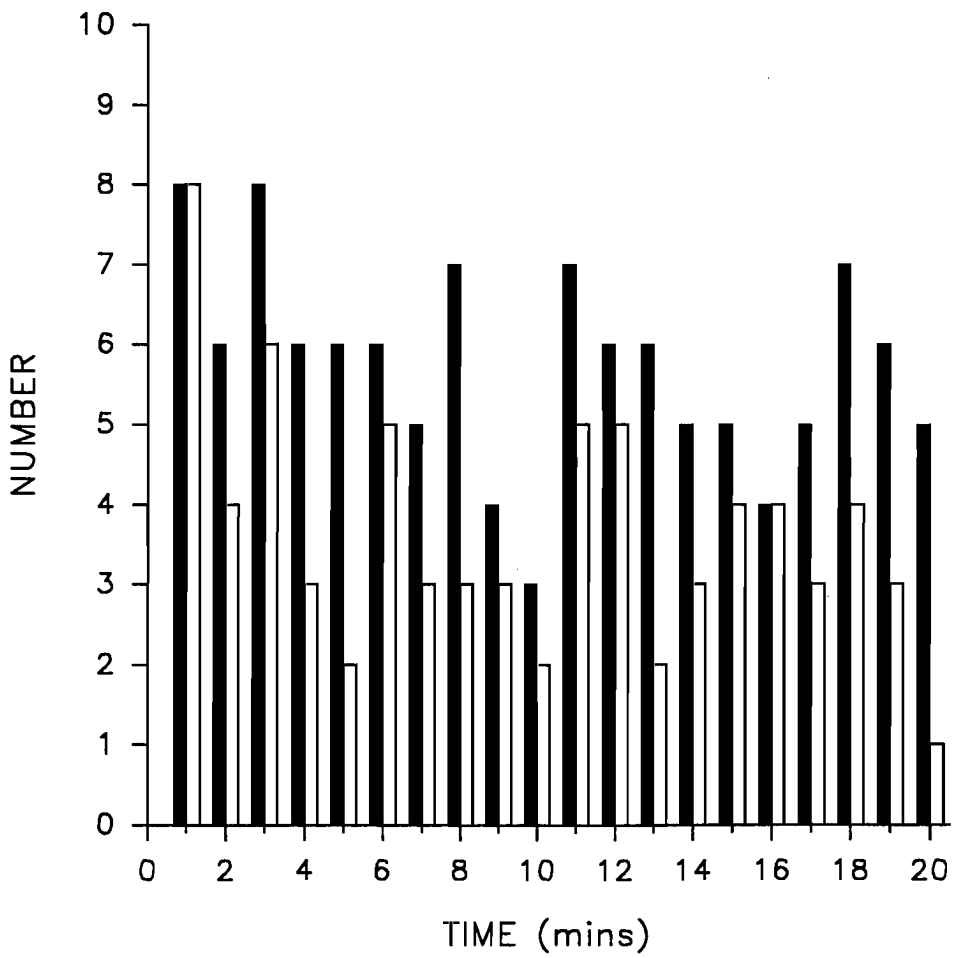

10
9
8
7
6
5
4
3
2
1
0
NUMBER
0
2
4
6
8
10
12
14
16
18
20
TIME (mins)

Figure 4.2 Mean number of approaches (solid bar) and attacks (open bar) made by sooty grunter during the trial period on day 1. Vertical bars indicate + 1 standard deviation.

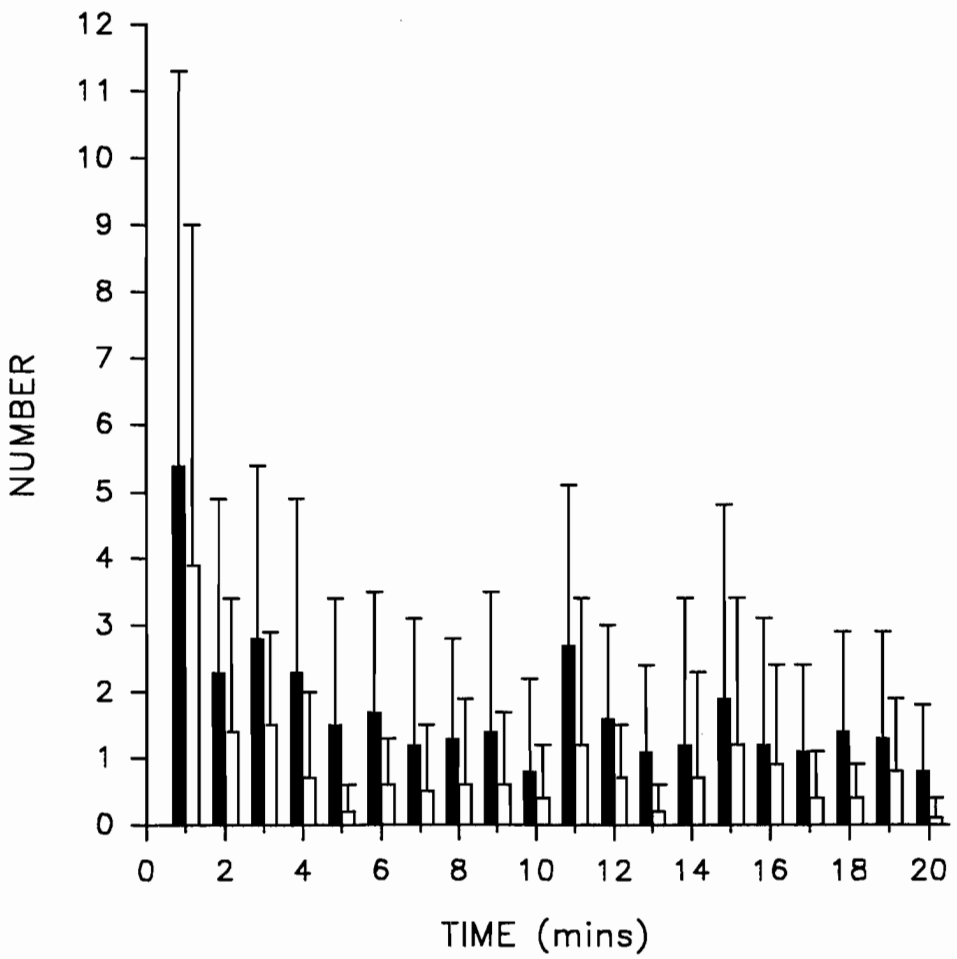

12
11
10
9
8
7
6
5
4
3
2
1
0
NUMBER
0
2
4
6
8
10
12
14
16
18
20
TIME (mins)

Figure 4.3 Number of barramundi that approached (solid bar) and attacked (open bar) *B. marinus* tadpoles during the trial period on day 1.

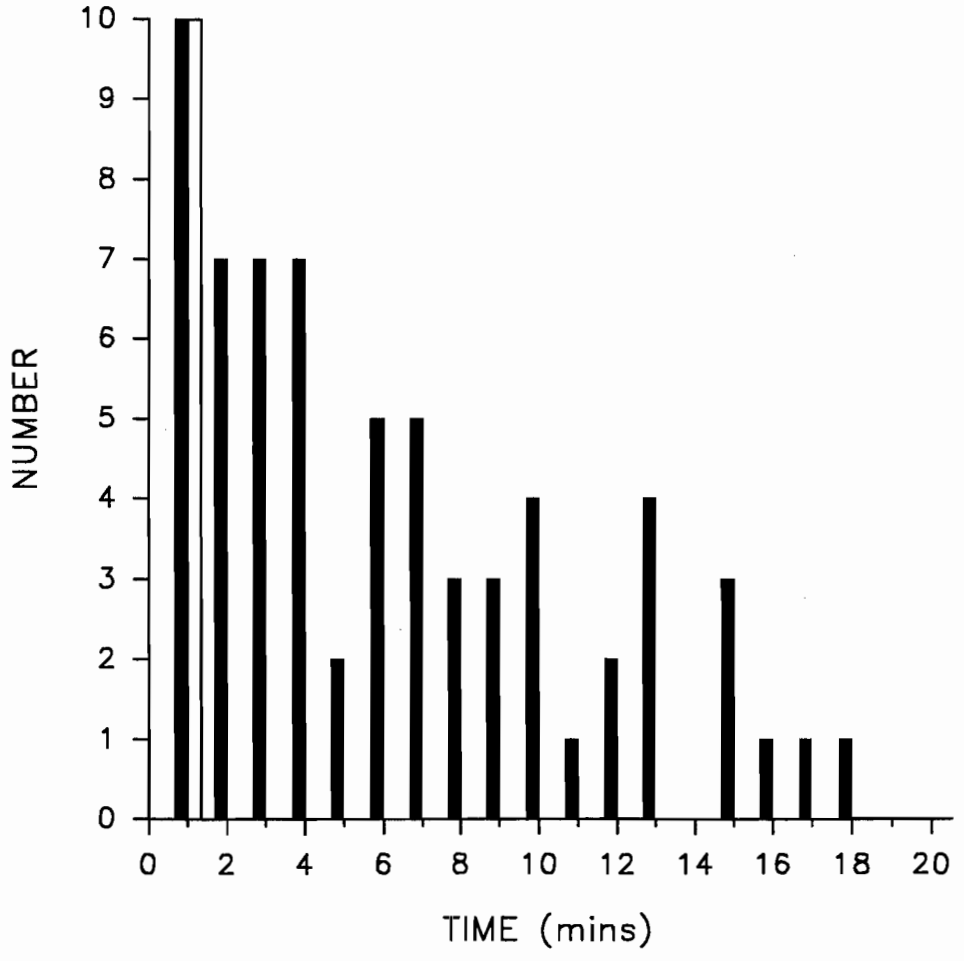

NUMBER
10
9
8
7
6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
TIME (mins)

Figure 4.4 Mean number of approaches (solid bar) and attacks (open bar) made by barramundi during the trial period on day 1. Vertical bars indicate + 1 standard deviation.

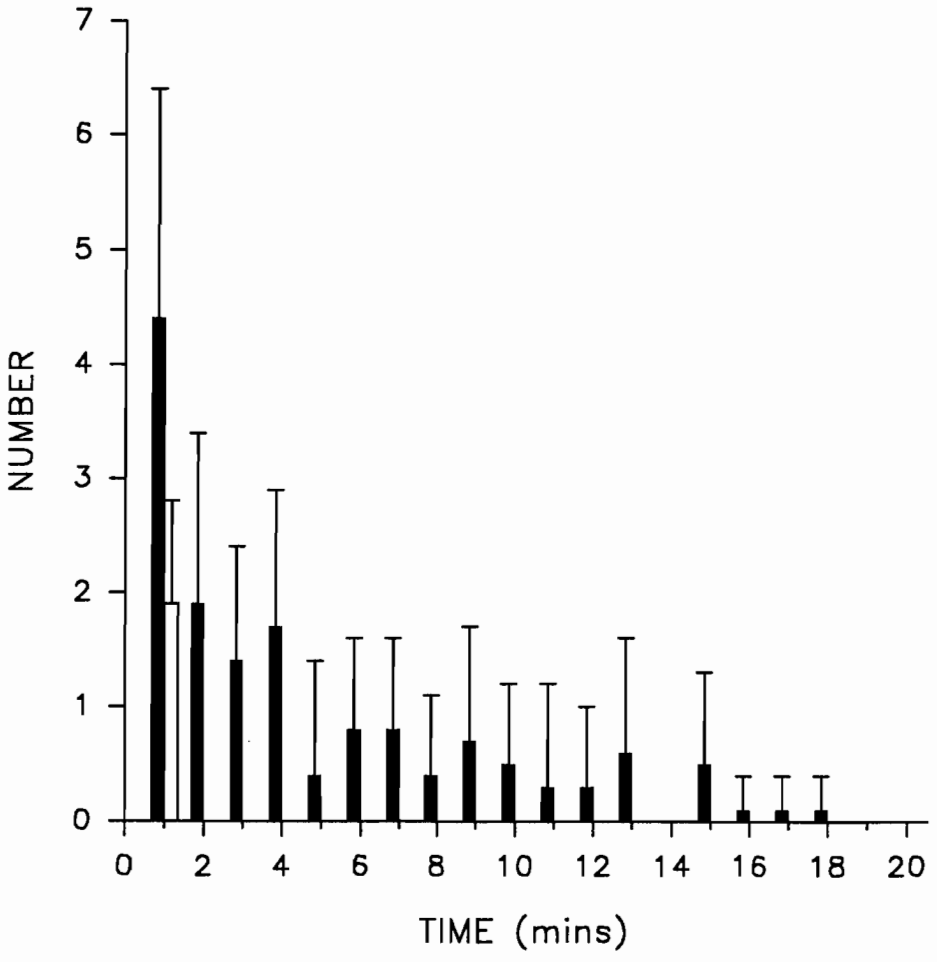

NUMBER
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16
18
20
TIME (mins)

Figure 4.5 Mean number of approaches (solid bar) and attacks (open bar) made by sooty grunter on days 1 and 2. Vertical bars indicate + 1 standard deviation.

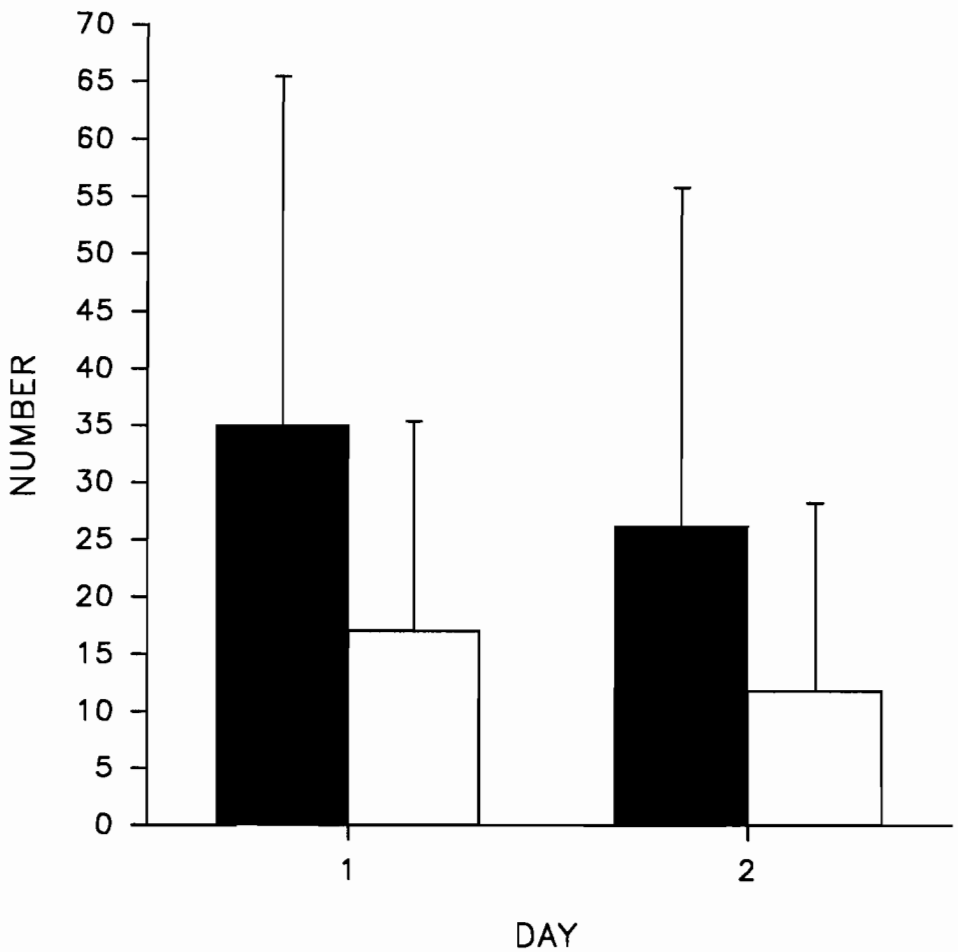

70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
NUMBER
1
2
DAY

Figure 4.6 Mean number of approaches made by sooty grunter prior to the first attack on *B. marinus* tadpoles on days 1 and 2. Vertical bars indicate + 1 standard deviation.

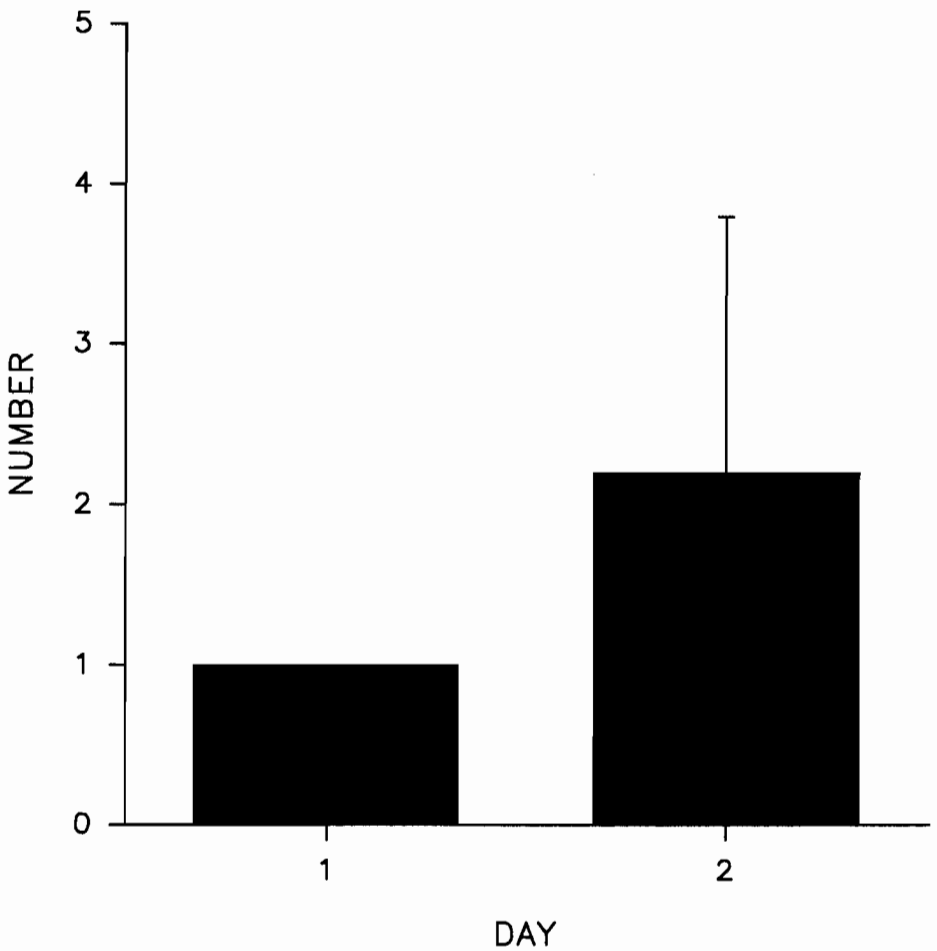

5
4
3
2
1
0
NUMBER
1
2
DAY

Figure 4.7 Mean time that elapsed before *B. marinus* tadpoles were first attacked by sooty grunter on days 1 and 2. Vertical bars indicate + 1 standard deviation.

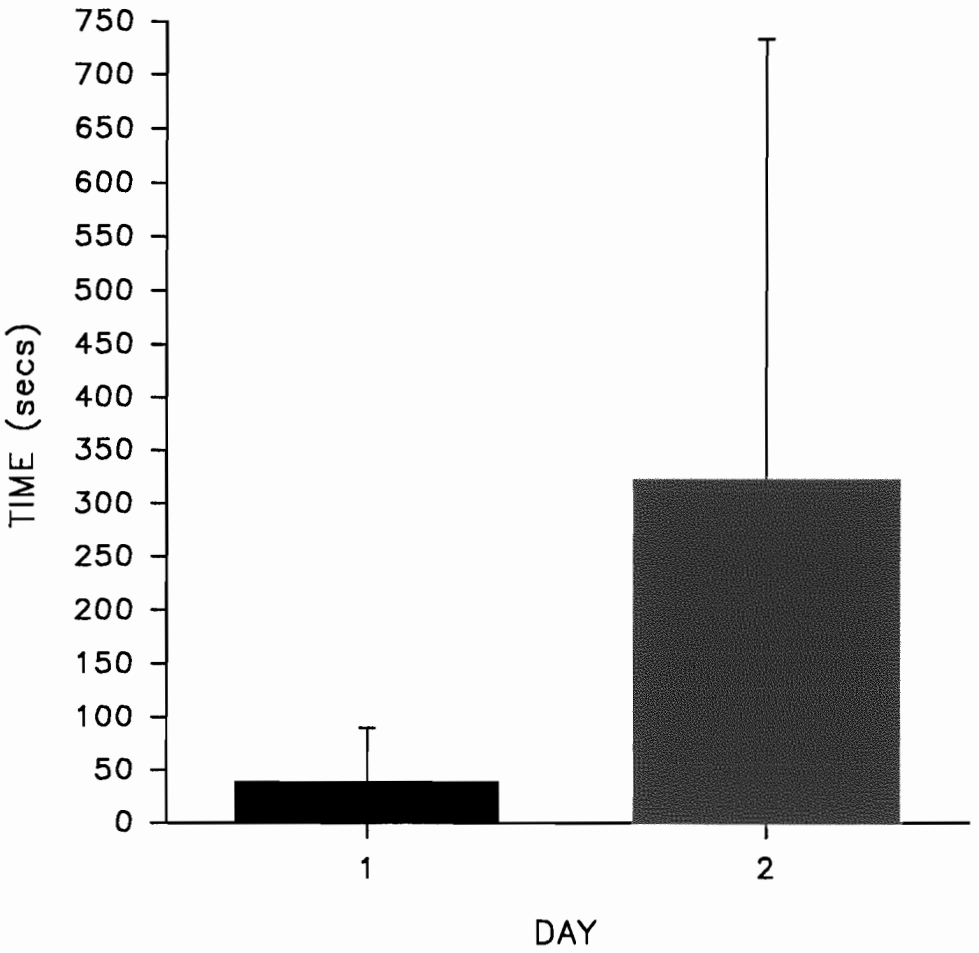

750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
TIME (secs)
1
2
DAY

Figure 4.8 Mean number of approaches (solid bar) and attacks (open bar) made by barramundi on days 1 and 2. Vertical bars indicate + 1 standard deviation.

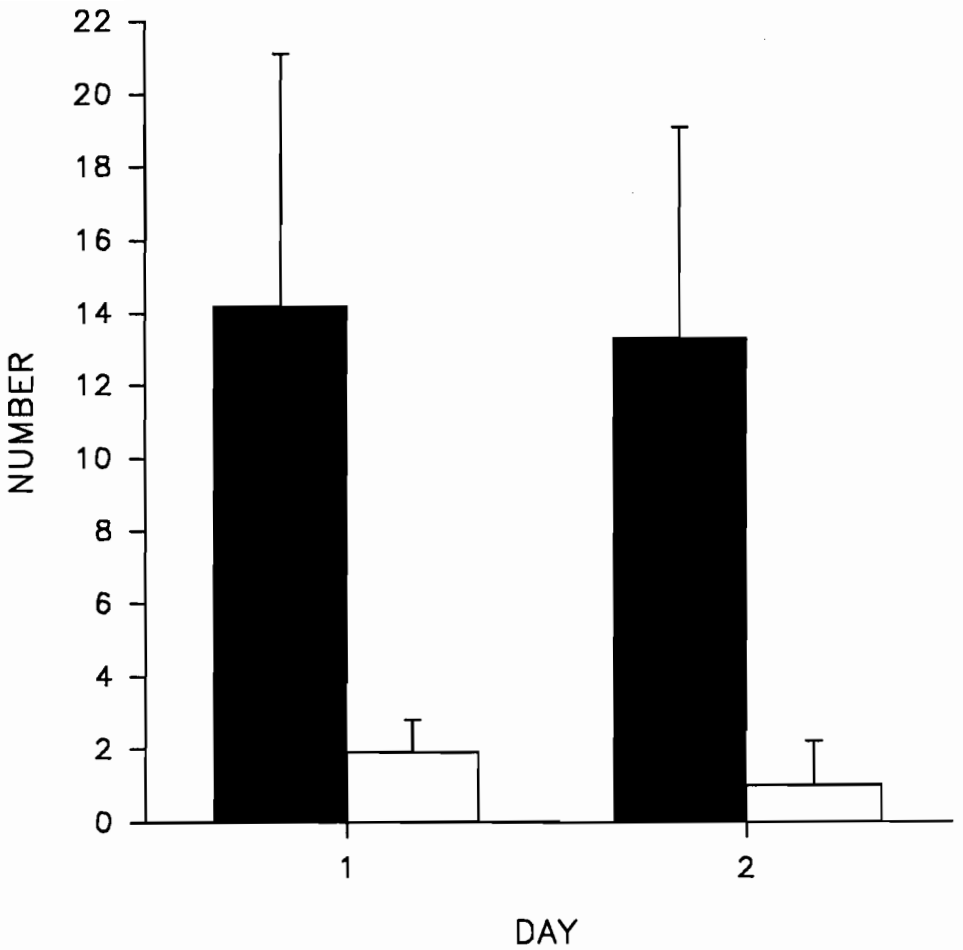
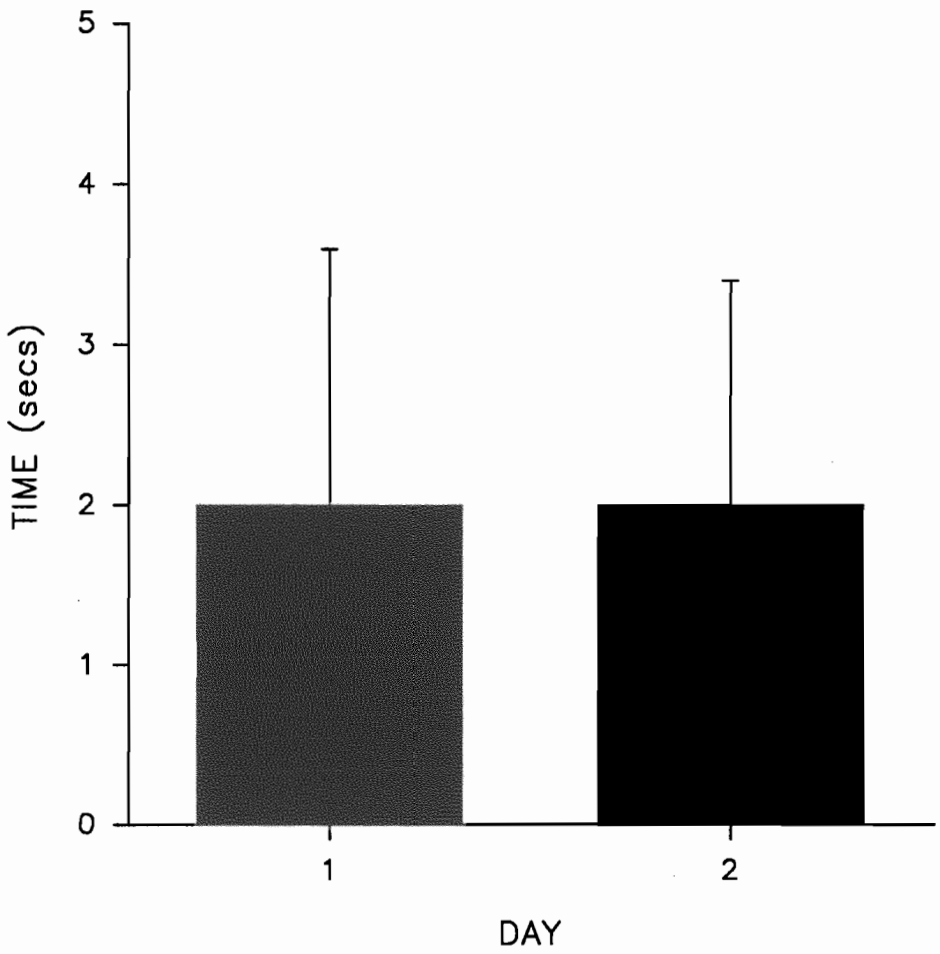

22
20
18
16
14
12
10
8
6
4
2
0
NUMBER
1
2
DAY

Figure 4.9 Mean time that elapsed before *B. marinus* tadpoles were first attacked by barramundi on days 1 and 2. Vertical bars indicate + 1 standard deviation.


5
4
3
2
1
0
TIME (secs)
1
2
DAY

4.4 Discussion

Previous studies have reported that *B. marinus* tadpoles are unpalatable to a variety of native Australian fish (Pearse 1980; Hearnden 1991). Similarly, the low predation rates on *B. marinus* tadpoles by native fish in Chapter 3 are also likely to be due to the unpalatability of *B. marinus* tadpoles. The responses of sooty grunter and barramundi to *B. marinus* tadpoles in this study clearly demonstrate that these fish also find *B. marinus* tadpoles unpalatable. All sooty grunter and barramundi readily consumed food pellets before and after being offered *B. marinus* tadpoles, demonstrating that they were feeding at the time of the *B. marinus* trials. In addition, all fish readily consumed *L. ornatus* tadpoles which were of similar size and developmental stage to the *Bufo* tadpoles, indicating that tadpole size or shape are unlikely to account for the minimal predation on *B. marinus* tadpoles. Sooty grunter and barramundi readily captured *B. marinus* tadpoles, but usually rejected them immediately. After rejecting the *B. marinus* tadpole, fish displayed vigorous "mouthing" and/or "head shaking" behaviour which was not observed following attacks on food pellets or *L. ornatus* tadpoles. From these observations, it can be concluded that *B. marinus* tadpoles are unpalatable to both sooty grunter and barramundi.

Although *Bufo* tadpoles are unpalatable to many fish, few published data exist on the ability of fish to learn to avoid unpalatable *Bufo* tadpoles. Voris and Bacon (1966) observed that bluegills (*Lepomis macrochirus*)

attacked and rejected *B. americanus* tadpoles as many as three times before eating or ignoring them. Kruse and Stone (1984) reported that largemouth bass (*Micropterus salmoides*) engulfed and expelled *B. woodhousei* tadpoles up to 24 times before ultimately accepting or rejecting them.

In the present study, sooty grunter exhibited considerable intraspecific variation in their ability to learn to avoid *B. marinus* tadpoles. One fish avoided the *B. marinus* tadpole after only attacking it twice during the early stages of the trial period. In contrast, another fish continued to attack and reject the *B. marinus* tadpole throughout the entire trial period (N=57 attacks). Several explanations may account for this variability. Some sooty grunter may simply be "better learners" than others (e.g. Coble *et al.* 1985). Alternately, differences in the avoidance of *B. marinus* tadpoles among sooty grunter may reflect differences in fish hunger levels. Kerfoot *et al.* (1980) found that predation on unpalatable water mites by guppies (*Lebistes reticulatus*) increased if fish were starved for several days. Similarly, Kruse and Stone (1984) found that the acceptability of unpalatable *Bufo woodhousei* tadpoles as food items by largemouth bass (*Micropterus salmoides*) increased as hunger levels increased. Thus, the sooty grunter which persisted in attacking *B. marinus* tadpoles throughout the trials may, by chance, have been hungrier than those individuals which only attacked *B. marinus* tadpoles a few times. Finally, there may be intraspecific variation in the

palatability of *B. marinus* tadpoles, with some tadpoles being extremely unpalatable while others are only "mildly" unpalatable. Previous studies have reported intraspecific variation in the palatability of other chemically defended taxa (e.g. sponges: Pawlik *et al.* 1995), and data from Chapter 3 suggest there may be intraspecific variation in the toxicity of *B. marinus* tadpoles (see Sections 3.3.1.3 and 3.3.2).

In contrast to sooty grunter, most barramundi learned to avoid *B. marinus* tadpoles after few (1-4) attacks. Since the *B. marinus* tadpoles offered to each fish species were of similar size and developmental stage, ontogenetic variation in tadpole palatability does not account for differences in the learned avoidance of *B. marinus* tadpoles by sooty grunter and barramundi. Barramundi may, in general, be "better learners" than sooty grunter (e.g. Coble *et al.* 1985). Alternately, the *B. marinus* tadpoles offered to barramundi and sooty grunter may have differed in their palatability, or there may have been interspecific differences in the hunger levels of barramundi and sooty grunter (e.g. Kerfoot *et al.* 1980; Kruse and Stone 1984).

Interestingly, one of the barramundi consumed a *B. marinus* tadpole on both days 1 and 2 without any apparent ill effect, demonstrating that the consumption of *B. marinus* tadpoles is not always fatal to native Australian fish (c.f. Pearse 1980; Hearnden 1991). Although the behaviour of this fish (excessive "mouthing" following consumption)

suggests that it found the *B. marinus* tadpole unpalatable, it did not regurgitate the tadpole. This individual was the largest of the barramundi tested, and its consumption of the *B. marinus* tadpole may have resulted from higher hunger levels than the other smaller barramundi.

Few data exist regarding the ability of aquatic predators to remember to avoid unpalatable amphibian prey which they have previously encountered. Brodie and Formanowicz (1981) found that larvae of the diving beetle *Dytiscus verticalis* continue to avoid pieces of unpalatable newt (*Notophthalmus viridescens*) for up to 96 hours after exposure. Similarly, dragonfly larvae (*Anax junius*) avoid unpalatable *B. americanus* tadpoles two days after being exposed to these tadpoles (Brodie and Formanowicz 1987). As far as I am aware, there are no published data on the ability of fishes to remember to avoid unpalatable amphibian prey. Many fishes have both short-term and long-term memory processes (Kerfoot *et al.* 1980; Csányi *et al.* 1989; Miklósi *et al.* 1992). Encounters with prey are usually remembered by fishes for only short periods of time, ranging from a matter of hours to several months (Bryan and Larkin 1972; Croy and Hughes 1991; Tullrot and Sunberg 1991; Milinski 1994).

The responses of sooty grunter and barramundi during the day 2 trials indicate that at least some individuals of both species remembered their

encounter with *B. marinus* tadpoles from the previous day. On day 2, five sooty grunter approached the *B. marinus* tadpole as soon as it was added to the tank, but halted their approach approximately 2 cm from the tadpole. These fish viewed the tadpole for up to 5 seconds before turning away from the tadpole. This behaviour was repeated up to three times before the *B. marinus* tadpole was attacked. Similarly, three barramundi approached to within 3 cm of the *B. marinus* tadpoles on day 2 but did not attack the tadpoles. Presumably, all of these fish approached *B. marinus* tadpoles until they were able to recognise the tadpole, either by visual or olfactory cues, and associate it with their experience from the previous day. The eventual attack of *B. marinus* tadpoles by all sooty grunter on day 2 may have resulted from high hunger levels of these fish (e.g. Kerfoot *et al.* 1980; Kruse and Stone 1984). The lack of avoidance behaviour by some sooty grunter and barramundi during the day 2 trials may have been due to intraspecific differences in memory (e.g. Miklósi *et al.* 1992), or intraspecific differences in hunger levels (e.g. Kerfoot *et al.* 1980; Kruse and Stone 1984).

The larvae of *B. marinus* are black in colour and frequently form large, dense, conspicuous aggregations in nature. The conspicuousness of *Bufo* tadpoles, coupled with their distastefulness, suggests that schooling may serve an aposematic function in deterring predators (Wassersug 1973; Waldman and Adler 1979; Waldman 1982). One

assumption of this hypothesis is that predators are able to learn to avoid *Bufo* tadpoles after consuming or attempting to consume them (Endler 1991). Kruse and Stone (1984) provided the first evidence in support of this hypothesis by demonstrating that largemouth bass were able to learn to avoid unpalatable *B. woodhousei* tadpoles. The present study provides further support for the proposed aposematic function of *Bufo* tadpole aggregations. Native fish learned to avoid *B. marinus* tadpoles, and, in some instances, remembered to avoid *B. marinus* tadpoles during subsequent encounters. This learning was usually achieved without killing the *B. marinus* tadpole. Kruse and Stone (1984) reported similar results: largemouth bass usually learned to avoid unpalatable *B. woodhousei* tadpoles without killing the tadpole. Since predator training may be more efficient when prey are at higher densities of fewer and more similar colour patterns (Endler 1991), native fish which encounter aggregations of similarly coloured *B. marinus* tadpoles in nature may learn to avoid *B. marinus* tadpoles more quickly than demonstrated in the present study, where fish only encountered one *B. marinus* tadpole at a time.

The range of *B. marinus* in Australia continues to expand (Freeland and Kerin 1988; Sabath *et al.* 1981; Freeland and Martin 1985; Seabrook 1991; Sutherst *et al* 1996), and the species is believed to pose a significant threat to native wildlife. Predatory native fish are potentially at risk as they are susceptible to the toxins present in *B. marinus* eggs

(Chapter 3) and tadpoles (Pearse 1980; Hearnden 1991). Since predatory fish play an important role in structuring many freshwater communities (e.g. Brooks and Dodson 1965; Hutchinson 1971; Hurlbert *et al.* 1972; Macan 1977; Anderson 1980; Zaret 1980; Elser and Carpenter 1988; Petranka 1983; Mittelbach *et al.* 1995), any impact of *B. marinus* on the survival of native predatory fish may also alter the composition of native aquatic communities at lower trophic levels (e.g. Hurlbert *et al.* 1972; Elser and Carpenter 1988; Mittelbach *et al.* 1995). The results of this study demonstrate that naive sooty grunter and barramundi are capable of learning to avoid *B. marinus* tadpoles with minimal trauma. Consequently, populations of these fishes are unlikely to experience significant declines in areas which have been colonised, or are currently being colonised by *B. marinus*.

CHAPTER 5. IMPACT OF *BUFO* ON NATIVE ANURAN LARVAE: PREDATION ON *BUFO*

5.1 Introduction

Anuran larvae often experience high mortality rates between egg hatching and metamorphosis (Savage 1952; Turner 1962; Calef 1973; Wilbur and Collins 1973; Licht 1974; Cecil and Just 1979; Yorke 1983). This mortality may result from biotic factors such as predation (Calef 1973; Licht 1974; Cecil and Just 1979; Wilbur 1980; Morin 1987; Stangel 1988) and competition (Alford and Wilbur 1985; Wilbur and Alford 1985; Morin 1987; Wilbur 1987; Griffiths 1991; Warner *et al.* 1993). In addition, abiotic factors such as habitat desiccation (Heyer *et al.* 1975; Wilbur 1977, 1984, 1987; Scott 1990), water acidification (Grant and Licht 1993) and ultraviolet radiation (Grant and Licht 1995) may also determine the survival of larval anurans. In nature, biotic and abiotic factors are likely to interact to regulate larval anuran survival (Wilbur 1987; Dunson and Travis 1991; Warner *et al.* 1993).

One possible source of mortality for anuran larvae which has received little attention to date is the consumption of toxic food items. For example, the eggs, hatchlings and tadpoles of many species of *Bufo* possess noxious or toxic chemicals (Voris and Bacon 1966; Licht 1967a, 1968, 1969; Wassersug 1971; Walters 1975; Wells 1979; Flier *et al.* 1980; Pearse 1980; Kruse and Stone 1984; Brodie and Formanowicz 1987; Kats *et al.* 1988; Henrikson 1990; Denton and Beebee 1991;

Hearnden 1991; Akizawa *et al.* 1994), and tadpoles of many other species may prey upon one or more of these life history stages (review by Alford in press). Consequently, the consumption of *Bufo* eggs, hatchlings and tadpoles may potentially be a source of mortality for anuran larvae. Several studies have documented predation by anuran larvae on *Bufo* eggs, hatchlings and/or tadpoles. *Bufo calamita* eggs are preyed upon by tadpoles of *Rana temporaria* (Heusser 1970; Banks and Beebee 1987), *B. bufo* (Beebee 1977; Banks and Beebee 1987), *Pelobates cultripes* (Tejedo 1991) and *Pelodytes punctatus* (Tejedo 1991). Heusser (1970) also reported predation on *B. bufo* eggs by *Rana temporaria* tadpoles. *Leptodactylus pentadactylus* tadpoles prey upon *B. typhonius* eggs (Wells 1979) as well as *B. marinus* hatchlings and tadpoles (Heyer *et al.* 1975). *Rana tigerina* tadpoles are predators of *B. melanostictus* tadpoles (Hota and Dash 1983). *Bufo marinus* tadpoles readily consume conspecific eggs (Hearnden 1991), while *Rana sylvatica* tadpoles are voracious predators of *B. americanus* eggs and hatchlings (Petranka *et al.* 1994). None of these studies mention any post-ingestion ill effects to tadpoles following the consumption of *Bufo*. However, experiments conducted in Chapter 3 demonstrated that the consumption of *B. marinus* eggs, hatchlings or dead tadpoles is a source of mortality for native Australian tadpoles (see Sections 3.3.1.1, 3.3.1.2, 3.3.3).

The behavioural responses of native tadpoles to *B. marinus* eggs and dead *B. marinus* tadpoles suggest that they are unable to detect and avoid the toxins present in these stages (see Sections 3.3.1.1 and 3.3.3). In this chapter, I investigate two factors likely to determine the impact of *B. marinus* eggs, hatchlings and tadpoles on native tadpoles in nature: (1) the ability of native tadpoles to detect and avoid the toxins present in *B. marinus* eggs, hatchlings and tadpoles, and (2) native tadpole size. Anuran larvae are generally considered to be indiscriminate feeders (Jenssen 1967; Nathan and James 1972; Heyer *et al.* 1975; Wassersug 1975; Seale and Beckvar 1980; but see Taylor *et al.* 1995) and it has been suggested that they may have limited ability to taste food items (Heyer *et al.* 1975). However, as far as I am aware, no published data exist regarding the ability of tadpoles to assess the palatability or toxicity of their food items. If native anuran larvae cannot detect *B. marinus* toxins, they will not avoid consuming *Bufo* and are therefore likely to experience reduced survival in water bodies where they co-occur with *B. marinus*. Tadpole size may also be an important factor determining survival of native tadpoles which cannot detect *B. marinus* egg toxins. Previous studies have found that small tadpoles are often less effective predators of anuran eggs than are large tadpoles due to their small mouth sizes (Crump 1983; Tejedo 1991; Petranka and Thomas 1995). Thus, size classes of native tadpoles which are constrained from consuming *B. marinus* eggs by their small mouth sizes are likely to experience higher survival rates in the presence of *B.*

marinus eggs than size classes of native tadpoles which are capable of consuming *B. marinus* eggs.

All of the native anuran species included in the following experiments (*Litoria alboguttata, L. bicolor, L. gracilenta, L. nigrofrenata, L. rubella, Cyclorana brevipes, Limnodynastes ornatus*) reproduce throughout the wet season in northern Queensland in temporary and semi-permanent water bodies which are often also used by *B. marinus* as breeding sites (pers. obs.). Thus, larvae of all these species are exposed to *B. marinus* eggs, hatchlings and tadpoles in nature.

5.2 Methods

The following experiments were conducted at James Cook University and Heathlands Reserve using either small (440 ml-10 l) plastic containers in the laboratory, or artificial plastic ponds (200-1000 l) located in outdoor enclosures. Containers in laboratory experiments were exposed to a 12HL:12HD photoperiod and 23-30°C air temperatures. Artificial ponds were exposed to natural photoperiod and air temperatures. Except for experiment 5.2.1.2, all of the experiments were randomised block design experiments where treatments were allocated randomly to containers or ponds within blocks with the constraint that no adjacent units had the same treatment. During all experiments, native anuran larvae and *Bufo* were randomly allocated to containers or ponds within treatments. All random allocations were

made using a random number table. All densities of native anuran larvae and *Bufo* used in the artificial pond experiments are within estimates of densities in natural ponds (R. Alford pers. comm.; M. Hearnden pers. comm.).

All native tadpoles and *B. marinus* eggs and tadpoles used in the experiments were collected from local temporary water bodies using dipnets. Unless otherwise stated, native tadpoles were maintained in the laboratory in 10 l buckets filled with 6 l water for up to 2 weeks before being included in an experiment. During this time they were fed frozen lettuce and tadpole chow (3:1 ground lucerne pellets and Tetra-Min^{tm} Tropical Fish Flakes passed through a 250 μm sieve) *ad libitum*. *Bufo marinus* eggs were collected early (0600-0800 hours) on the morning of deposition and were included in experiments on that day. *Bufo marinus* tadpoles were also included in experiments the same day they were collected.

5.2.1 Ability of Native Tadpoles to Detect and Avoid Bufotoxins

5.2.1.1 Avoidance of *Bufo marinus* Eggs

The following experiment was designed to investigate whether native tadpoles (*L. ornatus*) avoid eating *B. marinus* eggs when alternate non-toxic food is available. The experiment was conducted in the laboratory at James Cook University on 31 January 1993. Thirty 700 ml plastic containers were randomly positioned on a benchtop in a 3 X 10 array

and filled with 400 ml aged tap water. A single *L. ornatus* tadpole (11.4-14.1 mm SVL; Gosner 1960 stages 30-40; N = 30) was allocated to each container and exposed to one of three diet treatments:

(1) 5cm^2 frozen lettuce (control treatment),

(2) 50 *B. marinus* eggs (alternate food absent), or

(3) 50 *B. marinus* eggs plus 5cm^2 frozen lettuce (alternate food present).

Each treatment was replicated ten times.

All tadpoles were measured (SVL) and staged (Gosner 1960) prior to the experiment. The number of eggs consumed and the condition of *L. ornatus* tadpoles in containers were monitored at 12 hour intervals until hatchings began to emerge from the *B. marinus* eggs (48 hours).

5.2.1.2 Avoidance of Dead *Bufo marinus* Tadpoles

The following trials were designed to investigate whether native tadpoles (*L. alboguttata*, *L. gracilenta*, *L. rubella*, *C. brevipes*, *L. ornatus*; Table 5.1) avoid eating dead *B. marinus* tadpoles when alternate non-toxic food is available. The trials were conducted in the laboratory at James Cook University between January and March 1994. Larvae of each anuran species were tested in separate trials. For each trial, ten 10 l containers filled with 2 l aged tap water were randomly positioned on a benchtop in a 2 X 5 array. A single tadpole was placed in each container and simultaneously offered five dead *B. marinus* tadpoles and

five dead native (*L. ornatus*) tadpoles ($N = 10$ replicates). All dead *B. marinus* and *L. ornatus* tadpoles were of similar size and developmental stage (see Table 5.1). Previous observations indicated that dead *L. ornatus* tadpoles are non-toxic to all of the species of native anuran larvae tested (pers. obs.). All live tadpoles and five randomly chosen dead tadpoles of each species were measured (SVL) using vernier calipers and staged (Gosner 1960) prior to each trial.

Live tadpoles were starved for 12 hours prior to being offered dead tadpoles. The number of dead tadpoles consumed and the condition of live tadpoles were monitored at 12 hour intervals for a period of 24 hours.

5.2.2 Effect of Size on Ability of Native Tadpoles to Consume *Bufo marinus* Eggs

Results from experiments conducted in Section 5.2.1 indicated that *L. ornatus* tadpoles are unable to detect the toxins present in *B. marinus* eggs and tadpoles (see Section 5.3.1). The following experiment was designed to investigate whether size affects the ability of *L. ornatus* tadpoles to prey upon *B. marinus* eggs, and therefore determines survival of *L. ornatus* tadpoles in the presence of *B. marinus* eggs.

The experiment was conducted in the laboratory at James Cook University on 22 December 1992. Forty 440 ml plastic containers were

randomly positioned on a benchtop in a 4 X 10 array and filled with 350 ml aged tap water. The experiment consisted of four treatments:

(1) 1 small tadpole per container (small tadpole control treatment),

(2) 1 small tadpole plus 25 *B. marinus* eggs per container (small tadpole plus *Bufo* egg treatment),

(3) 1 large tadpole per container (large tadpole control treatment), and

(4) 1 large tadpole plus 25 *B. marinus* eggs per container (large tadpole plus *Bufo* egg treatment).

Each treatment was replicated ten times.

Prior to each experiment, all tadpoles were measured (SVL) using vernier calipers and staged (Gosner 1960). The sizes and developmental stages of the tadpoles used are as follows: small *L. ornatus* 5.0-6.1 mm SVL, stage 25; large *L. ornatus* 8.0-12.9 mm SVL, stages 30-37. Tadpole survival and the number of eggs consumed by each tadpole were monitored at 12 hour intervals until *Bufo* hatchlings began emerging from egg strings (36 hours).

Differences between the numbers of small and large *L. ornatus* tadpoles that consumed *B. marinus* eggs were tested for using a 2 X 2 Fisher's Exact Test. During the experiment, several of the small and large *L. ornatus* tadpoles which were exposed to *B. marinus* eggs did not consume eggs. Therefore, differences between the survival of small and

large *L. ornatus* tadpoles exposed to *B. marinus* eggs were tested for by comparing the numbers of live and dead tadpoles in each of the following categories: (1) small tadpoles that were exposed to eggs and ate eggs, (2) small tadpoles exposed to eggs that did not eat eggs, (3) large tadpoles exposed to eggs that ate eggs, and (4) large tadpoles exposed to eggs that did not eat eggs, using a 2 X 4 Fisher's Exact Test.

Limnodynastes ornatus tadpoles of similar size and developmental stage to the tadpoles used in the experiment were collected from local temporary water bodies in February 1993 and preserved in 10% formalin. The snout-vent length, maximum oral disc width, maximum beak width and Gosner (1960) stage of each tadpole was recorded. Snout-vent lengths were measured using vernier calipers. Dimensions of oral discs and beaks were measured using an ocular micrometer. Three *B. marinus* egg strings were collected from local water bodies in March 1993 and preserved in 10% formalin. For each clutch, the diameter of the egg string and one fertilised egg was measured at 10 haphazardly chosen positions using an ocular micrometer.

5.2.3 Impact of *Bufo* on the Survival of Native Tadpoles

5.2.3.1 *Bufo marinus* Eggs and Hatchlings

Limnodynastes ornatus tadpoles are highly susceptible to the toxins in *B. marinus* eggs and hatchlings (see Sections 3.3.1.1, 3.3.1.2) but have

limited ability to detect and avoid these toxins (see Section 5.3.1.1). Consequently, *L. ornatus* tadpoles are likely to experience reduced survival in water bodies where they co-occur with *B. marinus* eggs and hatchlings. I conducted an artificial pond experiment to quantify the impact of *B. marinus* eggs and hatchlings on the survival of *L. ornatus* tadpoles under naturalistic conditions. Plastic ponds (1000 l) were arranged in a 3 X 4 array in an outdoor compound with no obvious environmental gradient at the James Cook University campus. Two thermometers were placed in a centrally located pond to record daily bottom and surface maximum and minimum water temperatures.

The experiment consisted of three treatments:

(1) 50 *L. ornatus* tadpoles per pond (*L. ornatus* control treatment),

(2) 50 *L. ornatus* tadpoles plus 300 *B. marinus* eggs per pond (low density *Bufo* treatment), and

(3) 50 *L. ornatus* tadpoles plus 600 *B. marinus* eggs per pond (high density *Bufo* treatment).

Each treatment was replicated four times.

On 10 February 1994, the ponds were filled with 900 l tap water and allowed to stand for 6 days. All ponds were covered with lids comprised of birdwire (15 mm x 20 mm mesh size) and 70% shadecloth to prevent colonisation by frogs and predatory aquatic insects. On 16 February, 500 g dry leaf litter (previously collected from the overflow

area of a local dam: Mt Margaret dam) and 20 g lucerne pellets were randomly added to each pond to provide spatial complexity and a source of nutrients for the aquatic community. A 300 ml aliquot of well-mixed freshwater plankton collected from three local temporary ponds was added randomly to each pond on 19 February.

The ponds were stocked at a density of 50 *L. ornatus* tadpoles per pond at 0730 hrs on 23 February. This was achieved by haphazardly allocating groups of 10 *L. ornatus* tadpoles to 440 ml plastic containers, and then randomly combining groups of five containers into 10 l buckets. Buckets of 50 *L. ornatus* tadpoles were then allocated to ponds (one bucket per pond). Prior to being added to the ponds, one bucket from each treatment was randomly chosen and ten haphazardly netted tadpoles were measured (SVL) and staged (Gosner 1960). The *L. ornatus* tadpoles used in the experiment (10.3-12.9 mm SVL; stages 30-40) were of sufficient size to consume *B. marinus* eggs (see Sections 5.2.2, 5.3.2).

A freshly-laid clutch of *B. marinus* eggs was collected early (0600 hrs) on the morning of 23 February and returned to the laboratory. To account for any possible variation in toxicity within the clutch, segments of 100 eggs were cut along the length of the egg string and placed in 440 ml plastic containers (one segment per container). Containers were then randomly combined until there were four containers with 300 eggs,

and four containers with 600 eggs. The eggs were then added to ponds at 0930 hrs on 23 February.

The ponds were checked daily for minimum and maximum water temperatures and for metamorphosing *L. ornatus* tadpoles. On 27 February, free swimming (stage 25) *B. marinus* tadpoles were observed in the ponds. The experiment was stopped at this point as *L. ornatus* tadpoles do not prey upon free swimming *B. marinus* tadpoles (see Section 3.3.3.3). All live *L. ornatus* tadpoles present in each pond were removed by exhaustive sampling with a dipnet and counted on 27 February. The ponds were then drained through a pipe covered with 70% shadecloth, and any remaining *L. ornatus* tadpoles were collected and counted.

5.2.3.2 Dead *Bufo marinus* Tadpoles

Experiments conducted in Section 5.2.1.2 demonstrated that some native tadpoles have limited ability to detect and avoid the toxins present in dead *B. marinus* tadpoles (see Section 5.3.1.2). Therefore, the presence of dead *B. marinus* tadpoles may also pose a risk to the survival of such native tadpoles in water bodies where they co-occur with *Bufo*. I performed an artificial pond experiment to quantify the impact of dead *B. marinus* tadpoles on the survival of tadpoles of *L. nigrofrenata* and *L. bicolor*. Although these species were not included in the necrophagy feeding preference trials (Section 5.2.1.2), larvae of

both species are highly susceptible to bufotoxins and readily consume dead *B. marinus* tadpoles (Table 3.5).

Plastic ponds (200 l) were positioned in a 6 X 4 array in a field with no obvious environmental gradient at Heathlands Reserve. A thermometer was placed in a centrally located pond to record daily maximum and minimum water temperatures.

The experiment consisted of four treatments:

(1) 30 *L. nigrofrenata* tadpoles per pond (*L. nigrofrenata* control treatment),

(2) 30 *L. nigrofrenata* tadpoles per pond plus 5 dead *B. marinus* tadpoles added daily (*L. nigrofrenata* plus *Bufo* treatment),

(3) 25 *L. bicolor* tadpoles per pond (*L. bicolor* control treatment), and

(4) 25 *L. bicolor* tadpoles per pond plus 5 dead *B. marinus* tadpoles added daily (*L. bicolor* plus *Bufo* treatment).

Each treatment was replicated six times.

The ponds were filled with 180 l creek water on 2 March 1993 and allowed to stand for 7 days. Each pond was covered with a lid of birdwire (5 cm mesh size) to deter colonisation by predatory aquatic insects. Shadecloth was not incorporated in the lids as it was not available at Heathlands when the experiment was conducted. On 9

March, 200 g dry leaf litter (collected from the overflow area of a local creek: Bertie Creek) and 5 g lucerne pellets were added randomly to each pond to provide spatial complexity and nutrients for the aquatic community. Immediately prior to the addition of these nutrients, the ponds were checked visually to ensure that no predatory aquatic insects were present; no insects were found in any of the ponds. A 100 ml aliquot of well-mixed freshwater plankton collected from two local temporary ponds was randomly added to each pond on 11 March.

Native tadpoles were allocated to ponds in the following manner. For each species, groups of five tadpoles were initially allocated to 440 ml plastic containers (five tadpoles per container). Containers were then randomly combined into 10 l buckets until the appropriate density was attained (i.e. 30 *L. nigrofrenata* tadpoles per bucket or 25 *L. bicolor* tadpoles per bucket). Buckets of tadpoles were then allocated to ponds. Before being added to ponds, one bucket from each treatment was randomly chosen and all tadpoles in it were measured (SVL) and staged (Gosner 1960). The *L. nigrofrenata* tadpoles used in the experiment were 6.2-9.1 mm SVL, stages 26-28, while the *L. bicolor* tadpoles used were 8.0-11.1 mm SVL, stages 27-32.

I simulated mortality of *B. marinus* tadpoles by placing five freshly-killed *B. marinus* tadpoles (9.3-10.9 mm SVL, stages 30-36) in treatment 2 and 4 ponds each day at 1200 hrs. *Bufo* tadpoles were sacrificed as per

Section 3.2.3. Dead *B. marinus* were then haphazardly allocated to 5 ml plastic dishes (one tadpole per dish). Groups of five plastic dishes were randomly chosen and combined into 440 ml plastic containers, resulting in 5 *B. marinus* tadpoles per 440 ml container. Containers of dead *B. marinus* tadpoles were then added to ponds.

The experiment commenced on 13 March and continued for 10 days. Ponds were checked daily for maximum and minimum water temperatures, and for metamorphosing *L. nigrofrenata* and *L. bicolor* tadpoles. Each pond was also searched visually for a five minute period each day and night (night searches using a spotlight) to determine whether they had been colonised by predatory aquatic insects. On 23 March, the ponds were exhaustively sampled using a dipnet. All live tadpoles present in each pond were collected and counted. After being dipnetted, each pond was visually searched until a five minute search failed to locate any more live tadpoles.

5.2.4 Survival Advantages for Native Anurans Which Breed Synchronously with *Bufo marinus*

The experiment conducted in Section 5.2.3.1 demonstrated that, under naturalistic conditions, *B. marinus* eggs and hatchlings cause significant mortality of *L. ornatus* tadpoles (see Section 5.3.3.1). *Limnodynastes ornatus* tadpoles are significant predators of the eggs and hatchlings of many native anurans (see Chapter 6), and may therefore play an

important role as predators in structuring native larval anuran populations. The following experiment was designed to investigate whether there are survival advantages for native anurans which breed synchronously with *B. marinus* in water bodies which contain predatory *L. ornatus* tadpoles.

The experiment was conducted in 1000 l plastic ponds which were arranged in a 3 X 3 array in an outdoor compound with no obvious environmental gradient at the James Cook University campus. Two thermometers were placed in a centrally located tank to record daily bottom and surface maximum and minimum water temperatures.

The experiment consisted of three treatments:

(1) 100 *L. rubella* eggs per pond (*L. rubella* control treatment),

(2) 100 *L. rubella* eggs plus 80 *L. ornatus* tadpoles per pond (*L. rubella* plus predator treatment), and

(3) 100 *L. rubella* eggs plus 80 *L. ornatus* tadpoles plus 400 *B. marinus* eggs per pond (*L. rubella* plus predator plus *Bufo* treatment).

Each treatment was replicated three times.

The ponds were filled with 900 l tap water on 4 February 1995 and were allowed to stand for 7 days. All ponds were covered with lids comprised of birdwire and shadecloth as per Section 5.2.3.1. On 11

February, 500 g dry leaf litter (previously collected from the overflow area of a local dam: Mt Margaret dam) and 20 g lucerne pellets were randomly added to each pond to provide spatial complexity and a source of nutrients for the aquatic community. A 300 ml aliquot of well-mixed freshwater plankton collected from two local temporary ponds was randomly added to each pond on 14 February.

The *L. ornatus* tadpoles used in the experiment were initially collected as eggs from a local temporary pond and were raised in 1000 l artificial ponds located in an outdoor compound at James Cook University campus. These rearing ponds were stocked with leaf litter and freshwater plankton as described above. *Limnodynastes ornatus* tadpoles included in the experiment (N=480) were haphazardly netted from the rearing ponds when required. Only *L. ornatus* tadpoles which were 9 mm SVL or greater were used in the experiment to ensure that they were able to prey upon *B. marinus* eggs (see Section 5.3.2). After being netted from the rearing ponds, *L. ornatus* tadpoles were haphazardly allocated to 400 ml plastic dishes (10 tadpoles per container). Groups of eight containers were then randomly combined into 10 l buckets to yield 80 *L. ornatus* tadpoles per bucket. Buckets of tadpoles were then allocated to ponds at 0800 hrs on 23 February. Prior to being added to ponds, one bucket each from treatments 2 and 3 were randomly chosen. Ten *L. ornatus* tadpoles from each bucket were haphazardly netted. Each tadpole was measured (SVL) using vernier

calipers and staged (Gosner 1960). Tadpoles in treatment 2 were 9.4-11.8 mm SVL, stages 28-34, while tadpoles in treatment 3 were 9.7-13.1 mm SVL, stages 29-37.

A single clutch each of *L. rubella* eggs and *B. marinus* eggs were collected from a local temporary pond early (0600 hrs) on 23 February and returned to the laboratory. *Litoria rubella* eggs were haphazardly allocated to 5 ml plastic dishes (20 eggs per dish). Groups of 5 dishes were randomly chosen and combined into 440 ml plastic containers to yield 100 *L. rubella* eggs per container. The *B. marinus* egg string was cut into segments of 50 eggs, and egg segments were placed in 100 ml plastic dishes (one segment per container). Groups of eight containers were randomly chosen and combined into 440 ml plastic containers to yield 400 *B. marinus* eggs per container. Containers of *L. rubella* and *B. marinus* eggs were then allocated to ponds between 0830-0930 hrs on 23 February.

The ponds were checked daily for maximum and minimum water temperatures, and for metamorphosing tadpoles. On 28 February, free swimming (stage 25) *B. marinus* tadpoles were observed in the ponds. The experiment was stopped at this point as *L. ornatus* tadpoles are not significant predators of free swimming *B. marinus* or *L. rubella* tadpoles (see Section 3.3.3.3; Chapter 6). All live *L. rubella* and *L. ornatus*

tadpoles present in each pond were removed by exhaustive sampling as per Section 5.2.3.1 and counted.

5.3 Results

None of the ponds were colonised by native frogs or predatory aquatic insects during any of the artificial pond experiments (Sections 5.3.3.1, 5.3.3.2, 5.3.4). In addition, no native tadpoles included in these experiments metamorphosed prior to the completion of the experiment. All statistical analyses were performed using SAS (SAS Institute Inc. 1988) and Statistix (Version 3.0, Analytical Software). Hypothesis tests were conducted at $\alpha = 0.05$.

5.3.1 Ability of Native Tadpoles to Detect and Avoid Bufotoxins

5.3.1.1 *Bufo marinus* Eggs

Survival of *L. ornatus* tadpoles exposed to *B. marinus* eggs when alternate food was present and absent is plotted in Figure 5.1. No statistical tests were performed due to the obvious trend in the data. *Limnodynastes ornatus* tadpoles did not avoid eating *B. marinus* eggs when alternate non-toxic food was available. All of the *L. ornatus* tadpoles in the control treatment (treatment 1) consumed frozen lettuce without ill effect. However, all of the *L. ornatus* tadpoles offered *B. marinus* eggs (treatment 2) consumed eggs and died. Similarly, all of the *L. ornatus* tadpoles offered *B. marinus* eggs and frozen lettuce (treatment 3) consumed eggs and died. Excess frozen lettuce was

present in all of the treatment 3 containers at the completion of the experiment, indicating that none of the *L. ornatus* tadpoles had consumed all of the lettuce available to them prior to eating *B. marinus* eggs.

5.3.1.2 Dead *Bufo marinus* Tadpoles

The degree of avoidance of dead *B. marinus* tadpoles by native tadpoles in the presence of alternate food is plotted in Figure 5.2. Many tadpoles died after partially consuming dead *B. marinus* tadpoles. Since it was usually not possible to quantify the number of *B. marinus* tadpoles consumed, no hypothesis tests were performed on the data.

There was considerable variation in the degree of avoidance of dead *B. marinus* tadpoles by native tadpoles. Tadpoles of *L. alboguttata*, *L. gracilenta* and *L. rubella* displayed a high degree of avoidance of dead *B. marinus* tadpoles, and consequently experienced high survival rates. In contrast, tadpoles of *C. brevipes* and *L. ornatus* failed to avoid dead *B. marinus* tadpoles and therefore experienced low survival rates. None of the tadpoles that fed on dead *B. marinus* tadpoles had consumed all of the available dead *L. ornatus* tadpoles prior to feeding upon dead *B. marinus* tadpoles.

5.3.2 Effect of Size on Ability of Native Tadpoles to Consume *Bufo marinus* Eggs

There was a significant difference between the number of small and large *L. ornatus* tadpoles which consumed *B. marinus* eggs (2 X 2 Fisher's Exact Test $P=0.0011$). While all (N=10) of the small *L. ornatus* tadpoles were observed grazing on *B. marinus* egg strings during the experiment, only one individual penetrated an egg string and consumed eggs (0.5 eggs eaten). In contrast, nine of the ten large *L. ornatus* tadpoles penetrated *B. marinus* egg strings and consumed eggs (0.5-4 eggs eaten). All *L. ornatus* tadpoles which consumed *B. marinus* eggs died within 12 hours. There was also a significant difference between the survival of small and large *L. ornatus* tadpoles exposed to *B. marinus* eggs (2 X 4 Fisher's Exact Test $P<0.0001$), with large tadpoles experiencing lower survival than small tadpoles when exposed to *Bufo* (Figure 5.3). None of the small or large *L. ornatus* tadpoles died in control treatments during the experiment.

The oral disc and beak size data for small and large *L. ornatus* tadpoles is listed in Table 5.2. There was a significant difference between the oral disc widths (Mann-Whitney $P<0.0001$) and beak widths (Mann-Whitney $P<0.0001$) of small and large *L. ornatus* tadpoles. *Bufo marinus* egg string diameter ranged from 4.0-6.7 mm, while *B. marinus* egg diameter ranged from 1.3-1.6 mm.

5.3.3 Impact of *Bufo* on the Survival of Native Tadpoles

5.3.3.1 *Bufo marinus* Eggs and Hatchlings

The addition of *B. marinus* eggs to ponds had an immediate effect on the survival of *L. ornatus* tadpoles. Numerous dead *L. ornatus* tadpoles were observed floating in *Bufo* treatment ponds 24 hours after the experiment had commenced, but were no longer visible 48 hours after the start of the experiment. In contrast, no dead *L. ornatus* tadpoles were observed in control treatment ponds at any time during the experiment.

Survival of *L. ornatus* tadpoles differed significantly among treatments (Kruskal-Wallis ANOVA $H=9.9155$, $P=0.007$; Figure 5.4). Tadpoles in control treatment ponds experienced minimal mortality, while tadpoles exposed to *B. marinus* eggs and hatchlings experienced high mortality rates. Survival of *L. ornatus* tadpoles in treatment 2 and 3 ponds (300 and 600 *B. marinus* eggs per tank respectively) differed significantly (Mann-Whitney $P=0.0304$), with tadpole survival decreasing as *Bufo* density increased (Figure 5.4). Overall, survival of *L. ornatus* tadpoles was negatively correlated with *Bufo* density (Spearman rank correlation $r_s=-0.9494$, $N=12$, $P<0.001$). Water temperature in the ponds during the experiment ranged from 24-29°C.

5.3.3.2 Dead *Bufo marinus* Tadpoles

Survival of *L. nigrofrenata* and *L. bicolor* tadpoles in control and *Bufo* treatments is plotted in Figure 5.5. There was a significant difference between the survival of *L. nigrofrenata* ($t=3.5647$, d.f.$=10$, $P=0.0051$) and *L. bicolor* ($t=2.9542$, d.f.$=6$, $P=0.0255$) tadpoles in control and *Bufo* treatments. Tadpoles of both species experienced lower survival when exposed to dead *B. marinus* tadpoles than in their respective control treatments (Figure 5.5). Water temperature in ponds during the experiment ranged from 23-41°C.

5.3.4 Survival Advantages for Native Anurans Which Breed Synchronously with *Bufo marinus*

Survival of *L. rubella* eggs to the free swimming tadpole stage differed significantly among treatments (Kruskal-Wallis ANOVA $H=6.1609$, $P=0.0459$; Figure 5.6). No *L. rubella* survived in the presence of *L. ornatus* tadpoles alone (treatment 2). However, *L. rubella* did survive in control treatment ponds (treatment 1) and in ponds containing *L. ornatus* tadpoles plus *B. marinus* eggs (treatment 3). In fact, there was no significant difference between the survival of *L. rubella* in treatment 1 and 3 ponds (Mann-Whitney $P=0.3827$).

Survival of *L. ornatus* tadpoles in treatment 2 and 3 ponds differed significantly ($t=5.2167$, d.f$=4$, $P=0.0064$; Figure 5.6). Fewer *L. ornatus* tadpoles survived in ponds containing *L. rubella* eggs plus *B.*

marinus eggs (treatment 3) than in ponds containing just *L. rubella* eggs (treatment 2). Overall, survival of *L. rubella* at the completion of the experiment was negatively correlated with survival of *L. ornatus* tadpoles (Spearman Rank correlation $r_s = -0.8966$, $N = 9$, $0.005 > P > 0.002$; Figure 5.7). Water temperature in the ponds during the experiment ranged from 24-37°C.

Table 5.1 Size and developmental stage data (Gosner 1960) for tadpoles used during necrophagy feeding preference trials. N = number of replicates.

				Dead Tadpoles			
		Live Tadpoles		*B. marinus*		*L. ornatus*	
Species	N	SVL(mm)	Stage	SVL(mm)	Stage	SVL(mm)	Stage
Litoria alboguttata	10	21.2-31.0	30-38	5.6-6.3	25	5.9-6.4	25
Litoria gracilenta	10	11.3-13.0	30-37	5.0-6.2	25	5.0-6.9	25
Litoria rubella	10	9.5-12.8	34-38	4.7-5.1	25	4.6-5.1	25
Cyclorana brevipes	10	10.5-19.9	25-35	5.3-6.4	25	5.2-6.6	25
Limnodynastes ornatus	10	5.9-9.6	25-28	4.7-5.4	25	4.4-5.6	25

Table 5.2 Oral dimensions of small and large *L. ornatus* tadpoles. N = number of replicates. Stages are as per Gosner (1960).

Size Class	N	Mean SVL (mm) ± SD	Stage	Mean Oral Disc Width (mm) ± SD	Mean Oral Beak Width (mm) ± SD
Small	20	5.4 ± 0.4	25-26	1.3 ± 0.2	0.6 ± 0.8
Large	20	11.4 ± 1.5	31-38	3.0 ± 0.3	1.3 ± 0.2

Figure 5.1 Percent survival of *L. ornatus* tadpoles offered frozen lettuce (solid bar), *B. marinus* eggs (open bar), and frozen lettuce plus *B. marinus* eggs (hatched bar).

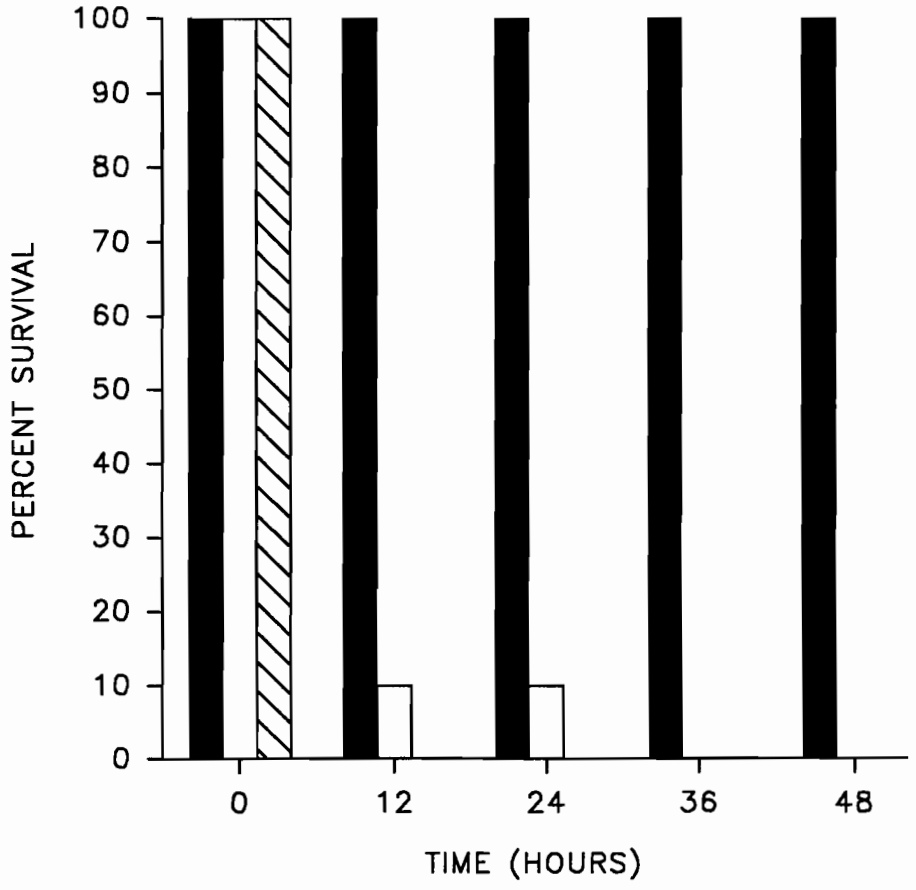

100
90
80
70
60
50
40
30
20
10
0
PERCENT SURVIVAL
0
12
24
36
48
TIME (HOURS)

Figure 5.2 Percent of feeding tadpoles which avoided dead *B. marinus* tadpoles (solid bar) and overall survival (open bar) of native tadpoles during the necrophagy feeding preference trials. Numbers indicate numbers of tadpoles which fed during the trials. Lr = *L. rubella*, Lg = *L. gracilenta*, La = *L. alboguttata*, Cb = *C. brevipes*, Lo = *L. ornatus*.

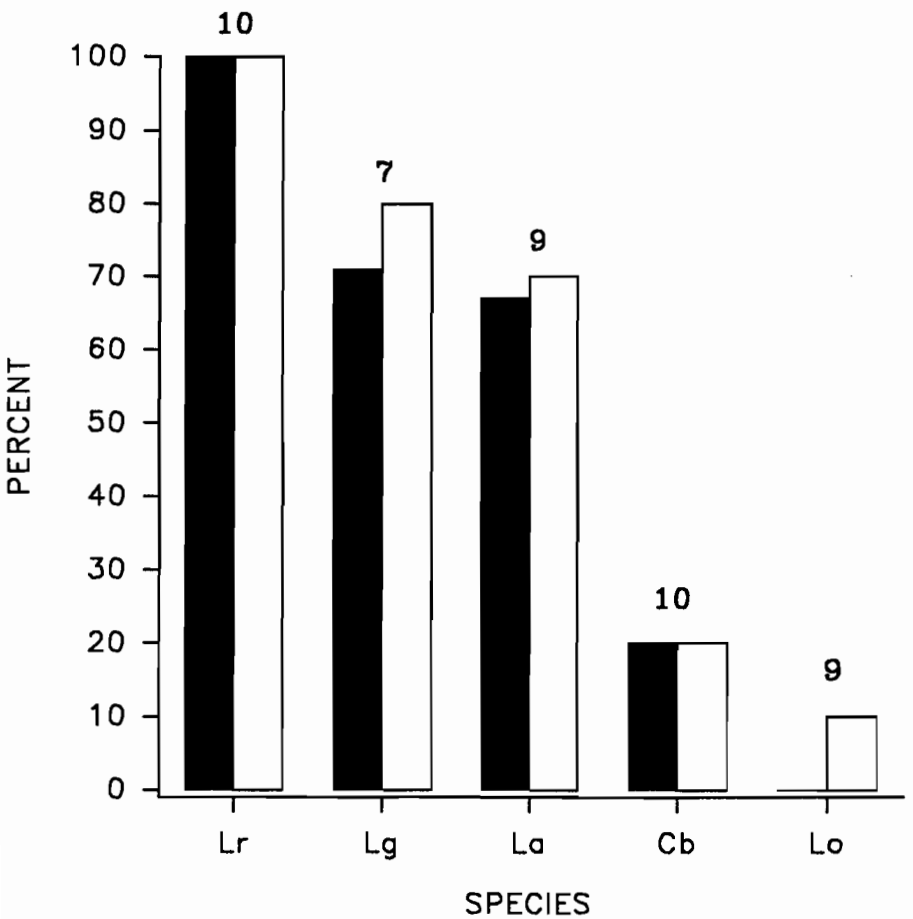

10
7
9
10
9
100
90
80
70
60
50
40
30
20
10
0
PERCENT
Lr
Lg
La
Cb
Lo
SPECIES

Figure 5.3 Percent survival of small and large *L. ornatus* tadpoles in control (solid bar) and *B. marinus* egg (open bar) treatments.

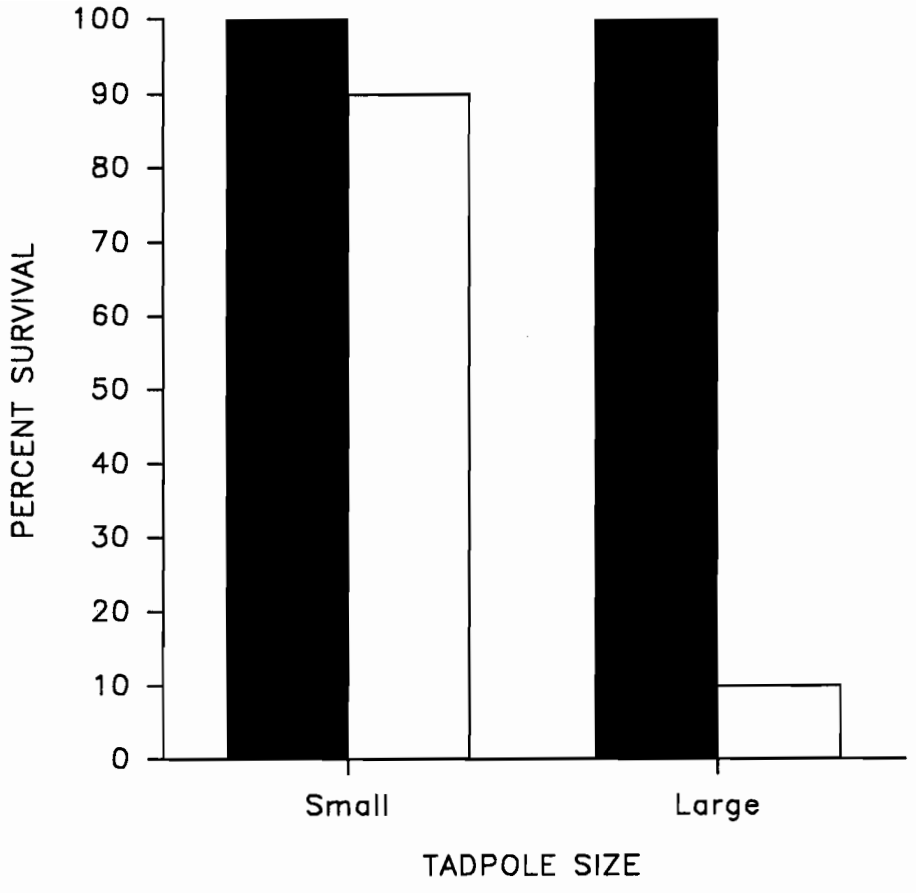

100
90
80
70
60
50
40
30
20
10
0
PERCENT SURVIVAL
Small
Large
TADPOLE SIZE

Figure 5.4 Mean percent survival of *L. ornatus* tadpoles exposed to *B. marinus* eggs and hatchlings. Vertical bars represent ± 1 standard deviation.

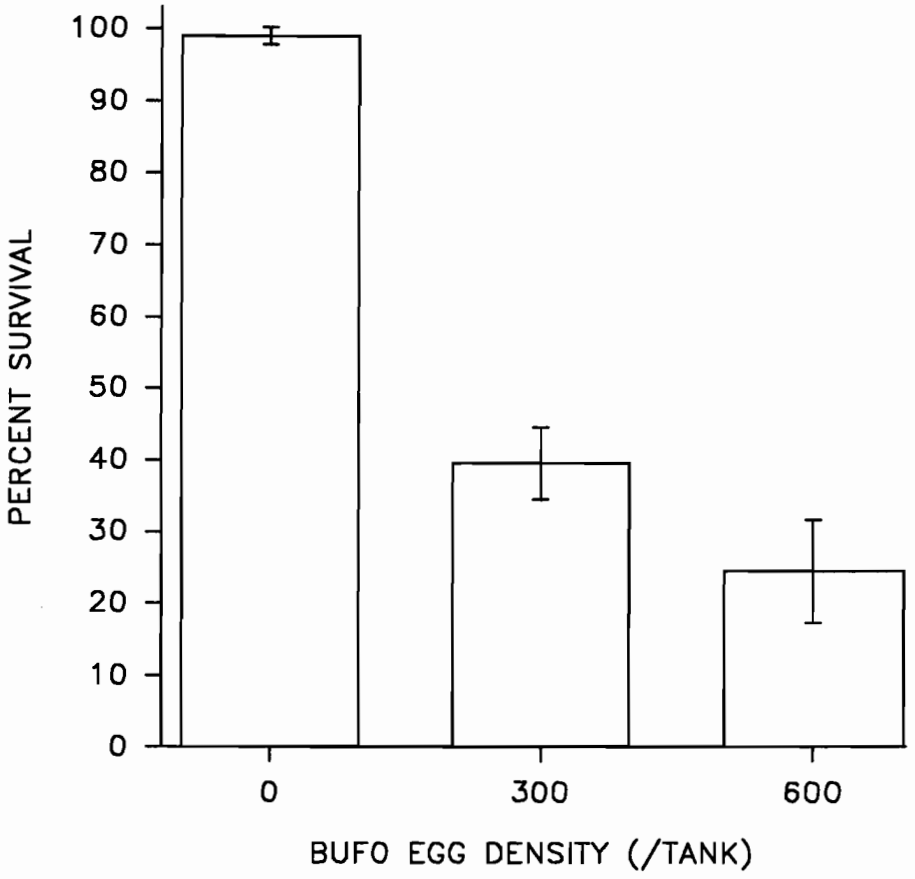
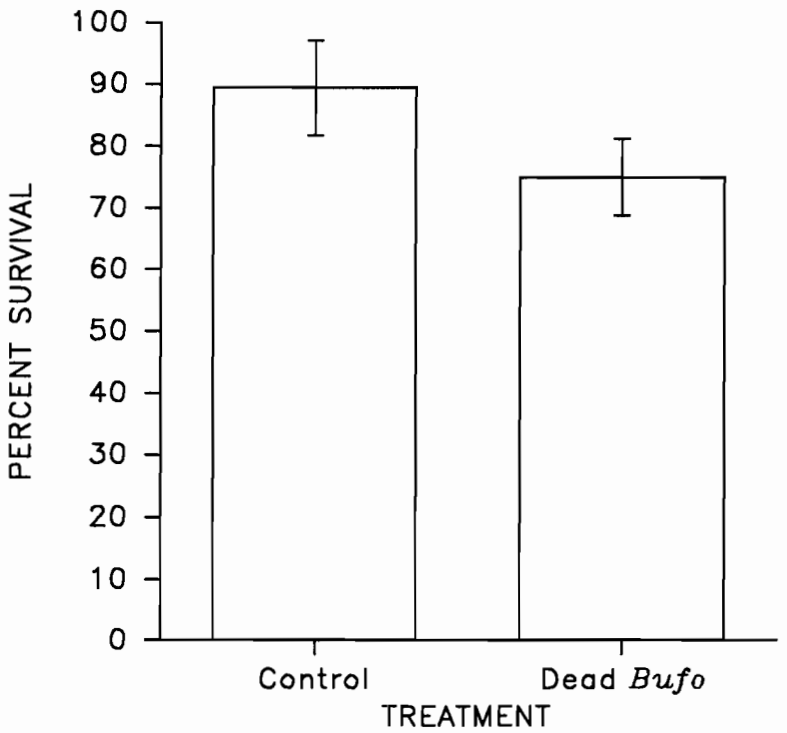

100
90
80
70
60
50
40
30
20
10
0
PERCENT SURVIVAL
0
300
600
BUFO EGG DENSITY (/TANK)

Figure 5.5 Mean percent survival of *L. nigrofrenata* and *L. bicolor* tadpoles in control and dead *B. marinus* tadpole ponds. Vertical bars represent ± 1 standard deviation.

L. nigrofrenata



L. bicolor

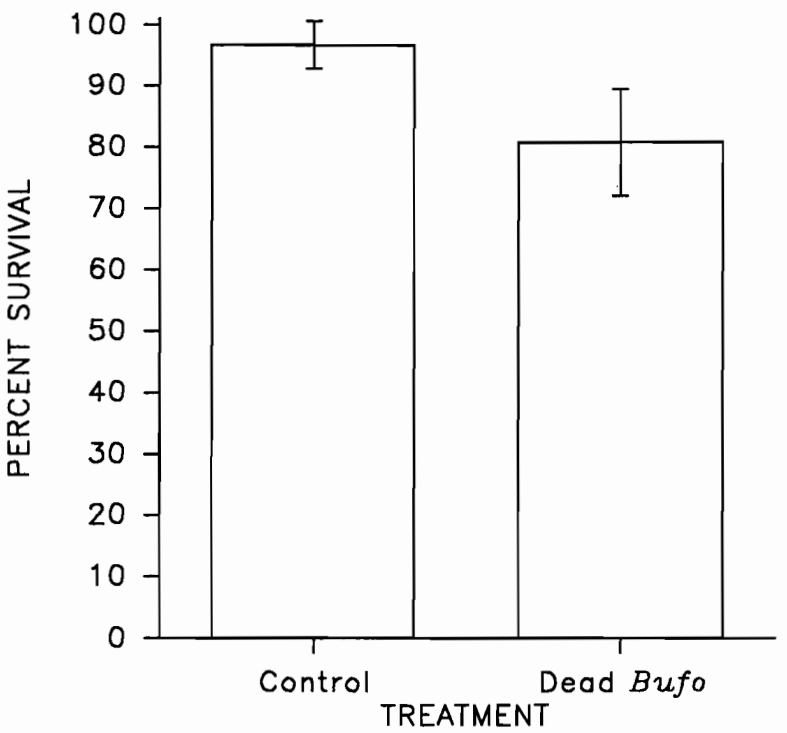


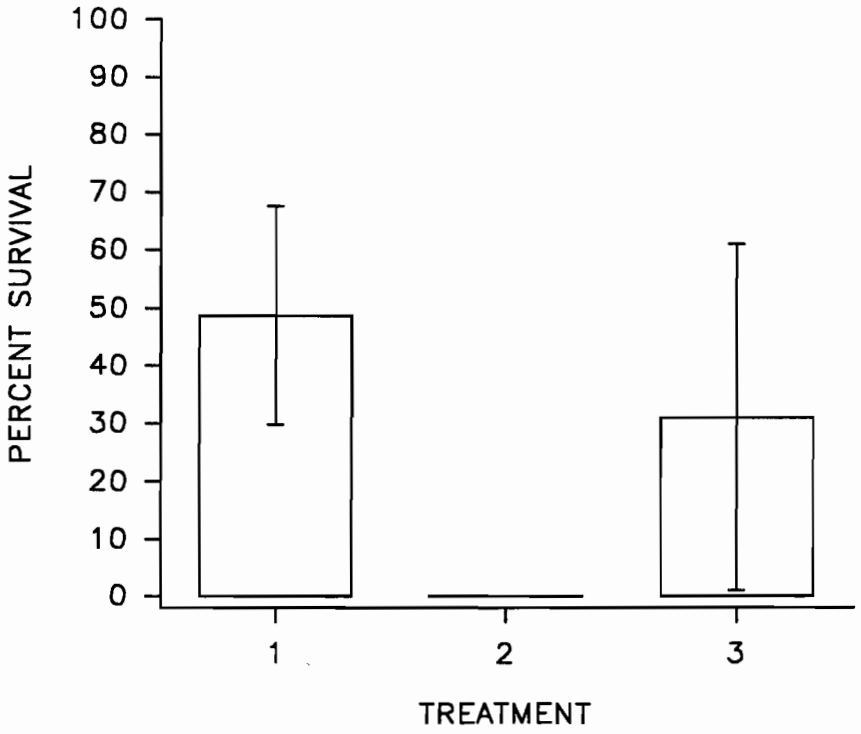
Figure 5.6 Mean percent survival of *L. rubella* and *L. ornatus* tadpoles. Vertical bars represent ± 1 standard deviation.

Treatment 1: 100 *L. rubella* eggs

Treatment 2: 100 *L. rubella* eggs plus 80 *L. ornatus* tadpoles

Treatment 3: 100 *L. rubella* eggs plus 80 *L. ornatus* tadpoles plus 400 *B. marinus* eggs

L. rubella



L. ornatus

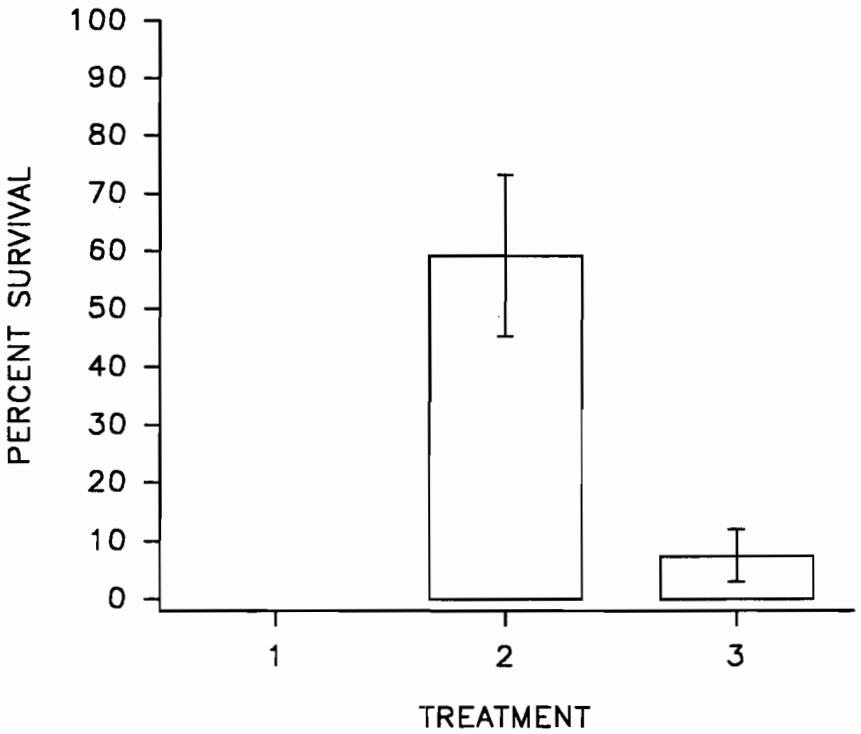
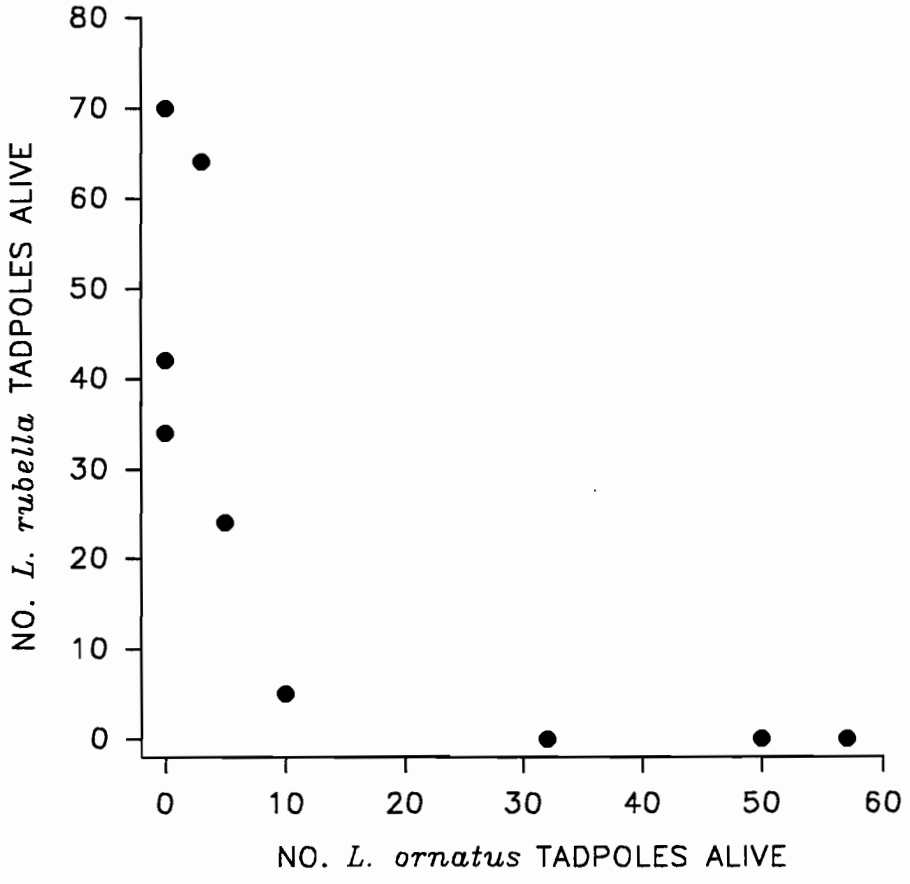


Figure 5.7 Number of *L. rubella* and *L. ornatus* tadpoles surviving in each tank at the completion of the experiment.


80
70
60
50
40
30
20
10
0
NO. L. rubella TADPOLES ALIVE
0
10
20
30
40
50
60
NO. L. ornatus TADPOLES ALIVE

5.4 Discussion

Native anuran larvae exhibited considerable interspecific variation in their ability to detect and avoid *B. marinus* toxins. Tadpoles of *L. alboguttata*, *L. gracilenta* and *L. rubella* generally avoided eating dead *B. marinus* tadpoles when alternate food (dead *L. ornatus* tadpoles) was available. In contrast, most *C. brevipes* and *L. ornatus* tadpoles readily consumed dead *B. marinus* tadpoles despite an abundance of non-toxic dead *L. ornatus* tadpoles. In addition, *L. ornatus* tadpoles also readily consumed *B. marinus* eggs despite the presence of an alternate non-toxic food supply (frozen lettuce). The dead *B. marinus* and *L. ornatus* tadpoles offered as food in the feeding choice trials were of similar size and "catchability" (i.e. dead), but differed in palatability and toxicity: *B. marinus* tadpoles are unpalatable and/or toxic to many predators (Wassersug 1971; Pearse 1980; Hearnden 1991; Chapter 3) while *L. ornatus* tadpoles are palatable and non-toxic to a variety of invertebrate and vertebrate predators (Crossland and Azevedo-Ramos in review; pers. obs.). Thus, the avoidance of *Bufo* by *L. alboguttata*, *L. gracilenta* and *L. rubella* may be interpreted as indicating that these species are able to detect and avoid *B. marinus* toxins. The lack of avoidance of *Bufo* by *C. brevipes* and *L. ornatus* indicates that these species have limited ability to detect and avoid *B. marinus* toxins. Previous studies have suggested that anuran larvae are indiscriminate feeders (Jenssen 1967; Nathan and James 1972; Heyer *et al.* 1975; Wassersug 1975; Seale and Beckvar 1980; but see Taylor *et al.* 1995) which may have limited ability to taste

their food items (Heyer *et al.* 1975). The results of this study indicate that anuran larvae may in fact be highly selective feeders, and that such discrimination may result from differences in the noxious or toxic qualities of food items. As far as I am aware, these are the first data to demonstrate that anuran larvae can assess the palatability or toxicity of their food items.

The ability of *L. alboguttata*, *L. gracilenta* and *L. rubella* tadpoles to detect and avoid *B. marinus* toxins may indicate that these species have adapted to these toxins. Adaptation could have occurred in the very short time interval since *Bufo* were introduced to Australia; the very high mortality rates experienced by native anuran larvae following the ingestion of *Bufo* (see Sections 3.3.1.1, 3.3.1.2, 3.3.3, 3.3.5, 5.3.1.1, 5.3.1.2, 5.3.2) would lead to rapid responses to such strong selective pressures if genetic variation existed. Alternately, these species may simply have a greater ability to assess the palatability or toxicity of their food items than do other native species such as *C. brevipes* and *L. ornatus*.

Survival of native anuran larvae in the presence of *Bufo* was determined by their ability to detect and avoid *B. marinus* toxins. In laboratory experiments, species that avoided consuming *Bufo* (*L. alboguttata*, *L. gracilenta* and *L. rubella*) experienced high survival rates when exposed to *Bufo*. In contrast, species that failed to avoid consuming *Bufo* (*C.*

brevipes, *L. ornatus*) experienced low survival rates when exposed to *Bufo*. These results suggest that tadpoles of *L. alboguttata*, *L. gracilenta* and *L. rubella* are likely to avoid consuming *Bufo* in nature, and are therefore unlikely to experience toxic effects in water bodies where they co-occur with *Bufo*. However, the limited ability of *C. brevipes* and *L. ornatus* tadpoles to detect and avoid *B. marinus* toxins suggests that these species may experience reduced survival in water bodies where they co-occur with *Bufo*. An artificial pond experiment confirmed this notion. Under naturalistic conditions, populations of *L. ornatus* tadpoles experienced significantly reduced survival when exposed to *B. marinus* eggs and hatchlings (Figure 5.4). A second artificial pond experiment demonstrated that populations of *L. bicolor* and *L. nigrofrenata* tadpoles experience significantly reduced survival when exposed to dead *B. marinus* tadpoles (Figure 5.5), indicating that these species also have limited ability to detect and avoid *B. marinus* toxins. Since toxins do not leach in toxic quantities from *Bufo* into solution (see Section 3.3.5), and native tadpoles that consume *Bufo* do not become toxic to necrophagous conspecifics (see Section 3.3.7.1), the mortality of native anuran larvae observed in these artificial pond experiments may be attributed to consumption of *Bufo*. These are the first quantitative data to demonstrate a significant negative impact of *B. marinus* on populations of any native Australian fauna, or on populations of native fauna in any country where *B. marinus* has been introduced. As far as I am aware, these are also the first data to demonstrate under naturalistic conditions

that the consumption of toxic anuran eggs, hatchlings and dead tadpoles are sources of mortality for anuran larvae.

For native anuran species which have limited ability to detect and avoid *B. marinus* toxins, the mortality risk posed by *B. marinus* eggs may change during tadpole ontogeny. *Bufo* eggs are surrounded by a gelatinous string which acts as a mechanical defence against some predators (Grubb 1972; Crump 1989). Small *L. ornatus* tadpoles were ineffective at penetrating the gelatinous string of *B. marinus* eggs and consequently experienced high survival rates when exposed to *B. marinus* eggs. In contrast, large *L. ornatus* tadpoles were more effective at penetrating *B. marinus* egg strings and consuming eggs. As a result, large *L. ornatus* tadpoles experienced low survival rates when exposed to *B. marinus* eggs. These differences in predation success are probably due to differences in the mouth sizes of small and large *L. ornatus* tadpoles. Large *L. ornatus* tadpoles have significantly larger oral discs and beaks than small *L. ornatus* tadpoles (Table 5.2) which may enable them to be more effective at preying upon *B. marinus* eggs (e.g. Crump 1983; Tejedo 1991). Thus, tadpoles which are unable to detect and avoid *B. marinus* toxins are only at risk from *B. marinus* eggs during the period of their larval life when they are effective predators of *B. marinus* eggs. Species which become effective predators of *B. marinus* eggs early in their larval development will be at risk from *B. marinus* eggs for the majority of their larval life. However, species which become

effective predators of *B. marinus* eggs later in their larval development will be at risk from *B. marinus* eggs for only part of their larval life.

The impact of *Bufo* on the survival of native tadpoles which have limited ability to detect and avoid *B. marinus* toxins will also depend on *Bufo* density. In the artificial pond experiment, survival of *L. ornatus* tadpoles was negatively correlated with *B. marinus* egg and hatchling density. This result was expected given the limited ability of *L. ornatus* tadpoles to detect and avoid *B. marinus* toxins. As the density of *B. marinus* eggs and hatchlings increased, the probability of *L. ornatus* tadpoles encountering and consuming *B. marinus* eggs and hatchlings also increased, resulting in reduced survival of *L. ornatus* tadpoles. In nature, *B. marinus* eggs and hatchlings may occur at very high densities. A single female *B. marinus* is capable of laying up to 35 000 eggs at a time (Straughan 1966; Zug and Zug 1979; Crump 1989; Hearnden 1991), and at least 20 pairs of *B. marinus* may reproduce in a given water body on the same night (pers. obs.). In such water bodies, the substrate may be covered with a "carpet" of *B. marinus* eggs and hatchlings for several days following *B. marinus* reproductive activity (pers. obs.; M. Hearnden pers. comm.). Under such conditions, one may expect catastrophic mortality of predatory native tadpoles which are able to consume *B. marinus* eggs but which cannot detect *B. marinus* toxins.

As far as I am aware, no published account exists of significant native tadpole mortality in water bodies where *B. marinus* also occur. This may in part be due to the fact that the toxic effects of *Bufo* on native tadpoles are likely to be very difficult to observe in nature. During the artificial pond experiments, the carcasses of *L. ornatus* tadpoles which had died after consuming *B. marinus* eggs or hatchlings only floated on the water surface for one day before sinking (see Section 5.3.3.1), while the carcasses of *L. bicolor* and *L. nigrofrenata* tadpoles which had died after consuming dead *B. marinus* tadpoles were never observed floating on the water surface (see Section 5.3.3.2). In addition, detritivores such as tadpoles, crustaceans, chironomid larvae and aquatic snails will readily consume dead tadpoles (Bragg 1940, 1962; Bragg and King 1960; Alcala 1962; Crump 1986; Table 3.5; pers. obs.), thereby removing evidence of native tadpole mortality. Nonetheless, mass mortality of native tadpoles (species unknown) has been observed on several occasions to coincide with *B. marinus* reproductive activity (D. James pers. comm.; R. Clerke pers. comm.).

Interestingly, the toxic effects of *B. marinus* eggs and hatchlings on *L. ornatus* tadpoles may indirectly facilitate the survival of other native anurans which reproduce at the same time as *Bufo*. Predation is a major factor determining the survival of many anuran eggs and hatchlings (review by Alford in press), and anuran larvae are often significant predators of these early life history stages (Wells 1979; Banks and

Beebee 1987; Tejedo 1991; Petranka *et al.* 1994; Petranka and Thomas 1995). Laboratory experiments have demonstrated that *L. ornatus* tadpoles are voracious predators of the eggs and hatchlings of many native anuran species (see Chapter 6). These results were verified by an artificial pond experiment (see Section 5.3.4). Under naturalistic conditions, survival of *L. rubella* eggs and hatchlings in ponds which contained *L. ornatus* tadpoles was nil (Figure 5.6). However, survival of *L. rubella* in ponds containing *L. ornatus* tadpoles plus *B. marinus* eggs and hatchlings did not differ significantly from survival of *L. rubella* in control ponds (Figure 5.6). These differences in *L. rubella* survival were due to the toxic effects of *Bufo* on *L. ornatus* tadpoles. *Limnodynastes ornatus* tadpoles experienced reduced survival in ponds containing *B. marinus* eggs and hatchlings (Figure 5.6). Consequently, predation pressure on *L. rubella* eggs and hatchlings by *L. ornatus* tadpoles in these ponds was reduced, resulting in increased survival of *L. rubella* (Figure 5.7). These results demonstrate that, when predatory tadpoles such as *L. ornatus* are present in a water body, native anurans may benefit if they breed synchronously with *B. marinus*.

The results of the artificial pond experiments suggest that *B. marinus* may be having a significant impact on adult populations of *L. ornatus*, *L. bicolor* and *L. nigrofrenata* via the toxic effects of *Bufo* on the larvae of these species. Quantitative population data for these native species prior to the introduction of *B. marinus* into Australia do not exist. Thus, there

are no baseline data with which to compare current adult populations. However, even if such data did exist, it would be impossible to separate the effects of *Bufo* from other factors such as habitat alteration and natural variation in anuran populations. Nonetheless, qualitative data indicate that *B. marinus* is not having a catastrophic impact on adult populations of these anurans. Both *L. bicolor* and *L. nigrofrenata* are very common at Heathlands Reserve (pers. obs.), although populations of these species at Heathlands have only been exposed to *Bufo* for a short period of time (since 1989/90, T. McLeod pers. comm.). In the Townsville region, *L. ornatus* has been exposed to *B. marinus* since 1944 (Easteal 1986), yet *L. ornatus* is still one of the most common frogs in this area (pers. obs.). Furthermore, no native anuran species which occur in sympatry with *B. marinus* are known to have gone extinct since the introduction of *Bufo* in Australia.

Several explanations may account for the lack of a catastrophic impact of *B. marinus* on adult *L. ornatus* populations in the Townsville region. Firstly, not all water bodies where *L. ornatus* tadpoles occur are used as breeding sites by *B. marinus* (pers. obs.). Thus, some *L. ornatus* tadpoles are never exposed to *B. marinus* eggs, hatchlings and tadpoles. Secondly, *L. ornatus* tadpoles grow very rapidly and are capable of completing development and metamorphosing 14 days after egg deposition (pers. obs.). Thus, in water bodies where *Bufo* and *L. ornatus* co-occur, there is only a short period of time during which *Bufo* can

affect populations of *L. ornatus* tadpoles. This time period is further reduced when considering the potential impact of *B. marinus* eggs on *L. ornatus* tadpoles as *L. ornatus* tadpoles must attain a minimum size before they can consume *B. marinus* eggs. In addition, *L. ornatus* tadpoles are voracious predators of conspecific eggs and hatchlings (see Chapter 6). Thus, the survival advantages demonstrated for *L. rubella* which breed synchronously with *B. marinus* in water bodies containing predatory *L. ornatus* tadpoles (Figure 5.6) may also apply to late breeding *L. ornatus*. If this is so, *B. marinus* eggs and hatchlings may reduce the survival of tadpoles of early breeding *L. ornatus*, but may enhance the survival of tadpoles of later breeding *L. ornatus*. Further possible explanations will be discussed in Chapter 7.

In summary, these experiments demonstrate that the early life history stages of *B. marinus* may significantly affect the survival of populations of native anuran larvae which have limited ability to detect and avoid *B. marinus* toxins. The impact of *Bufo* on such populations will depend upon (1) the size of the native tadpoles present in the water body at the time of *Bufo* reproductive activity, and (2) the density of *Bufo*. Since these factors are likely to be highly variable among water bodies, so the impact of *Bufo* on populations of these native anuran larvae will also be highly variable.

CHAPTER 6. IMPACT OF *BUFO* ON NATIVE ANURAN LARVAE: *BUFO* AS PREDATORS

6.1 Introduction

The majority of anuran larvae are opportunistic omnivores (Alford in press). Although primarily herbivorous (Kenny 1969; Wassersug 1974, 1975; Wilbur 1980), many tadpoles also prey upon a variety of aquatic organisms including anuran eggs (Heusser 1970; Beebee 1977; Wells 1979; Crump 1983; Banks and Beebee 1987; Magnusson and Hero 1991; Tejedo 1991; Petranka *et al.* 1994; Petranka and Thomas 1995), hatchlings (Crump 1983; Petranka *et al.* 1994; Petranka and Thomas 1995) and tadpoles (Bragg 1940, 1962a, 1964; Bragg and Nelson 1965; Heyer *et al.* 1975; Ruibal and Thomas 1988), as well as mosquito larvae (Bragg 1962b; Heyer *et al.* 1975), microcrustacea (Bragg 1962b; Sokal 1962; Altig *et al.* 1975) and even fish (Ruibal and Thomas 1988). In most instances such predation is opportunistic, although tadpoles of a few species are obligate carnivores (review by Crump 1983).

Previous studies have demonstrated that the feeding activities of anuran larvae may regulate the structure of freshwater algal communities (Dickman 1968; Seale 1980; Osborne and McLachlan 1985; Morin *et al.* 1990). In addition, the ability of many tadpoles to facultatively shift from a herbivorous to a predatory mode of life means that they may also play an important role as predators in structuring aquatic communities. In particular, anuran larvae may be major predators of the eggs and

hatchlings of con- and heterospecifics. Wells (1979) observed that predation by *Leptodactylus pentadactylus* tadpoles resulted in almost 100 percent mortality of *Bufo typhonius* eggs. Woodward (1982) demonstrated that *Scaphiopus multiplicatus* tadpoles preyed upon and eliminated *Rana pipiens* tadpoles in experimental pond communities. Banks and Beebee (1987) observed that survival of *B. calamita* eggs in ponds containing predatory *R. temporaria* tadpoles was often nil. Tejedo (1991) found that tadpoles of *Pelobates cultripes* and *Pelodytes punctatus* were major predators of *B. calamita* eggs. Petranka *et al.* (1994) observed that survival of *B. americanus* eggs in ponds containing predatory *R. sylvatica* tadpoles was usually nil, while Petranka and Thomas (1995) demonstrated that *R. sylvatica* tadpoles are also major predators of conspecific eggs and hatchlings. These results demonstrate that tadpoles may play a significant role as predators in determining the survival of the early life history stages of anurans.

In this chapter I investigate whether *B. marinus* tadpoles are significant predators of the eggs, hatchlings or tadpoles of native anurans. Crump (1989) recorded the diet of *B. marinus* tadpoles as consisting of detritus and suspended organic material. However, few studies have investigated the role of *B. marinus* tadpoles as predators of aquatic organisms. Crossland and Azevedo-Ramos (unpubl. data) found that *B. marinus* tadpoles did not prey upon *B. granulosus* eggs. Hearnden (1991), however, demonstrated that *B. marinus* tadpoles are significant

predators of conspecific eggs, but ineffective predators of conspecific hatchlings. No published data exist regarding predation by *B. marinus* tadpoles on native Australian aquatic fauna. However, the results of Hearnden (1991) indicate that *B. marinus* tadpoles may have a significant impact as predators on the survival of the early life history stages of native anurans.

Bufo marinus tadpoles were offered eggs, hatchlings and tadpoles of five native anuran species (*Litoria alboguttata*, *L. gracilenta*, *L. rubella*, *Limnodynastes ornatus* and *Cyclorana brevipes*) in a series of controlled laboratory experiments. Larvae of two native anuran species (*L. rubella* and *L. ornatus*) were also tested as predators for comparative purposes. In northern Queensland, all of these anuran species reproduce throughout the wet season, and often utilise the same temporary water bodies as breeding sites (pers. obs.). Thus, *B. marinus*, *L. rubella* and *L. ornatus* tadpoles have the opportunity to prey upon the eggs, hatchlings and tadpoles of the aforementioned anuran species in nature. Since tadpole size may influence the ability of tadpoles to prey upon anuran eggs and/or hatchlings (Crump 1983; Tejedo 1991; Petranka and Thomas 1995; Chapter 5), both small and large tadpoles were included in the experiments where possible.

6.2 Methods

Tadpoles of *B. marinus*, *L. ornatus* and *L. rubella* were collected from local temporary water bodies using dipnets. All tadpoles were maintained in 10 l buckets filled with 8 l aged tap water in the laboratory for a maximum of two weeks prior to being included in an experiment. During this time the tadpoles were fed *ad libitum* on frozen lettuce and tadpole chow (3:1 ground lucerne pellets and Tetra-Min™ Tropical Fish Flakes passed through a 250 μm sieve).

Amplectant pairs of adult *L. alboguttata*, *L. gracilenta*, *L. rubella*, *L. ornatus* and *C. brevipes* were collected at night from local temporary water bodies and allowed to spawn in 20 l buckets which contained a small quantity of pond water and vegetation. The resultant eggs were reared through to the free swimming tadpole stage (Gosner 1960 stage 25) in the laboratory using 10 l buckets filled with 8 l aged tap water. Each species was reared in separate buckets. Eggs, hatchlings and tadpoles of each species were haphazardly netted from the rearing buckets and included in the experiments as required.

The experiments were conducted in the laboratory (air temperature 23-26°C; 10HL: 14HD photoperiod) at James Cook University between January and March 1994. Each experiment was a randomised block design using 440 ml plastic containers filled with 350 ml aged tap water. Where possible, both small and large tadpoles of *B. marinus*, *L. rubella*

and *L. ornatus* were tested as predators. However, in some instances only large tadpoles of these species were available to test as predators. Consequently, the number of treatments employed in each experiment varied depending upon the availability of small and large tadpoles.

Both small and large *B. marinus* and *L. ornatus* tadpoles were tested with native anuran eggs and hatchlings. These experiments therefore consisted of three treatments:

(1) 10 eggs or hatchlings per container (control treatment),

(2) 1 small *B. marinus* or *L. ornatus* tadpole plus 10 eggs or hatchlings per container (small tadpole treatment), and

(3) 1 large *B. marinus* or *L. ornatus* tadpole plus 10 eggs or hatchlings per container (large tadpole treatment).

Only large *B. marinus* and *L. ornatus* tadpoles were tested with native tadpoles. These experiments therefore consisted of two treatments:

(1) 10 tadpoles per container (control treatment), and

(2) 1 large *B. marinus* or *L. ornatus* tadpole plus 10 tadpoles per container (tadpole treatment).

Only large *L. rubella* tadpoles were tested with native anuran eggs, hatchlings and tadpoles. Thus, the *L. rubella* experiments consisted of two treatments:

(1) 10 eggs, hatchlings or tadpoles per container (control treatment), and

(2) 1 large *L. rubella* tadpole plus 10 eggs, hatchlings or tadpoles per container (tadpole treatment).

All treatments were replicated 8-10 times.

For each experiment, containers were randomly positioned on a benchtop in an array which was predetermined by the number of treatments and replicates. Treatments were randomly assigned to containers within blocks with the constraint that no adjacent containers had the same treatment. All predators and prey were added randomly to containers within treatments. Random allocations were made using a random number table. All predators and prey were only used once during the experiments.

Prior to each experiment, all "predator" tadpoles were measured (SVL) using vernier calipers and staged (Gosner 1960). The sizes and developmental stages of the tadpoles used in the experiments were: small *B. marinus* 3.8-6.0 mm, stage 25; large *B. marinus* 6.9-12.0 mm, stages 28-40; small *L. ornatus* 4.2-6.7 mm, stage 25; large *L. ornatus* 7.5-14.5 mm, stages 27-40; *L. rubella* 8.9-13.8 mm, stages 30-36. Ten eggs, hatchlings and tadpoles of each prey species were haphazardly chosen and preserved in 10% formalin. The yolk and capsule diameter

of each egg were measured using an ocular micrometer (Table 6.1). The total length of each hatchling was also measured using an ocular micrometer, while tadpoles were measured from snout to vent using vernier calipers (Table 6.1). Eggs, hatchlings and tadpoles were staged according to Gosner (1960).

Survival of eggs, hatchlings and tadpoles in each container during the experiments was monitored at 12 hour intervals. The egg predation experiments ceased when hatchlings began emerging from the egg string (24 hours). The hatchling predation experiments ceased when hatchlings reached Gosner (1960) stage 25 (48 hours). The tadpole predation experiments continued for 48 hours.

6.3 Statistical Analyses

Hypothesis tests were not performed for experiments where there was no mortality in control and predator treatments. When mortality did occur, the number of live eggs, hatchlings or tadpoles in each treatment at the completion of the experiment was analysed in the following manner. Differences between the survival of native anuran eggs, hatchlings and tadpoles in experiments which consisted of two treatments (control vs. large tadpoles) were tested for using Student's *t*-tests. Differences between the survival of native anuran eggs, hatchlings and tadpoles in experiments which consisted of three treatments (control vs. small tadpoles vs. large tadpoles) were tested for

using one-way analysis of variance (ANOVA). No *a posteriori* tests were performed when ANOVAs revealed significant treatment effects due to obvious trends in the data.

Data sets which did not include zero values were square-root transformed prior to analysis. Data sets which did include zero values were square-root transformed after adding 0.5 to all variates (Sokal and Rohlf 1981), and then subjected to analysis. To account for the increased probability of committing a Type I error due to the large number of analyses performed, significant ANOVA and *t*-test *P* values were compared to an overall Bonferroni *P* value which was calculated for the total number of analyses performed (N = 33).

6.4 Results

All statistical analyses were performed using SAS (SAS Institute Inc. 1988). Hypothesis tests were performed at $\alpha = 0.05$

Results from the predation experiments are listed in Tables 6.2-6.4. Block and treatment were tested as main effects in all ANOVAs. Since block *P* values were never significant, only ANOVA treatment *P* values are presented in the tables.

Significant treatment effects were detected in 10 of the 33 analyses performed (Tables 6.2-6.4). Comparison with the Bonferroni *P* value

verified that eight of these results were valid (Bonferroni $P=0.001515$). However, two results (*L. ornatus* tadpoles vs. *L. gracilenta* eggs, *L. ornatus* tadpoles vs. *L. gracilenta* hatchlings; Table 6.4) were marginally non-significant when compared with the Bonferroni *P* value. Although not statistically significant, these results are nonetheless likely to be biologically significant given the large numbers of *L. gracilenta* eggs and hatchlings consumed by *L. ornatus* tadpoles (Table 6.4) and will therefore be discussed.

Eggs, hatchlings and tadpoles in control treatments experienced minimal mortality during most experiments. Egg mortality in such instances was apparently due to fungal infection or developmental failure. The causes of hatchling and tadpole mortality in control treatments are unknown. While consumption of anuran eggs was always the result of active predation by tadpoles, it is possible that some hatchlings and tadpoles exposed to "predator" tadpoles may have died from causes not related to predation, and their carcasses were subsequently consumed as detritus by tadpoles.

Predation on native anuran eggs, hatchlings and tadpoles by *B. marinus* tadpoles was minimal. Consequently, no native species experienced significantly reduced survival when exposed to either small or large *B. marinus* tadpoles (Table 6.2). Predation by *L. rubella* tadpoles on the eggs, hatchlings and tadpoles of most species was also minimal. *Litoria*

rubella tadpoles were, however, significant predators of conspecific eggs (Table 6.3).

In contrast to *B. marinus* and *L. rubella*, *L. ornatus* tadpoles were voracious predators of native anuran eggs and hatchlings. Survival of the eggs and/or hatchlings of *L. alboguttata*, *L. rubella*, *L. ornatus* and *C. brevipes* was significantly reduced in the presence of *L. ornatus* tadpoles. Although not statistically significant, *L. ornatus* tadpoles also readily preyed upon *L. gracilenta* eggs and hatchlings. In all instances, large *L. ornatus* tadpoles were more effective predators than small *L. ornatus* tadpoles (Table 6.4). However, neither small or large *L. ornatus* tadpoles were significant predators of free swimming tadpoles of any of the native anuran species tested (Table 6.4).

Table 6.1 Size and developmental stage (Gosner 1960) data for native anuran eggs, hatchlings and tadpoles offered to *B. marinus*, *L. ornatus* and *L. rubella* tadpoles. Measurements are explained in the text.

Species		N	Size(mm)	Stage
Litoria alboguttata	Egg Capsule	10	2.0-2.6	8-10
	Egg Yolk	10	1.2-1.3	8-10
	Hatchling	10	2.7-3.0	17-18
	Tadpole	10	3.2-4.5	25
Litoria gracilenta	Egg Capsule	10	1.9-2.1	12-15
	Egg Yolk	10	1.4-1.6	12-15
	Hatchling	10	3.6-4.2	19-20
	Tadpole	10	3.0-4.2	25
Litoria rubella	Egg Capsule	10	1.6-2.1	8-10
	Egg Yolk	10	0.9-1.0	8-10
	Hatchling	10	3.2-3.7	19-20
	Tadpole	10	2.4-4.5	25
Limnodynastes ornatus	Egg Capsule	10	1.5-1.9	12-15
	Egg Yolk	10	1.2-1.5	12-15
	Hatchling	10	3.2-4.3	18-20
	Tadpole	10	4.0-6.4	25
Cyclorana brevipes	Egg Capsule	10	4.1-6.5	9-14
	Egg Yolk	10	1.3-1.5	9-14
	Hatchling	10	3.0-3.7	18-19
	Tadpole	10	3.5-4.8	25

Table 6.2 ANOVA and Student's *t*-test results for the effect of *B. marinus* tadpoles on the survival of native anuran eggs (E), hatchlings (H) and tadpoles (T). Numbers in parentheses indicate the maximum number of eggs, hatchlings or tadpoles eaten. All treatments were replicated 10 times.

		Mean No. Alive ± SD					
Species	Stage	Control	+ Small Tadpole	+ Large Tadpole	*F*	*t*	*P*
Litoria alboguttata	E	10 ± 0	10 ± 0(0)	9.8 ± 0.4(0)	2.25	-	0.1248
	H	10 ± 0	10 ± 0(0)	8.8 ± 0.2(7)	2.51	-	0.1000
	T	9.7 ± 0.4	-	9.5 ± 0.7(2)	-	0.99	0.3367
Litoria gracilenta	E	9.8 ± 0.4	10 ± 0(0)	9.7 ± 0.5(0)	1.70	-	0.2012
	H	10 ± 0	10 ± 0(0)	10 ± 0(0)	-	-	-
	T	10 ± 0	-	10 ± 0	-	-	-
Litoria rubella	E	9.6 ± 0.5	9.3 ± 0.9(1)	9.8 ± 0.4(1)	2.16	-	0.1347
	H	10 ± 0	10 ± 0(0)	9.9 ± 0.3(1)	1.00	-	0.3811
	T	10 ± 0	-	10 ± 0(0)	-	-	-
Limnodynastes ornatus	E	10 ± 0	9.7 ± 0.6(0)	9.8 ± 0.4(1)	1.33	-	0.2803
	H	10 ± 0	9.8 ± 0.4(1)	9.4 ± 1.0(2)	2.55	-	0.0965
	T	10 ± 0	-	10 ± 0(0)	-	-	-
Cyclorana brevipes	E	10 ± 0	10 ± 0(0)	10 ± 0(0)	-	-	-
	H	9.9 ± 0.3	9.7 ± 0.7(2)	9.3 ± 0.9(2)	1.94	-	0.1634
	T	10 ± 0	-	10 ± 0	-	-	-

Table 6.3 Student's *t*-test results for the effect of *L. rubella* tadpoles on the survival of native anuran eggs (E), hatchlings (H) and tadpoles (T). Numbers in parentheses indicate the maximum number of eggs, hatchlings or tadpoles eaten. All treatments were replicated 10 times.

		Mean No. Alive ± SD			
Species	Stage	Control	+ Large Tadpole	*t*	*P*
Litoria alboguttata	E	10 ± 0	10 ± 0(0)	-	-
	H	10 ± 0	9.8 ± 0.4(1)	1.5000	0.1510
	T	9.8 ± 0.4	9.7 ± 0.5(1)	0.4932	0.6278
Litoria gracilenta	E	9.9 ± 0.3	9.9 ± 0.3(0)	0.0000	1.0000
	H	10 ± 0	9.9 ± 0.3(1)	1.0000	0.3306
	T	10 ± 0	10 ± 0(0)	-	-
Litoria rubella	E	9.0 ± 0.7	4.0 ± 2.8(10)	4.6087	0.0012
	H	9.9 ± 0.3	9.8 ± 0.4(1)	0.6000	0.5560
	T	7.7 ± 3.4	6.5 ± 3.4(2)	0.6111	0.5488
Limnodynastes ornatus	E	10 ± 0	10 ± 0(0)	-	-
	H	10 ± 0	9.9 ± 0.3(1)	1.0000	0.3306
	T	10 ± 0	10 ± 0(0)	-	-
Cyclorana brevipes	E	10 ± 0	9.9 ± 0.3(0)	1.0000	0.3306
	H	9.8 ± 0.4	9.8 ± 0.4(1)	0.0000	1.0000
	T	10 ± 0	10 ± 0(0)	-	-

Table 6.4 ANOVA and Student's *t*-test results for the effect of *L. ornatus* tadpoles on the survival of native anuran eggs (E), hatchlings (H) and tadpoles (T). Numbers in parentheses indicate the maximum number of eggs, hatchlings or tadpoles eaten. All treatments were replicated 10 times except * N = 8 replicates.

		Mean No. Alive ± SD					
Species	Stage	Control	+ Small Tadpole	+ Large Tadpole	*F*	*t*	*P*
Litoria alboguttata	E	10 ± 0	10 ± 0(0)	1.7 ± 3.5(10)	48.92	-	0.0001
	H	9.9 ± 0.3	10 ± 0(0)	0.1 ± 0.3(10)	1630.73	-	0.0001
	T	9.8 ± 0.4	-	8.4 ± 2.2(6)	-	1.9172	0.0859
Litoria gracilenta	E*	9.9 ± 0.4	10 ± 0(0)	7.0 ± 3.3(8)	5.12	-	0.0215
	H*	10 ± 0	10 ± 0(0)	6.1 ± 4.6(10)	5.11	-	0.0216
	T	10 ± 0	-	9.8 ± 0.6(2)	-	1.0000	0.3306
Litoria rubella	E	9.3 ± 1.0	8.4 ± 3.3(8)	1.2 ± 3.2(10)	65.12	-	0.0001
	H	9.9 ± 0.3	9.9 ± 0.3(0)	5.4 ± 2.8(10)	71.14	-	0.0001
	T	7.9 ± 2.2	-	8.4 ± 2.4(6)	-	0.4324	0.6706
Limnodynastes ornatus	E	10 ± 0	10 ± 0(0)	4.0 ± 3.9(10)	14.05	-	0.0002
	H	10 ± 0	10 ± 0(0)	9.9 ± 0.3(1)	1.00	-	0.3874
	T	10 ± 0	-	10 ± 0(0)	-	-	-
Cyclorana brevipes	E	9.9 ± 0.3	10 ± 0(0)	2.4 ± 3.7(10)	33.10	-	0.0001
	H	9.6 ± 0.7	9.1 ± 0.9(3)	1.1 ± 3.1(10)	71.74	-	0.0001
	T	10 ± 0	-	9.9 ± 0.3(1)	-	1.0000	0.3306

6.5 Discussion

Many tadpoles prey upon anuran eggs, hatchlings and/or tadpoles (Bragg 1940, 1964; Bragg and Nelson 1965; Heusser 1970; Heyer *et al.* 1975; Beebee 1977; Crump 1983; Banks and Beebee 1987; Ruibal and Thomas 1988; Magnusson and Hero 1991; Tejedo 1991; Petranka 1994; Petranka and Thomas 1995). Consequently, tadpoles may play a significant role as predators in determining the survival of the early life history stages of anurans (Wells 1979; Tejedo 1991; Banks and Beebee 1987; Petranka *et al.* 1994; Petranka and Thomas 1995). The present experiments demonstrated that, under laboratory conditions, neither small or large *B. marinus* tadpoles are significant predators of the eggs, hatchlings or tadpoles of *L. alboguttata*, *L. gracilenta*, *L. rubella*, *L. ornatus* or *C. brevipes*. These results suggest that *B. marinus* tadpoles are unlikely to reduce the survival of the early life history stages of these native anurans via predation in nature.

As far as I am aware, only two previous studies have investigated predation on anuran eggs, hatchlings or tadpoles by *B. marinus* tadpoles. Crossland and Azevedo-Ramos (unpubl. data) found that *B. marinus* tadpoles did not consume *B. granulosus* eggs. However, Hearnden (1991) found that *B. marinus* tadpoles preyed upon conspecific eggs and, to a lesser extent, conspecific hatchlings. Thus, *B. marinus* tadpoles are behaviourally capable of consuming anuran eggs and hatchlings.

Bufo marinus tadpoles are ineffective predators of mobile prey (Hearnden 1991). Consequently, the low predation rates experienced by native anuran hatchlings and tadpoles exposed to *B. marinus* tadpoles may be attributed to the mobility of these early life history stages. However, the reasons why *B. marinus* tadpoles were not significant predators of native anuran eggs are not apparent. *Bufo marinus* tadpoles that were not included in the experiments, but which were maintained under experimental conditions (i.e. one tadpole per 440 ml container filled with 350 ml aged tap water) in the same room and at the same time as the predation experiments readily fed on frozen lettuce (pers. obs.). Thus, it is unlikely that the experimental conditions imposed can account for the minimal predation by *B. marinus* tadpoles on native anuran eggs. Similarly, the inclusion of both small and large *B. marinus* tadpoles in the experiments should have accounted for any possible ontogenetic variation in the ability of *B. marinus* tadpoles to consume native anuran eggs (e.g. Crump 1983; Tejedo 1991; Petranka and Thomas 1995; Chapter 5). In addition, egg size should not have prevented *B. marinus* tadpoles from preying upon native anuran eggs. The native anuran eggs included in the experiments were of a similar size or smaller than *B. marinus* eggs (*B. marinus* egg string diameter 4.0-6.9 mm, N = 5, unpubl. data; c.f. Table 6.1) which are known to be consumed by *B. marinus* tadpoles (Hearnden 1991).

One possible explanation for the low predation rates on native anuran eggs by *B. marinus* tadpoles is that native anuran eggs may contain chemicals which are noxious to *B. marinus* tadpoles. Experiments conducted in Chapter 5 demonstrated that some anuran larvae can select food items on the basis of palatability and/or toxicity (see Section 5.3.1.2). Although the tadpoles (Bradfield 1995) and adults (Madsen and Shine 1994) of some native anurans are known to possess noxious or toxic chemicals, no published data exist regarding the palatability or toxicity of the eggs of any native anuran species. However, this explanation seems unlikely as eggs of all of the native anuran species tested are readily consumed by fish (*Ambassis agrammus*, pers. obs.) which usually reject unpalatable food items (e.g. *B. marinus* tadpoles, Chapter 3). Alternatively, some physical attributes of native anuran eggs may minimise predation by *B. marinus* tadpoles. Whatever the mechanism, the results indicate that *B. marinus* tadpoles are unlikely to be significant predators of the eggs, hatchlings or tadpoles of native anurans in nature.

There was considerable variation in predation rates on native anuran eggs, hatchlings and tadpoles by native tadpoles. *Litoria rubella* tadpoles were not significant predators of the eggs, hatchlings or tadpoles of *L. alboguttata*, *L. gracilenta*, *L. ornatus* or *C. brevipes*. In addition, *L. rubella* tadpoles were not significant predators of conspecific hatchlings or tadpoles. As with *B. marinus* tadpoles, *L. rubella* tadpoles may be

ineffective predators of mobile anuran hatchlings and tadpoles. However, *L. rubella* tadpoles were significant predators of conspecific eggs, demonstrating that they are behaviourally capable of consuming anuran eggs. The reasons why *L. rubella* tadpoles did not prey upon the eggs of *L. alboguttata*, *L. gracilenta*, *L. ornatus* or *C. brevipes* are unclear. It is possible that the large size of *C. brevipes* eggs may have precluded predation by *L. rubella* tadpoles (Table 6.1). However, the eggs of *L. alboguttata*, *L. gracilenta* and *L. ornatus* were of comparable size to *L. rubella* eggs (Table 6.1) which were readily consumed by *L. rubella* tadpoles. Consequently, egg size does not account for the lack of predation by *L. rubella* tadpoles on these eggs. Possibly other physical or chemical attributes of the eggs of these species may minimise predation by *L. rubella* tadpoles.

Limnodynastes ornatus tadpoles were also ineffective predators of mobile native anuran tadpoles. However, in contrast to *L. rubella*, *L. ornatus* tadpoles were voracious predators of the eggs and/or hatchlings of all of the native anuran species tested. In accordance with previous studies (Crump 1983; Tejedo 1991; Petranka and Thomas 1995; Chapter 5), large *L. ornatus* tadpoles were more effective predators than small *L. ornatus* tadpoles. This was probably due to differences in the oral disc and beak sizes of small and large *L. ornatus* tadpoles (Crump 1983; Tejedo 1991; Chapter 5). These results suggest that *L. ornatus* tadpoles may play a significant role as predators in structuring

populations of native anuran larvae. The results of an artificial pond experiment confirm this notion: under naturalistic conditions, survival of *L. rubella* eggs and hatchlings in the presence of *L. ornatus* tadpoles was nil (see Section 5.3.4).

In conclusion, the experiments indicate that the tadpoles of native anuran species such as *L. ornatus*, and in some instances *L. rubella*, are likely to have a greater impact as predators on the survival of the early life history stages of native anurans than *B. marinus* tadpoles.

CHAPTER 7. IMPACT OF *BUFO* ON NATIVE ANURAN LARVAE: INTERACTIONS OF COMPETITION AND TOXICITY

7.1 Introduction

The distributions and abundances of many species are determined by the outcome of interspecific competition (see reviews by Connell 1983; Schoener 1983; Gurevitch *et al.* 1992). Numerous studies have demonstrated that competition is a major factor affecting larval amphibian populations (Wilbur 1972; Morin 1983a, 1987; Alford and Wilbur 1985; Wilbur and Alford 1985; Wilbur 1987; Alford 1989a, b; Scott 1990; Griffiths 1991; Van Buskirk and Smith 1991; Lawler and Morin 1993; Warner *et al.* 1993). Anuran larvae may compete with a variety of aquatic taxa including other anuran larvae (Wiltshire and Bull 1977; Morin 1987; Wilbur 1987; Griffiths 1991; Lawler and Morin 1993; Alford 1989a, b, in press; Werner 1994; Pehek 1995), aquatic insects (Morin *et al.* 1988), zooplankton (Alford 1989a) and snails (Brönmark *et al.* 1991). Such competitive interactions may arise from exploitation and/or interference mechanisms (Savage 1952; Rose 1960; Licht 1967b; Wilbur 1977; Steinwascher 1978, 1979; Semlitsch and Caldwell 1982; Berven and Chadra 1988; Griffiths *et al.* 1993; Alford in press).

The intensity and outcome of competition among anuran larvae may be mediated by a variety of factors. Competition among anuran larvae becomes more intense as competitor density increases (Brockelman

1969; Wilbur 1972, 1976, 1977; Collins 1979; Morin 1986, 1987; John-Alder and Morin 1990; Alford 1994; Schmuck *et al.* 1994; Werner 1994). However, predators may preclude or reverse the outcome of competition among anuran larvae by preying differentially upon competitively dominant species (Morin 1981, 1983a, 1986, 1987; Morin *et al.* 1983; Wilbur *et al.* 1983; Wilbur 1987). Differences in activity levels and body size may also influence the outcome of competition among anuran larvae. Tadpoles with higher activity levels often outcompete tadpoles with lower activity levels (Woodward 1982, 1983; Werner 1992, 1994; Lawler and Morin 1993; Werner and Anholt 1996), while large tadpoles often outcompete smaller tadpoles (Steinwascher 1978, 1979; Breden and Kelly 1982; Morin and Johnson 1988; Werner and Anholt 1996). In addition, abiotic factors such as pH may interact with biotic factors to alter the intensity of competitive interactions among anuran larvae (Warner *et al.* 1993; but see Pehek 1995).

Recent studies have demonstrated that differences in reproductive phenology may also play a crucial role in determining the outcome of interspecific competition among anuran larvae. Species which appear early in ponds often gain a competitive advantage over species which appear later (Alford and Wilbur 1985; Wilbur and Alford 1985; Alford 1989c; Morin 1987; Lawler and Morin 1993). Such priority effects may be due to size-specific competition, nutrient loss through transforming metamorphs of early arriving species, or alteration of pond trophic

structure by early arriving species (Alford and Wilbur 1985; Wilbur and Alford 1985; Alford 1989c). In contrast, some anuran larvae perform better when appearing later in ponds rather than earlier (Alford and Wilbur 1985), while others do not respond to differences in reproductive phenology at all (Alford 1989b; Lawler and Morin 1993). The intensity of these priority effects may in turn be mediated by predators (Morin 1981, 1983a, 1987; Alford 1989a), differences in tadpole activity levels (Werner 1994) and adult parentage (Alford 1989c).

In this chapter I investigate whether the outcome of interspecific competition between native anuran tadpoles (*Limnodynastes ornatus*) and *B. marinus* tadpoles is altered by differences in reproductive phenology. Previous studies have demonstrated that *B. marinus* tadpoles compete intensely with conspecifics (Hearnden 1991; Alford 1994). However, few data exist regarding interspecific competition between *B. marinus* tadpoles and native Australian aquatic taxa. In a laboratory experiment, Alford (pers. comm.) found that *L. ornatus* tadpoles were competitively superior to *B. marinus* tadpoles; *B. marinus* tadpoles raised with *L. ornatus* tadpoles of similar initial size grew slowly and always died before metamorphosis. However, it is not known whether these effects are sensitive to the relative body sizes or timing of reproduction of these species. If such competitive priority effects do exist, they may in turn be mediated by the toxic effects of late arriving *B. marinus* eggs and hatchlings on tadpoles of early arriving *L. ornatus* (see Chapter 5).

7.2 Methods

The experiment was a randomised block design and was conducted using 1000 l plastic ponds. The ponds were arranged in a 5 X 4 array in an outdoor compound with no obvious environmental gradient at the James Cook University campus. Ponds were thus exposed to natural photoperiod and temperatures. Two thermometers were placed in a centrally located pond to record daily bottom and surface water temperatures.

Each pond was stocked with 100 *B. marinus* and 100 *L. ornatus* as described below. The letters in parentheses following each treatment (see below) describe the addition of each species to ponds. The first and fourth letters identify the species (B=*B. marinus*, L=*L. ornatus*). The second and fifth letters refer to whether that species was added early (E) or late (L) to ponds, while the third and sixth letters refer to whether that species was added to ponds as eggs (E) or tadpoles (T). The orders of introduction were as follows:

(1) both species added simultaneously as tadpoles (BETLET),

(2) *B. marinus* added early as tadpoles, *L. ornatus* added late as eggs (BETLLE),

(3) *L. ornatus* added early as tadpoles, *B. marinus* added late as eggs (BLELET),

(4) *B. marinus* added early as tadpoles, *L. ornatus* added late as tadpoles (BETLLT), and

(5) *L. ornatus* added early as tadpoles, *B. marinus* added late as tadpoles (BLTLET).

Treatments were assigned randomly to ponds within blocks with the constraint that no adjacent ponds had the same treatment. Each treatment was replicated four times. The densities and orders of introduction of *B. marinus* and *L. ornatus* used in the experiment mimic the densities and reproductive phenologies of these species in natural water bodies (Hearnden 1991; R. Alford pers. comm.; pers obs.).

On 20 January 1995, the ponds were filled with 900 l tap water and covered with lids comprised of birdwire and shadecloth as per Section 5.2.3.1. The water was then allowed to stand for 15 days. On 4 February, 500 g dry leaf litter (previously collected from the overflow area of a local dam: Mt Margaret dam) and 20 g lucerne pellets were randomly added to each pond to provide spatial complexity and a source of nutrients for the aquatic community. A 300 ml aliquot of well-mixed freshwater plankton, collected from three local temporary ponds, was randomly added to each pond on 6 February.

A single clutch each of *B. marinus* eggs and *L. ornatus* eggs were collected from a local temporary water body on 5 February. Eggs of each species were raised to early tadpole stage (Gosner 1960 stage 25) in the laboratory using 10 l buckets filled with 8 l aged tap water. Each

species was raised in separate buckets. Tadpoles of each species included at the start of the experiment were haphazardly netted from the rearing buckets and allocated to 5 ml plastic containers (5 tadpoles per container). Groups of twenty containers were then randomly chosen and combined into 10 l buckets to yield 100 tadpoles per bucket. Buckets of tadpoles were then randomly allocated to treatments, and then to ponds within treatments, on 8 February (i.e. treatment 1 ponds (BETLET): tadpoles of both species added; treatments 2 (BETLLE) and 4 (BETLLT) ponds: *B. marinus* tadpoles added; treatments 3 (BLELET) and 5 (BLTLET) ponds: *L. ornatus* tadpoles added).

A second group of *B. marinus* eggs (1 clutch) and *L. ornatus* eggs (1 clutch) were collected from a local temporary water body on 12 February. Eggs were haphazardly allocated to 5 ml plastic containers in the laboratory (5 eggs per container). Groups of twenty containers were then randomly chosen and combined into 440 ml containers to yield 100 eggs per container. Eggs of each species were then randomly added to treatment 2 (BETLLE: *L. ornatus* eggs) and treatment 3 (BLELET: *B. marinus* eggs) ponds on 12 February.

Eggs collected on 12 February that were not included in the experiment on that day were raised to Gosner (1960) stage 25 tadpoles in the laboratory using 10 l buckets filled with 8 l aged tap water. Each species was reared in separate buckets. As soon as hatchlings reached

Gosner (1960) stage 25 (16 February), groups of 100 tadpoles of each species were allocated to 10 l buckets as described above, and then randomly added to ponds within treatments (i.e. treatment 4 (BETLLT) ponds: *L. ornatus* tadpoles added; treatment 5 (BLTLET) ponds: *B. marinus* tadpoles added).

Prior to the addition of eggs and tadpoles to ponds, one pond from each treatment was randomly chosen and twenty haphazardly netted tadpoles were measured (SVL) and staged (Gosner 1960). Ponds where *B. marinus* and *L. ornatus* were added late as eggs were monitored daily. As soon as Gosner (1960) stage 25 tadpoles were observed, one pond from each treatment was randomly chosen and twenty individuals were haphazardly netted, measured (SVL) and staged (Gosner 1960). All random allocations and selections were made using a random number table.

The ponds were checked daily for emerging metamorphs. Metamorphosis was defined as the emergence of at least one forelimb. Metamorphs were easily observed as they perched in conspicuous positions on pond walls or on pieces of floating styrofoam which I had provided as additional emergence sites. After capture, metamorphs were brought to the laboratory and held for several days without food in 440 ml plastic containers with a small quantity of water until tail resorption

was complete. Metamorphs were then blotted dry and weighed to the nearest milligram.

The last metamorph was captured on 24 March. Searches of ponds for the next 10 days failed to find any more metamorphs. I visually searched each pond for tadpoles on 3 April. Most ponds were devoid of tadpoles; the few remaining tadpoles looked starved and were still in early developmental stages. The experiment was stopped at this point as these tadpoles were not close to metamorphosis and, in nature, would probably die from habitat desiccation before completing development.

7.3 Statistical Analyses

To determine whether there was any intraspecific variation in the sizes of *B. marinus* and *L. ornatus* tadpoles between clutches, the sizes (SVL) of twenty immediate post-hatchling (Gosner 1960 stage 25) tadpoles from each treatment were compared using Kruskal-Wallis analysis of variance (ANOVA). Significant size differences among treatments might reflect genetic variation between clutches which would be confounded with any priority effects observed in the experiment.

The response variables for *B. marinus* and *L. ornatus* tadpoles in the experiment were survival to metamorphosis, length of larval period and mass at tail resorption. Length of larval period for an individual was counted as the number of days from hatching to metamorphosis (as

defined above). Since individuals within a tank cannot be treated as statistically independent observations (Wilbur 1987) and distributions of metamorphic parameters are often skewed (Wilbur and Collins 1973), biasing mean values, statistical analyses for larval period and mass at tail resorption are based on population median values rather than means or individual values.

Survival, length of larval period and body mass at metamorphosis in amphibians are often correlated within populations (Wilbur 1971; Shoop 1974; Collins 1979; Travis 1980; Morin 1986). Therefore, multivariate analysis of variance (MANOVA) was used to test for significant treatment effects. Hypothesis tests used randomisation as this procedure is robust to violations of the assumptions of parametric MANOVA (Manly 1991). When MANOVAs revealed significant treatment effects, pairwise comparisons were used to test for significant differences between all possible treatment combinations.

7.4 Results

Statistical analyses were performed using SAS (SAS Institute Inc. 1988), Statistix (Version 3.0, Analytical Software) and RAMAN (randomization MANOVA program by R.A. Alford). All hypothesis tests were conducted at $\alpha = 0.05$.

The sizes of immediate post-hatchling (Gosner 1960 stage 25) *B. marinus* ($H=3.3296$, $P=0.5043$) and *L. ornatus* ($H=5.4436$, $P=0.2447$) tadpoles did not differ significantly among treatments, indicting that any priority effects observed are unlikely to be confounded with variation in tadpole size between clutches. Size and developmental stage (Gosner 1960) data for *B. marinus* and *L. ornatus* tadpoles present in ponds when the other species was introduced are listed in Table 7.1. *Limnodynastes ornatus* tadpoles exposed to *B. marinus* eggs (BLELET ponds) were large enough to prey upon *B. marinus* eggs (see Table 7.1, Section 5.3.2). Water temperature minima and maxima were relatively constant throughout the experiment: minimum water temperatures ranged from 26-29°C while maximum water temperatures ranged from 32-37°C.

The addition of *B. marinus* eggs to treatment 3 (BLELET) ponds on 12 February had an immediate effect on the survival of *L. ornatus* tadpoles: numerous dead *L. ornatus* tadpoles were observed floating on the water surface of these ponds on the afternoon of 12 February. No dead *L. ornatus* or *B. marinus* tadpoles were observed in any other treatment ponds at any stage during the experiment.

There was a significant difference in the overall responses of *B. marinus* among treatments (Table 7.2, Figure 7.1). *Bufo marinus* experienced increased survival, decreased larval period and increased mass at tail

resorption when *L. ornatus* were added late to ponds as eggs (BETLLE) or tadpoles (BETLLT) as compared to ponds where both species were added simultaneously as tadpoles (BETLET; Figure 7.1). However, the difference was only statistically significant when *L. ornatus* were added late as tadpoles (BETLLT, Table 7.2). In contrast, *B. marinus* added late to ponds as tadpoles (BLTLET) experienced decreased survival, increased larval period and increased mass at tail resorption as compared to when added to ponds simultaneously with *L. ornatus* (BETLET), although the difference was not statistically significant (Figure 7.1, Table 7.2). However, when added late to ponds as eggs (BLELET), *B. marinus* experienced significantly increased survival, reduced larval period and increased mass at tail resorption as compared to ponds where they were added at the same time as *L. ornatus* (BETLET, Figure 7.1, Table 7.2). In fact, there was no significant difference between the responses of *B. marinus* in ponds where *L. ornatus* were added late as eggs (BETLLE) and in ponds where *B. marinus* were added late as eggs (BLELET, Table 7.2). *Bufo marinus* experienced similar survival and larval periods in both of these treatments, however, *B. marinus* added to ponds late as eggs (BLELET) metamorphosed at almost twice the size of conspecifics which preceded *L. ornatus* eggs into ponds (BETLLE, Figure 7.1).

There was also a significant difference in the overall responses of *L. ornatus* among treatments (Table 7.3, Figure 7.2). *Limnodynastes ornatus* which preceded *B. marinus* into ponds (BLELET, BLTLET)

exhibited significantly different responses than conspecifics which were added to ponds at the same time as *B. marinus* (BETLET, Table 7.3). When *B. marinus* were added late to ponds as tadpoles (BLTLET), *L. ornatus* experienced increased survival, increased larval period and increased mass at tail resorption as compared to ponds where both species were added simultaneously to ponds as tadpoles (BETLET, Figure 7.3). When *B. marinus* were added late to ponds as eggs (BLELET), *L. ornatus* experienced similar survival and larval periods to ponds where both species were added simultaneously as tadpoles (BETLET), but metamorphosed at much larger sizes (Figure 7.2). The difference between responses of *L. ornatus* when *B. marinus* were added late as eggs (BLELET) and tadpoles (BLTLET) is marginally non-significant (Table 7.3). When *B. marinus* were added late to ponds as eggs (BLELET), *L. ornatus* experienced reduced survival, reduced larval period and increased mass at tail resorption as compared to ponds where *B. marinus* were added late as tadpoles (BLTLET). None of the *L. ornatus* which were added late to ponds as either eggs (BETLLE) or tadpoles (BETLLT) metamorphosed during the experiment.

Table 7.1 Size and developmental stage (Gosner 1960) data for *B. marinus* and *L. ornatus* tadpoles when exposed to eggs or tadpoles of the other species during the competition experiment. Treatment codes are explained in the text.

	B. marinus		*L. ornatus*	
Treatment	Mean SVL (mm) ± SD	Gosner Stage	Mean SVL (mm) ± SD	Gosner Stage
BETLET	4.7 ± 0.2	25	4.6 ± 0.3	25
BETLLE	9.5 ± 0.6	28-32	EGGS	1-10
BLELET	EGGS	1-10	10.2 ± 1.4	27-32
BETLLT	11.1 ± 0.7	32-37	4.6 ± 0.3	25
BLTLET	4.7 ± 0.3	25	12.4 ± 1.4	28-37

Table 7.2 MANOVA and pairwise comparison results for responses of *B. marinus* tadpoles in the competition experiment. Treatment codes are explained in the text.

	Wilk's Lambda	*P*
Overall MANOVA	0.179984	0.039000
Pairwise Comparisons		
BETLET vs. BETLLE	0.437041	0.281800
BETLET vs. BLELET	0.071591	0.028100
BETLET vs. BETLLT	0.049004	0.029600
BETLET vs. BLTLET	0.506812	0.733333
BETLLE vs. BLELET	0.434905	0.255300
BETLLE vs. BETLLT	0.791486	0.874700
BETLLE vs. BLTLET	0.375699	0.400000
BLELET vs. BETLLT	0.170330	0.028700
BLELET vs. BLTLET	0.028489	0.133333
BETLLT vs. BLTLET	0.008843	0.066667

Table 7.3 MANOVA and pairwise comparison results for responses of *L. ornatus* tadpoles in the competition experiment. Treatment codes are explained in the text.

	Wilk's Lambda	*P*
Overall MANOVA	0.025019	0.000200
Pairwise Comparisons		
BETLET vs. BLELET	0.041511	0.028571
BETLET vs. BLTLET	0.089967	0.013600
BLELET vs. BLTLET	0.083443	0.057143

Figure 7.1 Median percent survival, median mass at tail resorption, and median larval period for *B. marinus* tadpoles in the competition experiment. Treatment codes are explained in the text.

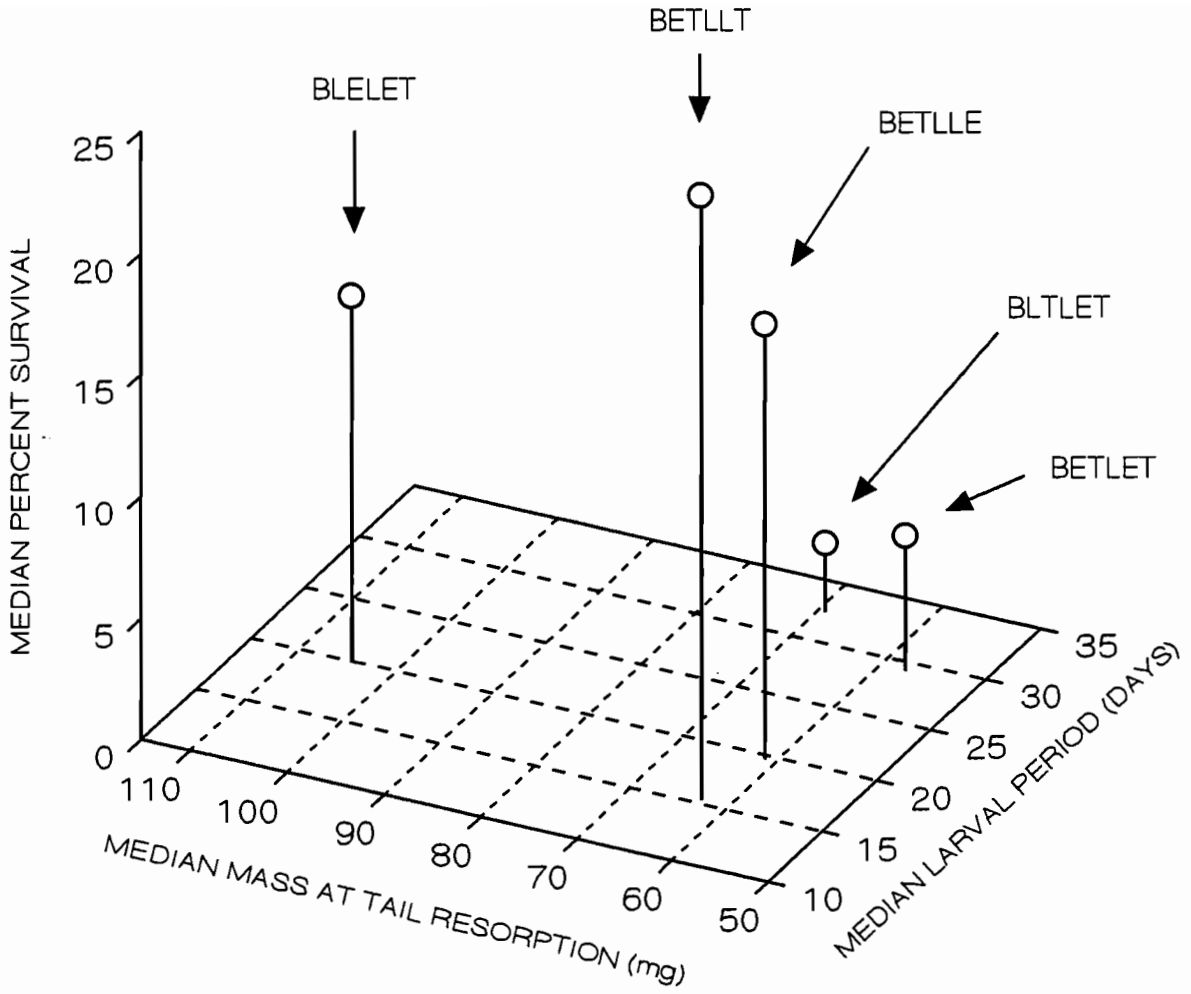
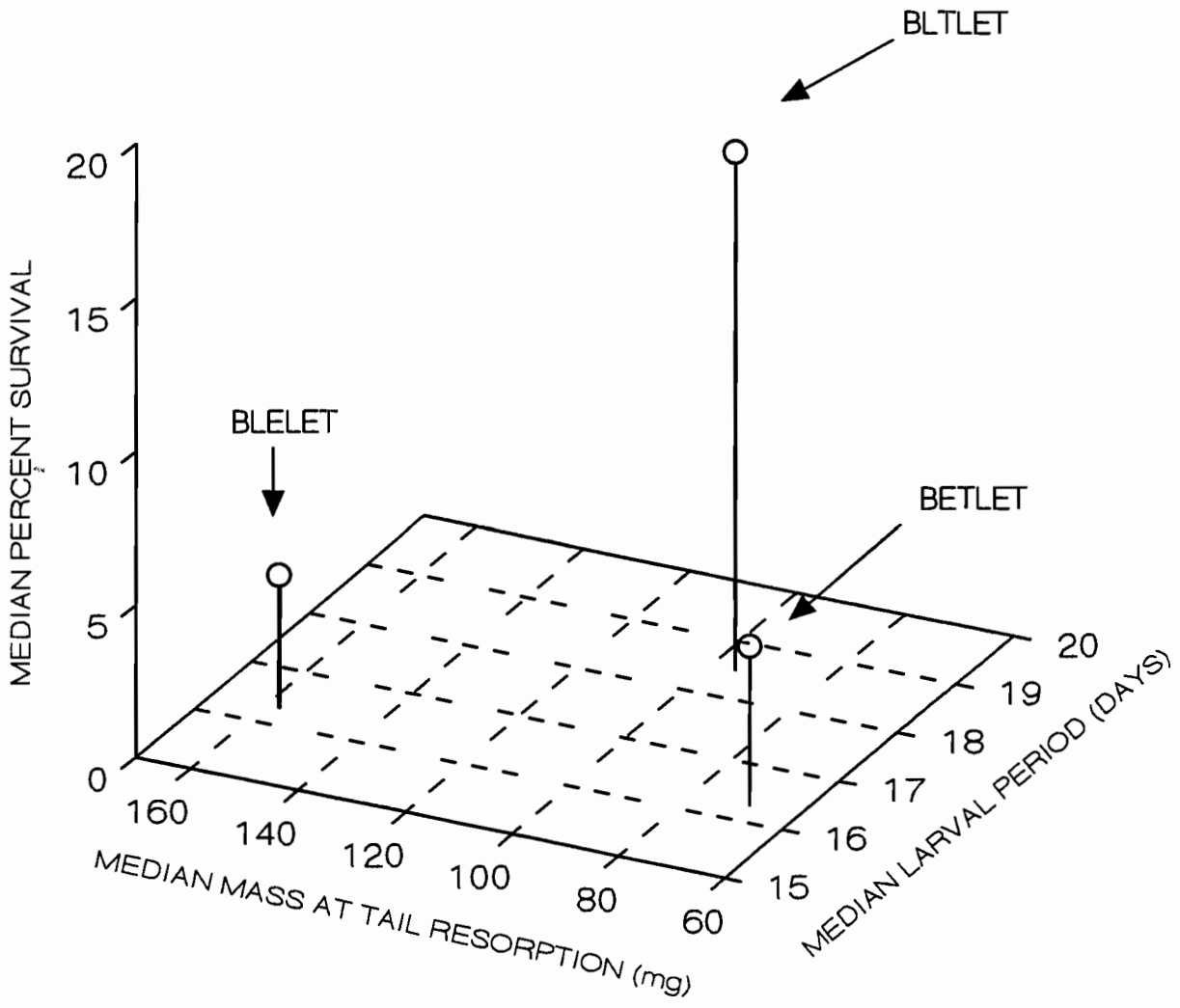

BETLLT
BLELET
BETLLE
BLTLET
BETLET
MEDIAN PERCENT SURVIVAL
25
20
15
10
5
0
110
100
90
80
70
60
50
MEDIAN MASS AT TAIL RESORPTION (mg)
10
15
20
25
30
35
MEDIAN LARVAL PERIOD (DAYS)

Figure 7.2 Median percent survival, median mass at tail resorption, and median larval period for *L. ornatus* tadpoles in the competition experiment. Treatment codes are explained in the text.


BLTLET
BLELET
BETLET
MEDIAN PERCENT SURVIVAL
20
15
10
5
0
160
140
120
100
80
60
MEDIAN MASS AT TAIL RESORPTION (mg)
15
16
17
18
19
20
MEDIAN LARVAL PERIOD (DAYS)

7.5 Discussion

Intensities of competition experienced by anuran larvae are negatively correlated with survival and/or mass at metamorphosis, and positively correlated with the duration of the larval period (Wilbur 1976; Morin 1983a). Thus, in the present experiment it may be inferred that competition between *B. marinus* and *L. ornatus* was more intense in populations exhibiting reduced survival, prolonged larval period and/or reduced mass at tail resorption relative to populations with increased survival, reduced larval period and/or larger metamorphs (e.g. Morin 1987).

The outcome of interspecific competition between *B. marinus* and *L. ornatus* varied depending on the timing of arrival of each species to ponds relative to the other. *Bufo marinus* which preceded *L. ornatus* into ponds performed better (increased survival, reduced larval period and increased mass at tail resorption) than when added to ponds simultaneously with *L. ornatus*. This increased performance of early arriving *Bufo* was further enhanced as *L. ornatus* were added progressively later to ponds: *B. marinus* in ponds where *L. ornatus* were added late as tadpoles (*L. ornatus* added 16 February) experienced increased survival and reduced larval period as compared to ponds where *L. ornatus* were added late as eggs (*L. ornatus* added 12 February). This trend probably resulted from the greater number of "competitor-free" days experienced by *Bufo* prior to the introduction of *L. ornatus*. In

contrast, *B. marinus* added to ponds as tadpoles after *L. ornatus* performed worse (reduced survival, increased larval period) as compared to when added to ponds simultaneously with *L. ornatus*. However, the few *Bufo* which did survive when added late to ponds as tadpoles metamorphosed at larger sizes than conspecifics which were added to ponds at the same time as *L. ornatus*. This larger size probably resulted from a reduction in intraspecific competition in these ponds, although interspecific competition may, to some extent, also have been reduced as some *L. ornatus* tadpoles probably fed on dead *B. marinus* tadpoles and subsequently died (see Chapters 3 and 5). These observed trends of enhanced performance when *B. marinus* precede *L. ornatus* into ponds and diminished performance when *B. marinus* follow *L. ornatus* into ponds, as compared to when both species were added to ponds together, are consistent with a mechanism of size-specific competition (e.g. Alford and Wilbur 1985; Alford 1989c).

In direct contrast to this trend, *B. marinus* added late to ponds as eggs performed better (increased survival, decreased larval period and increased mass at tail resorption) than *B. marinus* which were added either simultaneously to ponds with *L. ornatus*, or late to ponds as tadpoles. This reversal in competitive priority effects resulted from the toxic effects of *B. marinus* eggs and hatchlings on *L. ornatus* tadpoles. When *B. marinus* were added late to ponds as eggs, survival of both *L. ornatus* and *B. marinus* was reduced: *B. marinus* eggs and hatchlings

were preyed upon by *L. ornatus* tadpoles, and those *L. ornatus* tadpoles which consumed *Bufo* died (as per Sections 5.3.3.1 and 5.3.4). The resulting decrease in inter- and intraspecific competition allowed the remaining *B. marinus* to develop rapidly and metamorphose at very large sizes. Previous studies have demonstrated that predators can alter the intensity or outcome of competitive priority effects among anuran larvae (Morin 1981, 1983a, 1987; Alford 1989a). However, as far as I am aware, this is the first study to demonstrate that the consumption of toxic anuran eggs and hatchlings by early breeding, competitively superior anuran larvae can produce similar results.

Size at metamorphosis is positively related to fitness in amphibians. Larger juveniles often have higher survival rates (Berven and Gill 1983; Smith 1987) and an earlier age of first reproduction (Turner 1962; Clarke 1974; Smith 1987; Semlitsch *et al.* 1988) than smaller juveniles. In addition, female clutch size is often positively correlated with body size (Oplinger 1966; Pettus and Angleton 1967; Clarke 1974; Howard 1978a) and larger body size in males often leads to increased mating success (Howard 1978b; Wilbur *et al.* 1978; Ryan 1980; Berven 1981). Furthermore, larger juveniles have increased locomotory ability and stamina compared to smaller metamorphs which may enhance their ability to escape predators and also increase their likelihood of successful dispersal (John-Alder and Morin 1990). *Bufo marinus* metamorphs from ponds where *Bufo* were added late as eggs were almost twice the size of

Bufo metamorphs from any other treatment. Thus, the toxic effects of *B. marinus* eggs and hatchlings on *L. ornatus* tadpoles not only reversed the competitive priority effects between these species, they also resulted in fitter *Bufo* metamorphs emerging from ponds than when *Bufo* either preceded *L. ornatus* into ponds, or arrived at ponds at the same time as *L. ornatus*.

In general, the responses of *L. ornatus* tadpoles to different times of introduction of *B. marinus* were also consistent with size-specific competition. *Limnodynastes ornatus* tadpoles performed better (increased survival and increased mass at tail resorption) when *B. marinus* were added late to ponds as tadpoles, and performed worse (nil survival) when *Bufo* preceded them into ponds, as compared to when both species were added to ponds simultaneously. The impact of *B. marinus* tadpoles on late arriving *L. ornatus* was catastrophic; none of the *L. ornatus* added late to ponds as eggs or tadpoles metamorphosed during the experiment. Visual censuses of ponds verified that very few of these late arriving *L. ornatus* were alive at the completion of the experiment. Since *B. marinus* tadpoles are not significant predators of *L. ornatus* eggs, hatchlings or tadpoles (see Chapter 6), this impact can be attributed to competitive exclusion by early arriving *B. marinus* tadpoles. Previous studies have also found that intense interspecific competition among anuran larvae may result in the competitive exclusion of certain species (e.g. Wiltshire and Bull 1977; Morin 1981, 1987). In natural

water bodies, *B. marinus* tadpoles may occur at much higher densities than used in this experiment (up to 800 tadpoles/m^2: Hearnden 1991). At such densities, one would expect survival of late breeding *L. ornatus* in ponds which already contain *B. marinus* tadpoles to be nil.

The toxic effects of *B. marinus* eggs and hatchlings on *L. ornatus* tadpoles reduced the competitive dominance of early arriving *L. ornatus* tadpoles by reducing their survival. However, there were some positive effects for the *L. ornatus* tadpoles which survived in the presence of *Bufo* eggs and hatchlings: reduced intensities of inter- and intraspecific competition allowed those *L. ornatus* tadpoles which did not eat *Bufo* eggs or hatchlings to complete their development in a short period of time and metamorphose from ponds at very large sizes. In the Townsville region, *L. ornatus* tadpoles often occur in temporary water bodies which dry up before development is completed (pers. obs.). Competition among anuran larvae may reduce growth rate and consequently increase the risk of desiccation in such habitats (Woodward 1982; Wilbur 1987). Previous studies have shown that predation may reduce such competitive effects and allow survivors to grow rapidly enough to metamorphose before water bodies dry up (e.g. Wilbur 1987). The toxic effects of *Bufo* eggs and hatchlings may provide similar benefits for surviving *L. ornatus* tadpoles in natural water bodies. The reduced larval period of surviving *L. ornatus* tadpoles would also benefit *L. ornatus* by reducing the likelihood of predation in the

aquatic habitat (e.g. Wilbur 1987). Finally, the *L. ornatus* which metamorphosed from ponds where *B. marinus* were added late as eggs were up to two and a half times the size of *L. ornatus* metamorphs from any other treatment. As explained above, these large metamorphs can be considered the fittest of all the *L. ornatus* metamorphs collected in the experiment. These observations may help to explain why *B. marinus* does not appear to be having a catastrophic effect on adult *L. ornatus* populations in the Townsville region. Although *B. marinus* eggs and hatchlings may have significant impacts on larval populations of *L. ornatus*, the reduction in density releases the surviving *L. ornatus* tadpoles from competitive restraints and allows them to metamorphose early from ponds, thereby reducing the chances of habitat desiccation or predation, and to metamorphose as much fitter individuals than they would have if their larval population had not been reduced by *Bufo*. Thus, the negative impact of *B. marinus* eggs and hatchlings on *L. ornatus* larval populations is offset by positive effects on the surviving tadpole, metamorph and adult populations.

The inability of *L. ornatus* tadpoles to detect and avoid toxic *B. marinus* eggs and hatchlings (see Chapters 3 and 5) allowed late arriving *B. marinus* to reverse the competitive priority effects which exist between these species. In the present experiment, the *L. ornatus* tadpoles exposed to *Bufo* eggs were large enough to consume *Bufo* eggs (Table 7.1, Chapter 5). However, if these tadpoles had been smaller and

unable to consume *Bufo* eggs (see Chapter 5), it is unlikely that the observed reversal in competitive priority effects would have occurred. Thus, the size of *L. ornatus* tadpoles present in a water body at the time of *Bufo* reproductive activity is likely to be an important factor determining the outcome of competitive interactions between these species. In contrast to *L. ornatus*, some native anuran larvae which often co-occur with *Bufo* (*L. alboguttata*, *L. gracilenta*, *L. rubella*) can detect and avoid *Bufo* toxins (see Chapter 5). Therefore, if similar competitive priority effects exist between tadpoles of these species and *B. marinus* larvae, it may be expected that late arriving *B. marinus* would not be able to reverse such priority effects because these species would probably avoid consuming *B. marinus* eggs and hatchlings.

The results indicate that *B. marinus* tadpoles can have a significant impact on native larval anuran communities via competitive effects. The intensity and outcome of these competitive interactions in natural water bodies will vary depending on: (1) the degree to which *Bufo* and native tadpoles compete, (2) the timing of arrival of native species to ponds relative to *Bufo*, (3) the ability of native species to avoid consuming *Bufo*, and (4) the size of native tadpoles already present in a water body when *Bufo* breed.

CHAPTER 8. GENERAL DISCUSSION

Humans have accidentally or deliberately introduced many species into non-native habitats throughout the world. The impacts of these introductions on native biota range from subtle changes which are not easily discerned (e.g. McIntosh and Townsend 1995) through to large scale effects which have altered the composition and dynamics of native communities (e.g. Elton 1958; Zaret and Paine 1973; Bond and Slingsby 1984; Vitousek *et al.* 1987a; Nichols *et al.* 1990; Mackie 1991) and, in some instances, have changed properties of entire native ecosystems (e.g. Vitousek 1990).

The introduced cane toad, *Bufo marinus*, is popularly believed to adversely affect native Australian fauna. However, few quantitative data exist to support or refute this notion. Studies to date have concentrated primarily on the effects of adult *B. marinus* on native terrestrial fauna (Covacevich 1974; Covacevich and Archer 1975; Shine and Covacevich 1983; Easteal *et al.* 1985; Freeland and Kerin 1988). Little is known of the effects of the aquatic life history stages of *B. marinus* on native aquatic species. *Bufo marinus* breeds in temporary, semi-permanent and permanent water bodies in northern Queensland, Australia. Thus, a wide variety of invertebrate and vertebrate native aquatic species may be exposed to *Bufo* eggs, hatchlings and tadpoles in

nature. The aim of the present study was to investigate the impact of these early life history stages on native Australian aquatic fauna.

There are several mechanisms by which native aquatic fauna may be adversely affected by the early life history stages of *B. marinus*. *Bufo marinus* eggs, hatchlings and tadpoles possess toxins which might adversely affect native species that consume them. These toxins could also be released from *Bufo* into solution, or sequestered by native aquatic predators and ultimately affect other native species at higher trophic levels. *Bufo* tadpoles might also prey upon, or compete with, a variety of native aquatic fauna. Finally, there could be higher-order effects produced by *Bufo* on other trophic interactions within native aquatic communities. Aspects of all of these mechanisms were investigated in this study.

8.1 Review of Findings

Laboratory experiments identified dytiscid larvae, notonectids, belastomatids, leeches, snails, anuran larvae and fish as being susceptible to *B. marinus* toxins. These taxa usually died after preying upon live *B. marinus* eggs, hatchlings or tadpoles. In addition, snails and anuran larvae usually died after feeding upon the carcasses of *B. marinus* tadpoles which had recently (less than 96 hours) died. Some of these "susceptible" taxa were only affected by certain developmental stages of *Bufo*. For example, belastomatids (*Lethocerus insulanus*) were

unaffected by the consumption of *B. marinus* eggs and early developmental stage tadpoles, but usually died after preying upon mid and late developmental stage *Bufo* tadpoles. These results indicate that the toxicity of *B. marinus* increases as the tadpoles approach metamorphosis. Other species (e.g. snails, anuran larvae), however, died regardless of the developmental stage of *Bufo* they consumed. There was no evidence that toxins are released from *Bufo* eggs, hatchlings or tadpoles into solution in quantities large enough to have any major negative effects on "susceptible" species under natural conditions. Thus, "susceptible" native aquatic species are only affected by *B. marinus* eggs, hatchlings or tadpoles if they consume these stages.

Although *Bufo* eggs, hatchlings and tadpoles were highly toxic to some native aquatic species, other species (e.g. nepids, odonate larvae, adult dytiscids, crustaceans, turtles) readily consumed these early life history stages without any apparent ill effect. This result was surprising given the known toxicity of *Bufo* eggs and larvae, and the mechanism(s) which allow these species to consume *Bufo* remain to be determined. The large numbers of *Bufo* consumed by many "non-susceptible" native species suggests that they may be major predators of *Bufo* in nature. *Bufo* eggs, hatchlings and tadpoles may thus provide an important food source for "non-susceptible" native aquatic predators in natural water bodies. This may be particularly so during the dry season (May-November); at this time of year *Bufo* eggs and larvae are usually the only

anuran eggs and larvae present in the semi-permanent water bodies where many of these predators occur (pers. obs.).

The initial toxicity experiments performed in this study were designed to identify lethal effects which result from the consumption of *B. marinus* eggs, hatchlings and tadpoles. It is also possible that some native aquatic species may survive after consuming *Bufo* but experience sublethal effects (e.g. reduced activity: Blau *et al.* 1978; reduced growth rates: Feeny 1970; Reese and Beck 1976a, b, c; Bernays 1978; Hough-Goldstein *et al.* 1993; Leather and Walsh 1993) which may affect their survival in nature. Casual observations made during the experiments suggested that there was no difference between the activity of any of the "non-susceptible" native species in control and *Bufo* treatments, although this was not quantified. Whether "non-susceptible" native species experience other sublethal effects such as reduced growth rates following the consumption of *Bufo* remains unknown and should be addressed in any future studies. It is also possible that some "non-susceptible" native species may accumulate *B. marinus* toxins and subsequently become noxious or toxic to native taxa at higher trophic levels. A preliminary experiment indicated that dragonfly larvae (*Pantala flavescens*) do not accumulate *B. marinus* toxins in levels sufficient to cause lethal effects in higher trophic level predators (purple-spotted gudgeon: *Mogurnda adspersa*). However, given the large numbers of native aquatic predators which consumed *Bufo* without apparent ill

effect, any future research should also investigate the possibility of bioaccumulation of *B. marinus* toxins by other "non-susceptible" native species.

Since *B. marinus* eggs, hatchlings and tadpoles are only toxic to native aquatic species when ingested, the ability of "susceptible" species to detect *B. marinus* toxins and avoid consuming *Bufo* will be a major factor determining their survival in water bodies where they co-occur with *Bufo*. Two "susceptible" native aquatic taxa (fish and anuran larvae) were chosen to investigate their ability to detect and avoid *Bufo*. Native fishes (barramundi: *Lates calcarifer*, sooty grunter: *Hephaestus fuliginosus*) found *B. marinus* tadpoles unpalatable and usually learned with minimal trauma to avoid them. This is in agreement with previous studies which have shown that fishes readily learn to avoid unpalatable or toxic prey (Voris and Bacon 1966; Kerfoot *et al.* 1980; Kruse and Stone 1984; McClintock and Janssen 1990; Tullrot and Sunberg 1991; Tullrot 1994). The ability of barramundi and sooty grunter to detect and avoid *Bufo* means that populations of these species are unlikely to decline in water bodies where they co-occur with *Bufo*.

Native anuran larvae exhibited considerable interspecific variation in their ability to detect and avoid *Bufo*. Under laboratory conditions, tadpoles of *Litoria alboguttata*, *L. gracilenta* and *L. rubella* generally avoided consuming *Bufo* when alternate food was available. Consequently, these

species are unlikely to experience toxic effects in water bodies where they co-occur with *Bufo* because they will probably avoid consuming *Bufo* in favour of alternate non-toxic food items. However, the larvae of other native anuran species (*L. bicolor*, *L. nigrofrenata*, *Cyclorana brevipes*, *Limnodynastes ornatus*) exhibited limited ability to detect and avoid *B. marinus* toxins. Artificial pond experiments demonstrated that, under naturalistic conditions, populations of *L. bicolor*, *L. nigrofrenata* and *L. ornatus* tadpoles experienced significant mortality when exposed to *B. marinus* eggs, hatchlings or dead tadpoles. These are the first quantitative data to demonstrate a significant negative impact by *B. marinus* on populations of any native Australian species. They are also the first data to demonstrate that the consumption of toxic anuran eggs, hatchlings and dead tadpoles may be sources of mortality for anuran larvae. In natural water bodies, the impact of *Bufo* on populations of such native anuran larvae should be positively correlated with *Bufo* density. However, since native tadpoles must attain a minimum size before they can prey upon *Bufo* eggs, the size of native tadpoles present in the water body at the time of *Bufo* reproductive activity will also be a major factor determining the impact of *Bufo* eggs on these tadpoles.

The toxic effects of *Bufo* on native tadpoles may indirectly affect other native aquatic species. For example, *L. ornatus* tadpoles are voracious predators of the eggs and hatchlings of many native anurans. An artificial pond experiment demonstrated that *L. ornatus* tadpoles can play

a significant role as predators in structuring native larval anuran populations: survival of *L. rubella* eggs and hatchlings in the presence of predatory *L. ornatus* tadpoles was nil. However, the toxic effects of *Bufo* on *L. ornatus* tadpoles indirectly facilitated the survival of eggs and hatchlings of later-breeding *L. rubella* by reducing the intensity of predation on these early life history stages by *L. ornatus* tadpoles. Thus, while *Bufo* may have a direct negative impact on some native tadpole populations via toxic effects associated with the consumption of *Bufo*, they may also have indirect positive effects on other native tadpole populations by decreasing the intensity of predation on these populations by native anuran larvae. As far as I am aware, these are the first data to demonstrate that the toxic effects of a species may alter predatory priority effects within aquatic communities.

Bufo tadpoles were not significant predators of the eggs, hatchlings or tadpoles of native anurans in laboratory experiments and are therefore considered unlikely to significantly affect the survival of these early life history stages via predation in natural water bodies. However, *Bufo* tadpoles did compete with native tadpoles (*L. ornatus*). The outcome of competition between *Bufo* tadpoles and *L. ornatus* tadpoles was determined by their order of introduction into ponds. Generally, each species performed better (increased survival, decreased larval period and/or increased mass at tail resorption) when added to ponds before the other species, and performed worse (reduced survival, increased larval

period and/or decreased mass at tail resorption) when added to ponds after the other species, as compared to when both species were added to ponds simultaneously. These trends are consistent with a mechanism of size-specific competition (e.g. Alford and Wilbur 1985; Alford 1989c). However, the toxic effects of *Bufo* eggs and hatchlings on *L. ornatus* tadpoles allowed late arriving *Bufo* to reverse these competitive priority effects. Consequently, *Bufo* added late to ponds as eggs performed as well as, or better than, conspecifics which preceded *L. ornatus* into ponds. However, there were benefits for those early-arriving *L. ornatus* tadpoles that survived in the presence of *Bufo* eggs and hatchlings. The reduction in inter and intraspecific competition allowed surviving *L. ornatus* to complete their larval development in a short period of time, thereby reducing the likelihood of mortality via predation or habitat desiccation, and to metamorphose from ponds as larger (i.e. fitter) metamorphs. Previous studies have shown that predators can mediate priority effects among competing species (Morin 1981, 1983a, 1987; Alford 1989a). However, this is the first study to demonstrate that toxic species may also alter competitive priority effects.

Further studies are required to determine the extent to which *Bufo* tadpoles compete with native aquatic species other than *L. ornatus* tadpoles (e.g. other tadpoles, aquatic snails, herbivorous insects). If competitive priority effects also exist between these native species and *Bufo*, and these species are susceptible to *Bufo* toxins, their ability to

detect and avoid consuming the eggs or hatchlings of later-breeding *Bufo* should play a significant role in determining the outcome of competitive interactions with *Bufo*. It is also possible that the toxic effects of *Bufo* on native tadpoles may alter the intensity and/or outcome of interspecific competition between these tadpoles and other native aquatic species if the competing species differ in (1) their susceptibility to *Bufo* toxins, or (2) their ability to detect and avoid *Bufo* toxins. These interesting possibilities also warrant further investigation.

The role of toxic chemicals in providing protection from predators has been demonstrated for a variety of taxa (e.g. Voris and Bacon 1966; Kerfoot *et al.* 1980; Kruse and Stone 1984; Pawlik *et al.* 1986; McClintock and Janssen 1990; Tullrot and Sunberg 1991; Hough-Goldstein *et al.* 1993; Tullrot 1994; Cronin *et al.* 1995; Rowell-Rahier *et al.* 1995). However, the extent to which these toxins also affect competitors has received little attention. The present study demonstrated that the toxins present in the early life history stages of *B. marinus* favour *Bufo* by (1) providing protection from some aquatic predators, and (2) reducing competition between *Bufo* and other aquatic species. As far as I am aware, these are the first data to demonstrate that the possession of toxic chemicals may benefit a species by reducing both predation and competition.

In addition to documenting the impact of the early life history stages of *B. marinus* on native aquatic fauna, this project has also provided new information on the processes that structure aquatic communities. Previous studies have identified predation, competition and abiotic factors as the primary factors determining the structure of aquatic communities (e.g. Brooks and Dodson 1965; Zaret and Paine 1973; Dodson 1974; Alford and Wilbur 1985; Morin 1987; Wilbur 1987; Elser and Carpenter 1988; Alford 1989a; Wissinger 1989; Dunson and Travis 1991; Hart 1992; Reice 1994). The present study demonstrated for the first time that toxic anuran eggs, hatchlings and tadpoles may also structure aquatic communities by reducing the survival of certain species and thereby altering predatory and competitive interactions within aquatic communities. Whether such toxic effects occur within a particular community may depend on the evolutionary history of exposure of that community to these toxins (e.g. Ehrlich and Raven 1964; Licht and Low 1968; Krieger *et al.* 1971; Whittaker and Feeny 1971; Blau *et al.* 1978; Scriber 1978; Ryan and Byrne 1988; Speiser *et al.*1992; Gilbert 1994; Crossland and Azevedo-Ramos in review). Consequently, while the toxic effects of *Bufo* on anuran larvae demonstrated in this study may also occur in other countries where *B. marinus* has been introduced, they may not occur within the natural distribution range of *B. marinus* where anuran larvae have co-evolved with these toxins (e.g. Crossland and Azevedo-Ramos in review). Such potential evolutionary patterns are worthy of further investigation.

8.2 Conclusions

The results of this study demonstrate that *B. marinus* eggs, hatchlings and tadpoles may have a significant impact on native aquatic communities. In particular, *Bufo* may alter the composition and dynamics of native larval anuran communities via: (1) toxic effects associated with the consumption of *Bufo*, (2) competitive interactions, and (3) higher-order effects produced by *Bufo* on other trophic interactions within native tadpole communities. As a result, *Bufo* may have both positive and negative effects on native larval anuran populations.

Clearly, there remains a great deal of scope for further research on the impact of *B. marinus* eggs, hatchlings and tadpoles on native aquatic fauna. For example, the present study concentrated on the impact of *Bufo* on two "susceptible" native aquatic taxa: fish and anuran larvae. The ability of other "susceptible" native aquatic species (dytiscid larvae, notonectids, belastomatids, leeches, snails) to detect and avoid *B. marinus* toxins remains to be determined. If these species have limited ability to detect and avoid *Bufo*, then they are also likely to experience significant population declines in water bodies where they co-occur with *Bufo*. Such impacts may in turn affect other native aquatic species within the same trophic level and at other trophic levels as well. The extent to which "non-susceptible" native aquatic species either bioaccumulate *Bufo* toxins or experience sublethal effects after

consuming *Bufo* also remains to be determined. Similarly, the competitive impact of *Bufo* tadpoles on many native aquatic fauna remains largely unknown.

The range of *B. marinus* within Australia continues to expand (Van Beurden and Grigg 1980; Sabath *et al.* 1981; Van Beurden 1981; Easteal *et al.* 1985; Freeland and Martin 1985; Seabrook 1991; Sutherst *et al.* 1996) and the species is popularly believed to pose a significant threat to native fauna. The present study is the first detailed investigation of the impact of *B. marinus* eggs, hatchlings and tadpoles on native aquatic fauna. The results demonstrate that the aquatic life history stages of *B. marinus* may have a significant impact on the structure and dynamics of native aquatic communities, and in particular, on native larval anuran communities.

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