THE REPRODUCTIVE AND LARVAL ECOLOGY OF *Bufo marinus* (ANURA: BUFONIDAE)

Thesis submitted by

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"These foul and loathsome animals are distinguished by a heart with a single ventricle and a single auricle, doubtful lungs, and a double penis. Most Amphibia are abhorrent because of their cold body, pale colour, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive smell, harsh voice, squalid habitation, and terrible venom: and so their creator has not exerted his powers to make many of them."

Carolus Linnaeus, *Systema Naturae* (1758).

Abstract

The reproductive and larval ecology of the introduced species *Bufo marinus* was studied at two breeding sites in tropical northern Australia. Reproductive activity, recruitment patterns, larval growth and survival, and predation by conspecifics and by insect, crustacean and fish species were looked at in detail.

Reproduction at both sites occurred from December to June. Recruitment from reproductive events was sporadic. It was not concentrated during any particular period of the breeding season. During breeding periods, individuals at oviposition sites were predominantly males, with a population sex ratio of 10:1. Less than 30 percent of the estimated male population and 5 percent of the estimated female population were active (found in transect searches) on any one night during the breeding period. The proportion of active individuals in amplexus at any time was low. Total numbers of active individuals declined by the end of the dry season and sex ratios approached 1:1. No breeding was observed during the late dry season although histological examination of gonads indicated that reproductively active males and females (with mature sperm and ova) were present all year round.

Sampling of larval populations indicated that densities recorded at both sites varied temporally and spatially. High variance-mean ratios for sample densities indicated that larval distributions within transects were aggregated. Peak densities were typically between 600 to 800 per m^2. Larval density was used to determine population size but estimates showed no consistent association with recruitment of eggs from observed breeding.

Abstract

Experimental studies indicated that during the embryonic stages (Gosner stages 1 to 24) predator-free survival was approximately 70 percent (90 percent survival/day) over a mean developmental period of 72 hours. Highest mortality occurred between three days (hatching) and twelve days (stage 25) after laying. Mean larval periods of 22 to 54 days were recorded. Shorter larval periods were associated with lower larval density. Survival to metamorphosis ranged from 0.1 to 10 percent (97 percent mean survival/day). Survival and growth rates in most experiments were density dependent. Growth of pre-hatching stages was linear but growth was exponential or decreasing exponential during the tadpole stages, controlled either by temperature or density. Growth of larvae is food limited, with advantages to older larvae demonstrated in an artificial pond experiment with two overlapping cohorts of different age. Growth was reduced for both cohorts but the effect was greater for younger cohorts. Individuals that survived egg predation by older cohorts grew larger because of reduced competition.

Egg predation by older cohorts of *Bufo* larvae appeared to be a major source of mortality. Intraspecific predation was restricted to pre-swimming embryo stages and greatly reduced pre-hatching survival in field and experimental populations (1.67 percent mean survival/day). No other predators of eggs were recorded.

Few species were active predators of hatched tadpoles. Only two of eleven potential predators examined in feeding rate experiments consumed tadpoles. These were an adult dytiscid beetle, *Homeodytes scutellaris* (0.28 tadpoles/hour), and a freshwater

crayfish, *Cherax quadricarinatus* (0.48 tadpoles/hour). None of the native fish species examined successfully consumed eggs or larvae.

A predation experiment was conducted in experimental ponds with aeschnid dragonfly larvae, *Hemianax papuensis*, and high and low densities of *Bufo* larvae. Predation significantly reduced survival of larvae at both densities and growth was significantly lower for larvae in predator treatments at high density. Slower growth for larvae in predator treatments probably resulted from larvae altering their foraging behaviour in response to the presence of predators. Tadpoles appeared to be less conspicuous in predator treatments. In predator treatments with a low initial density of *Bufo*, initial predation decreased densities to a level where encounter rates with predators were low and tadpole food resources were probably not limiting. Final mass and larval period for tadpoles at low density in predator treatments were similar to controls.

Larval population size is most likely to be influenced by differences in cohort overlap resulting from adult breeding phenology. A small overlap will result in generations with narrow size differences and will produce increased competition between cohorts. A large overlap may result in large size differences in cohorts and may lead to heavy predation of eggs by older cohorts. Invertebrate predators are likely to affect the survival of larval *Bufo marinus* populations during colonisation and periods of habitat deterioration.

Declaration

I declare that this thesis is my own work and has not been submitted in any other form for another degree or diploma at any University or other institute 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.

19/12/91

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December 1991

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Chapter 1. General Introduction.

For anurans, the aquatic larval phase of their complex life cycle represents an opportunity to exploit transient opportunities for rapid growth (Wassersug 1975). The ecological performance of larvae, as measured by survival and growth to metamorphosis, may have a direct bearing on the fitness of individuals in subsequent phases of the life history (Smith 1987, Berven 1990). Larval growth and survival may be influenced by the amount of resources available (Wilbur 1977a), the co-occurrence and density of conspecific larvae of similar or disparate age or size (Sauer and Slade 1987), and the presence and density of interspecific competitors and predators (Morin and Johnson 1988, Fauth 1990). These, in turn, will be affected by phenological patterns of recruitment that result from adult reproduction. Knowledge of any of these influences assists in more comprehensive understanding of the population dynamics of species and provides information necessary for modelling these processes.

1.1 A Review of the Literature

1.1.1 Reproductive Strategies

Studies of the reproductive biology of anurans have concentrated on the characterisation of reproductive success and behaviour, sexual selection and mate choice, gonad condition and vocalisation behaviour. Wells (1977) noted two broad

categories of temporal patterns in reproductive behaviour in anurans that represented either end of a broad continuum: prolonged (a few to many months) and explosive (a few days) breeding. These patterns tended to influence the type of male-male competition for mates. On one hand, explosive breeding in dense aggregations resulted in "scramble" competition among mobile, calling males. Alternatively, prolonged breeding was distinguishable by within-male spacing and calling from stationary positions. Male spacing can be territorial, resembling lekking behaviour observed in other vertebrates (Sullivan 1987, Howard 1988a).

Prolonged breeding is more common, particularly among tropical species where reproduction may occur year round (Inger and Greenberg 1963, Brown and Alcala 1970, Crump 1974) or throughout the wet season (Lee 1967, Crump 1974). Howard (1978) showed that in *Rana catesbeiana*, female reproductive success was higher in territories controlled by larger males. Reproductive success was measured as the percentage of eggs surviving to hatching. These premium sites tended to be at an optimum temperature for egg development. This led to faster development and decreased the risk from leech predation on eggs. The establishment of territories and maintenance of spacing between *Hyla regilla* males may be mediated by neighbour-call amplitudes, aggressive "encounter" calls, and physical encounters (Brenowitz 1989). Fights among male *Rana virgatipes* were usually determined by larger size although territory residents usually won aggressive calling interactions with intruders (Given 1988).

Vocalisation over a prolonged breeding season is an energetically demanding behaviour and can account for periodic variations often noted in chorus sizes (Sinsch 1988, Forester et al. 1989). Wells and Taigen (1989) demonstrated that due to the energetic cost of calling, male *Hyla microcephala* reduced call rates when only a few males were active but increased calling effort when vocal competition became more intense. In dense populations of calling males, neighbours tend to maximise the effectiveness of calls by actively avoiding acoustic overlap (Brush and Narins 1989). This also plays a role in facilitating inter-male spacing (Schwartz 1987). This type of "partitioning" is represented in the wider anuran community. Duellman and Pyles (1983) surveyed 621 call groups of 214 individuals representing thirty-nine different hylid species. With few exceptions, calls of closely related species were similar only when the species were from different communities. When closely related sympatric species had similar acoustic properties, they exhibited differences in breeding patterns and call times.

Explosive reproduction patterns are more common in ephemeral habitats where extremely short breeding periods provide little opportunity for mate-choice (Wells 1977, Howard 1988b). This reproductive strategy has been shown to incur a substantial energetic cost. Both sexes of *Rana temporaria* lost twenty-nine to thirty-three percent of body weight due to gonad production, spawning behaviour and the cessation of feeding (Ryser 1989). Calling and non-calling males may be successful in amplexing with females as calling probably only acts as a long-distance

attraction for females (Wells 1979, Fairchild 1984). However, subsequent studies have demonstrated patterns of non-random mating in explosive breeders. Patterns observed in *Bufo boreas* and *Bufo americanus* demonstrate a mating advantage for large males and no particular relationship between male-female sizes (Gatz 1981, Olson et al. 1986). This suggests that either females may choose mates on the basis of size (Woodward 1982) or that larger males are more successful in displacing smaller, amplexed males before fertilisation occurs (Wilbur et al. 1978, Wells 1979). Morris (1989) noted that within a breeding population of *Hyla chrysoscelis*, females initiated amplexus with larger males. Lee (1986) demonstrated that for explosive-breeding anurans, large body size *per se* may not be the mechanism for observed non-random mating patterns. Within populations of *Bufo marinus* and *Bufo terrestris*, advantages were conferred to individuals with longer forelimbs that were better able to resist displacement during amplexus (Howard and Kluge 1985).

Some studies indicated that male body size was inconsequential in aquiring females for mating (Kruse 1981, Gerhardt et al. 1987). Large and small male *Bufo bufo* employed different tactics to secure females (Loman and Madsen 1986). Large males occupied the pond early and relied on size to acquire females. Smaller males intercepted the later arriving females before they reached the water and depended on the short period the female spends at the pond before spawning to remain in amplexus. Female choice has been shown to occur on the basis of acoustic cues (Schmidt 1971, Ryan et al. 1990). Mating and vocalisation studies in

Eleutherodactylus coqui demonstrate that all mated males called earlier in the evening and at a rate greater than the mean call rate for unmated males and the overall male population. Furthermore, mated males were not necessarily the loudest or the largest males (Lopez and Narins 1991). Call rate has also been shown to be important in mate aquisition for other species; *Hyla chrysoscelis* (Morris and Yoon 1989), and *Bufo woodhousei* (Sullivan and Wallsberg 1985, Sullivan 1989a).

The issue of predation risk to individuals while breeding is still being debated (Ryan et al. 1981, Endler 1987, Gwynne 1989, Crowley et al 1991) but aggregation involved in explosive breeding may decrease the risk of predation to participants (Olsen 1989).

Brown and Alcala (1983) studied the reproductive strategies of fifty-six of the sixty-five anuran species in tropical communities of the Philippines. They used the classification scheme of Salthe and Mecham (1974) to index type of development, but recognised more specific modes. Observations demonstrated that two general modes (aquatic larval development and terrestrial direct development) and eighteen specific modes existed among the species. Specific modes incorporated factors such as egg size and pigmentation, number of clutches, type of nest construction and positioning, larval nutrition and degree of parental care. Of the species reviewed, they noted that there was little or no intrageneric diversity of mode except in *Rana* species where three specific modes were identified.

Larger egg size in anurans is associated with larger hatchling and larval size, and faster growth rates that can reduce predation risk (Reading 1986, Kaplan 1989, see later discussion on predation). Advanced embryo development in anurans is exhibited by species that oviposit on vegetation above water, where eggs hatch, and larvae then drop into the water. This strategy may have evolved in response to heavy predation pressure in the more vulnerable egg stage. However, this strategy requires a higher energy investment per egg, therefore clutch sizes tend to be smaller than species with more aquatic reproductive modes (Crump and Kaplan 1979). To facilitate growth through the early larval stages without feeding, a greater energy input per egg is required.

The habitat chosen during ovipostion can have a large influence on the subsequent survival rates of the larvae (Newman 1988a, 1988b, 1989). Female *Hyla pseudopuma* have been shown to discriminate in choice of preferred oviposition sites based on the number of eggs and tadpoles already present (Crump 1991). Similar strategies have been shown for *Hyla chrysocelis* (Resetarits and Wilbur 1989). Characteristics of the habitat such as availability of food resources and persistence of water levels can operate to exert strong control on the growth of larval anurans.

1.1.2 Larval Growth and Development

Alford (1991) summarises the diversity of factors controlling growth and development of larval anurans. These include a variety of types and sources of competition and environmental influences. Competition may be either exploitative (Wilbur 1977a, 1977b, Semlitsch and Caldwell 1982, Smith 1983, Travis 1984, Scott 1990) or interference (Wassersug 1986, Steinwascher 1987, Woodward 1982, 1987, Petranka 1989a). These may occur within or between cohorts of a species and between different species or phyla exploiting a common resource pool (Morin et al. 1990, Griffiths 1991). Larval survival may be influenced by an integrating of the effects of competition with other environmental influences such as the composition of the predatory fauna (Heyer et al. 1975, Cecil and Just 1979, Woodward 1983, Holomuzki 1986, Cortwright 1988, Alford 1989, Fauth 1990, Magnusson and Hero 1991) and habitat duration and quality (Black 1974, Wilbur 1976, Harris 1980, Travis and Trexler 1986, Semlitsch 1987).

Present models of amphibian metamorphosis consider two of the main features mentioned above. These are patterns of resource availability (that determine levels of competition) and habitat stability. In the original model, proposed by Wilbur and Collins (1973), it was hypothesised that larvae could adjust their developmental (=differentiation) rates, and therefore the length of their larval period, in response to changes in realised growth rate. This would allow larvae that experience increased

growth rates in response to an abundant resource, to decrease developmental rate. This would prolong time spent in conditions favourable to growth and maximise body size before a larvae metamorphosed. If conditions were unfavourable or deteriorated, therefore slowing growth rates, then developmental rate could be increased. Larvae under these conditions would grow to some minimum size necessary for metamorphosis to occur thereby minimising the time spent in a habitat where mortality risks were high. Wilbur and Collins suggested that an evolutionary advantage would be gained by larvae that evolved this pattern.

Two direct mechanisms have been proposed that may act as controls on developmental rate, a feature not considered by Wilbur and Collins' model or that modified by Collins (1979). Crump (1981) suggested that the accumulation of energy reserves that occurs under conditions of favourable growth may act to regulate developmental rate. If body fat decreases in response to deterioration of the resource pool, then this may act as a feedback mechanism to increase developmental rates. Similarly, Wassersug (1986) suggested that hormonal controls may be operating to govern development. He suggested that larger amounts of prostaglandins ingested with increased feeding rates would accompany increased growth. This would slow development of metamorphic characters (here, gut development) and prolong larval periods.

When Travis (1984) tested the Wilbur-Collins theory for evidence of "competitive release", he noted that *Hyla gratiosa* larvae did not appear to alter developmental rates on release from competition. Travis (1984) hypothesised that differentiation rates were determined early in the larval period of anurans and were fixed despite increases that may occur in growth rate. Instead of a minimum body size requirement as predicted by Wilbur and Collins, growth was constrained within minimum and maximum larval periods. Variation in body size would result from changes in growth rates imposed by competition.

Two common obstacles encountered by larvae growing in ephemeral habitats are decreasing availability of food resources and shrinking of the habitat through drying. Alford and Harris (1988) designed a study to test assertions of the Wilbur-Collins and Travis models. They examined the premise that larvae experiencing deteriorating resource levels would accelerate developmental rate relative to those at constant resource levels. They grew *Bufo woodhousei fowleri* at a uniform density with low and high food levels. After 12, 18 and 30 days they switched the food levels for each treatment and compared these with larvae on constant high and constant low food levels. The Travis model predicted that with decreased food, growth rates would also decrease but due to a fixed developmental rate, larvae would persist until a minimum larval period length was reached. However larvae in Alford and Harris' experiment responded by decreasing growth rate, increasing developmental rate and metamorphosing as predicted by Wilbur and Collins. Crump

(1989) demonstrated that habitat controls on growth and development, inherent in the Wilbur-Collins model, are also applicable. She showed that *Hyla pseudopuma* tadpoles responded differently to habitats with constant high, constant low and diminishing water levels. Individuals in constant high level regimes metamorphosed at larger sizes than those in constant low or diminishing treatments. Tadpoles in the diminishing water-level treatments had the shortest larval periods.

Physiological models that invoke proximal mechanisms have been developed by Smith-Gill and Berven (1979) for temperature and Pandian and Marian (1985) for energetic parameters. Smith-Gill and Berven found that temperature was a major factor in determining larval growth and differentiation patterns. Temperature decreased developmental rate differently from growth rate. At lower temperatures, developmental rate was decreased, while growth rate was reduced proportionately less. At lower temperatures, growth was extended within each developmental stage such that body size at metamorphosis was larger than for larvae raised at higher temperatures.

In a different approach to the problem, Werner (1986) discussed amphibian metamorphosis within an ecological framework that considers lifetime fitness. Trade-offs between growth rates and mortality risks in the larval and terrestrial habitats interact with timing and size at metamorphosis. Werner argues that growth in the terrestrial phase is probably a more important consideration and has been

underestimated by many studies. Body size of metamorphosed bufonids and hylids represent 0.1 to 10 percent of the adult median size. Thus the size at which metamorphosis should occur will be influenced by the potential for growth in the terrestrial phase. Therefore, metamorphosis should take place at a size where mortality risk might increase without a corresponding increase in size, hence fitness.

An extension of this theory is presented by Rowe and Ludwig (1991) who added optimal time trajectories to the model. These were linked to the growth and mortality rates in each growth phase. For amphibians, the decision to metamorphose is an assessment of the growth-time trajectories from hatching to metamorphosis, then to time-at-first-reproduction. Metamorphosis is predicted to occur when a decline in growth rate is coupled with increasing mortality risk for the larvae (Ludwig and Rowe 1990). Tadpoles in low risk environments thus spend longer as a larvae and grow larger. Because fitness advantages exist for earlier reproduction, advantages will be conferred to metamorphs of increased mass that require less time to reach maturity. The larvae of individuals breeding first will have access to a greater resource pool, be at less risk to mortality from transient and size limited predators, and may be exposed to less competition than later larvae (Alford and Wilbur 1985, Wilbur and Alford 1985). Although the model is applicable to insect and semelparous amphibian species, it is compatible with those of Wilbur and Collins (1973) and Werner (1986) in its consideration of growth and mortality trade-offs for amphibians in general.

1.1.3 Studies of Tadpole Competition

Most studies of intraspecific competition in anurans consider the effects of population density and food resources on a range of larval characters. These include body size at metamorphosis, development rates, and proportion surviving to a particular stage. In 1980, Wilbur reviewed the literature covering aspects of intraspecific competition in anurans. Alford (1991) expanded on the issues covered by Wilbur and synthesised the results of subsequent studies that have improved our knowledge of competition.

Early studies in tadpole ecology focused on survival and sources of mortality of natural populations. Herreid and Kinney (1966), Calef (1973), Licht (1974) and Cecil and Just (1979) all studied populations of *Rana* species. In each study, mortality of the larval stages was constant over time (see Petranka 1985) and resulted in low (less than 10 percent) rates of survival to metamorphosis. Significant mortality was attributed to vertebrate and invertebrate predation in all studies. Licht also associated egg mortality with desiccation after falling water levels exposed oviposition sites. In a study of *Pseudacris triseriata*, Smith (1983) found that survival of larval populations present in ephemeral pools was determined by pool size and predation. Larvae in large pools were eliminated by newt and dragonfly naiad predation, while small pools dried up before most of the larvae could metamorphose. Recruitment was controlled by intraspecific competition in pools that did not contain predators. Field experiments in pools suggested that the level of

competition was density dependent and involved food limitation. Berven (1990) studied a natural population of *Rana sylvatica* for a seven year period. He found premetamorphic survival and size at metamorphosis was negatively correlated with the number of eggs deposited in ponds while larval period was positively correlated. The effect of density on these larval characteristics appeared to be the major factor regulating the size of adult populations.

Brockelman (1969) modified the natural system approach by using *in situ* pens to regulate factors such as predators and tadpole density. He found that increases in pen densities of *Bufo americanus* were associated with decreases in metamorph body size and increases in larval period. Survival was related to density, being highest at the lowest densities. Predation from dragonfly larvae and leeches negated the effect of density but decreased overall survival. Brockelman supplemented pens with food (as periphyton on plant litter) and found a density dependent increase in growth rate.

Field-pens were also used by Wilbur (1976) for studies with *Rana sylvatica*. He showed that larvae possessed unequal competitive abilities by demonstrating that the relationship between survival and density fitted a negative exponential model. This produced small numbers of successfully metamorphosing individuals at high densities when equal competitive ability would have predicted no survivors. Travis (1983) investigated variation in growth and survival of seven sibships of larval *Hyla gratiosa*

in experimental enclosures. Cages were colonised by a suite of insect predators but larger vertebrate predators were excluded. Survival was positively correlated with the mean body size of the caged larvae. Travis concluded that size-limited predation resulted in these observed patterns of survival. Therefore survival was enhanced for faster growing larvae that were able to reach a size refuge. Travis and Trexler (1986) caged different densities and different size-classes of *Bufo terrestris* in sections of a pond that represented differences in environmental harshness. Increased density retarded growth rates only in more "harsh" environments (low circulation and oxygen levels) with no effect in benign areas. Larger larvae had an increased rate of survival to metamorphosis in all environments.

Laboratory studies controlling the effect of food and density on larval growth have been conducted by several authors. Wilbur's (1977b) study of *Bufo americanus* revealed that population density had a significant influence on the proportion that successfully metamorphosed. However, this result was interpreted as an effect of growth rate, rather than survival. At the higher densities, a few individuals grew at the expense of the majority thereby lowering the probability of metamorphosis overall. Other studies of the effect of density on growth have been conducted on *Rana sylvatica* (Wilbur 1977a), *Rana tigrina* (Dash and Hota 1980, Hota and Dash 1981), and *Scaphiopus holbrooki* (Semlitsch and Caldwell 1982). Always, density was significantly correlated with decreases in growth rate, increases in larval period, or both.

Mechanisms of interference competition were first proposed in relation to extreme crowding of larvae. Retardation of growth in larvae at high densities was demonstrated by Richards (1958) and in subsequent studies by Rose (1959, 1960). They attributed this response to the unresolved inhibiting properties of unicellular material found in the faeces of larger tadpoles. Licht (1967) also noted these "alga-like" cells appeared to slow growth in all but one of seventeen species he surveyed. Of the species examined, growth was actually enhanced in aggregating *Bufo woodhousei*, and the inhibition displayed by other *Bufo* species was less pronounced than in other genera. These "alga-like" cells were later shown to be a *Chlorella* species and do not appear to have any inhibitory effect on growth (Pelaz 1987). Steinwascher (1978, 1979) identified a yeast, *Candida humicola*, in larval *Rana clamitans* and *R.utricularia*. *C.humicola* formed a mutualistic relationship with the large tadpoles as it retarded the growth of smaller larvae only. This indirectly increased the growth rate of the larger tadpoles by reducing the demand on resources. Petranka (1989b) studied a series of systems, assaying tadpole growth in water conditioned by previous populations of varying densities of tadpoles. Experiments were designed to test for the presence of chemicals that alter growth. He noted that the growth of *Rana utricularia* could be inhibited by water conditioned by high densities of conspecifics and other species but only in certain circumstances. These were usually when dense aggregations occurred or when habitat desiccation increased regional densities.

Cannibalism is a common form of intraspecific competition between cohorts of different age classes (Eickwort 1973, Fox 1975, Polis 1981, Kusano et al. 1985, Polis and Myers 1985). It can be controlled by several factors that govern food supply and intraspecific aggression. These include density (Wright and Giles 1987, Van Buskirk 1989, Panjunen and Panjunen 1991) and size differences (Crump 1986, Semlitsch and West 1988, Orr et al. 1990). The most common form of cannibalism in anurans is egg and hatchling predation (Crump 1983, Polis and Myers 1985). Spawn predation is an important form of interference competition between and within anuran species (Crump 1983, Banks and Beebee 1987) and can account for 80 to 100 percent of egg and hatchling mortality.

Tadpoles that prey on their own or other species of tadpoles are often associated with ephemeral ponds where desiccation is the major cause of mortality (Heyer et al. 1975, Bragg 1946, 1964). Examples are spadefoot toads of the genus *Scaphiopus*, in which some species are polyphenic with a cannibalistic morph (Bragg 1964). The expression of polyphenism in species was shown to be controlled by environmental influences (Hampton and Volpe 1963, Collins and Cheek 1983). For these species, feeding on conspecifics greatly reduces time spent in larval habitats where mortality risks are high due to the ephemeral nature of the habitat (Bragg 1965). Intraspecific predation has also been reported for tadpoles of *Osteopilus septentrionalis* preying on older metamorphosing larvae at Gosner stages 42 to 43 (Crump 1986). At this stage of development, larvae are more vulnerable than either tadpoles or fully

metamorphosed juveniles (Wassersug and Sperry 1977, Arnold and Wassersug 1978).

For cannibalism to be selectively favoured, Eickwort (1973) suggested that there should be a nutritional benefit and predation should occur at a phase in the life cycle where age-specific mortality is high. Pre-hatchling mortality of anuran egg and hatchling stages can be high. Bragg and Bresler (1951) reported a hatching rate of 34 percent for *Bufo cognatus*. Crump (1990) has experimentally demonstrated that oophagy enhanced the growth of *Hyla pseudopuma* tadpoles. In any form of cannibalism, growth advantages could accrue to both survivors and cannibals as the number of competitors exploiting a finite resource are reduced (Semlitsch and Caldwell 1982, Wilbur 1988). Cannibalism is less likely to be advantageous if the chance of eating relatives, thus reducing inclusive fitness, is high (Crump 1990). This suggests that intraspecific predation should be more prevalent in systems that involve explosive breeding of large numbers of pairs and where juveniles or adults disperse away from their natal ponds. It should be less common in species where breeding is prolonged, oviposition sites are limiting, and adults breed repeatedly at the same site.

1.1.4 Tadpole Aggregations - A Response to Predation ?

The effect that crowding has on tadpole growth and survival presents an enigma when the densities involved in aggregation behaviour are considered. Aggregation or gregariousness is considered to an characteristic of species for which predation risk is a major source of mortality (Turner and Pitcher 1986, Sillén-Tullberg and Leimar 1988). The formation of social aggregations similar to fish schools or asocial aggregations in response to environmental gradients is a feature of tadpoles of many anuran species (Wassersug 1973, Caldwell 1989). The maintenance of aggregations can involve visual (Wassersug and Hessler 1971) or chemical cues (Blaustein and O'Hara 1982). Bragg (1954, 1960, 1968) observed various aggregations that contained individuals feeding in similar manner or were composed of non-feeding metamorphosing individuals. He assumed from these observations that formation of aggregations was influenced by food or preferred thermal regimes. Aggregation modes of anurans are discussed by Wassersug (1973) and Beiswenger (1975). Asocial aggregations may form in response to gradients of temperature (Brattstrom 1962, Mares 1972), water current (Orton 1953, Inger 1966), light intensity (Brattstrom and Warren 1955, Ashby 1969, Beiswenger 1977), olfaction associated with food sources (Mares 1972), oxygen concentrations (Savage 1961), and habitat type (Weins 1970). Social aggregation is behavioural trait and is not dependent on environmental influences. They can be influenced and maintained by a variety of factors. Mares (1972) noted that tail thrashing by individuals in *Bufo marinus*

aggregations assisted feeding by stirring up detrital deposits. Beiswenger (1975) also observed this, and found relationships between the mobility and density of aggregations: stationary aggregations included groups of non-feeding metamorphosing tadpoles at a range of densities. Stationary necrophagous groups feeding on dead tadpoles or carrion were smaller and more dense than groups feeding on periphyton. Groups either "streamed" (moved in elongate processions) in low densities while feeding infrequently or "schooled" (slower movement in compact groups) at higher density and consisted primarily of feeding tadpoles. The principal function of these aggregations was suggested to be efficient location and utilisation of food resources (see also Turchin 1989). Beiswenger argued that food resource location and utilisation operates synergistically with increased protection from predators (Caldwell 1989) and faster development through selection of warmer regions of the habitat.

Tadpole aggregations can also be formed by the association of tadpoles with their siblings (Waldman and Adler 1979, Blaustein and O'Hara 1981, Waldman 1982). Sibling cohorts appear to have chemically mediated behavioural recognition that allows preferential association. However this association does not result in genotypically 'pure' aggregations, suggesting that sibling recognition may not be error-free, or that the benefits of aggregation behaviour may be independent of relatedness. Waldman's (1982) analysis of sibling association in *Bufo americanus* demonstrated specific advantages to individuals in a group. Enhancement of

inclusive fitness was gained through more efficient responses to predation, such as aposematic advertisement and alarm signalling. Predators may find eating individuals in an aggregation distasteful, as in many *Bufo* species (Brodie et al. 1978). Thus the group becomes a visual signal to the predator not to pursue future attacks (Sillén-Tullberg and Leimar 1988). Avoidance behaviour may be elicited by alarm substances released by tadpoles in response to a predatory attack (Petranka 1989c). These substances may be detected disproportionately better by kin when they are aggregated (*sensu* Maynard Smith 1965) thus enhancing the survival of kin groups (Turner and Pitcher 1986). Similarly, the action of growth retarding or enhancing substances may also be affected by relatedness (Licht 1967, Schvarts and Pyastolova 1970).

Breden et al. (1982) studied the orientation and size composition of *Bufo* schools and compared them with the structure of fish schools. They were also interested in the mechanism that resulted in the preferential grouping of siblings. They concluded that although size sorting and orientation occurred in laboratory and natural populations, they were less precise other commonly schooling species such as teleosts. The more interesting result was that if members of a sibling cohort with similar growth histories remain comparable in size, the tendency for similarly sized individuals to associate would lead to the patterns of sibling association observed by Waldman and Adler (1981) and Blaustein and O'Hara (1981). Thus, kin selection does not necessarily imply kin recognition and vice versa (O'Hara and Blaustein 1985). The

stimulus for non-random size association may be preferences for certain environmental gradients that change with development (Wassersug 1973) or ontogenetic shifts in specific habitat preference (Alford and Crump 1982, Alford 1986).

Interestingly, kinship recognition and association is retained for a short period after metamorphosis has been completed (Lillywhite 1974, Blaustein et al. 1984, Waldman 1989). This would be a potential restraint on dispersal but kin-recognition ability is be lost soon after metamorphs are fully transformed (Lillywhite 1974). Increased survival of *Bufo boreas* metamorphs was noted by Arnold and Wassersug (1978) when metamorphosing larvae actively formed aggregations. At this developmental stage, larvae were particularly vulnerable to predation by garter snakes (*Thamnophis* sp.). Metamorphically synchronous groups were able to satiate predators and increase individual survival probabilities. Thus, aggregation of metamorphs acts similarly to aggregation in tadpoles by increasing the probability of survival for individuals in the group.

If aggregation behaviour is a strategy that has developed in species of anurans at least partially as a defence against predation, what then are the anti-predator characteristics of larvae that do not aggregate?

1.1.5 Tadpole-Predator Interactions

Predation is a major influence on population and community structure (Connell 1983, Sih et al. 1985, Thorp 1986). It has been suggested that the higher predation pressure on larval stages in larger, more permanent systems has been a major cause of the evolutionary shift of anuran oviposition sites to small bodies of water (Roth and Jackson 1987). In anurans, predation can limit the colonisation of otherwise suitable habitats (Woodward 1983, Kats et al. 1988). Predation can alter the outcome of competitive interactions between species by removing otherwise dominant groups, thus reversing competitive hierarchies (Morin 1981). The major predators present in anuran communities are insects, in particular, dragonfly larvae and dytiscid beetle adults and larvae (Heyer et al. 1975, Heyer and Muedeking 1976, Smith 1983, Sheratt and Harvey 1989), crayfish (Fauth 1990), and vertebrate predators including salamanders (Morin 1983a, 1983b, Wilbur et al. 1983, Brodie and Formanowicz 1987), and fish (Duellman and Trueb 1986, Kats et al. 1988). Recent studies have also shown that tadpoles can be at risk from wading bird species (Crump and Vaira 1991).

Many characteristics of anuran larvae can reduce their vulnerability to predation. These include fast rates of growth to particular sizes and developmental stages that represent a size refuge or enable better swimming capabilities for escape (Wassersug and Sperry 1977, Crump 1984). Some larvae possess cryptic behaviour (Woodward

1983, see also discussion on aggregations above), are toxic and unpalatable (Wassersug 1971), or shift habitat preferences in the presence of predators (Morin 1986, Formanowicz and Bobka 1989). At least some of these characters are genetically determined, and can therefore evolve (Alford 1986a).

The evolution of unpalatability and aposematic colouration has been linked to the development of gregariousness in insects (Treisman 1975, Sillén-Tullberg and Leimar 1988). Similar advantages have been proposed for anuran species that display these traits (Waldman 1982). Toxicity and unpalatability are correlated and are important antipredator mechanisms in several anuran species (Formanowicz and Brodie 1982). Members of the Bufonidae, in particular the genus *Bufo*, have evolved highly toxic compounds that are present in all developmental stages from ovarian tissue to adults (Licht 1968, Wassersug 1971). Toxicity can vary with developmental stage (Walters 1975). Formanowicz and Brodie (1982) demonstrated that premetamorphic larvae of five anuran species (*Bufo americanus*, *Hyla crucifer*, *Rana sylvatica*, *R.palaustris*, and *R.clamitans*) were palatable to larval dytiscids. Palatability then decreased at metamorphosis, possibly due to the development of glandular toxins. Brodie and Formanowicz (1987) later found that hatchling stages of *Bufo americanus* were unpalatable to newts (*Notophthalamus*) and insect predators (*Belostoma* sp. bugs and *Anax junius* larvae). Interestingly, when larvae became more palatable with development, they were actively avoided by predators that had been exposed to the hatchling stages and probably associated them with distastefulness.

A second strategy for predator avoidance is the detection of chemical cues that indicate the presence of a predator. Petranka et al. (1987) showed experimentally that larval *Hyla chrysocelis* alter their activity in water that has been conditioned by predatory sunfish, *Lepomis cyanellus*. In the presence of chemical cues, tadpoles significantly increased their use of refuges. Morin (1986) demonstrated that *Hyla crucifer* larvae detect chemical cues; when newts are present, *Hyla crucifer* tadpoles shift their habitat use. This was either a response to chemical substances from newts or from tadpoles emitting alarm substances. Kats et al. (1988) demonstrated that unpalatability and chemical detection of predators are more prevalent in species that regularly occur in permanent ponds with fish. Temporary pond species not usually exposed to fish predation were rarely unpalatable, and did not react to predator-conditioned water by increasing time spent in refuges. This suggests that antipredator defences are most likely an evolutionary response to long term coexistence with fish predators. Skelly and Werner (1990) point out that this shift in spatial distribution to avoid predators may lead to increased times spent in sub-optimal habitats. Consequently, growth is affected, either reducing the size of the metamorphosing larvae or lengthening the developmental period (Wilbur and Collins 1973).

Several studies point out that rapid growth to large body sizes increases tadpole survival by making then too large to be handled by gape-limited predators such as fish and salamanders (Formanowicz and Brodie 1982, Travis et al. 1985,

Formanowicz 1986, Semlitsch and Gibbons 1988). Larger size also confers a better swimming ability and a greater capacity to escape capture (Semlitsch and Gibbons 1988, Richards and Bull 1990). Other predators such as dytiscids, crayfish and dragonfly larvae that either rip and chew prey or pierce and suck body fluids are not limited by prey size but tend to be restricted more by size independent characteristics such as avoidance behaviour and palatability.

Wassersug and Sperry (1977) noted that the metamorphosing larvae of *Pseudacris triseriata* were particularly vulnerable to predation by garter snakes (*Thamnophis sirtalis*). This was related to poor ability to escape from predators during this transition stage. The swimming ability of pre-metamorphic tadpoles and the mobility of post-metamorphic froglets was more effective than metamorphosing individuals. Consequently, the time spent in this stage was minimised to decrease vulnerability.

Larvae of *Hyla gratiosa* reduce predation pressure from salamanders and dragonfly larvae using a combination of the mechanisms mentioned above; rapid growth to a size refuge, different habitat preferences, bahavioural reponses to predators (immobility), and cryptic colouration while in the smallest, most vulnerable size classes (Caldwell et al 1980). In addition, regular deposition of eggs by laying females ensures that the smaller, more vulnerable tadpoles are in high abundance that can saturate predators.

Genetic control of vulnerability to predation has been investigated by Alford (1986b) and Semlitsch and Gibbons (1988). Alford noted that the effect of the male parent and an interaction between female parent, male parent and newt sex affected predation rate of *Hyla chyroscelis* tadpoles by newts (*Notophthalamus viridescens dorsalis*). Genotype appeared to control factors such as tadpole behaviour or palatability, and body mass. Semlitsch and Gibbons studied the same hylid species but with bluegill sunfish, *Lepomis macrochirus*. They also detected differences due to genotype but considered these as secondary to the effect of relative tadpole body size.

1.1.6 Summary

It can be concluded from the above review that the study of population dynamics even for single species systems is complex. For larval anurans, it involves integrating adult reproductive biology and larval ecology, which respond to many factors. The major effects on larvae are often related to density; both competition and predation are affected by it. To detect and quantify the effects of these factors, it is necessary to investigate a system using many approaches (Roughgarden 1983, Tilman 1987). The study of competition requires a multifaceted approach that is both observational and experimental. Experiments designed to test for the presence of specific mechanisms should be used to predict possible outcomes that can be

confirmed through observational and behavioural studies. Together, these help to derive hypotheses about particular responses that are testable through manipulation of population structure.

To develop a holistic theory for the patterns and processes that are observed in communities, we need to draw on models at individual and population levels. Research that integrates this approach to the study of competition (eg, Morin and Johnson 1988, Wilbur and Fauth 1990) are providing starting points for this step.

Chapter 2. Aims, Research Background, and Study Sites

2.1 Research Aims

This study considers aspects of the population dynamics of reproductive and larval populations of *Bufo marinus* (Bufonidae). It concentrates on aspects anuran ecology included in the previous review, namely breeding, larval growth, and predation. This species was chosen initially for a comparative study of two distinct populations (see section 2.3).

Specifically, this study:

(1) describes the structure and reproductive strategy of two populations of reproductive individuals in geographically isolated sites and produces quantitative estimates for recruitment patterns into aquatic habitats;

(2) describes the patterns of survival and growth of larval populations through all stages of development in the aquatic phase of the life history, with particular reference to the modifying effects of density, and;

(3) examines the effects of intraspecific competition including predation by conspecifics, and predation by other species.

This research is the first completed section of a long-term project set up to describe the population dynamics of introduced populations of *Bufo marinus* in Australia. Studies of the aquatic phase of the life-history are intended to be coupled with studies of the dynamics of the terrestrial phase. Knowledge of the rates of survival of each phase in the life-history and factors that have a controlling influence are required to derive simulation models of the populations studied.

2.2 Background to *Bufo marinus* Research in Australia

The toad, *Bufo marinus*, is a native inhabitant of lowland forest margins and grassland savanna habitats from 27°N latitude in Central America (Mexico) to 10°S latitude in central Brasil, South America (Zug and Zug 1979). Studies of *Bufo marinus* outside Australia have been conducted in Papua New Guinea (Zug et al. 1975) and within its native distribution in Panama (Zug and Zug 1979). These studies found that it is a nocturnal species. The adults are sexually dimorphic and mature at approximately 85 to 90mm snout vent length but can reach 230mm. They feed on an extremely wide range of prey items from carrion to juvenile conspecifics, but numerically dominant components of the litter fauna, such as ants and beetles, tend to contribute most to the diet biomass (Strüssmann et al. 1984). However, these studies did not document reproductive output or the larval ecology of the species.

Toads were introduced to Australian habitats on 22 June 1935 to control beetle pests associated with commercial sugar-cane crops (Mungomery 1935). Freeland (1984) reviewed the literature on *Bufo marinus* including studies on toads in Australia. He also assessed the likely impact that toads were having on Australian native fauna.

Studies conducted on introduced populations of *Bufo marinus* in Australia include an histological investigation of reproductive cycles (Wilhoft 1965) and a series of technical reports to the Australian National Parks and Wildlife Service (Van Beurden 1978, 1979, 1980a, 1981). These covered ecological and physiological aspects of populations in Queensland and New South Wales. Shortly thereafter, a series of physiological studies were published on thermal requirements of larvae (Van Beurden 1880b, Floyd 1983, 1984, 1985). In addition, further research documented the rate of colonisation since introduction (Easteal 1981, Sabath et al. 1981, Easteal et al. 1985, Freeland 1986, Freeland et al. 1986a) and recorded parasitic infections in adult toads (Freeland et al. 1986b). There have also been studies of the ecology of toad interactions with adult tropical native frog populations (Freeland and Kerin 1988) and ontogenetic changes in habitat use of juvenile toads (Freeland and Kerin 1991). Nothing has been published on the ecology and population dynamics of the larval stage except a minor contribution from Van Beurden (1979, 1980a) who looked at fecundity and noted development times of tadpoles in natural pools.

2.3 Study Sites

Two sites, Mt Margaret Station near Townsville, and Calvert Hills Station near Borroloola, were chosen for this study. Both sites were subject to grazing by cattle but differed in the time since *Bufo marinus* had colonised. Toads were first recorded from the Townsville district around 1939 (Freeland 1986) and from the Calvert River in 1986 (RA Alford, pers comm).

Mt Margaret Station is located 25km west of Townsville, Queensland, at the base of Hervey's Range (19°21' S 145°36' E, Figure 2.1). The study population was centered around a dam on the property in habitat consisting of dry, open sclerophyll and eucalypt forest with a grassy understorey. The dam embankment was constructed prior to 1987 and blocked the flow of a concourse of smaller creek channels. The region behind the wall was excavated to create a pond, five metres deep in the centre, and roughly rectangular in shape, approximately 60 by 30 metres. During the wet season the dam fills and floods the surrounding catchment, creating a large region of shallow flooded grasslands. This slowly recedes throughout the dry season until it becomes a series of deep channels (0.5 to 2 metres) terminating in a pool behind the dam embankment. The margin of the dam along the wall slopes steeply into the water for a 50 metre distance but elsewhere margins are shallow for up to 5 metres.

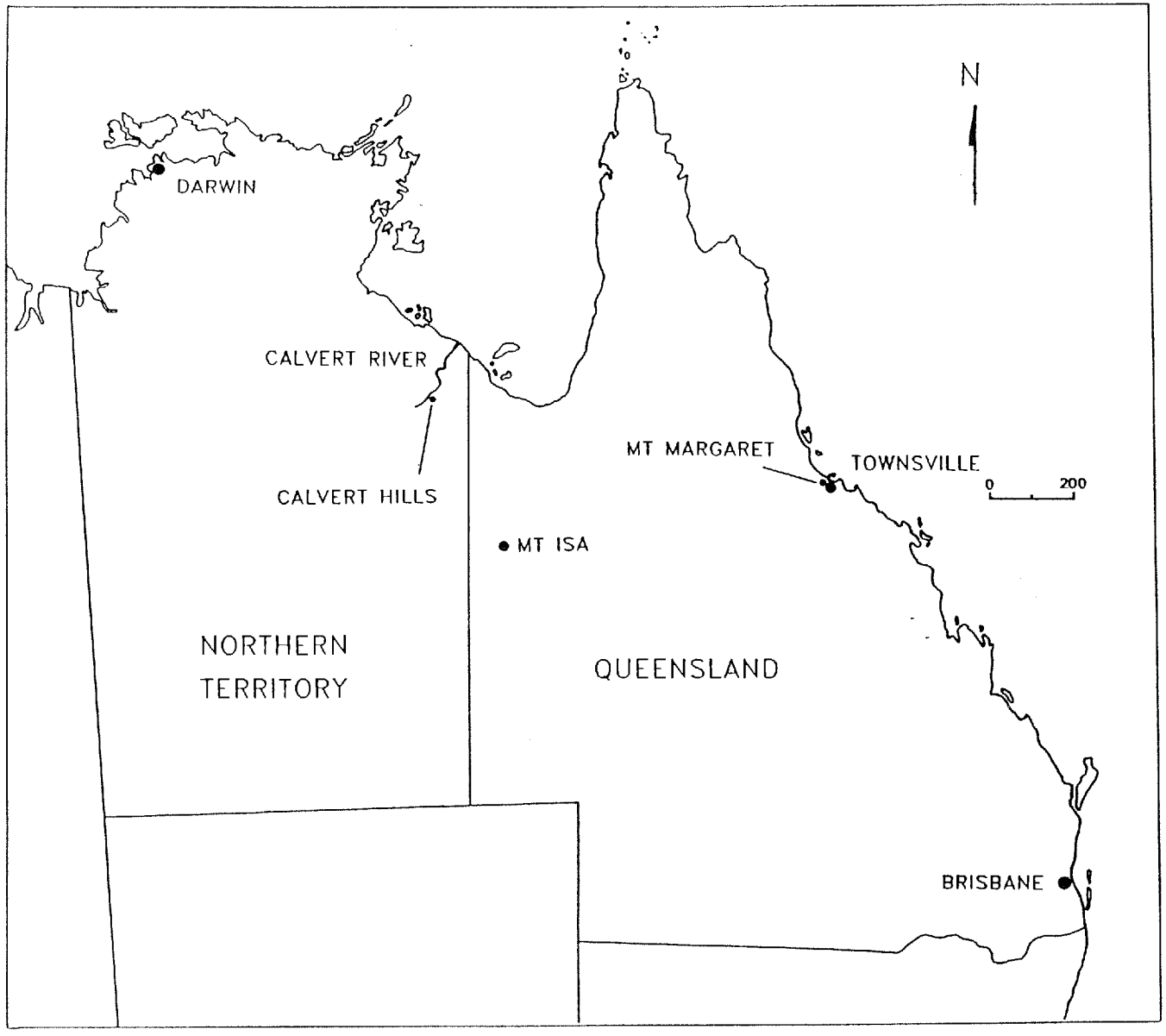
Calvert Hills Station is located on the Calvert River in the Northern Territory (17°45' S 137°19' E, Figure 2.1). The banks of the river are forested with *Melaleuca* and *Pandanus*. Surrounding habitat is predominantly dry, open sclerophyll and eucalypt forest and grassland. At most times of the year except when in spate, the section of the river used as a study site experienced little or no flow. By early dry season, river levels contracted to leave a series of elongate ponds, roughly 100 to 200 metres long and 5 to 20 metres across. The ponds were usually delineated up and downstream by regions of exposed rock strata. Margins were generally shallow sloping on one bank and at either end, and steeper on the opposite bank.

Other anuran species recorded during preliminary work at both study sites are shown in Table 2.1. Laboratory and experimental pond studies conducted in Townsville were carried out at the James Cook University of North Queensland's Zoology Department and the experimental aquaculture compound located on University grounds.

Table 2.1 List of adult anuran species recorded at both study sites.

Species	Mt Margaret	Calvert Hills
Crinia remota	*	
Cyclorana australis		*
Cyclorana brevipes	*	
Cyclorana maculosis		*
Cyclorana novaehollandiae	*	
Limnodynastes ornatus	*	*
Limnodynastes tasmaniensis	*	
Litoria alboguttata	*	
Litoria bicolor	*	*
Litoria caerulea	*	*
Litoria gracilenta	*	
Litoria inermis	*	*
Litoria nasuta	*	*
Litoria pallida		*
Litoria rothi	*	*
Litoria rubella	*	*
Litoria wotjulumensis		*
Uperoleia lithomoda	*	*
Uperoleia mimula	*	

Figure 2.1 Map of Queensland and the Northern Territory showing the location of each of the study sites, Mt Margaret Station (Q), and Calvert Hills Station (NT).


N
DARWIN
CALVERT RIVER
CALVERT HILLS
MT MARGARET
TOWNSVILLE
0
200
MT ISA
NORTHERN
TERRITORY
QUEENSLAND
BRISBANE

Chapter 3. Reproductive Ecology

3.1 Introduction.

The activity patterns of *Bufo marinus* have been studied with respect to reproductive behaviour (Zug and Zug 1979), foraging (Freeland and Kerin 1988) and male mating success (Lee 1986). Activity of mature individuals at water, either foraging or reproductive, is almost exclusively nocturnal, concentrated in the first few hours after dusk. Populations near water are frequently male biased, particularly during periods of breeding. Only a fraction of the total population (31-50% for Panama, Zug and Zug 1979) of adults is active on any one night even under the most ideal conditions.

Members of the genus *Bufo* are typically monobrachyandrous (oviposit all eggs in a single spawning), laying large numbers of eggs (in 'strings') in shallow water. Eggs develop into feeding larvae that eventually metamorphose into the terrestrial habitat. This is the most common reproductive mode in anurans (Salthe and Duellman 1973, Salthe and Mecham 1974).

Many tropical bufonids breed explosively (Brown and Alcala 1983, Wells 1977). This involves a short breeding period (a few days), usually during the first rains of the warmer, wetter months in the tropics. High densities of males collect at breeding sites such as ponds and ephemeral pools. Opportunistic breeding is common. Opportunistic species breed over longer periods, ranging from weeks to months. Some may reproduce at any time conditions are favourable.

Studies of breeding populations of *Bufo marinus* are few but have shown that opportunistic behaviour, with males calling over extended seasons, is the predominant pattern. In the Philippines where bufonids display three distinct reproductive patterns (Brown and Alcala 1983), *Bufo marinus* used rain-filled puddles and slow moving streams for egg deposition. In Panama, breeding activity was sporadic but showed definite bimodal concentrations with season (Zug and Zug 1979). Ponds were used during the early wet when they were full, but activity switched to streams in the early dry after the cessation of flow. Similarly, in a study at Panguana in the Amazon Basin, *Bufo marinus* used streams during the early dry when flow was limited or had ceased (Aichinger 1987). Studies of *Bufo marinus* reproduction in Japan indicate year-round breeding (Maeda and Matsui 1989, cited in Kusano et al. 1991).

Within Australia, the breeding characteristics of *Bufo marinus* were studied initially by Wilhoft (1965) who investigated the histological condition of the gonads of males and females over a twelve month period. The testes contained gametes in a continuous cycle of spermatogenesis (some mature sperm) with a pool of males in each sample possessing predominantly mature spermatocytes. Females were similar; individuals with mature ovaries occurred in all samples. Over 50 percent of females were gravid in eight of the twelve months. This pattern of gamete maturation is also evident for populations in Papua New Guinea (Zug *et al.* 1975) and within the natural distribution of *Bufo marinus* (Zug and Zug 1979).

Studies of *Bufo marinus* fecundity are few. Zug and Zug (1979) demonstrated a power relationship with snout vent length (egg number=0.0006 $SVL^{3.497}$) but a small sample (n=8) and a limited range of female body size make this relationship tenuous. Van Beurden (1980a) revealed a very similar relationship (egg number=0.001 $SVL^{3.4}$) which explained 50 percent of the variation in egg number.

No studies have ever attempted to estimate the recruitment of eggs from specific breeding populations into the larval habitat for any bufonids.

This study aimed to examine the reproductive ecology of *Bufo marinus* at Calvert Hills and Mt Margaret using several techniques. First, to determine the size and sex structure of breeding adult populations while monitoring egg recruitment into the aquatic stage. Second, to determine the timing of reproductive cycles and the relationships of body size by histological examination of females. Third, to examine seasonal rates of recruitment by relating observed breeding events with fecundity and activity.

3.2 Methods.

3.2.1 Reproductive Activity.

Adult populations were monitored in 250 x 10 m transects along water bodies. At the Calvert River, populations were monitored during January-February and May, 1988. (For August and November 1988, all larval habitat was dry or nearly dry and on preliminary observation, no reproductive activity was observed. For these periods, a population estimate was determined over a three night period). At Mt Margaret Dam, populations were monitored during March, June, and November, 1989. Similarly for November 1989, populations were estimated over a three-night sampling period. In each sampling period, transects were walked, starting approximately one hour after sunset, nightly for 15-30 nights.

During each transect walk the activity (amplexus, calling, sitting) and sex of each adult toad (SVL $\geq$ 90mm) encountered in the water or within 10 metres of the water were noted. Toads were captured, marked, their SVL was measured, and they were then released. Females were also inspected for gross reproductive condition. The presence of gravid or near gravid ovaries in females was detected by inducing them to inflate their lungs and holding them upside-down over a strong torch beam. The darker ovary tissue was visible over the more translucent tissue of the lungs. All individuals encountered for the first time were toe-clipped with a unique number. Recaptured individuals were noted. Jolly-Seber (Jolly 1965, Seber 1965) and

Petersen estimates (Caughley 1977) were used to calculate mark-recapture model estimates of population size. The transects were walked a second time each morning after sunrise, and the water margin checked for the presence of egg strings. Their locations were recorded and marked for larval studies (see Chapter 4).

3.2.2 Gonad Condition and Fecundity.

During most sampling periods, at least 20 males and 20 females were collected from breeding habitats adjacent to the transect site where activity was recorded. For Mt Margaret Dam during July 1989, only eleven females were found, and during November 1989, only fourteen males could be found. All animals were weighed and their SVL measured. They were then dissected and their gonads transferred to potassium-buffered, 10 percent formalin. These were processed in the laboratory to determine a number of parameters.

For each individual, each gonad was blotted dry, weighed and measured for volume. Ovaries were scored on a visual maturity index (VMI) according to the scheme presented in Table 3.1. For males, an entire testis, or for females, a portion of the ovary was sectioned and stained in haematoxylin and eosin, for histological examination. For testes, the presence of mature sperm and the condition of the interstitial epithelial cells were noted. In addition, the diameter of ten seminiferous tubules from each individual was measured using an eyepiece micrometer. The

Table 3.1. Definition of Visual Maturity Indices 1-5 for ovary examinations.

Gonad stage (VMI)	Description
1	ovary transparent, small, oocytes not discernible unless under a dissecting microscope.
2	ovary white, small, oocytes discernible within tissue, no pigmentation.
3	ovary mottled, small to large oocytes larger and easily discernible black, brown and cream in colour.
4	ovary dark, large, oocytes large, approximately 50 percent pigmented black.
5	ovary dark, large, oocytes large, approximately 95 percent pigmented black.

condition of the tubules and interstitial cells has been reported to correlate with sperm production in *Bufo bufo* (Obert 1977). In ovary tissue, the condition of the eggs (presence or absence of yolk and pigment) was noted.

Ovaries at VMI stage 5 were examined using the following procedure. First, a subsample of approximately 10 percent of the total weight was removed from the ovary. The sample was weighed and individual eggs were separated from the mesentery. The eggs in the subsample were counted using a dissecting microscope. This number was used to determine total number for the ovary by proportion. Second, ten eggs were selected haphazardly from the subsample and their diameter measured using an optical micrometer. Diameter was used to calculate the volume for each egg estimated from the formula:

$$Egg\ Volume = \frac{4}{3}\pi r^3$$

where r is the radius of the egg in millimetres.

Amplexing females commence laying prior to dawn and in some cases are still laying 3 to 4 hours after sunrise (Floyd 1983b; personal observation). To show that females release all eggs in a single spawning event, a sample of females was collected from a site in Townsville after a period of heavy rain. Females were monitored and collected immediately after completing egg-laying. Individuals were collected if they were observed in the final stages of amplexus; usually still laying eggs or in the immediate vicinity of egg strings. In some cases, females were

observed resting next to an egg string with the terminal segments of the string being released from the cloaca. Ten females were collected and transferred to the laboratory for dissection and examination of the ovaries.

3.2.3 Recruitment Estimates.

Recruitment was determined on a daily basis for each site and season, using data collected from the activity and fecundity sections of this study. The number of egg masses deposited was observed for each night during the sampling period. For any particular sampling night, the mean snout-vent length of recorded females was calculated, and the exact snout vent length of females observed breeding was known. Recruitment was calculated from these data by multiplying the number of egg masses found and the fecundity for the mean body length of females in the sample from the previous night.

3.3 Results.

For analysis, the months during which sampling took place were categorised into seasons. Samples taken between January and April were deemed wet-season, those taken between May and July deemed early dry season, and those between August and December deemed late dry season. The dry season samples were pooled in some analyses.

3.3.1 Reproductive Activity

Mark-Recapture Estimates

The data used to estimate the population parameters using the mark recapture models and estimates for the Petersen model are presented in Table 3.2. Estimates from these data using the Jolly-Seber and Bailey's Triple Catch models are given in Appendix 1. Data are presented separately for males and females due to seasonal variables acting independently on the activity patterns of each sex (R.Alford and M.Cohen, pers. comm). The Jolly-Seber model for all female populations at Mt Margaret returned non-estimates due to the small proportion of the marked females recaptured. For Calvert Hills, with the exception of the November sample, the standard error of almost all Petersen or Triple-catch estimates are 50 to 140% of the

Table 3.2 Data and parameters for mark-recapture models and population estimates using the Petersen Model. Data for males and females presented separately as shown.

Parameters are:

ni - total captures
ri - total released
mi - total marked in the sample
yi - total subsequent recaptures
zi - previous releases that were not captured on day i but recaptured subsequently
Mi - total marked animals in the sample immediately preceding the ith sample

N(est) - Petersen estimate (times 3..n use weighted mean technique)
SE(N) - standard error of the estimate

Site: Calvert Hills

Males										Females									
Date	Day	ni	ri	mi	yi	zi	Mi	N(est)	SE(N)	Date	Day	ni	ri	mi	yi	zi	Mi	N(est)	SE(N)
20/01/88	1	29	29		22					20/01/88	1	2	2		0				
21/01/88	2	36	36	6	19	16	36	153.29	48.80	21/01/88	2	1	1	0	0	0	0		
22/01/88	3	71	71	12	21	23	90	275.42	66.93										
23/01/88	4	60	60	9	24	35	97	439.75	86.33										
24/01/88	5	20	20	9	9	49	118	424.14	71.74										
25/01/88	6	3	3	2	0	56	0	416.23	68.47										
26/01/88	7	6	6	3	4	53	83	412.36	65.23										
27/01/88	8	6	6	3	1	54	327	409.40	62.46	27/01/88	3	2	2	0	0	0	0		
28/01/88	9	4	4	1	1	54	217	416.76	62.85	28/01/88	4	1	1	0	0	0	0		
30/01/88	10	25	25	5	7	50	184	469.04	67.03	30/01/88	5	6	6	0	0	0	0		
31/01/88	11	28	28	13	8	44	167	465.64	59.15	31/01/88	6	11	11	0	0	0	0	NON EST	
01/02/88	12	18	18	7	3	45	277	476.77	57.41	01/02/88	7	6	6	0	0	0	0		
09/02/88	13	62	62	21	22	27	97	526.99	55.56	09/02/88	8	2	2	0	1	0	0		
10/02/88	14	15	15	8	5	41	131	526.38	53.18										
11/02/88	15	8	8	2	3	43	117	538.33	53.84	11/02/88	9	1	1	0	0	1	0		
12/02/88	16	16	16	5	1	41	661	556.54	54.32										
13/02/88	17	25	25	11	3	31	269	568.43	52.78										
14/02/88	18	20	20	6	1	28	566	591.73	53.58	14/02/88	10	5	5	0	0	1	0		
15/02/88	19	25	25	7	4	22	144	622.90	54.85	15/02/88	11	8	8	0	0	1	0		
16/02/88	20	17	17	6	3	20	119	638.68	54.97	16/02/88	12	9	9	0	2	1	5		
17/02/88	21	24	24	14	3	9	86	636.36	52.14	17/02/88	13	11	11	1	0	2	0	931.50	1232.26
18/02/88	22	26	26	12				648.21	51.09	18/02/88	14	7	7	2				577.75	395.56
06/05/88	1	46	46		20					06/05/88	1	5	5		2				
07/05/88	2	44	44	4	28	16	29	414.00	159.35	07/05/88	2	2	2	0	0	2	0	NON EST	
08/05/88	3	40	40	13	20	31	75	303.56	76.04	08/05/88	3	7	7	1	4	1	3	29.50	39.02
09/05/88	4	47	47	21	20	30	91	276.28	45.45	09/05/88	4	4	4	0	0	5	0	55.50	73.42
10/05/88	5	34	34	17	18	32	77	276.80	37.68	10/05/88	5	5	5	4	2	1	7	32.67	16.33
12/05/88	6	34	34	16	12	34	112	288.96	34.54	12/05/88	6	1	1	0	0	3	0	35.67	17.83
13/05/88	7	24	24	6	6	40	166	320.27	36.74	13/05/88	7	2	2	1	0	2	0	36.00	16.15
14/05/88	8	22	22	14	10	32	84	317.45	33.47	14/05/88	8	2	2	1	0	1	0	36.50	14.96
15/05/88	9	30	30	10	8	32	130	345.15	34.52	15/05/88	9	1	1	0	0	1	0	39.13	16.04
16/05/88	10	25	25	10	6	30	135	363.44	34.66	16/05/88	10	1	1	1	1	0	1	37.22	14.13
18/05/88	11	24	24	14	9	22	73	376.82	33.03	18/05/88	11	1	1	0	0	1	0	39.67	15.05
19/05/88	12	35	35	21	3	10	138	373.61	31.03	19/05/88	12	3	3	1	0	0	0	42.60	15.12
20/05/88	13	15	15	13				376.53	29.24	20/05/88	13	1	1	0				45.10	16.01
10/08/88	1	17	17							10/08/88	4	4							
11/08/88	2	19	19				21	68.00	24.04	11/08/88	9	9					6	20.00	10.33
12/08/88	3	14	14					51.40	14.29	12/08/88	8	8						33.00	22.59
01/11/88	1	20	20							01/11/88	51	51							
02/11/88	2	17	17				15	51.43	14.21	02/11/88	46	46					33	149.81	29.51
03/11/88	3	21	21					66.07	18.37	03/11/88	41	41						172.97	31.09

Table 3.2 : continued.

Site: Mt Margaret

Males										Females									
Date	Day	ni	ri	mi	yi	zi	Mi	N(est)	SE(N)	Date	Day	ni	ri	mi	yi	zi	Mi	N(est)	SE(N)
05/03/89	1	21	21		10														
06/03/89	2	31	31	3	9	7	27	168.00	70.28										
07/03/89	3	27	27	7	6	9	48	179.45	60.04	07/03/89	1	1	1		0				
08/03/89	4	7	7	3	1	12	87	175.50	50.81										
09/03/89	5	2	2	0	0	13	0	185.93	53.83										
10/03/89	6	2	2	1	0	12	0	183.53	51.04										
12/03/89	7	1	1	0	0	12	0	188.60	52.45	11/03/89	2	1	1	0	0	0	0		
13/03/89	8	2	2	1	1	11	23	186.44	49.95										
14/03/89	9	2	2	2	2	10	12	174.39	43.69									NON EST	
15/03/89	10	1	1	1	1	11	12	169.32	41.14										
16/03/89	11	18	18	5	4	7	37	192.54	41.10	16/03/89	3	2	2	0	0	0	0		
17/03/89	12	32	32	7	4	4	39	243.00	45.16	17/03/89	4	3	3	0	0	0	0		
18/03/89	13	14	14	4	1	4	60	261.63	45.57	18/03/89	5	1	1	0	0	0	0		
19/03/89	14	12	12	4	0	1	0	NON EST											
21/03/89	15	1	1	1				NON EST		20/03/89	6	1	1	0					
21/06/89	1	5	5		5					21/06/89	1	1	1		0				
22/06/89	2	7	7	0	4	5	9	NON EST		22/06/89	2	1	1	0	0	0	0		
25/06/89	3	2	2	1	2	8	9	29.50	39.02	25/06/89	3	1	1	0	0	0	0		
26/06/89	4	4	4	2	3	8	13	27.75	19.00	26/06/89	4	3	3	0	0	0	0		
27/06/89	5	3	3	2	2	9	15	26.00	13.00	27/06/89	5	4	4	0	1	0	0		
28/06/89	6	11	11	6	5	5	17	27.67	8.78	28/06/89	6	2	2	0	0	1	0		
30/06/89	7	5	5	2	2	8	22	31.21	9.04	30/06/89	7	1	1	0	0	1	0		
01/07/89	8	5	5	1	2	9	23	37.13	10.33	01/07/89	8	1	1	0	0	1	0		
02/07/89	9	17	17	6	5	5	23	49.19	11.30	02/07/89	9	4	4	1	0	0	0	68.50	92.62
03/07/89	10	16	16	8	1	2	40	57.14	11.01	03/07/89	10	3	3	0	0	0	0	94.20	124.35
04/07/89	11	9	9	3				65.00	11.88	04/07/89	11	1	1	0				104.00	137.58
01/11/89	1	8	8							01/11/89	1	13	13						
02/11/89	2	26	26				1	108.00	60.00	02/11/89	2	16	16				2	73.67	33.43
03/11/89	3	13	13					159.25	109.03	03/11/89	3	10	10					159.33	140.52

total number. Thus minimum female population size is best inferred from the total observed numbers of females encountered during the sampling period.

Jolly-Seber estimates for male numbers at all sites vary greatly from night to night. The probability of capturing individual males (and females) almost certainly remains the same over the period of the sample, so one would expect similar estimates given the short periods over which sampling occurred (15, 16, 18 and 30 nights). The Petersen models for the same data have standard errors of 25% or less of the estimate and are used for comparisons.

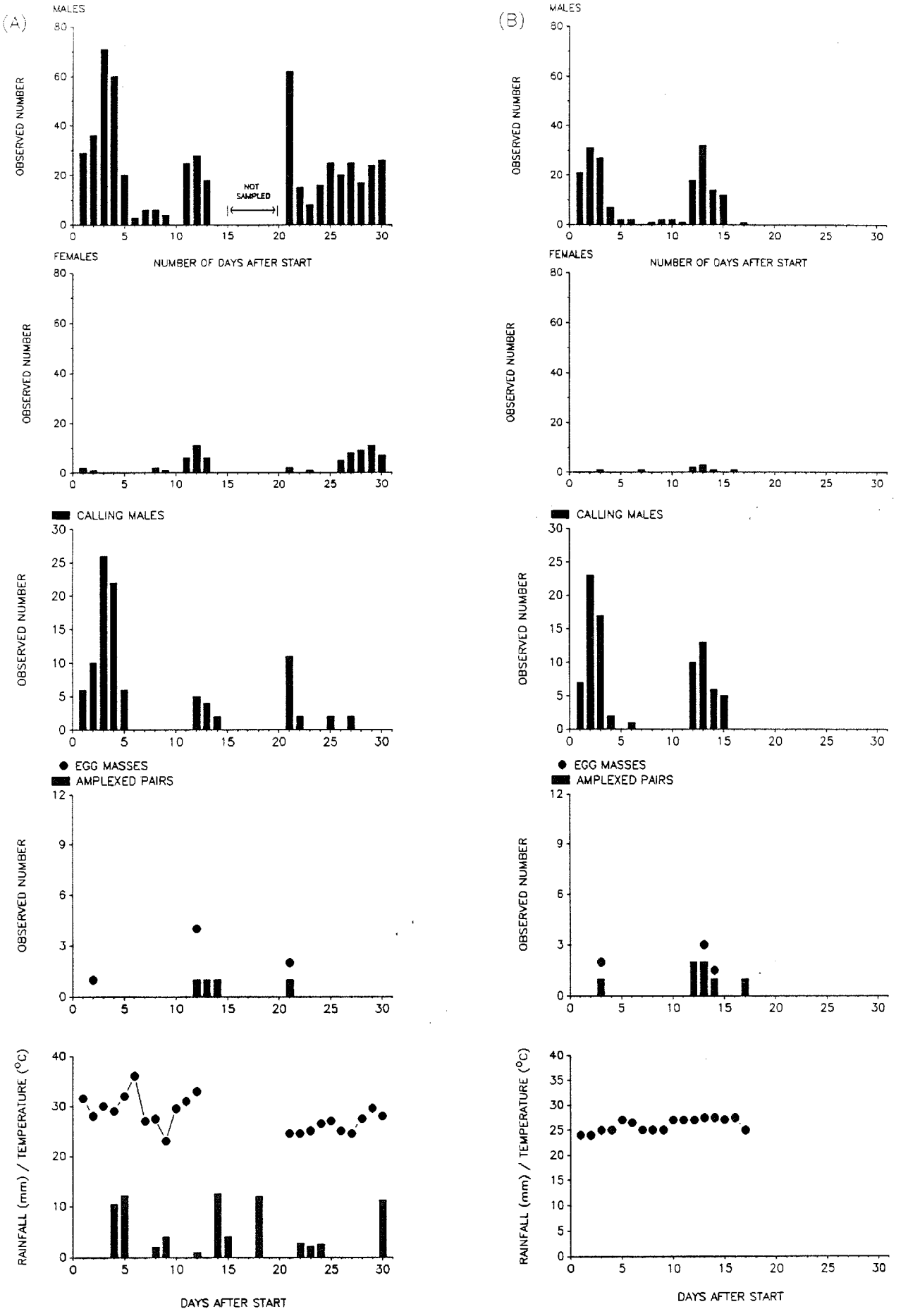
Population sizes of males (per 250m transect of habitat) were approximately 2.5 times larger at Calvert Hills than at Mt Margaret during the wet season and up to 5 times larger in the early dry season. Late dry season estimates for Mt Margaret are higher those for Calvert Hills. Estimated male population sizes declined from the wet through the dry season while female populations tended to increase.

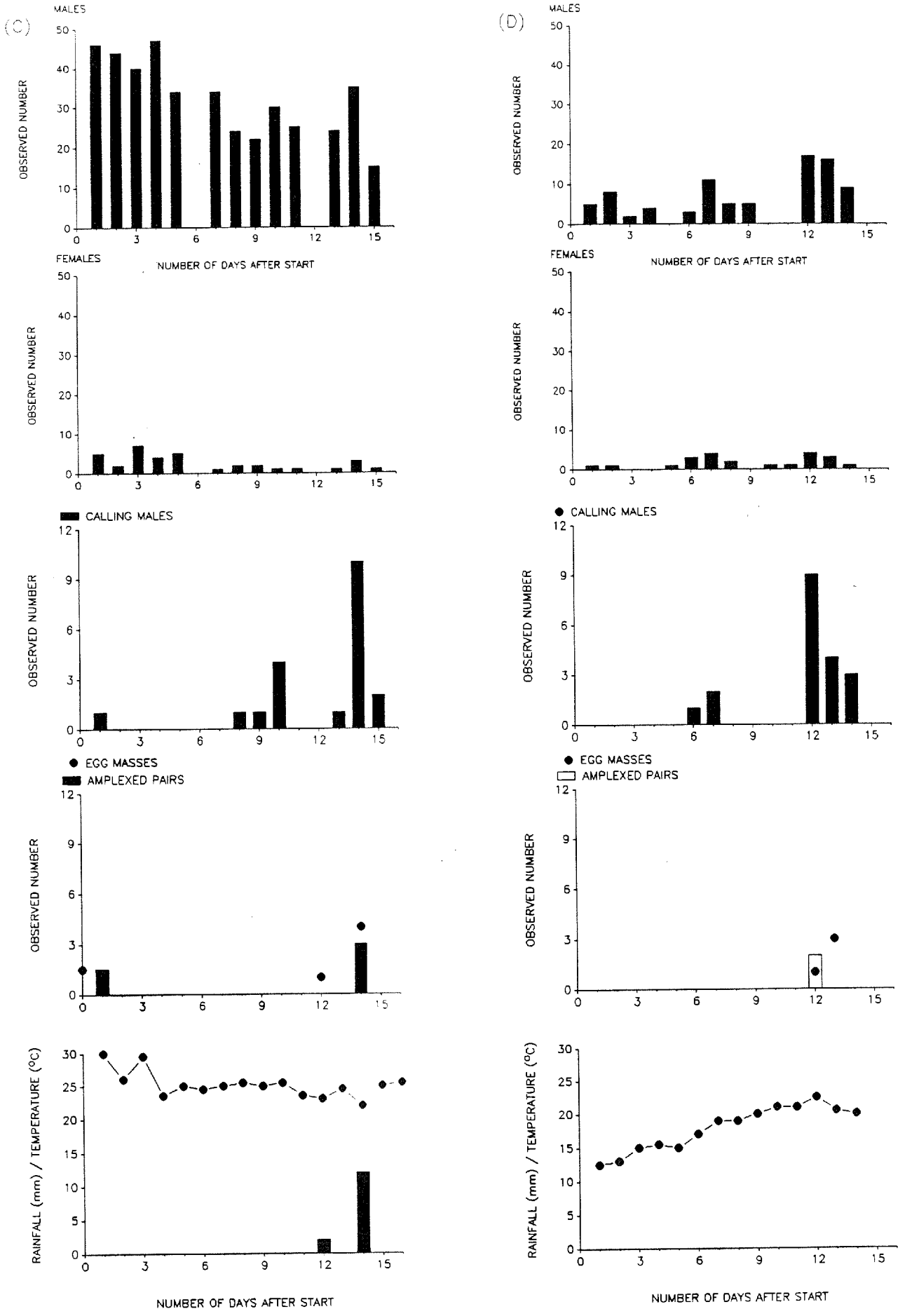
Numbers of males and females and numbers observed calling and amplexing during transect samples are presented in Figure 3.1 along with environmental data (rainfall and air temperature). Male numbers were higher in all sampling periods except the November sample (late dry season) at Calvert Hills (Figure 3.1E). Correlation analyses for all number comparisons and number-environmental variable comparisons are presented in Appendix 2. Relationships between variables discussed below apply principally to the data collected for Calvert Hills adults as sample periods were

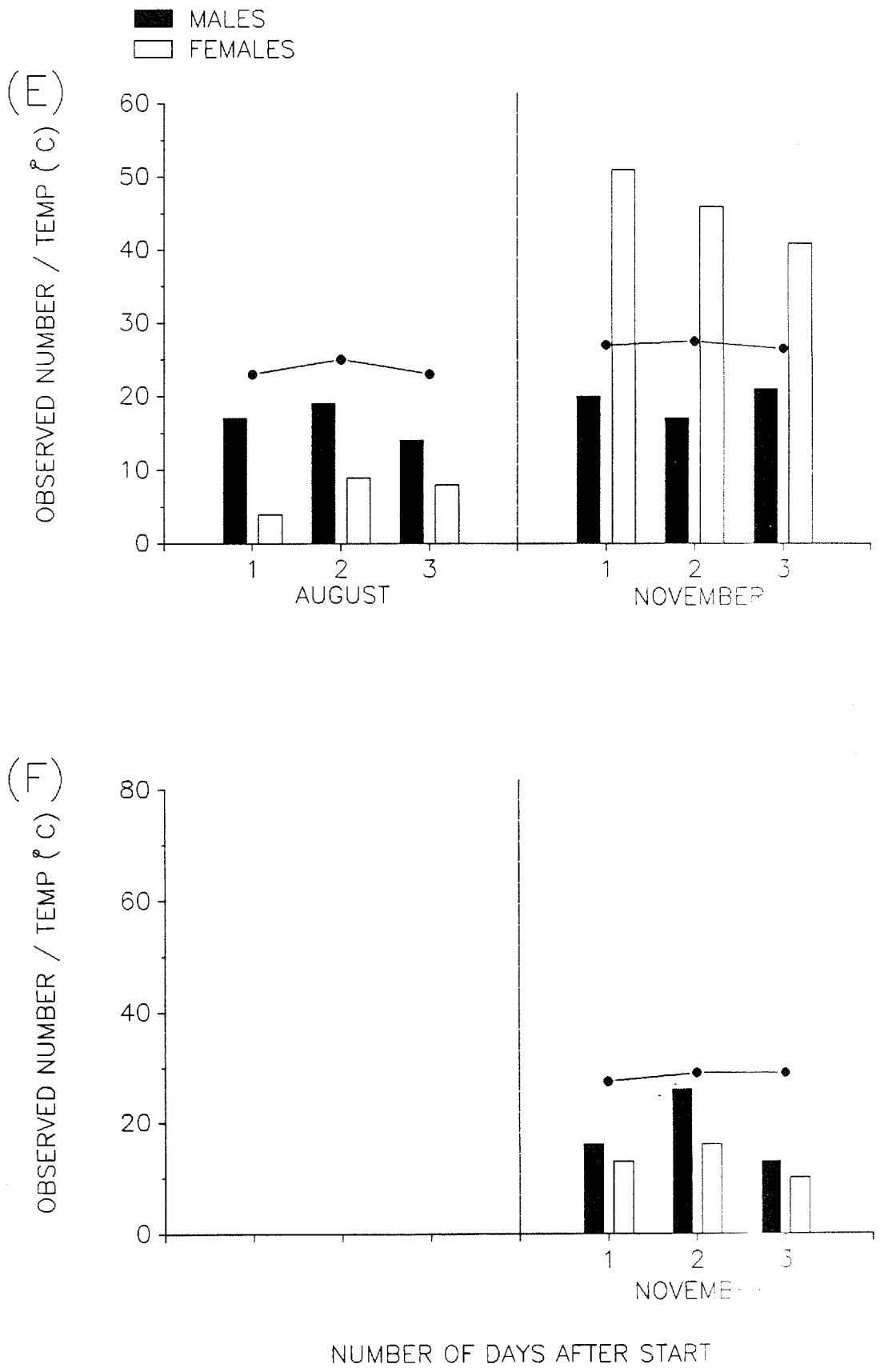
Figure 3.1 Observed numbers of individuals (males, females, calling males, amplexed pairs) and environmental data (rainfall, temperature) recorded during night transects. The x-axis is labelled as the number of days after the first night of the transect period, as indicated by (A) to (F) below.

(A) Calvert Hills: 21 January - 19 February 1988
(B) Mt Margaret: 03 March - 21 March 1989
(C) Calvert Hills: 05 May - 20 May 1988
(D) Mt Margaret: 21 June - 04 July 1989
(E) Calvert Hills: 10-12 August 1988 / 01-03 November 1988
(F) Mt Margaret: 01-03 November 1989

For Figures 3.1E and 3.1F, males and females are shown together with corresponding temperature recording. No rainfall, amplexus or spawning were observed.


(A)
MALES
OBSERVED NUMBER
NOT SAMPLED
FEMALES
NUMBER OF DAYS AFTER START
CALLING MALES
EGG MASSES
AMPLEXED PAIRS
RAINFALL (mm) / TEMPERATURE (°C)
DAYS AFTER START
(B)


(C)
MALES
OBSERVED NUMBER
FEMALES
OBSERVED NUMBER
CALLING MALES
OBSERVED NUMBER
EGG MASSES
AMPLEXED PAIRS
OBSERVED NUMBER
RAINFALL (mm) / TEMPERATURE (°C)
NUMBER OF DAYS AFTER START
(D)
MALES
OBSERVED NUMBER
FEMALES
OBSERVED NUMBER
CALLING MALES
OBSERVED NUMBER
EGG MASSES
AMPLEXED PAIRS
OBSERVED NUMBER
RAINFALL (mm) / TEMPERATURE (°C)
NUMBER OF DAYS AFTER START


MALES
FEMALES
(E)
OBSERVED NUMBER / TEMP (°C)
60
50
40
30
20
10
0
1
2
3
AUGUST
1
2
3
NOVEMBER
(F)
OBSERVED NUMBER / TEMP (°C)
80
60
40
20
0
1
2
3
NOVEMBER
NUMBER OF DAYS AFTER START

generally longer and numbers higher. However, due to the relatively short sampling periods that were possible in this study, the responses of toads to fine-scale environmental effects may have obscured meaningful seasonal and site comparisons.

Simple linear Pearson correlations of numbers with any environmental variable are poor for wet-season periods at both sites. The only significant relationship is the correlation between percent moon illumination and numbers of amplexing pairs ($r^2=0.8565$ $p=0.0001$), hence also the relationship with gravid females. This relationship is also highly significant for the early dry season (see below). For the early dry season when breeding was still occurring, temperature, rainfall and illumination all correlate with numbers. Moon illumination is of interest as both males ($r^2=0.7138$ $p=0.0061$) and females ($r^2=0.5905$ $p=0.0336$ for non gravid females) respond similarly to this factor but differently to temperature and rainfall. Increases in male numbers are more closely associated with declining temperatures ($r^2=-0.6322$ $p=0.0274$) and previous 24-hour rainfall ($r^2=-0.5565$ $p=0.0482$) whereas female numbers increase with higher (but not necessarily increasing) air temperatures ($r^2=0.5625$ $p=0.0224$ for all females and $r^2=0.6838$ $p=0.0001$ for non gravid females). Numbers of males calling ($r^2=0.8303$ $p=0.0004$) and the number of amplexing pairs ($r^2=0.9463$ $p=0.0001$) both correlate highly with rainfall within a 48 hour period.

An analysis of the size of males and females at each sampling period (recapture records excluded) are presented in Table 3.3. All main effects and interactions are

Table 3.3 Analysis of size (snout-vent length) for samples of males and females from each site and season.

Levels for each treatment:

Site : Calvert Hills / Mt Margaret
Sex : Male / Female
Season : Early Dry / Late Dry / Wet

Source of Variation	df	Sum of Squares	Mean Square	F	p
Model	11	8124.9667	738.6333	15.16	0.0001
Site	1	423.0708	423.0708	8.68	0.0033
Season	2	2631.0848	1315.5424	27.00	0.0001
Site*Season	2	572.7693	336.3846	6.90	0.0010
Sex	1	6142.5416	6142.5416	126.09	0.0001
Site*Sex	1	291.9786	291.9786	5.99	0.0145
Season*Sex	2	1126.7891	563.3945	11.56	0.0001
Site*Sex*Seas	2	860.6348	430.3174	8.83	0.0002
Residual	1709	83256.7776	48.7166		
Total	1720	91381.7443			

significant. Of the main effects, (Figure 3.2A), the overall mean size of females is larger than that of the males, mean sizes of both sexes decrease toward the late dry, and are larger at Mt Margaret (due mainly to the larger mean size of the female population, Figure 3.2B). At the highest level of interaction (Figure 3.2C), mean female sizes are smaller during the wet-season at Calvert Hills, and decline from wet to late dry season, though mean male sizes at both sites show little variation.

Sex Ratio

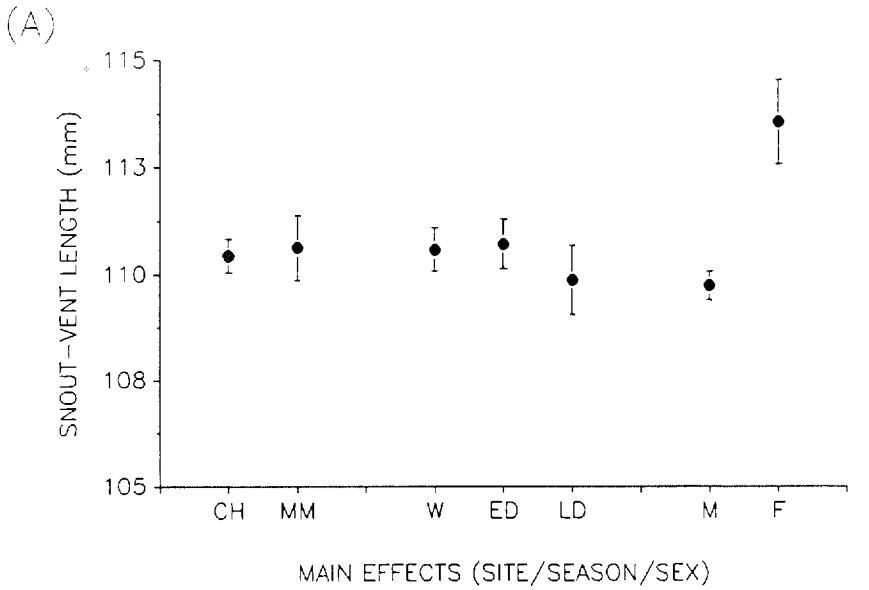
The proportion of individuals active within the transect that were males, shown in Figures 3.3A and 3.3B, varied between 0.70 and 1.00 for both wet and early dry season periods at both sites. The proportion of active females in the total sample increases toward the late dry season from a ratio of 0.1 of the population during the wet-season to 0.6 (Calvert Hills, χ^2 test, $p<0.0001$) and 0.4 (Mt Margaret, χ^2 test, $p<0.0001$) at the end of the dry season (Figure 3.3C).

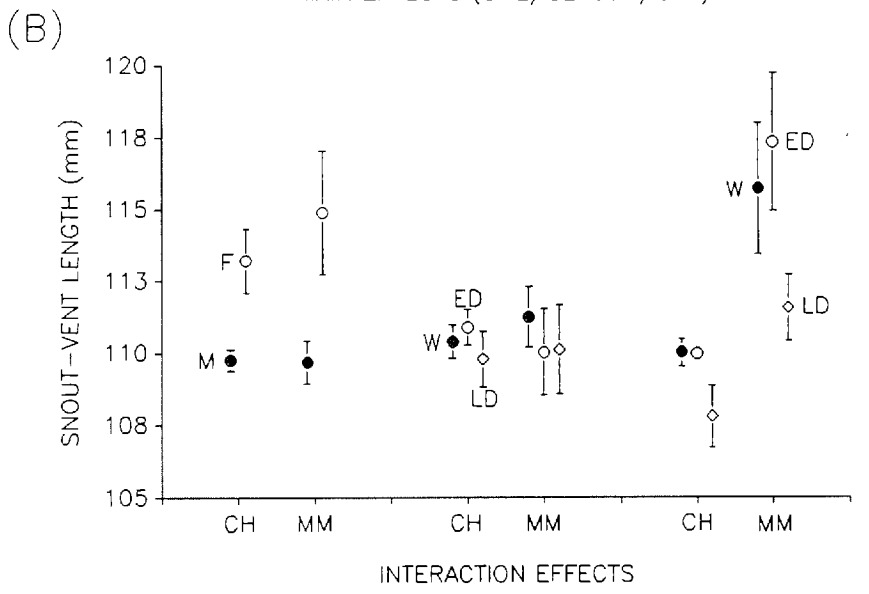
Behaviour

The percentages of adults in the samples displaying different behaviours (sitting, amplexing or calling) are shown in Figure 3.4. For males at both sites, calling and amplexus occur during the wet and early dry season, are more common during the wet season (Figure 3.4A). For females, reproductive activities are absent late in the dry season (Figure 3.4B). A feature of most sampling periods is the low percentage

Figure 3.2 **A.** Mean snout-vent lengths for each site, each season and each sex recorded during activity transect samples. **B.** Mean snout-vent lengths for site by sex, site by season and sex by season recorded during activity transect samples. **C.** Mean snout-vent length for each sex, at each site during wet, early dry and late dry seasons recorded during activity transect samples. Error bars indicate the 95% confidence interval for the mean. All figures represent significant effects shown in Table 3.3

Abbreviations: CH-Calvert Hills, MM-Mt Margaret, W-wet season, ED-early dry season, LD-late dry season, M-male, F-female.


(A)
SNOUT–VENT LENGTH (mm)
115
113
110
108
105
CH
MM
W
ED
LD
M
F
MAIN EFFECTS (SITE/SEASON/SEX)


(B)
SNOUT–VENT LENGTH (mm)
120
118
115
113
110
108
105
F
M
ED
W
LD
ED
W
LD
CH
MM
CH
MM
CH
MM
INTERACTION EFFECTS

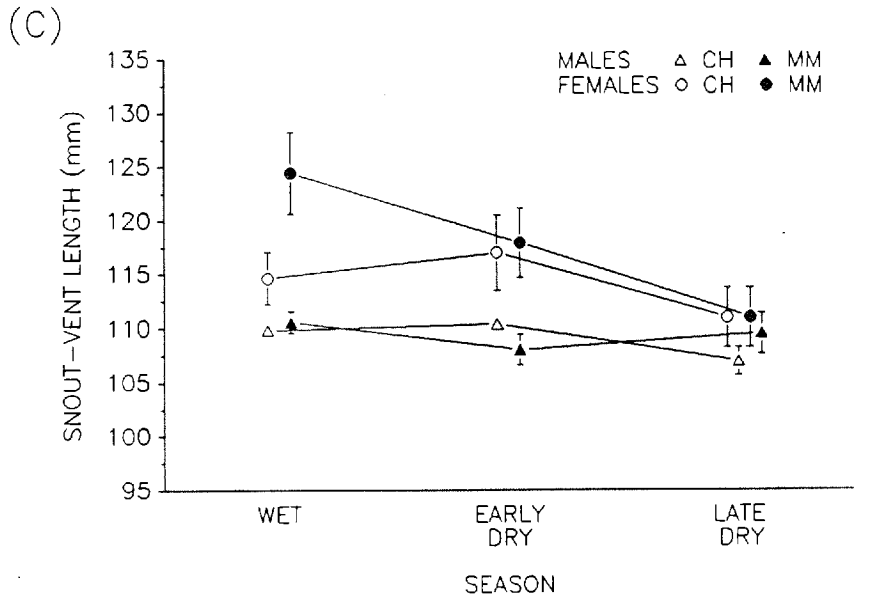
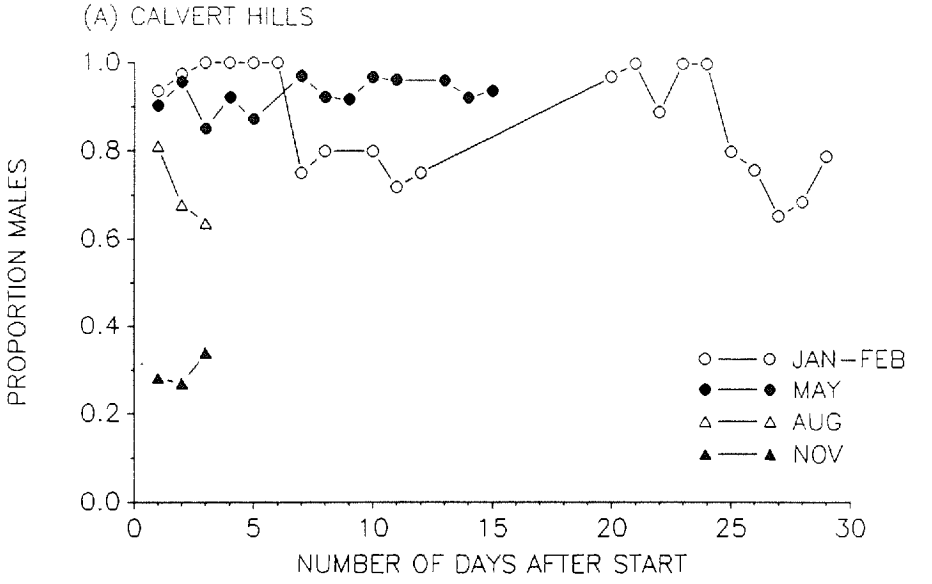
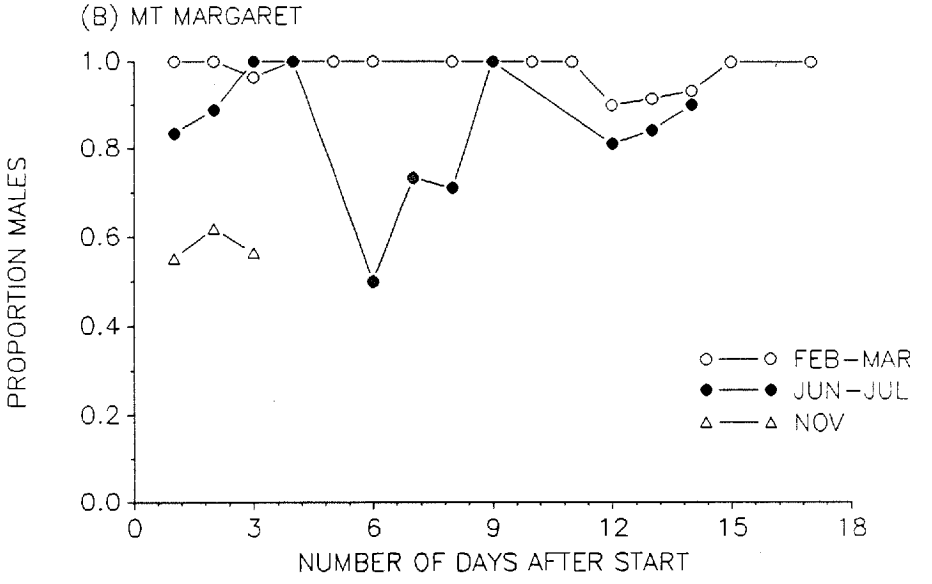

(C)
MALES △ CH ▲ MM
FEMALES ○ CH ● MM
SNOUT–VENT LENGTH (mm)
135
130
125
120
115
110
105
100
95
WET
EARLY DRY
LATE DRY
SEASON

Figure 3.3 Proportion of numbers in nightly activity samples for **(A)** Calvert Hills and **(B)** Mt Margaret that were males. **(C)** Proportion of numbers that were males in total sample for each season at each site. ($p < 0.0001$ for each site, $N_{CH}=1338$ $N_{MM}=383$)

Abbreviations shown on each figure.


(A) CALVERT HILLS
PROPORTION MALES
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
NUMBER OF DAYS AFTER START
JAN–FEB
MAY
AUG
NOV


(B) MT MARGARET
PROPORTION MALES
1.0
0.8
0.6
0.4
0.2
0.0
0
3
6
9
12
15
18
NUMBER OF DAYS AFTER START
FEB–MAR
JUN–JUL
NOV

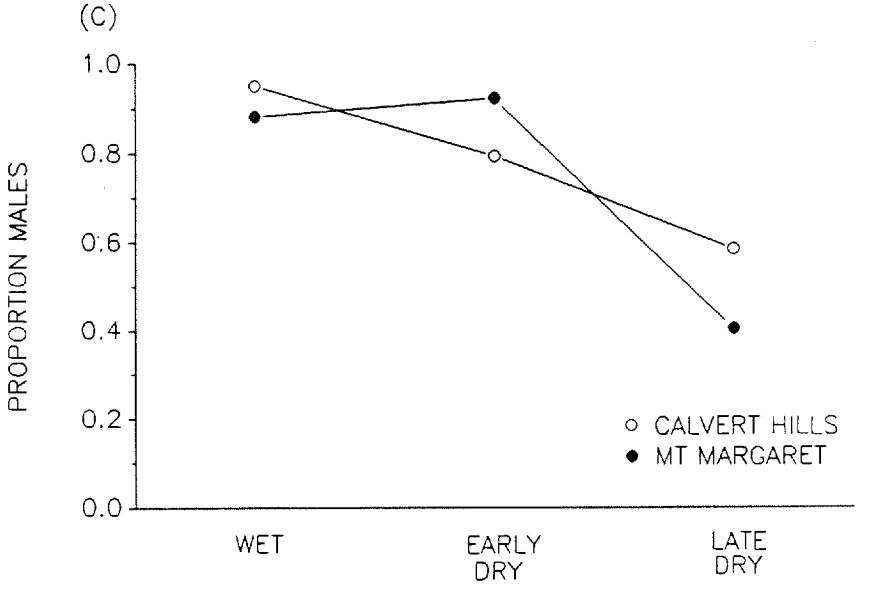
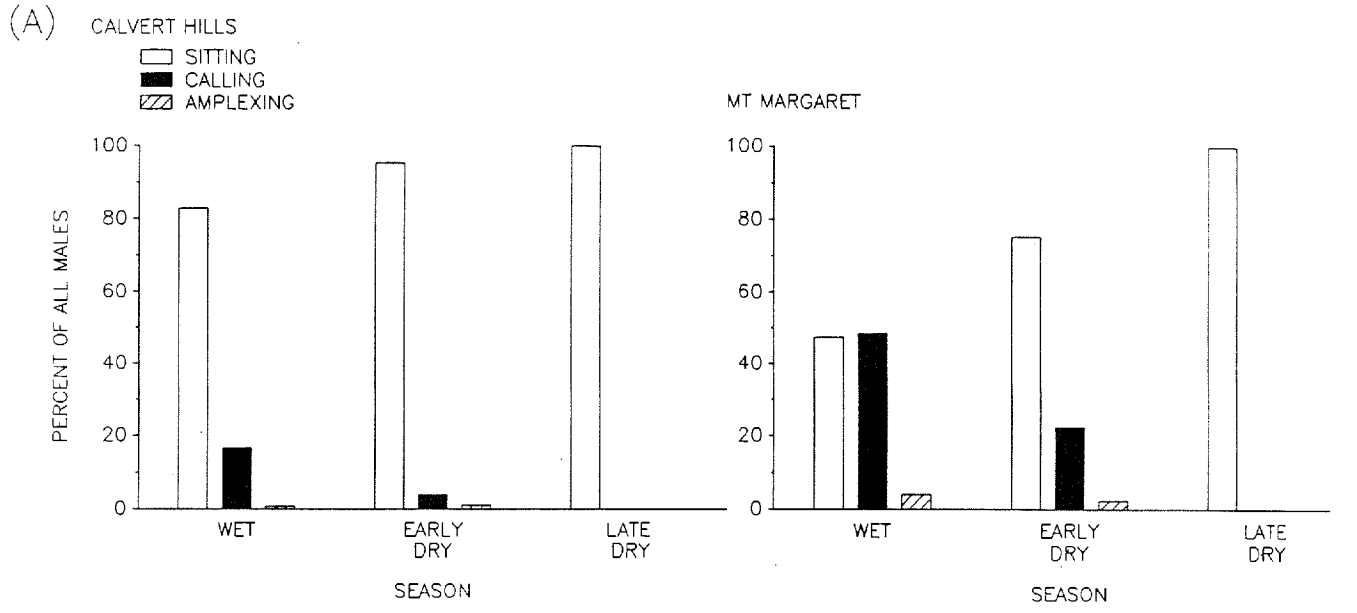
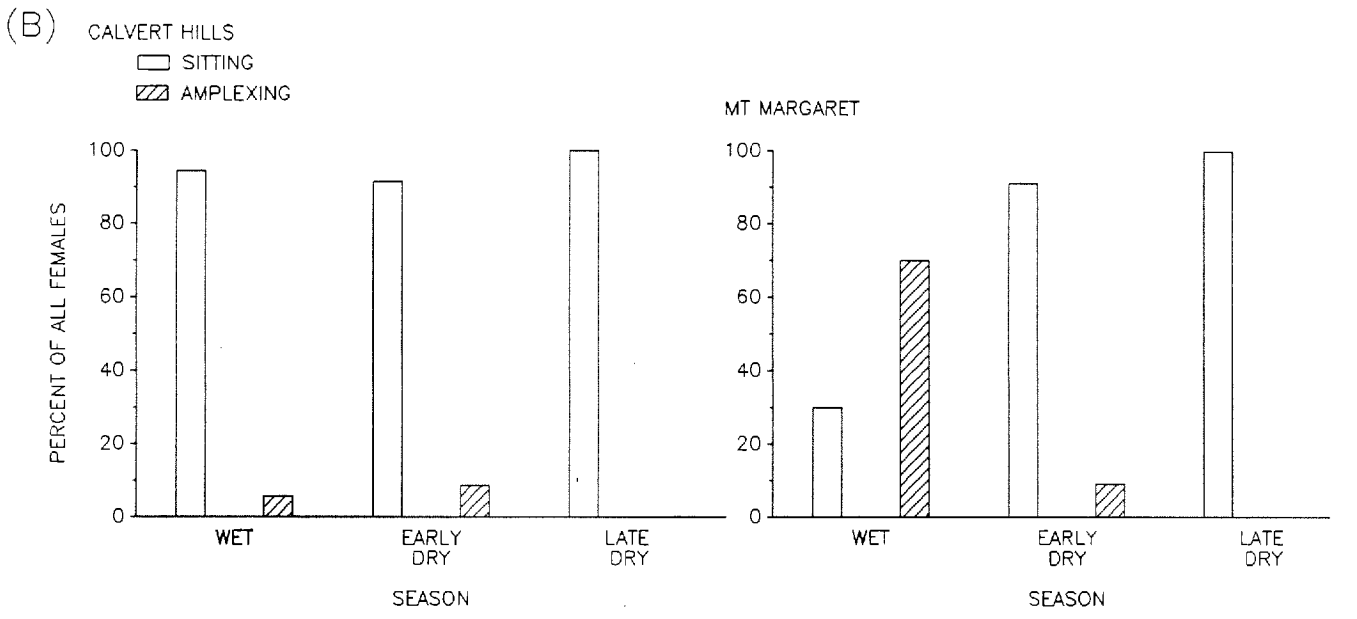

(C)
PROPORTION MALES
1.0
0.8
0.6
0.4
0.2
0.0
WET
EARLY DRY
LATE DRY
CALVERT HILLS
MT MARGARET

Figure 3.4 Percent of all males (A) and females (B) displaying each type of behaviour during each season for Calvert Hills and Mt Margaret.

(Calvert Hills $N_{males}=1072$, $N_{females}=266$; Mt Margaret $N_{males}=312$, $N_{females}=71$)


(A)
CALVERT HILLS
SITTING
CALLING
AMPLEXING
MT MARGARET
PERCENT OF ALL MALES
100
80
60
40
20
0
WET
EARLY DRY
LATE DRY
SEASON


(B)
CALVERT HILLS
SITTING
AMPLEXING
MT MARGARET
PERCENT OF ALL FEMALES
100
80
60
40
20
0
WET
EARLY DRY
LATE DRY
SEASON

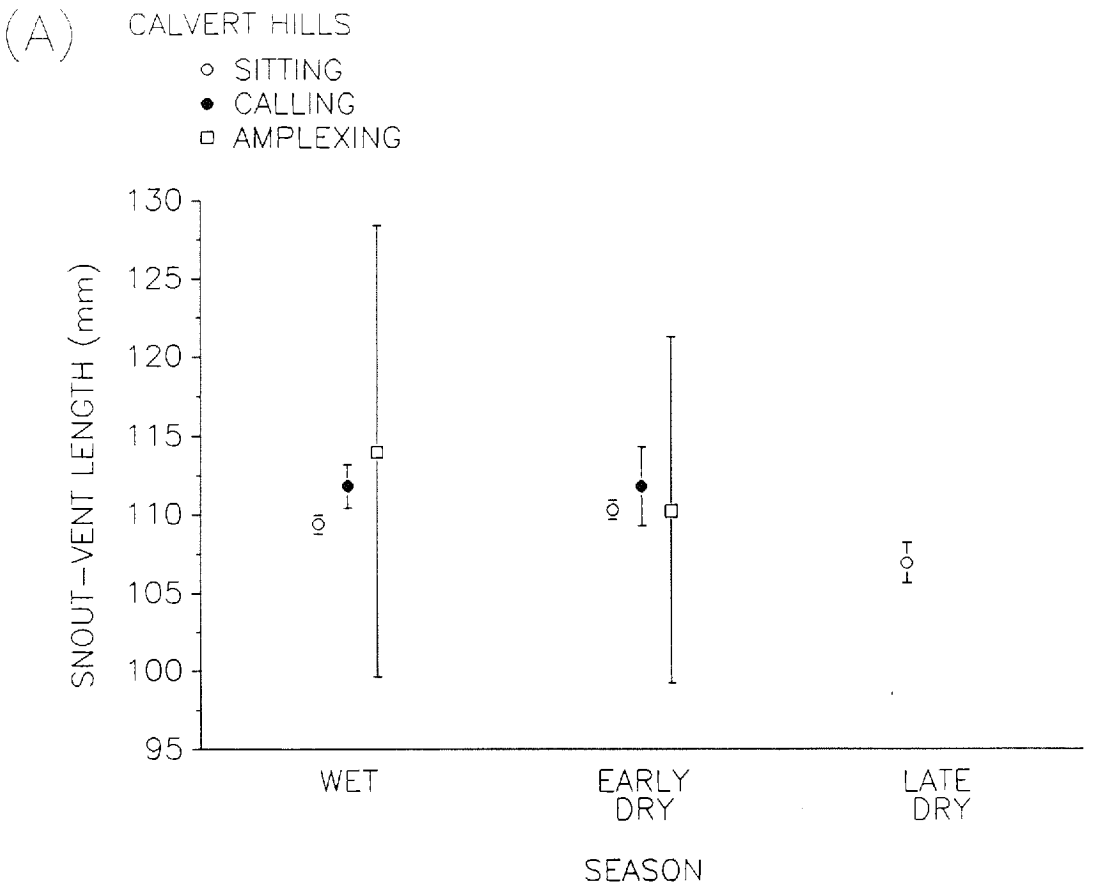
of the active adults involved in reproductive activities. The exception is at Mt Margaret in the wet season, when around 45% of males caught were calling and 7 of 10 females caught were amplexing.

The mean size of males displaying each type of behaviour is shown in Figure 3.5. For both seasons where reproductive activity occurs (wet and early dry), one or both of the reproductive classes are larger than males taking no apparent role in reproductive activity (ie. sitting). Analysis of the data (Table 3.4) for wet and early dry seasons show that there is no significant difference between amplexing and calling males but that these males are significantly larger in size than others (Figure 3.6). The interaction effect between season and behaviour is caused by a decrease in the mean size of males not involved in reproductive activity during each season, while the mean size of others increases (Figure 3.7).

To further clarify this trend, it was assumed that all amplexing males were involved in calling, so the two were grouped and compared with non-calling males (Table 3.5). Mean size was significantly larger for all calling males (Figure 3.8), associated with the larger mean size of calling males during the wet-season (Figure 3.9). Mean size did not differ between seasons but when the interaction with site is considered, mean size decreased from the wet to the early dry for Mt Margaret (Figure 3.10).

Figure 3.5 Mean snout-vent length of males at Calvert Hills (A) and Mt Margaret (B) displaying each type of recorded behaviour for each season.

Error bars indicate the 95% confidence interval for the mean.


(A)
CALVERT HILLS
SITTING
CALLING
AMPLEXING
SNOUT–VENT LENGTH (mm)
130
125
120
115
110
105
100
95
WET
EARLY DRY
LATE DRY
SEASON

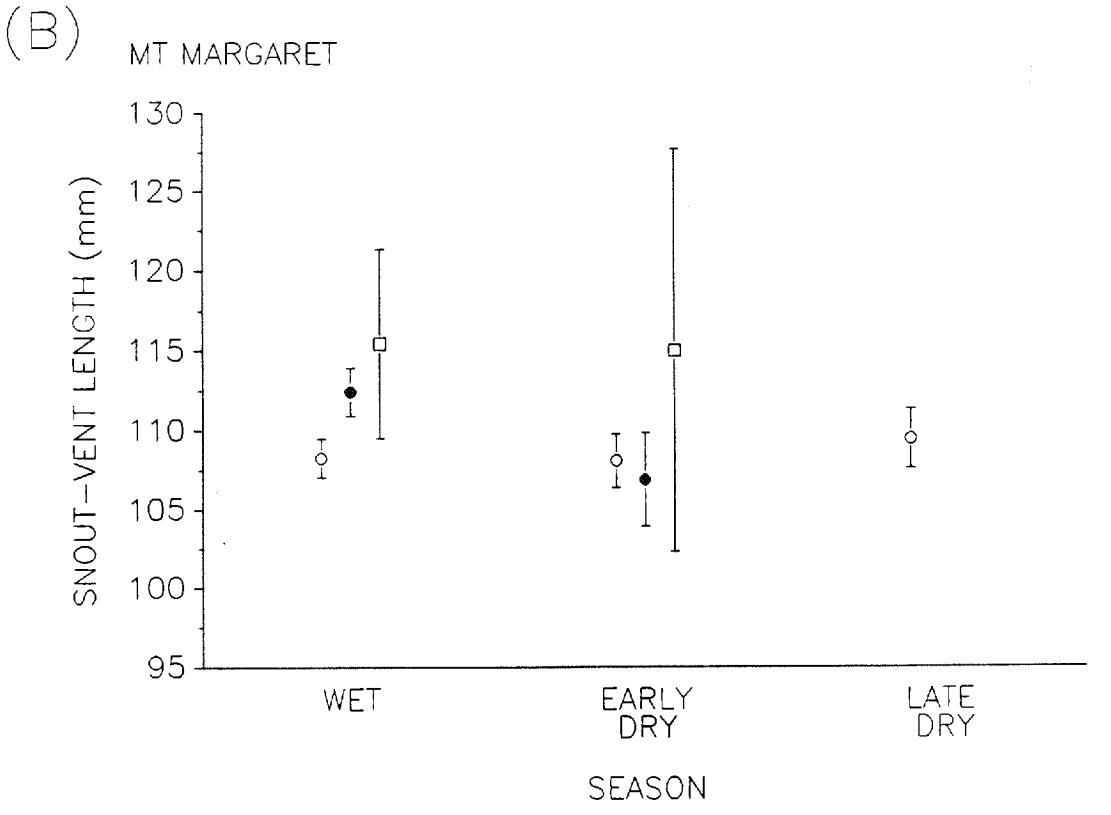

(B)
MT MARGARET
SNOUT–VENT LENGTH (mm)
130
125
120
115
110
105
100
95
WET
EARLY DRY
LATE DRY
SEASON

Table 3.4 ANOVA of mean snout vent length for males displaying calling, amplexing and non-reproductive behaviour.

Class Levels: Site: Calvert Hills / Mt Margaret
Season: Wet / Early Dry
Behaviour: Sitting / Calling / Amplexing

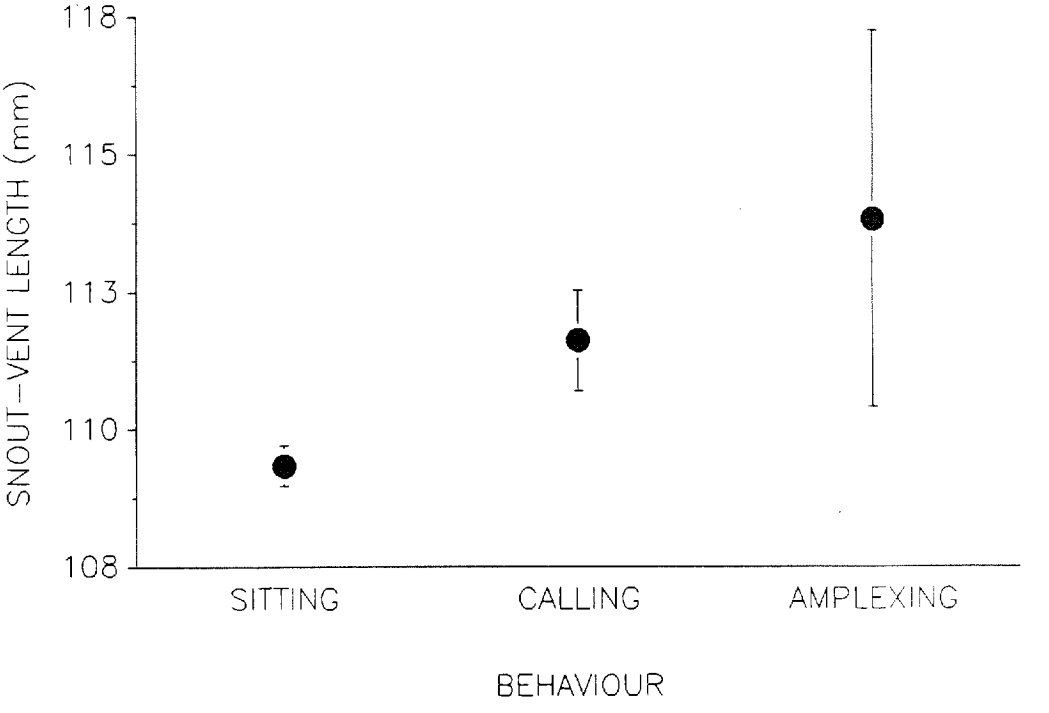
Source of Variation	df	Sum of Squares	Mean Square	F	p
Model	11	1945.6725	176.8793	4.43	0.0001
Site	1	1.0699	1.0699	0.03	0.8700
Season	1	56.8050	56.8050	1.42	0.2332
Site*Season	2	6.4799	6.4799	0.16	0.6871
Behaviour	2	497.2800	248.6400	6.23	0.0020
Site*Behav	2	86.7138	43.3569	1.09	0.3880
Season*Behav	2	268.5041	134.2520	3.36	0.0350
Site*Sea*Behav	2	152.6055	76.3027	1.91	0.1484
Residual	1209	48279.0907	39.9330		
Total	1220	50224.7633			

Figure 3.6 Mean snout-vent lengths of males displaying each type of recorded behaviour in all samples.

Error bars indicate the 95% confidence interval for the mean.

Figure 3.7 Mean snout-vent lengths of males displaying each type of recorded behaviour for samples taken during each season.

Error bars indicate the 95% confidence interval for the mean. Both figures represent significant effects shown in Table 3.5.


118
115
113
110
108
SNOUT–VENT LENGTH (mm)
SITTING
CALLING
AMPLEXING
BEHAVIOUR

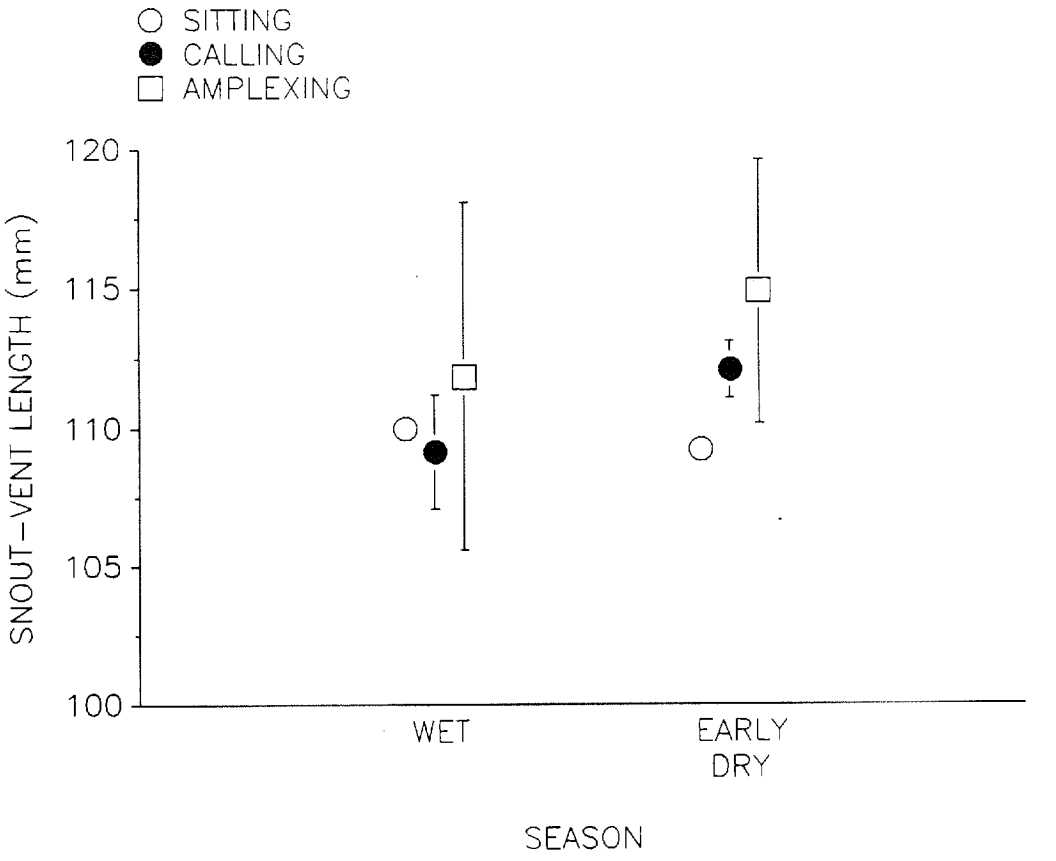

SITTING
CALLING
AMPLEXING
120
115
110
105
100
SNOUT–VENT LENGTH (mm)
WET
EARLY
DRY
SEASON

Table 3.5 ANOVA of mean snout vent length for males displaying reproductive and non-reproductive behaviour.

Class Levels: Site: Calvert Hills / Mt Margaret
Season: Wet / Early Dry
Behaviour: Calling / Non-Calling

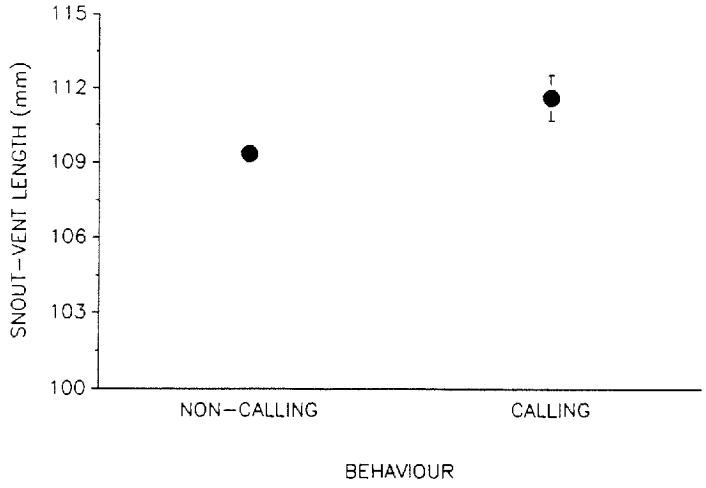
Source of Variation	df	Sum of Squares	Mean Square	F	p
Model	7	1741.9235	248.8462	6.23	0.0001
Site	1	247.9121	247.9121	6.20	0.0129
Season	1	123.0718	123.0718	3.08	0.0796
Behaviour	1	352.2356	352.2356	8.81	0.0031
Site*Season	1	184.4209	184.4209	4.61	0.0319
Site*Behav	1	0.0706	0.0706	0.00	0.9665
Seas*Behav	1	275.0605	275.0605	6.88	0.0088
Site*Seas*Behav	1	94.7355	94.7355	2.37	0.1239
Residual	1213	48482.8397	39.9693		
Total	1220	50224.7633			

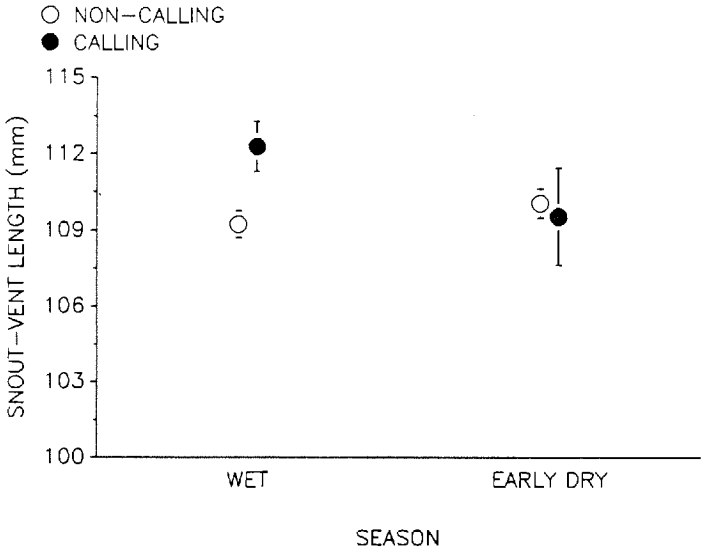
Figure 3.8 Mean snout-vent length for calling (includes both calling and amplexed males) and non calling males in all samples.

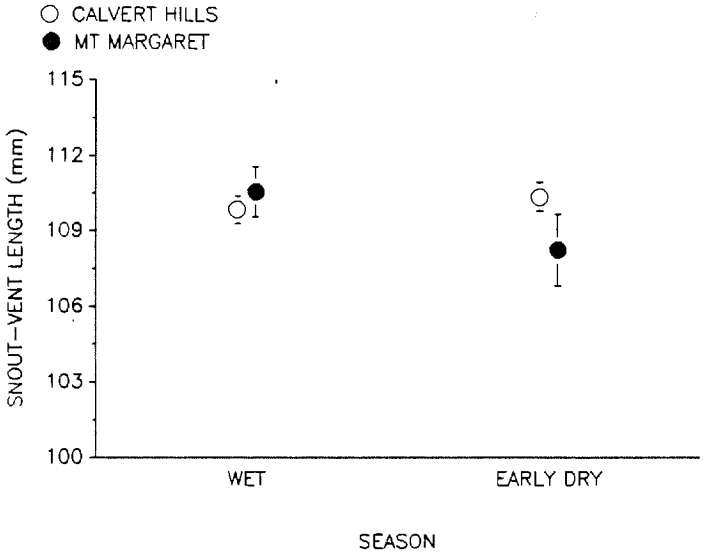
Figure 3.9 Mean snout-vent length for calling (includes both calling and amplexed males) for all samples taken during the wet and early dry seasons.

Figure 3.10 Mean snout-vent length for males in samples at each site taken during the wet and early dry seasons.

Each figure represents significant effects shown in Table 3.6
Error bars indicate the 95% confidence interval for the mean.


115
112
109
106
103
100
SNOUT-VENT LENGTH (mm)
NON-CALLING
CALLING
BEHAVIOUR


NON-CALLING
CALLING
115
112
109
106
103
100
SNOUT-VENT LENGTH (mm)
WET
EARLY DRY
SEASON


CALVERT HILLS
MT MARGARET
115
112
109
106
103
100
SNOUT-VENT LENGTH (mm)
WET
EARLY DRY
SEASON

The mean size of gravid females in amplexus is shown in Figure 3.11, against gravid females not in amplexus, and the overall mean for the sample. The mean size of all females differed between sites (Table 3.6, Calvert mean=115.411 95% CL=3.96; Mt Margaret mean=119.806 95% CL=5.3) but not between females amplexing and not amplexing. Gravid females however, differed in size between season, being larger during the wet and early dry seasons (Table 3.7, Figure 3.12).

Significantly higher proportions of gravid females were caught during the late dry season at Calvert Hills (Figure 3.13, χ^2 test $p<0.0001$), though proportions of each were equal during the wet-season. Higher proportions were also present for the late dry season and the wet-season at Mt Margaret though the difference was not significant (χ^2 test, $p=0.242$).

3.3.2 Gonad Condition and Fecundity

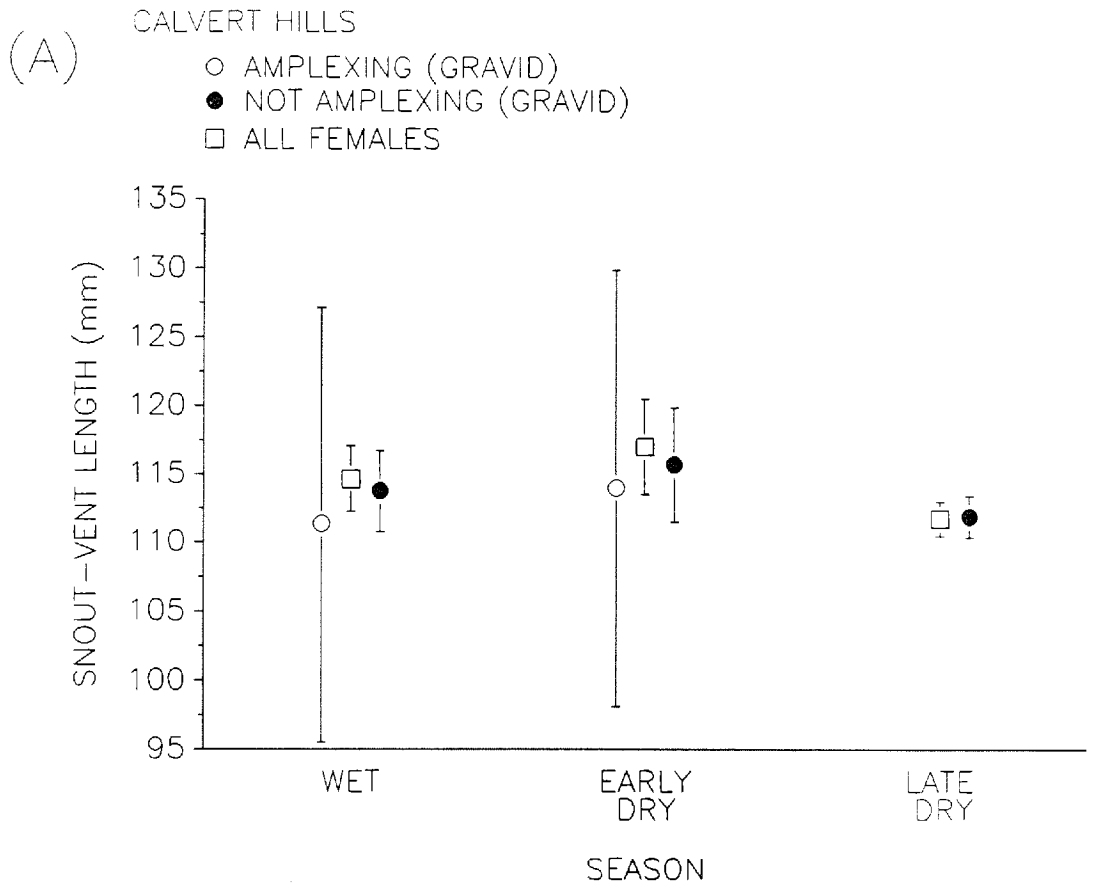
The following analyses refer to gonads extracted from adults collected in breeding habitats adjacent to the activity sampling sites (section 3.2.2).

Post-Spawning Ovary Condition

Autopsies conducted on ten females collected immediately after spawning in the field showed that all ova were released and that the condition of the ovary membrane resembled that of a VMI stage 1 (Table 3.1).

Figure 3.11 Mean snout-vent length of females amplexing, not amplexing and all females recorded during activity transects during each season at Calvert Hills (A) and Mt Margaret (B).

Error bars indicate the 95% confidence interval for the mean.


(A)
CALVERT HILLS
AMPLEXING (GRAVID)
NOT AMPLEXING (GRAVID)
ALL FEMALES
SNOUT–VENT LENGTH (mm)
135
130
125
120
115
110
105
100
95
WET
EARLY DRY
LATE DRY
SEASON

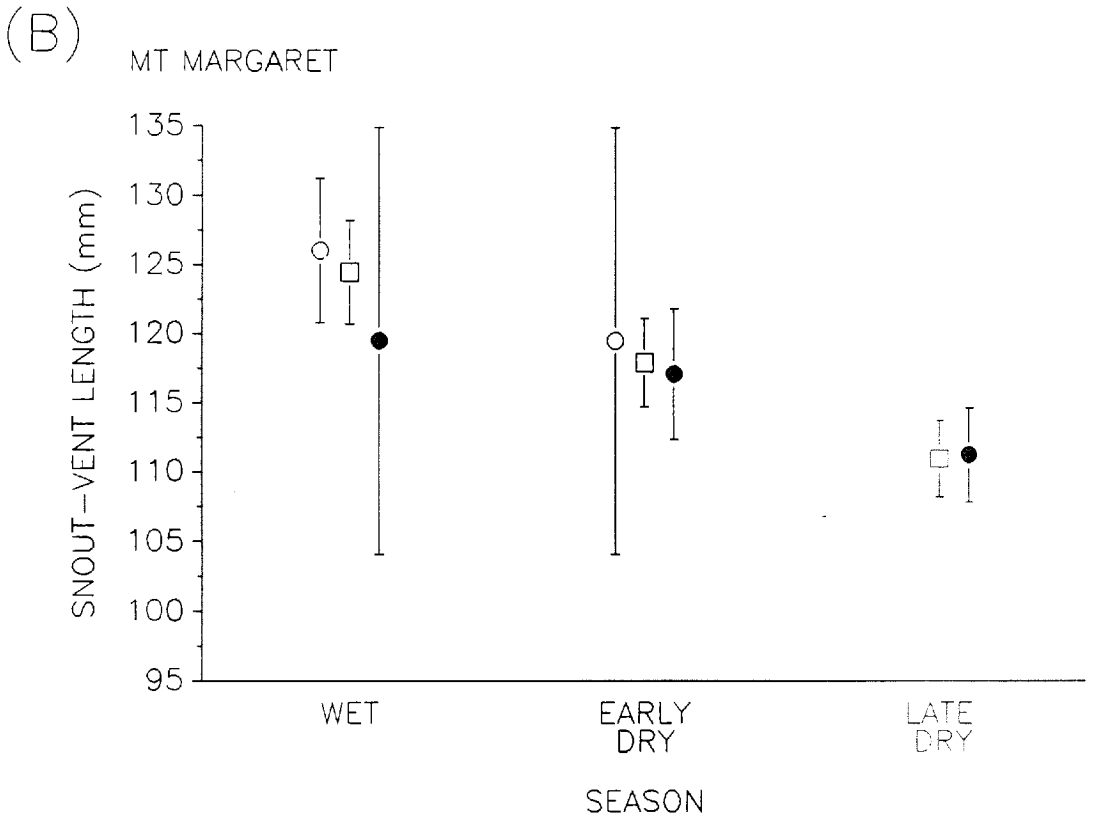

(B)
MT MARGARET
SNOUT–VENT LENGTH (mm)
135
130
125
120
115
110
105
100
95
WET
EARLY DRY
LATE DRY
SEASON

Table 3.6 ANOVA of mean snout vent length of gravid females in amplexus and not in amplexus between site and season.

Class Levels: Site: Calvert Hills / Mt Margaret
Season: Wet / Early Dry
Behaviour: Amplexus / Sitting

Source of Variation	df	Sum of Squares	Mean Square	F	p
Model	7	1082.2923	154.6131	1.64	0.1299
Site	1	499.5169	499.5169	5.30	0.0230
Season	1	2.2255	2.2255	0.02	0.8782
Behaviour	1	3.3468	3.3468	0.04	0.8509
Site*Season	1	197.9759	197.9759	2.10	0.1498
Site*Behav	1	137.6552	137.6552	1.46	0.2292
Seas*Behav	1	0.3499	0.3499	0.00	0.9515
Site*Seas*Behav	1	25.9385	25.9385	0.28	0.6009
Residual	130	12260.7873	94.3137		
Total	137	13343.0797			

Table 3.7 ANOVA of mean snout vent length for gravid females caught in samples for site and season.

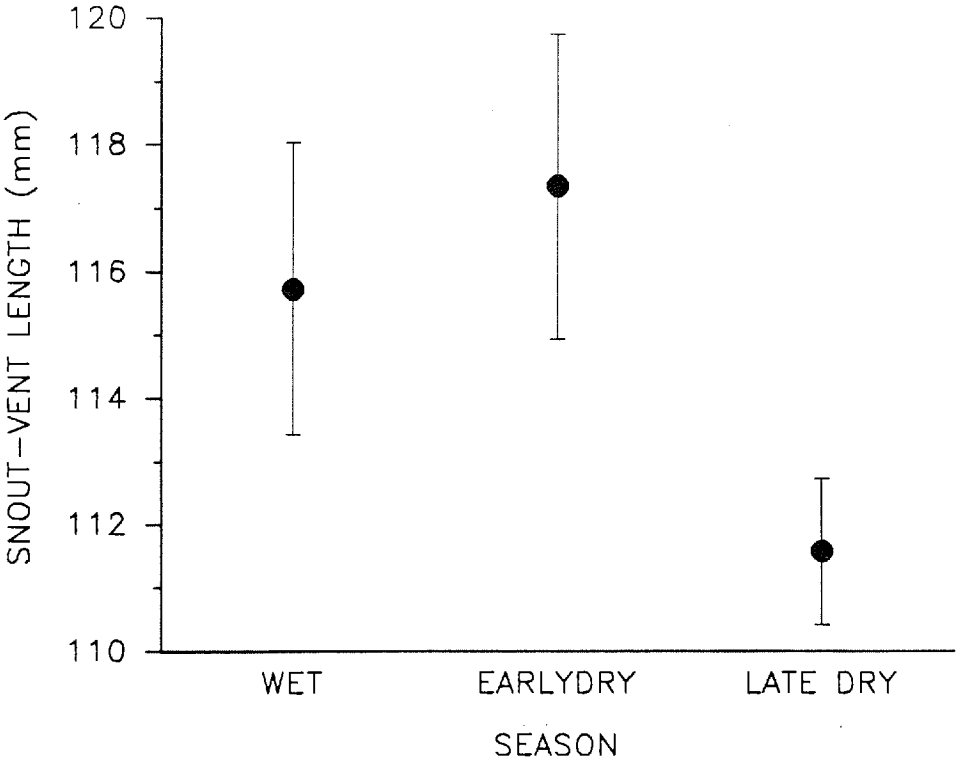
Class Levels: Site: Calvert Hills / Mt Margaret
Season: Wet / Early Dry / Late Dry
Grav: Gravid / Non-Gravid

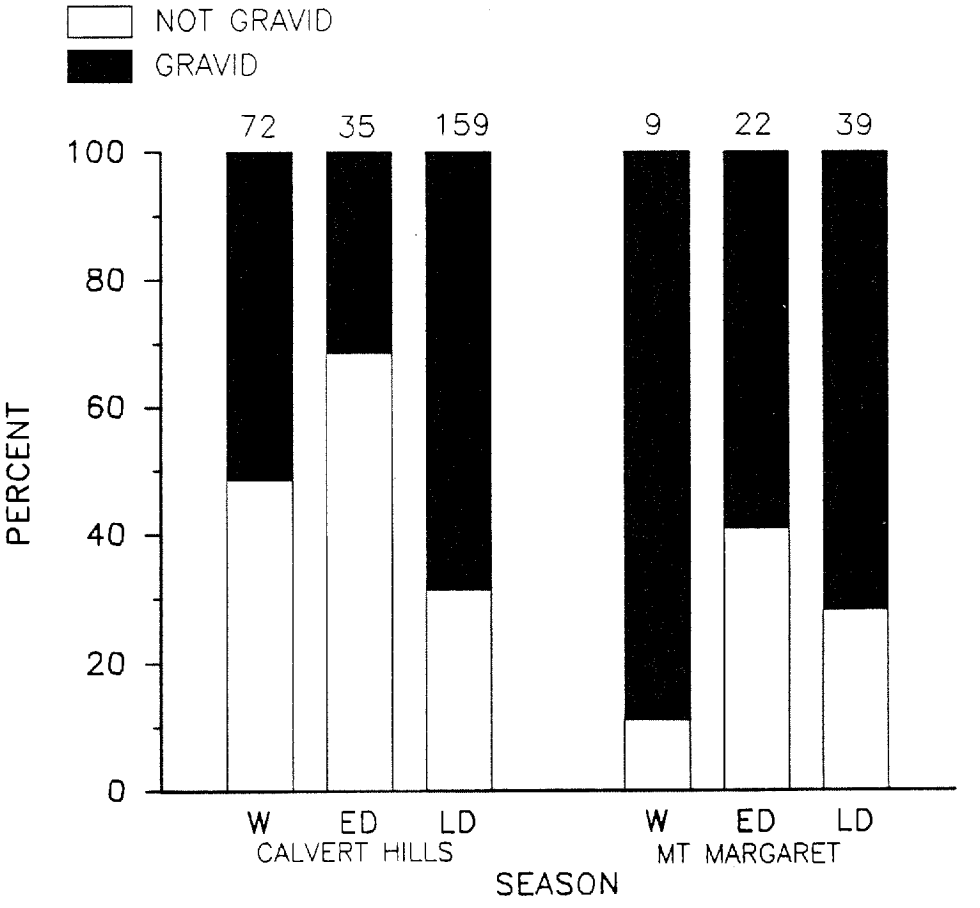
Source of Variation	df	Sum of Squares	Mean Square	F	p
Model	11	2935.1016	266.8274	3.66	0.0002
Site	1	271.2338	271.2338	3.41	0.0656
Season	2	1768.9605	884.4802	11.13	0.0001
Gravid	1	14.4122	14.4122	0.18	0.6705
Site*Season	2	364.3686	182.1843	2.29	0.1027
Site*Grav	1	6.9901	6.9901	0.09	0.7670
Seas*Grav	2	57.4507	28.7253	0.36	0.6970
Site*Seas*Grav	2	2.0147	1.0073	0.01	0.9874
Residual	342	25755.8239	79.4932		
Total	335	28690.9255			

Figure 3.12 Mean snout-vent length of the total female sample recorded during activity transects for each season. Error bars indicate the 95% confidence interval for the mean.

Figure 3.13 Percent of females gravid and non-gravid recorded in activity transects for each season at Calvert Hills and Mt Margaret.

Numbers above bars indicate total females recorded for each season.


120
118
116
114
112
110
SNOUT–VENT LENGTH (mm)
WET
EARLYDRY
LATE DRY
SEASON


NOT GRAVID
GRAVID
72
35
159
9
22
39
100
80
60
40
20
0
PERCENT
W
ED
LD
CALVERT HILLS
W
ED
LD
MT MARGARET
SEASON

Ovary Condition

Histological sections of ovaries from all females collected show that ova are all within the one developmental stage, indicating synchronous development. In mature ovaries (VMI 5) and VMI 1 ovaries, heavily pigmented, atretic eggs are present.

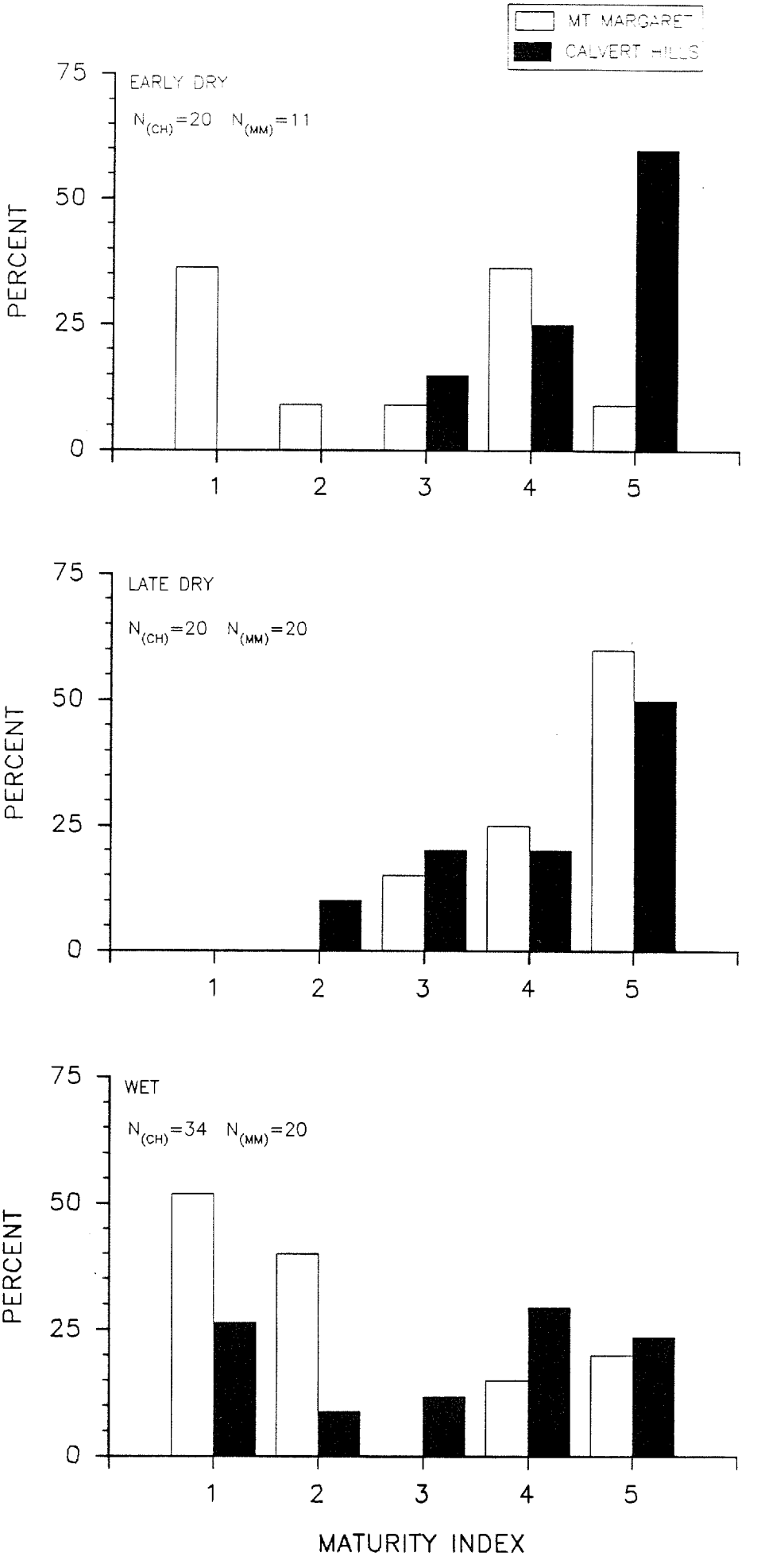
The proportion of females in each reproductive stage (VMI) is presented in Figure 3.14. There are distinct patterns of numbers in each stage for the different seasons (Fisher's Exact Test, Calvert $p=0.05$, Mt Margaret $p=0.0036$), though the small samples tend to obscure interpretation. Fully mature females are present on all sampling occasions at both sites, also indicated by the presence of gravid females in all activity transect samples. Larger proportions of females at the lower stages (1-3), most likely indicating post-spawning females, tend to occur during the wet-season periods. Higher proportions of stage 4 and 5 females occur near water during both dry season periods, with the stage 5 females generally being the largest group. During the wet season proportions in these two stages are roughly similar.

Testis Condition

Mature sperm occur in the testes of all males at all times of the year. The condition of the interstitial cells (flattened or rounded) in the testes is variable from individual to individual, numbers occurring in proportions not significantly different from 1:1

Figure 3.14 Percent of females in each reproductive stage (see Table 3.1) for samples collected during each season at Calvert Hills and Mt Margaret.

Numbers for each season indicated.


MT MARGARET
CALVERT HILLS
EARLY DRY
$N_{(CH)}$=20 $N_{(MM)}$=11
LATE DRY
$N_{(CH)}$=20 $N_{(MM)}$=20
WET
$N_{(CH)}$=34 $N_{(MM)}$=20
PERCENT
75
50
25
0
1
2
3
4
5
MATURITY INDEX

(χ^2 test, $p>0.05$) in most samples. Seminiferous tubule diameter, an indicator of sperm production, (Figure 3.15) does not depend on site or season (ANCOVA, $p=0.3877$, covariate=male mass $p=0.0894$).

Testis Mass

The mass of the testes for males at each site is presented in Figure 3.16. The regression relationships between testes mass and body mass are shown for both sites and seasons within sites, and for all data combined (Table 3.8). In each case the regression explained a significant amount of the variance. In all cases the optimum fit was linear, when compared with r^2 values for regressions where testes mass was log-transformed. An analysis of covariance was conducted on testes mass between sites and seasons, using body mass as a covariate (Table 3.9). Results show that the relationship between testes mass and body mass does not vary significantly among sites and seasons. The relationship obtained to describe the data is:

$$Testes\ Mass = 0.00242\,(Body\ Mass) - 0.003251$$

$$r^2 = 0.4721 \quad p < 0.0001$$

Size-Fecundity Relationships

Log_e-transformed values for total ovarian egg number provided the best fit for regression relationships with body mass (Table 3.10). Only one stage 5 female was

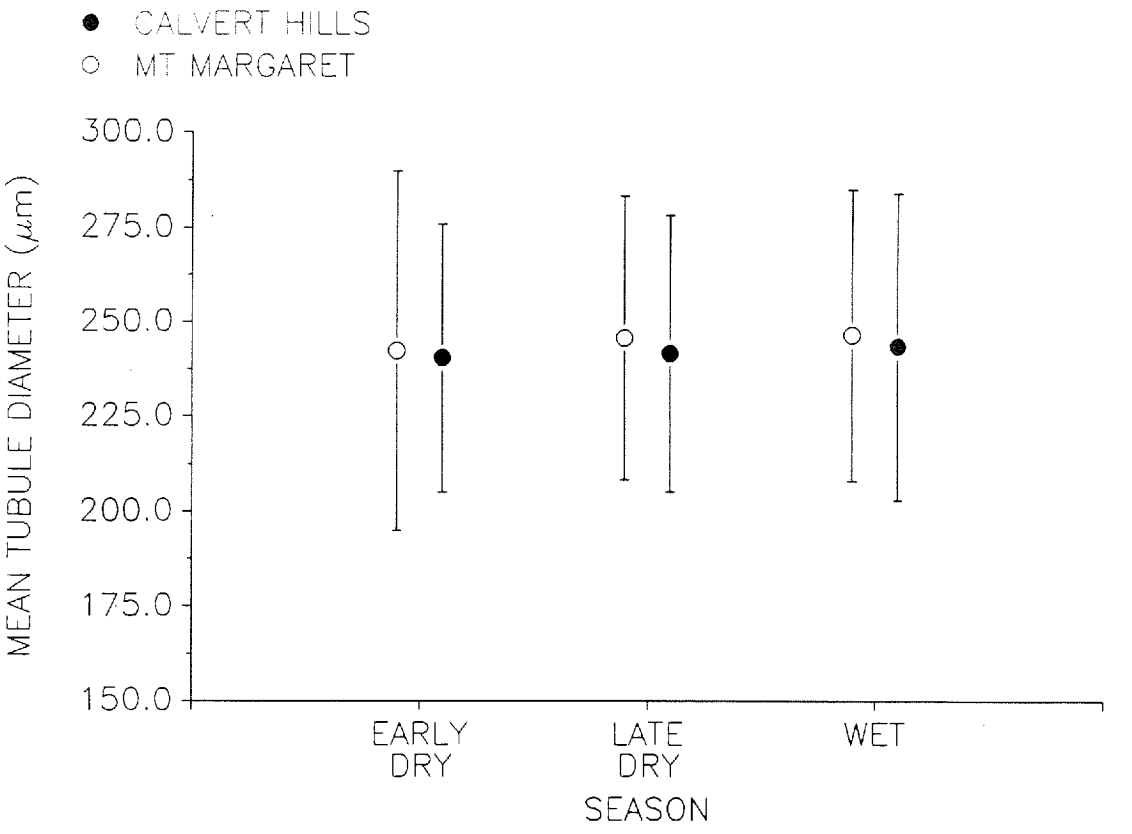
Figure 3.15 Mean diameter of seminiferous tubules in testes from males collected during each season at Calvert Hills and Mt Margaret.

Error bars indicate the 95% confidence interval for the mean.

Figure 3.16 Relationship between testes mass and body mass for toads collected at Calvert Hills and Mt Margaret. The line fitted to the data is:

$$\textit{Testes Mass} = 0.00242(\textit{Body Mass}) - 0.003251$$

$$r^2 = 0.4721 \quad p = 0.0001$$


CALVERT HILLS
MT MARGARET
MEAN TUBULE DIAMETER (μm)
300.0
275.0
250.0
225.0
200.0
175.0
150.0
EARLY DRY
LATE DRY
WET
SEASON

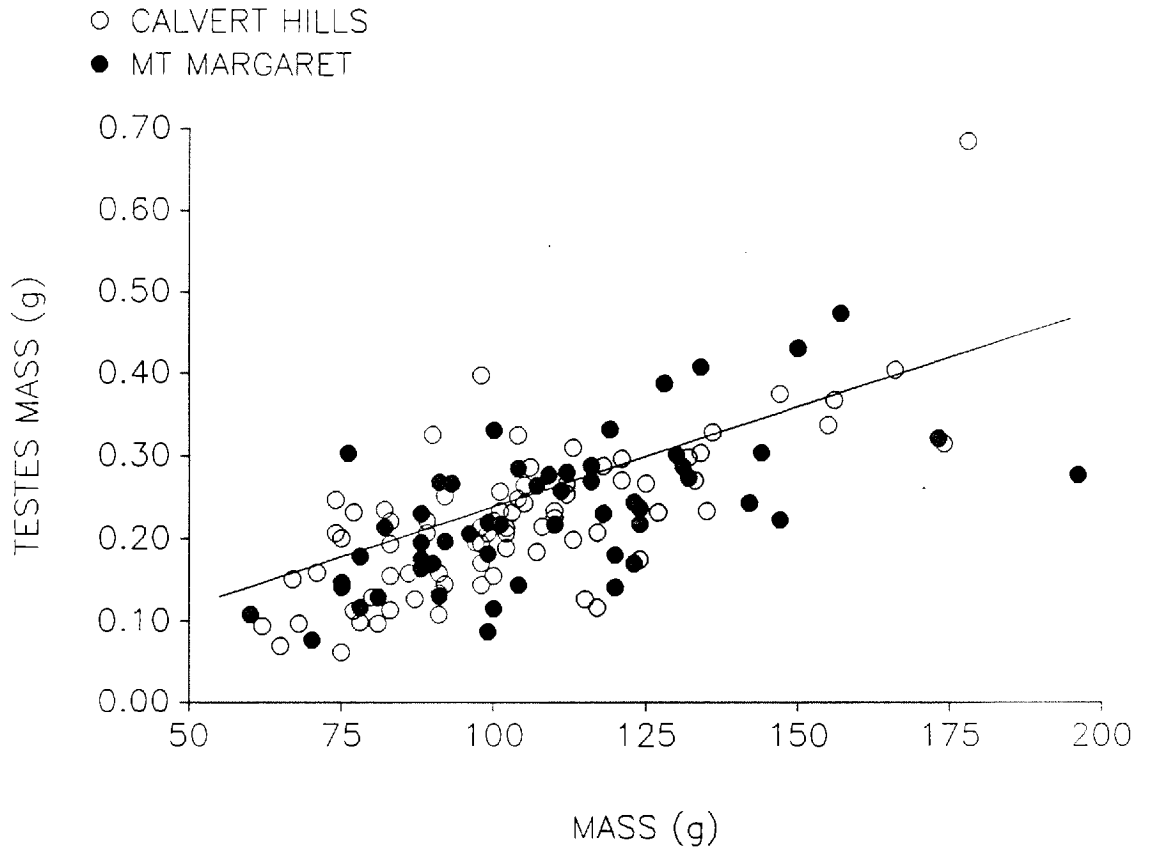

CALVERT HILLS
MT MARGARET
TESTES MASS (g)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
50
75
100
125
150
175
200
MASS (g)

Table 3.8 Linear regression relationships for testes mass and body mass (a=intercept b=slope) for both sites.

Equations are of the form Testes Mass= (b x Body Mass) + a

Site	Season	a	b	p	r^2
Calvert Hills	Wet	-0.118121	0.003175	0.0001	0.8879
	Early Dry	-0.057108	0.002677	0.0001	0.8106
	Late Dry	-0.055857	0.002743	0.0001	0.4085
	All	-0.066455	0.002783	0.0001	0.5382
Mt Margaret	Wet	-0.037901	0.002602	0.0029	0.3967
	Early Dry	-0.006495	0.002278	0.0004	0.5049
	Late Dry	-0.006778	0.001750	0.0005	0.6452
	All	0.015618	0.001982	0.0001	0.3860
All Sites/All Seasons		-0.030251	0.002420	0.0001	0.4721

Table 3.9 ANOVA of mean testes mass for site and season with body mass as a covariate.

Class Levels: Sites: Calvert Hills / Mt Margaret
Season: Wet / Early Dry / Late Dry

Covariate: Mass: Body Mass

Source of Variation	df	Sum of Squares	Mean Square	F	p
Model	11	0.5805	0.0527	13.13	0.0001
Site	1	0.0062	0.0062	1.52	0.2194
Season	2	0.0029	0.0014	0.37	0.6944
Mass	1	0.4702	0.4702	115.83	0.0001
Site*Seas	2	0.0002	0.0001	0.03	0.9687
Mass*Site	1	0.0078	0.0078	1.93	0.1676
Mass*Season	2	0.0052	0.0026	0.64	0.5305
Mass*Site*Seas	2	0.0014	0.0007	0.17	0.8419
Residual	122	0.5196	0.0043		
Total	133	0.5562			

Table 3.10 Curvilinear regression relationships for egg number and body mass (a=intercept b=slope) for both sites.

* indicates the relationship not significant (α=0.05)

Equations are of the form: Egg Number = a x $e^{b \times \text{Body Mass}}$

Site	Season	a	b	p	r^2
Calvert Hills	Wet	7557.7741	0.004415	0.0098	0.6980
	Dry	7336.1833	0.003110	0.0009	0.4139
	Early Dry	7191.8376	0.003187	0.0003	0.7399
	Late Dry	7393.2448	0.003123	0.1025*	0.2685
	All	6373.2001	0.004520	0.0001	0.5942
Mt Margaret	Wet	7807.3666	0.003273	0.2456*	0.5691
	Dry	5939.2135	0.004842	0.0005	0.6851
	All	5978.4881	0.004771	0.0001	0.6903
All Sites/All Seasons		6192.1956	0.004650	0.0001	0.6426

present in the early dry season sample for Mt Margaret so comparisons were made for both sites divided into wet and dry (early dry and late dry pooled) and for all three seasons within Calvert Hills. Analysis of total egg number per female for these classifications are presented in Table 3.11A and B. For the two site/two season comparison, only the covariate, body mass, was significant (Table 3.11A) indicating that number does not vary with either site or season. For Calvert Hills females where all three season categories can be examined, (Table 3.11B), mean egg number does not vary significantly among seasons. Pooling the data across seasons and comparing the sites revealed no significant difference between Calvert Hills and Mt Margaret (ANCOVA, $p=0.7125$).

The regression equation obtained to describe the data (Figure 3.17A) is:

$$Egg\ Number = 6192.1956\ e^{0.00465\,(Body\ Mass)}$$

$$r^2 = 0.6426\ p < 0.0001$$

Regression relationships were also derived for egg number and snout-vent length. These were required for estimates of recruitment from activity transect data where mass was not measured. Similar to the data for mass, the best fit for the data resulted from $\log_e$ transformed values. All r^2 values were lower for these data but all relationships significant for mass were significant for snout-vent length. The relationship to describe the data for both sites (Figure 3.17B) is:

Table 3.11 A. Analysis of covariance for egg number per gravid female (log_e transformed) by site and season with female body mass as a covariate.

Class Levels: Site: Calvert Hills / Mt Margaret
Season: Wet / Dry
Covariate: Mass: Body Mass

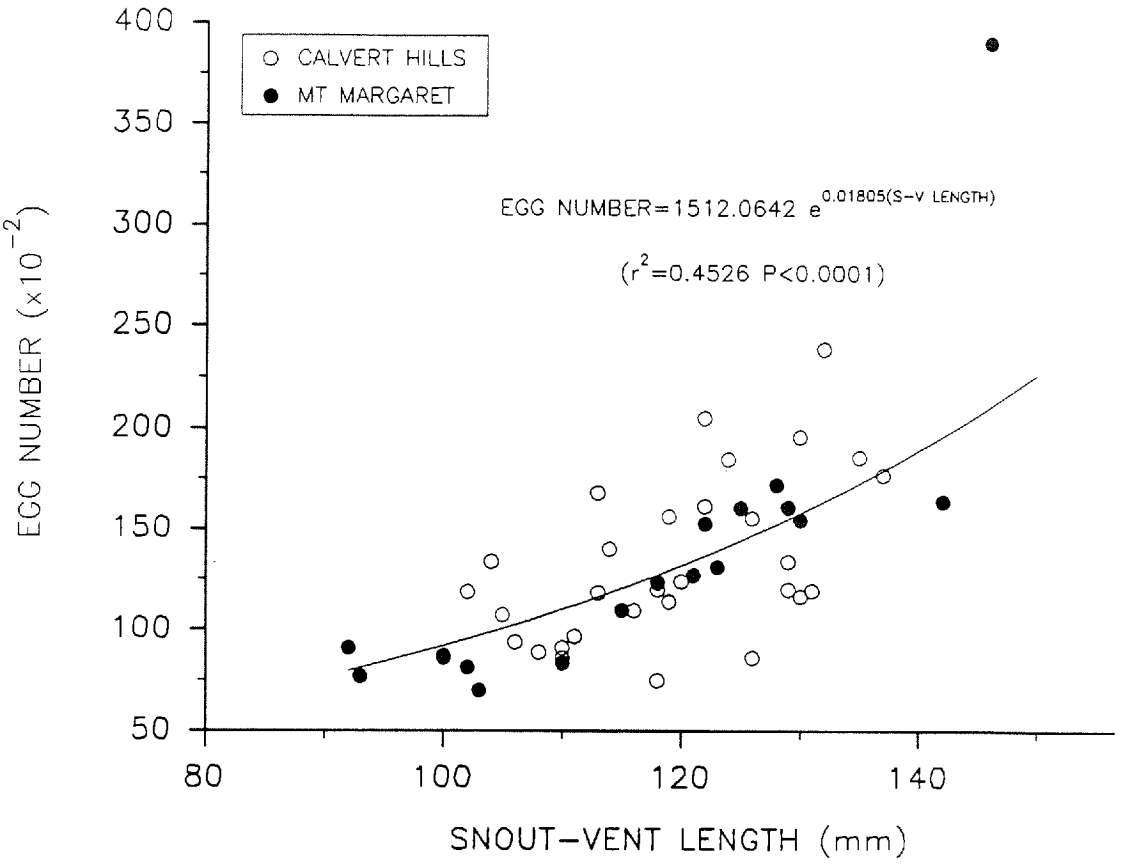
Source of Variation	df	Sum of Squares	Mean Square	F	p
Model	7	0.6679	0.0954	9.21	0.0001
Site	1	0.0003	0.0003	0.03	0.8649
Season	1	0.0007	0.0001	0.07	0.7875
Mass	1	0.0733	0.0733	7.08	0.0127
Site*Seas	1	0.0005	0.0005	0.05	0.8185
Mass*Site	1	0.0001	0.0001	0.01	0.9244
Mass*Seas	1	0.0001	0.0001	0.00	0.9646
Mass*Site*Seas	1	0.0024	0.0024	0.23	0.6318
Residual	28	0.2899	0.0104		
Total	35	0.9578			

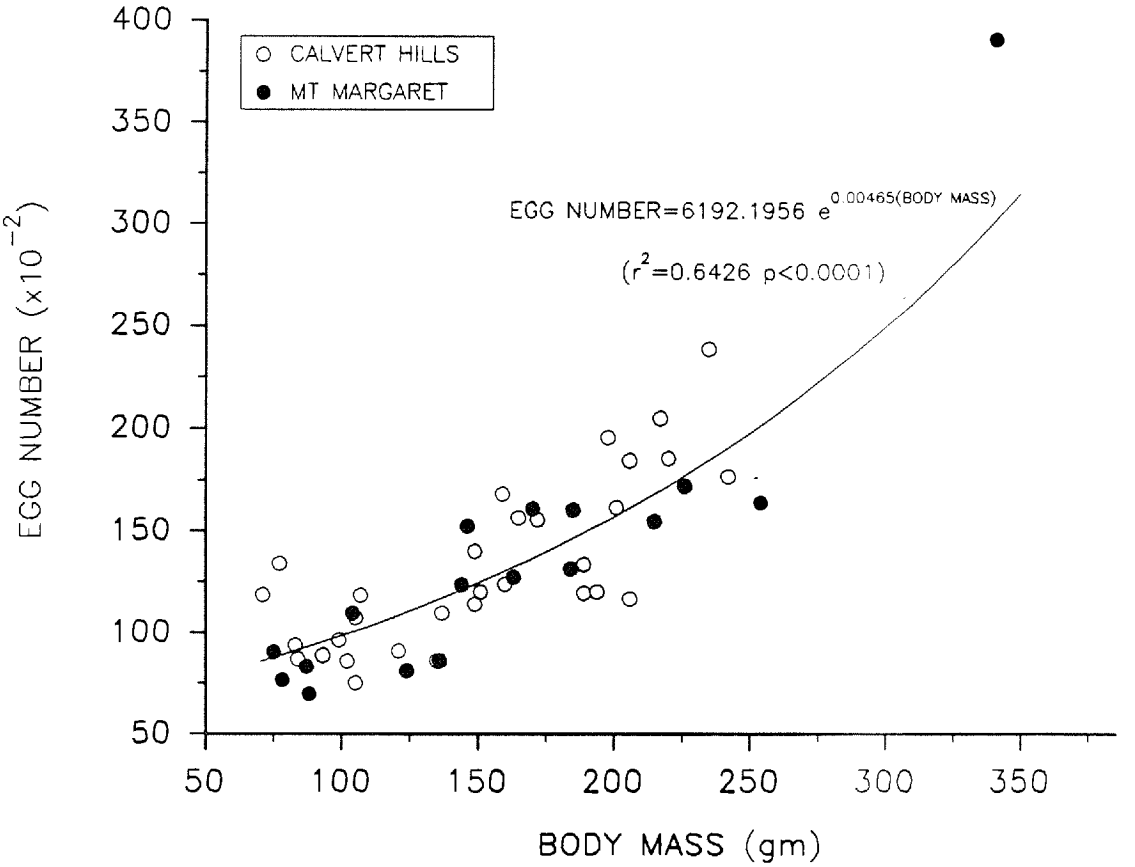
Table 3.11 B. Analysis of covariance for egg number per gravid female (log_e transformed) by season for Calvert Hills with female body mass as a covariate.

Class Levels: Site: Calvert Hills
Season: Wet / Early Dry / Late Dry
Covariate: Mass: Body Mass

Source of Variation	df	Sum of Squares	Mean Square	F	p
Model	5	0.3643	0.0728	13.07	0.0001
Season	2	0.0001	0.00005	0.01	0.9899
Mass	1	0.0824	0.0814	14.77	0.0007
Mass*Season	2	0.0015	0.0007	0.13	0.8750
Residual	25	0.1393	0.0056		
Total	30	0.5037			

Figure 3.17 Relationships for egg number and snout-vent length (**A**) and egg number and body mass (**B**) for females with stage 5 ovaries collected at Calvert Hills and Mt Margaret.


CALVERT HILLS
MT MARGARET
EGG NUMBER=1512.0642 e^0.01805(S-V LENGTH)
(r²=0.4526 P<0.0001)
EGG NUMBER (x10⁻²)
SNOUT-VENT LENGTH (mm)
400
350
300
250
200
150
100
50
80
100
120
140


CALVERT HILLS
MT MARGARET
EGG NUMBER=6192.1956 e^0.00465(BODY MASS)
(r²=0.6426 p<0.0001)
EGG NUMBER (x10⁻²)
BODY MASS (gm)
400
350
300
250
200
150
100
50
50
100
150
200
250
300
350

$$Egg\ Number = 1512.0642\ e^{0.018047\,(S\text{-}V\ Length)}$$

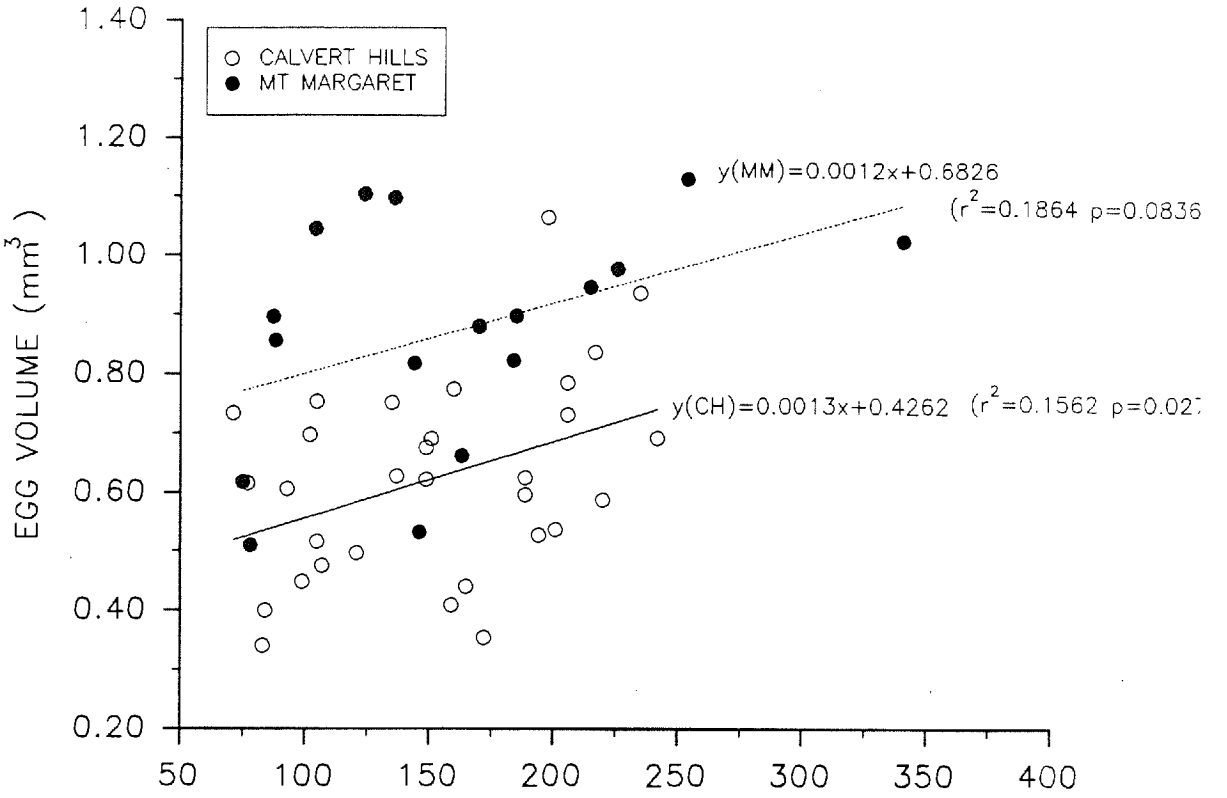
$$r^2 = 0.4526 \quad p < 0.0001$$

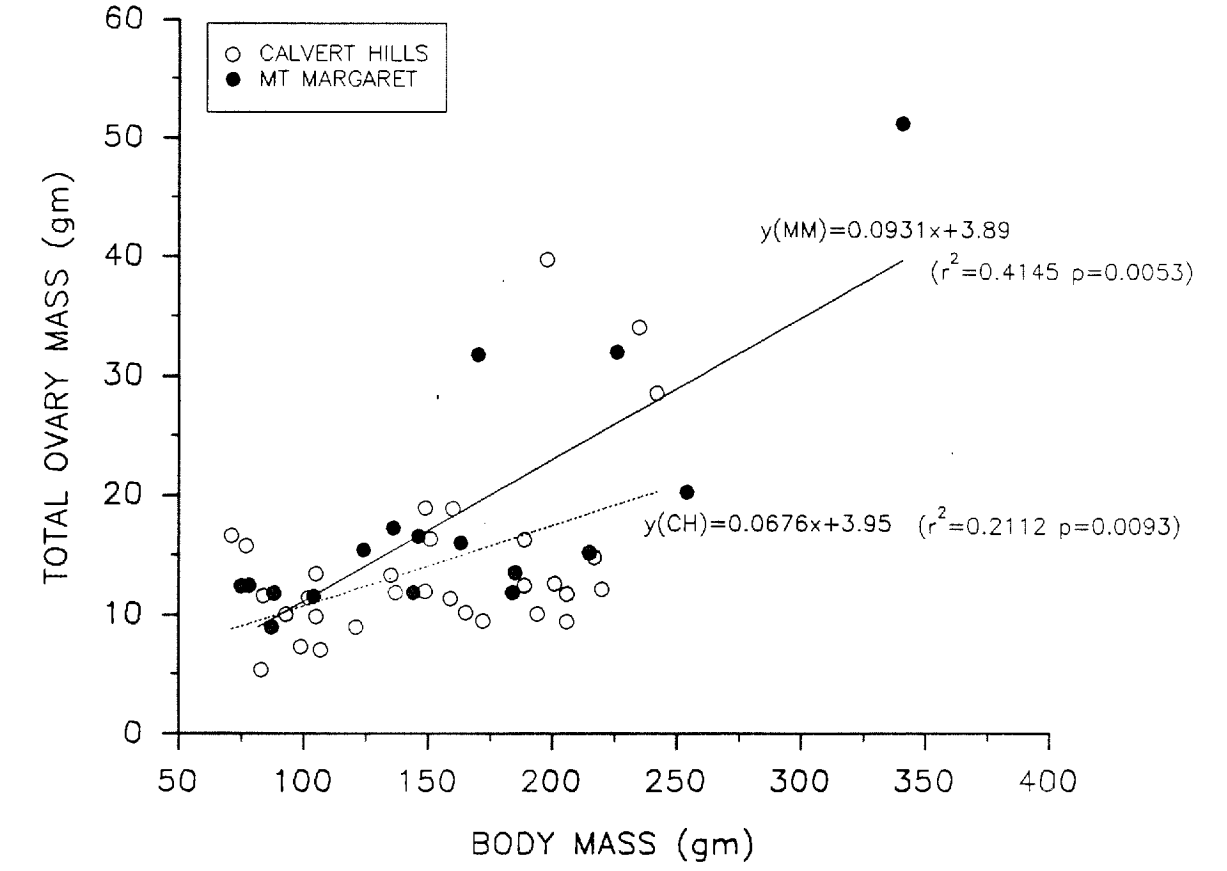
Egg Volume

Regression equations for mean egg volume from stage 5 females at both sites are shown in Figure 3.18A. This relationship was poor for Mt Margaret females. The effects of site and season on egg volume were analysed using ANCOVA with body mass as the covariate. Egg volume increased with female body size similarly for females at both sites. Season had no influence on egg size, however the effect of site ($F_{1,40}=4.37$, $p=0.0430$) shown by the difference in the regression intercepts indicate that egg volume was significantly larger for Mt Margaret females. Least-squares means for egg volume (the expected means for each site given a balanced design with the covariate at its mean value) and standard errors are: Mt Margaret, $0.85 \pm 0.06 mm^3$, and Calvert Hills, $0.59 \pm 0.06 mm^3$.

Regressions equations for total ovary mass of stage 5 females from each site are presented in Figure 3.18B. Both relationships were significant. The significantly higher slope of the equation for Mt Margaret (ANCOVA, covariate=body mass, $F_{2,44}=16.04$, $p<0.001$) indicates that with increasing size, females have significantly larger ovaries than Calvert Hills individuals.

Figure 3.18 **(A)** Regressions for mean egg volume in ovaries from stage 5 females collected at Calvert Hills and Mt Margaret. **(B)** Regressions for total ovary mass from stage 5 females collected at Calvert Hills and Mt Margaret.


CALVERT HILLS
MT MARGARET
y(MM)=0.0012x+0.6826
(r²=0.1864 p=0.0836
y(CH)=0.0013x+0.4262 (r²=0.1562 p=0.027
EGG VOLUME (mm³)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
50
100
150
200
250
300
350
400


CALVERT HILLS
MT MARGARET
y(MM)=0.0931x+3.89
(r²=0.4145 p=0.0053)
y(CH)=0.0676x+3.95 (r²=0.2112 p=0.0093)
TOTAL OVARY MASS (gm)
60
50
40
30
20
10
0
50
100
150
200
250
300
350
400
BODY MASS (gm)

A summary of fecundity and egg volume results indicate that fecundity of females is similar at both sites. However, for the same body size, egg volume is larger for Mt Margaret individuals. This larger volume is accounted for by a larger ovary mass for these females, relative to body size, which increases with increasing body mass (Figure 3.18B).

3.3.3 Recruitment Estimates

Recruitment rates for wet and early dry seasons are presented in Table 3.12. Due to the non-linear relationship between female body size and egg number, total eggs for the number of egg masses found was calculated by weighting for the proportion of females present in 10mm size classes above 90mm SVL. The fecundity of each size class was calculated from the midpoint. If the number of females in the sample was five or less, or the number of egg masses found equalled the number of females observed in amplexus then the estimate for egg number was calculated directly from observed snout vent lengths.

Daily recruitment rates were calculated for the period over which activity transect samples were taken. These were: Calvert Hills, wet-season=22 days, early dry season=16 days; Mt Margaret, wet-season=17 days, early dry season=14 days. Rate estimates are similar for both seasons at each site (Friedman's test, $p=0.1573$), though they tend to be less for Mt Margaret populations.

Table 3.12 Estimates for seasonal recruitment rates for Calvert Hills and Mt Margaret. Recruitment rate applies to input of eggs along the 250 metre transect of water margin where adult activity was monitored over the number of days in the period it was sampled. Dates refer to the actual night that egg masses were laid within the transect.

* refers to a single date observation at Mt Margaret not included in the Early Dry transect samples.

Date	Egg Masses	Range S.V.L.	Total Estimate	Cumulative Number	Rate (per day)
Calvert Hills - Wet Season					
12JAN88	4	96-109	38724	38724	
22JAN88	1	120	13185	51909	
31JAN88	4	101-122	49345	101254	
07FEB88	2	105-117	22549	123808	5627.41
Calvert Hills - Early Dry Season					
04MAY88	2	116-122	25348	25348	
17MAY88	1	117	12491	37839	
19MAY88	4	111-117	47956	85795	5362.19
Mt Margaret - Wet Season					
07MAR89	1	126	14693	14693	
17MAR89	3	119-130	43174	57867	
18MAR89	1	133	16672	74539	4384.65
Mt Margaret - Early Dry Season					
02JUL89	1	114-121	12732	12732	
03JUL89	3	103-118	34041	46773	3340.93
* 28MAY89	7	102-135	90044		

An additional estimate for recruitment is included in Table 3.12 for 28 May 1989 at Mt Margaret. On this occasion, breeding occurred after an isolated rainfall event which filled the dam to capacity after water levels had been decreasing over a 7 week period without rainfall. Females deposited 7 egg masses along a 15 metre section of the margin and in no other spot along the 250 metre transect. Recruitment was calculated for the mean female size from activity transects performed 30 days later, therefore it is probably an underestimate given that mean female size declines through the dry season (Figure 3.2C).

3.4 Discussion.

The results of this study indicate that there are very few differences between Calvert Hills and Mt Margaret in the reproductive ecology of adult populations. Though populations differed in size between the two regions, the sex ratio, physiological characteristics such as testes size and maturity, fecundity relationships, and timing of behavioural characteristics such as chorusing and amplexus were similar. One important difference was that egg volume differed between the two regions. The implications of this are discussed in more detail below. Despite the larger observed numbers of females active at Calvert Hills seasonal recruitment rates for eggs into the aquatic habitat were similar for both sites.

The larger population estimates for males and females at Calvert Hills (Table 3.2) cannot be explained within the context of this study but may be the result of a number of factors. Firstly, the Calvert Hills population may have been experiencing the explosive growth that often follows initial colonisation (Newsome and Noble 1986, Roughgarden 1986, Hengeveld 1988). Secondly, habitat differences, such as the quality and availability of shelter, or the abundance of predators may also affect population size. Predation by corvids on *Bufo boreas* has been shown to reduce the size of breeding male populations by twenty percent within a season (Olson 1989). High numbers (5 to 20/night within approximately 500m of shoreline) of male toads at Mt Margaret are commonly found eviscerated in a manner that indicates predation by native water rats, *Hydromys chrysogaster* (personal observations). Predation of

Bufo marinus by *Rattus rattus* has also been observed (Adams 1967, Fitzgerald 1990). Although the predation rate at Mt Margaret was not estimated, it appeared high. Sustained predation pressure, even from a small population of *Hydromys* may have reduced the toad population to a size where the highest estimate for male number was approximately two hundred and fifty. Adult mortality of this nature has not been observed in Calvert Hills populations.

While populations of adult males at breeding locations at Calvert Hills and Mt Margaret during the wet and early dry seasons differed in numbers, they both maintained male-biased sex ratios (Figure 3.3C) The sex ratios found at both sites are more biased than any previously reported. Male-female ratios within the natural distribution of *Bufo marinus* are commonly 2:1 (Zug and Zug 1979). A male biased breeding population has been reported for almost all studies of reproductive aspects of *Bufo* species that exhibit opportunistic breeding behaviour in ephemeral habitats (see Wells 1977 for review).

Similar to results reported by Zug and Zug (1979), only a small component of either the total male or female population were found active at water during the breeding period. The proportion of males in transects ranged from one to fifteen percent of the total breeding male population at both sites with the highest being twenty-five percent at Mt Margaret during the early dry season. Similarly for females, total proportions ranged from zero to fifteen percent. It is interesting to note that the number of recaptured individuals was far higher for males than for females. Some

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males were caught up to seven times within a sampling period. The lack of frequent recaptures of females explains the poor Petersen estimates for population size. Given that a high proportion of females caught in transects were gravid, it also indicates that females were approaching water to reproduce and when successful, may remain outside these areas for the duration of the season.

Female activity appears to change during the latter dry season when larger numbers were represented in transect samples. This was most likely related to rehydration. The large drop in Petersen estimates for the male population is presumably linked to the high proportion of non-active individuals remaining in shelter and not being represented in the sample (R Alford, pers comm). Therefore, population size was probably underestimated due to the unequal probability of capture for toads that remained in shelter. Thus estimates probably represent the active population only. Ninety to ninety-five percent of this active male population were sampled at Calvert Hills during the late dry period. This is likely to be linked to the local topography of the two sites. At Calvert Hills the total amount of water (and water margin) available to the population was small and consisted of widely separated, long, muddy pools of less than 0.2 metres in depth along the river course. In contrast, the Mt Margaret water source during the late dry period was considerably larger and was 0.5 to 1.0 metre in depth, though it was also contracting to a shallow pool. So while the same sized transects were sampled at both sites, toads at Mt Margaret may have had a larger choice of habitat along which to disperse during the evening.

The relationships between adult activity and environmental variables differed between sites and seasons. Although activity maxima of tropical species are usually associated with rainfall and photoperiod (Wolda 1989), only moon illumination was correlated with male and female activity during some sampling periods. It has been shown that increasing light improved prey-strike accuracy while foraging (Hailman 1984). It may also have a role in sex recognition in breeding aggregations. It is suspected that environmental data collected for this study are too sparse to represent realistic interpretations. Anuran activity may depend heavily on recent environmental histories such as changes in daily temperature and humidity (RA Alford, pers comm) but these could not be consistently recorded.

During the periods of breeding activity examined in this study, only a small percentage of the active male population is involved in calling and an even smaller proportion in amplexus. The latter behaviour is obviously restricted by the number of females present. A common feature in *Bufo* species is that a proportion of the males present at breeding aggregations are involved in chorusing behaviour while a higher number are silent. Silent males are actively searching for females close to those calling and attempt to intercept females as they approach (Wells 1977, Sullivan 1982a, Fairchild 1984, Jacobson and Vandenberg 1991). If presented with a male or a female, they will always try to amplex (personal observations). These are distinguished from the 'satellite' males of prolonged breeders that rarely clasp passing females but presumably wait for territorial males to vacate a calling site (Wells 1977). The proportion of males adopting this mate-searching strategy has

been shown to increase with increasing male density (Krupa 1989) though no evidence for this was shown in this study. Numbers of calling males correlate positively and significantly with numbers of males in three of the four breeding periods examined (Appendix 2). This is similar to choruses described for *Bufo valliceps* (Sullivan and Wagner 1988). Mate searching breeding strategies are associated with male-male competition and are most likely to be related to the high energetic cost of calling (Taigen and Pough 1985). Wells (1979) hypothesised that synchronous chorusing in breeding aggregations would not necessarily attract individual mates as calling by neighbours would interfere with each other and serve to decrease the individual's own call efficiency. Synchronous calling is more likely to be a long distance signal to females. The energetic constraints resulting in switching of behaviour between calling and searching (Fairchild 1983, Sinsch 1988) would also account for the periodic variation in the chorusing effort observed in all breeding seasons during this study.

Calling males were larger than those observed to be silent. Mean body size of amplexed males were larger than non-amplexed males (Figures 3.6 and 3.18). This corresponds with other studies that found calling males in breeding populations of various species were larger than silent males (Howard 1988b, Howard and Kluge 1985, Sullivan 1982a). Others suggest that there appears to be no selection for a larger male size (Wilbur et al 1978, Gatz 1981). There are no obvious phylogenetic or habitat-related patterns evident in these studies. Sullivan (1984b) suggests that size-assorted mating may involve a plethora of factors including sexual selection,

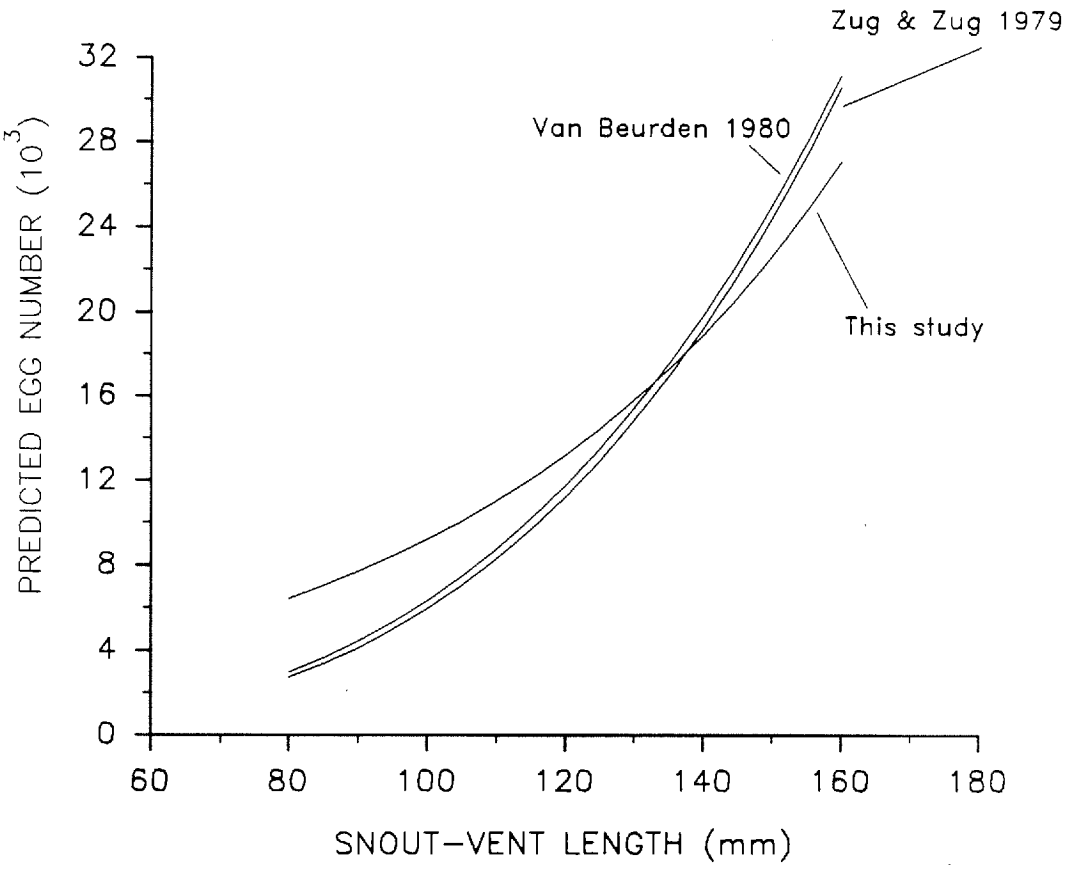
energetic contraints, size dimorphism and operational sex ratios. The likelihood of a female amplexing with the first male encountered is higher than initiating amplexus with a calling male in the chorus. The non-random mating patterns observed are probably the result of subsequent male-male competition rather than female choice. Initial amplexus may be random by size (Kruse 1981) but after amplexus, male-male competition, resulting in displacement of smaller males by larger ones, can produce a non-random pattern (Davies and Halliday 1977, 1978, Sullivan 1984, Loman and Madsen 1986, Höglund 1989). Further studies have shown that for *Bufo marinus*, a large male mating advantage was not a consequence of large body size *per se*, but that longer forelimbs (radio-ulnas), which was independent of body size, assisted in resisting displacement while in amplexus (Lee 1986). However, more intensive and detailed observations of behaviour are required to demonstrate the mechanism that produce the non-random pattern shown for this study.

The relationship between seasonal asynchrony in cycles of gonad maturation, synchronous growth of vitellogenic oocytes, and periods where high proportions of the population contain mature gametes resemble other reported studies of *Bufo marinus* (Wilhoft 1965, Zug *et al* 1975, Zug and Zug 1979) and other tropical populations of bufonids (*Bufo melanostictus*, Jørgensen 1986). The year-round presence of males and females with mature gametes, and the high proportion of females with fully mature ovaries in the pre-wet and early dry seasons indicate that breeding can occur whenever environmental conditions are favourable.

Testes mass and egg number relationships was not different between populations. The regression derived for fecundity (N=48 females) is compared to those described by Zug and Zug (1979, N=8 females) and Van Beurden (1980a, N=464) in Figure 3.19. Though the relationship described from this study is highly significant and identical for the two sites examined, the dissimilarity of these three relationships may be attributable to interpopulation variation. Estimates from previous studies indicate egg numbers from *Bufo marinus* in the range of 4,240 to 12,700 from Ecuador (Crump 1974), 6,050 to 23,000 from Panama (Zug and Zug 1979) and 1,800 to 54,000 from Australia (Van Beurden 1980a). The range recorded from this study was 6,970 to 36,100. No female was observed spawning twice in a sampling period but there has been a record of an individual spawning after an interlude of 74 days, laying an estimated 16,000 eggs in the first string and an undetermined number in the second (Buzacott 1936).

The seasonal recruitment rate estimates were similar between seasons and at both sites. It is interesting to note the extent of the variability that can occur when recruitment observed for a single event (1 night - 28 May 1989) can be larger than three of the four multiple-event periods (up to 30 nights) examined. This implies that there is a need for considerable caution when estimating these recruitment rates. If records for egg laying do not include the first few days or weeks of breeding during the wet season, then recruitment rates may be underestimated (personal observations).

Figure 3.19 Predicted fecundity relationships for snout-vent length as described by Zug and Zug (1979), Van Beurden (1980a) and this study.


Zug & Zug 1979
Van Beurden 1980
This study
PREDICTED EGG NUMBER (10^3)
32
28
24
20
16
12
8
4
0
60
80
100
120
140
160
180
SNOUT–VENT LENGTH (mm)

Egg size is often more variable within populations than fecundity, though both tend to vary from year to year (Cummins 1986, Ryser 1988, Kaplan 1989). Egg volume differed between the populations in this study. Reading (1986) found a trade-off between egg size and the number of eggs produced by female *Bufo bufo*. For the populations in this study, this trade-off did not occur. Females at Mt Margaret possessed larger eggs but also larger ovaries when compared with similar sized females from Calvert Hills. This accounted for the similar size-fecundity relationship in both populations. Therefore, the population at Mt Margaret appeared to be investing greater energy per body mass in egg production. The amount of investment appeared to increase with larger body size. It is possible that egg volume may also be related to female age (Ryser 1988). This would suggest that reproductively mature females in the Mt Margaret population may represent individuals older than females of a similar size at Calvert Hills.

Egg size may also be controlled by some form of sensitivity to environmental conditions detected by females, similar to larval perception of the environment in the timing of metamorphosis (sensu Wilbur 1980, Alford and Harris 1988). Calvert Hills and Mt Margaret females may be demonstrating a plasticity by producing different sized ova in response to different environmental conditions. Plasticity in individual mean ova size between breedings under different environmental conditions has been demonstrated for anuran species (Kaplan 1989). Perhaps conditions for breeding adults at Mt Margaret were more favourable, allowing a greater amount of investment for larger egg size without a trade-off in fecundity. However, Cummins

(1986) points out that as egg size can vary with proximate environmental effects, a single years' data may not be sufficient to accurately compare reproductive characteristics for separate populations.

In summary, an important result from this section of the study is the implication of variation exhibited in egg size for the two breeding populations. Both populations exhibit similarities in the timing of breeding and season duration, behavioural characteristics such as chorusing and proportion of the total male and female populations involved in breeding, operational sex ratios and changes with seasonal conditions, fecundity, and seasonal cycles of gonad maturation. However, investment in reproductive output (egg size) may be have been under the control of differing levels of environmental condition of mean female age. Previous studies of intraspecific variation in egg size in anurans have shown that it can affect fitness parameters such as size at hatching, larval growth rate, duration of larval period and size at metamorphosis (Kaplan 1980, Berven 1982). These larval characteristics are investigated in the following chapter.

Chapter 4. Population Biology.

4.1 Introduction

The density dependent effects of intraspecific competition are of principal importance in the dynamics of natural populations of anurans. Significant mortality during the embryonic and larval stages of amphibians has been demonstrated in several studies (Licht 1974, Cecil and Just 1979, Yorke 1983, Malone 1985, Stenhouse 1987, Ireland 1989). In studies examining competition between conspecifics and with other species, intraspecific competition is usually the dominant factor influencing growth and mortality (Connell 1983). In anurans, larval density has been identified as an important factor controlling growth and survival (Herreid and Kinney 1966, Brockleman 1969, Calef 1973, Smith-Gill and Berven 1979, Semlitsch and Caldwell 1982, Smith 1983, Scott 1990, reviews by Wilbur 1980 and Alford 1991). Growth and survival may be modified by interactions between density and factors such as food (Wilbur 1977a, Hota and Dash 1981, Steinwascher and Travis 1983), levels of social interaction (Breden and Kelly 1982, Sokol 1984), spatial complexity (John and Fenster 1975), habitat quality (Travis and Trexler 1986) or pH (Clarke and Hall 1985, Cummins 1989). Interference mechanisms appear to supplant exploitative mechanisms as the relative food level decreases, or levels of density increase (Wilbur 1977b, Steinwascher 1978).

Growth rates influence the survival and ultimate fitness of anuran larvae. Faster growth decreases the time spent in temporary habitats. In these habitats, the risk of mortality from predation, desiccation, and competition accumulate with increasing larval periods

(Wilbur and Collins 1973, Travis 1974, Heyer et al. 1975, Collins 1979, Wilbur 1980, Werner 1986, Ludwig and Rowe 1990, Rowe and Ludwig 1991).

This chapter examines aspects of the growth and survivorship of embryos and tadpoles of *Bufo marinus*. To determine the importance of intraspecific competition, the effects of density on growth and survival were determined for tadpoles in two experimental systems: small *in situ* experimental enclosures, and larger artificial ponds. Field sampling of uncaged populations in natural habitats was conducted to relate patterns of natural density to experimental procedures and to compare natural growth and survival patterns with experimental populations.

4.2 Methods

Larval life-history was examined in two stages. Growth and survival was monitored from the egg (Gosner stage 1) or very soon after stage 1, through embryogenesis to the free swimming larvae (Gosner stage 24). Larvae were then monitored for growth and survival to metamorphosis using experimental and field sampling techniques.

4.2.1 Field Sampling

Regular sampling of tadpoles was carried out during January-February 1988 and May 1988 at the Calvert River and December 1988, March 1989 and May-June 1989 at Mt. Margaret Dam. During the August to September period and the November period at Calvert Hills no eggs or larvae were found. All sites were substantially contracted or dry therefore no sampling was conducted. At Mt. Margaret, the wet season sampling period was interrupted by extensive rains associated with Cyclones Delilah (January), Harry (February) and Aivu (April). This additional rainfall regularly removed populations from the study area by flooding the margins of the dam. Water currents increased dispersal when these flooded areas drained into an adjacent water course. Sampling was not continued until breeding and subsequent recruitment occurred in May.

Sampling was carried out using a 'drop-box' sampler, constructed of galvanised steel. Dimensions of the box were 1.0m long, 0.5m wide and high with a 2 by 4cm wooden

rim around the top edge for handling. Due to their mobile nature and the aggregation behaviour frequently displayed by *Bufo* tadpoles (Mares 1972, Breden et al. 1982, Waldman 1982, Woodward 1987), a stratified sampling program was used. A 500m transect along the bank of an area of habitat was selected. This transect was selected after tadpoles were found in the habitat and inspection at night revealed that adults were present. On each sampling day, samples were taken in a 100m subset of the 500m transect. The placement of the 100m transect was decided after inspection of the 500m transect on each sampling occasion. If an aggregation was detected then this was selected as the centre of the 100m transect. If no aggregation was detected, the 0m mark of the 100m transect was placed where tadpoles were first spotted along the 500m. The positions at which samples were taken along the 100m transect were decided before sampling using a random number generator. At each sampling position, a coin toss was used to decide if the sample was to be approximately ankle-deep (150mm) or shin-deep (300mm) (see Appendix 3). Ten samples were conducted on each sampling occasion with the distance from the edge to a point where depth became greater than 300mm noted for each sample taken. Samples were taken by approaching the precise location to be sampled, dropping the box into the water, and pushing down to seal the sampler on the substrate. Water temperature was then recorded and the depth in the centre of the sampler noted. Dissolved oxygen concentrations (mg/L) just above the level of the substratum were recorded using a Titron dissolved oxygen meter. Any tadpoles present in the sample were removed using a 0.5m² net swept through the water in the sampler. Sweeps were continued until three consecutive sweeps contained no tadpoles. Tadpoles were separated into native species and *Bufo marinus*. Native species were released. All

Bufo marinus tadpoles were transferred to a plastic container and photographed with a millimetre scale. Tadpoles were then released back into the sampler, which was then lifted and carried to the next sampling position. Sampling was always carried out between three to five hours after sunrise to minimise the catch-per-unit-effort variation due to differences in time of day or water temperature that might affect activity patterns or the position of tadpoles (Beiswenger 1978, Floyd 1983a, Dupré and Petranka 1985, Wollmuth and Crawshaw 1988).

Population estimates for each sampling occasion were derived from mean sample density and the total area sampled. Total area was based on transect length (100m) and margin width. Margin width was measured as the perpendicular distance between the shore and the point where depth exceeds 300mm for each spot at which a sample was taken. The total area sampled on each occasion was then calculated as the product of the transect length (100m) and the mean margin width for that day. Sampling-area related estimates for density were also compared with estimates that characterise density taking into account the heterogeneity of resources (area of habitat) available to each individual (see Lewontin and Levins 1989). Resource weighted densities (D_R) involve the classical notion of the ratio between the number of individuals and the total area. Organism weighted densities (Do) as defined by Lewontin and Levins, involve a different concept of effective density, being the mean density experienced by tadpoles. This takes into account that a mean of density derived from a group of samples, some patches will contain very few individuals and others will contain considerably higher numbers of individuals.

Organism weighted density, Do, is defined by:

$$D_O = \sum_{i=1}^{n} \left(\frac{N_i}{R_i} \cdot \frac{N_i}{N_T} \right)$$

where n is the number of samples, N_i is the number of individuals in the ith sample, R_i is the area of the ith sample and N_T is the total number of individuals in n samples. Thus it calculates density at which individuals are sampled and weights these densities by the proportion of organisms living at that density. Densities were also calculated as mean numbers per litre.

Number of tadpoles per sample and the snout-vent length of each individual were recorded from the photographs for each sample using a dissecting microscope and microcomputer-digitiser.

4.2.2 Growth and Survival of Eggs.

Fertilised egg strings were collected from the field soon after laying and returned to a laboratory. Ten haphazardly selected replicates of 100 eggs were removed from the main string as continuous segments and placed in 400ml round polypropylene containers. For competition studies (see Chapter 5) an additional ten replicates were placed with five older *Bufo marinus* tadpoles (stage 26-30). Survival of the eggs was monitored at regular intervals until development reached Gosner stage 24. An extra replicate was set up for monitoring growth: at each census, five representative

individuals were removed from this replicate and preserved. Their total length was measured using a dissecting microscope and eyepiece micrometer. These experiments were conducted during January 1988 (two series) and May 1988 at Calvert Hills and January 1989 at Mt Margaret (sites described in Chapter 2).

In situ Survival of Eggs

In January 1988, the survival of eggs placed in the habitat from which they were collected was monitored. The site used was a section of non-flowing stream within the Little Calvert River approximately 15 metres wide and 75 metres long. The substratum consisted of sparse leaf litter over sand and loose rocks 10-60cm in diameter. Containers similar to those used in the laboratory experiments were buried so that the lip of the container was flush with the substrate. One treatment was open to colonisation by predators, the second had mesh-screened (400μm) lids to exclude predators. A segment of 100 eggs was placed in each container and survival was monitored at regular intervals until hatchlings became mobile enough to escape the unscreened containers. Ten replicates of each treatment were placed haphazardly in the stream bed. Survival of eggs in the unscreened treatment is examined in section 5.3.1.

Survival of eggs was analysed using a Profile Analysis MANOVA. Responses were percent larvae surviving (arcsine-squareroot transformed) at 0, 16, 38, 60 and 70 hours.

4.2.3 Growth and Survival of Swimming Larvae.

Tadpoles were reared from hatching to metamorphosis in three litre enclosures (pots) and 1000 litre artificial ponds for growth and survival experiments. Larger versions of suspended enclosures built on the same principle have been evaluated and recommended for larval fish mortality studies (De Lafontaine and Leggett 1987). The use of artificial ponds has been criticised (Jaeger and Walls 1989) and defended (Hairston 1989, Morin 1989, Wilbur 1989) but their benefits (greater precision and sophistication of design and analysis), their ability to complement the results of ecological observations and unrestricted field experiments, and their current wide usage by community ecologists demonstrate their value.

Enclosure Experiments

Larvae were reared in enclosures at densities of 15 larvae (5 per litre) and 30 larvae (10 per litre). Ten replicates of each density were used. Experiments were conducted during January and May 1988 at Calvert Hills and January 1989 at Mt Margaret. For January 1988, an additional 10 replicates of 50 larvae (16.7 per litre) was used. Enclosures were cylindrical pots made of white plastic (140mm diameter, 210mm height). Windows were cut into opposite sides (90mm x 130mm) and these were covered with 2 mm flyscreen mesh.

Lids were fitted with styrofoam blocks so that pots floated upright. Groups of four pots were tethered to wood posts driven into the substratum. Posts were placed 1 metre apart. Pots were placed at the intended site at least 5 days prior to larvae being introduced. This allowed settlement of detrital materials and colonisation of the surfaces by algae and microfauna. Pots were tied to the posts so that they were fully submerged with the base situated just off, or resting on the substrate. Posts were moved the shortest distance possible throughout the experiments to maintain this level when water levels varied due to rain or drying. Posts were placed in habitat where *Bufo marinus* tadpoles were found to commonly occur. This was usually immediately adjacent to sites where box-sampling was conducted (section 4.2.1).

Egg strings were collected from the region of the study site the morning after they were spawned. They were transferred to the laboratory and kept in lightly aerated aquariums at uniform densities until hatching took place. At approximately two days after hatching the tadpoles were at Gosner stage 24. A haphazard sample of twenty tadpoles was preserved for snout vent length measurement. Others were counted into groups of five, and these groups fully randomised into containers of 15, 30 and 50 tadpoles. These containers were transferred to the study site and haphazardly assigned to pots.

Bufo tadpoles cannot be tagged to obtain individual growth measurements. Thus, the mean snout vent length for individual pots at each interval was used to assess growth. Before introduction, the tadpoles in each replicate were photographed with a millimetre scale for length measurement. At intervals of three days, the pots were recensused and

number of survivors was recorded. Maximum and minimum water temperatures were recorded and tadpoles were rephotographed on each occasion. This procedure was repeated until at least half the tadpoles remaining had erupted fore-limbs (Gosner stage 42) and were deemed to have successfully metamorphosed. When larvae reached metamorphosis, they were transferred to individual containers until the completion of tail absorption, and photographed.

Mass at metamorphosis and larval period were analysed together using MANOVA as these effects are not considered as independent attributes of larval anuran life-histories (Wilbur and Collins 1973, Alford and Harris 1988). Proportion surviving after 3, 6, 12, 14, and 19 days of the larval period (arcsine-squareroot transformed) in each enclosure was analysed separately using a Profile Analysis MANOVA as some replicates produced no survivors, leaving metamorph mass and larval period undefined. Final percent survival of larvae (arcsine-squareroot transformed) for all experiments was analysed using ANOVA.

Appropriate growth curves were fitted to the measurements of mean snout vent length using a non-linear regression estimation procedure (NLIN, SAS Institute 1987). Curves were fitted for individual replicates within a treatment (each enclosure) and for the composite (all enclosures) to determine differences in terms of the model parameters. Comparisons between individual curves within a treatment and between composite curves were based on asymptotic F-tests calculated in a manner analogous to F-tests for nested linear-regression models (Delaney and De'ath 1990). To test for equality in

individual parameter values for curves within treatments and between composite curves, the following test was used;

$$\chi = \sum_{i=1}^{N} \sum_{j=1}^{n} \left(\frac{\overline{\beta}_i - \beta_{ij}}{SE_{(\beta_{ij})}} \right)^2 \sim \chi^2 \quad d.f. = \sum_{i} (n_j - 1)$$

where $\beta_{i\cdot}$ is the mean for all the parameter estimates in the treatment i, β_{ij} is estimate for replicate j in treatment i, n is the number of replicates and N is the number of treatments, and $SE_{(\beta ij)}$ is the standard error for the replicate parameter. This test compares the distribution of replicate values for a particular parameter against the normal distribution for the mean value of the treatment in a way that approximates the chi-squared distribution.

Experimental Ponds

Above-ground, 1000 litre plastic cattle-watering tanks were used as experimental ponds. Before an experiment, tanks were scrubbed clean and filled with water from the domestic supply. One kilogram of dried leaf litter was then added to each tank. The litter was collected from an existing aquatic habitat, usually the margin of a dam where the water level had receded. After being filled, each tank was covered with a lid to exclude colonisation of the pond by other frog species and dragonflies. Lids were constructed of a wood frame over which was stretched 70 percent shade-cloth (maximum mesh size 2mm). The tanks were then left for 24 hours to settle.

Macrophytes (0.5 kg of *Ceratophyllum demersum*) and a zooplankton inoculum were then added. Zooplankton samples were collected from Ross River using a 63μm net. Samples were concentrated, thoroughly mixed and 100ml subsamples added to each tank. To supplement the initial nutrient pool for each community, a 100g sample of crushed and mixed lucerne-Tetramin® fish food (3:1 respectively) was added. This settled as a detritus layer on the bottom of each tank. The tanks were then left for 48 hours before tadpoles were added.

The experiment was designed to investigate the effect of tadpole density on growth and survival. Four replicates of five densities (30, 60, 120, 240 and 480 per 1000l) were used. Larvae were raised initially from a single egg mass collected at Townsville on 5 July 1989. Eggs were kept in aerated aquaria at uniform densities until hatching occurred on 9 July 1989. Groups of five larvae were then selected and randomly assigned to the five density treatments. Ten larvae from each replicate were weighed, then all replicates were randomly assigned to ponds on day 9. Ponds were arranged in a randomised 5X4 blocked array with no obvious environmental gradients. On days 19 and 35, each pond was visually censused and ten larvae from each pond were weighed and returned. Metamorphs were then weighed as they emerged from each pond.

The responses of mass at metamorphosis and length of larval period were analysed together using MANOVA (Wilbur and Collins 1973, Alford and Harris 1988). Proportion of survivors (arcsine-squareroot transformed) from each experimental pond was analysed separately using ANOVA. The responses of mean mass and proportion

surviving (arcsine-squareroot transformed) on days 9, 19 and 35 were separately analysed using a Profile Analysis MANOVA.

4.3 Results.

4.3.1 Field-Sampling

Population Densities

The mean number per m^2 (log_{10} transformed) for each sampling date within each sample period is shown in Figure 4.1. Population and density estimates for these dates are shown in Table 4.1. Density estimates include resource weighted ratios (D_R) and organism weighted ratio (D_O) (see section 4.2.1). Population estimates were derived from the mean density (D_R) and the total area sampled (100m multiplied by the mean width of the transect margin, *m*). References to mean density below refer to D_R unless stated otherwise. Numbers per sample were $log_{10}(x+1)$ transformed to normalise the variances. Densities were analysed using a mixed-model ANOVA with site and sample period (month) as fixed effects. Sample date was included as a random effect and was nested within sample period. There was significant variation in density between sampling dates within a particular sampling period (ANOVA, $F_{12,17}=3.03$ $p=0.0005$). Mean density was significantly lower for the May sampling period at Calvert Hills (ANOVA, $F_{2,17}=16.95$ $p=0.0001$). There was also significant variation for density

Figure 4.1 $Log_{10}(x+1)$ transformed values for mean density per m^2 ($\pm$ 95% C.L.) caught in ten box-samples for each sampling period:

(A) Calvert Hills - 24 January to 17 February, 1988
(B) Calvert Hills - 6 May to 20 May, 1988
(C) Mt Margaret - 23 May to 30 June 1989.

Arrows indicate the night on which eggs were laid within the transect area.

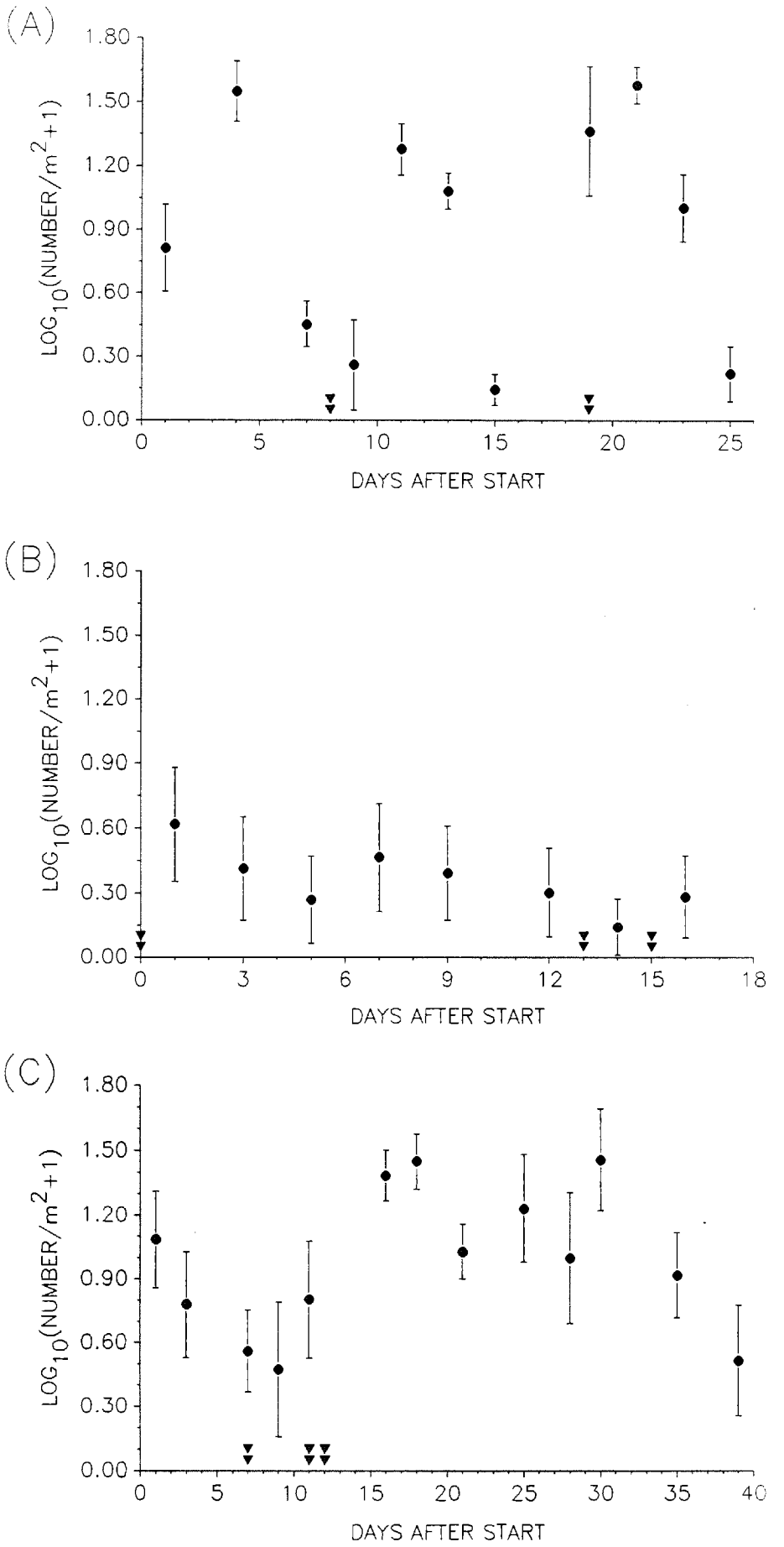

(A)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
LOG10(NUMBER/m2+1)
0
5
10
15
20
25
DAYS AFTER START
(B)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
LOG10(NUMBER/m2+1)
0
3
6
9
12
15
18
DAYS AFTER START
(C)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
LOG10(NUMBER/m2+1)
0
5
10
15
20
25
30
35
40
DAYS AFTER START

Table 4.1 Details of transect date, mean tadpole densities per m^2, maximum and minimum numbers per m^2, and predicted population estimates for transect (N) and variance-mean ratio for box samples taken at Calvert Hills (CH) and Mt Margaret (MM). Margin width is the mean distance from the edge to a depth of 300mm at each spot a sample was taken. D_R refers to the standard area related (ie resource-weighted) mean density per m^2, Do refers to an organism-weighted density (see text for explanation). Also shown are mean (Dv) and maximum numbers per litre.

(†, minor sampling dates - see section 4.3.1)
(*, observed recruitment event prior to sampling)

				TADPOLES										
SITE	DATE	SAMPLES	MARGIN	MEAN/m²								MEAN/l		
			(m)	DR	DO	DO/DR	SE(DR)	N	MAX	MIN	V.M.R	DV	MAX/l
CH	24/01/88	10	1.1	16.2	66.3	4.09	9.49	1782	100	0	55.663	0.435	0.90
	27/01/88	10	1.1	47.8	68.2	1.40	10.40	5258	110	2	22.658	0.359	0.55
	30/01/88	10	1.2	2.6	4.5	1.73	0.73	312	6	0	2.068	0.018	0.14
	01/02/88 *	10	1.3	13.8	134.1	9.72	13.57	1794	136	0	133.620	1.232	2.22
	03/02/88	10	1.4	25.0	44.8	1.79	7.42	3500	84	2	22.053	0.534	0.98
	05/02/88	10	1.4	13.4	19.2	1.43	2.98	1876	32	4	6.637	0.306	3.66
	07/02/88	10	1.7	0.6	2.0	3.33	0.30	102	2	0	1.556	0.055	0.79
	11/02/88 *	10	1.7	142.6	781.2	5.48	100.59	24242	1036	0	709.584	5.453	7.55
	13/02/88	10	1.9	46.2	80.3	1.74	13.23	8778	160	18	37.892	3.976	5.76
	15/02/88	10	1.9	18.0	45.5	2.53	7.41	3420	62	2	30.519	1.675	3.33
	17/02/88	10	2.0	2.0	13.2	6.60	1.57	400	16	0	12.444	0.174	0.45
CH	06/05/88 *	10	0.4	28.6	144.7	5.06	19.21	1144	176	0	129.046	0.234	1.22
	08/05/88	10	0.4	12.6	103.8	8.24	11.29	504	114	0	101.309	0.622	2.98
	10/05/88	10	0.4	10.0	65.7	6.57	7.86	400	78	0	61.867	0.786	1.87
	12/05/88	10	0.5	15.0	71.5	4.77	9.70	750	88	0	62.770	0.334	0.95
	14/05/88	10	0.5	11.0	78.6	7.15	9.08	550	92	0	75.091	0.223	0.54
	16/05/88	10	0.6	12.0	112.2	9.35	11.55	720	116	0	111.333	0.956	3.34
	18/05/88 *	10	0.6	2.6	26.0	10.00	2.60	156	26	0	26.000	0.143	0.91
	20/05/88 *	10	0.5	5.2	28.5	5.48	3.66	260	34	0	25.846	0.066	0.37
† MH	14/12/88	10	1.2	42.8	64.6	1.51	10.19	5136	118	6	24.240	0.215	0.44
	18/12/88	10	1.2	1.6	1.6	2.50	4.00	192	6	0	2.670	0.012	0.04
	22/12/88	10	0.8	0	0	-	0	0	0	0	-	-	-
	28/12/88	10	0.8	0	0	-	0	0	0	0	-	-	-
† MH	12/03/89	10	1.4	21.8	106.3	4.88	14.31	3052	150	0	93.932	0.104	0.71
	16/03/89	10	1.5	2.8	6.0	2.14	1.71	525	8	0	3.332	0.018	0.03
	19/03/89	10	0.9	0	0	-	0	0	0	0	-	-	-
	21/03/89	10	0.8	0	0	-	0	0	0	0	-	-	-
MH	23/05/89	10	1.2	28.4	71.5	2.52	11.66	3408	122	0	47.944	0.253	0.60
	25/05/89	10	1.3	22.8	92.0	4.04	13.23	2964	130	0	76.850	0.422	1.07
	29/05/89 *	10	1.3	6.6	15.2	2.30	2.51	858	20	0	9.569	0.052	0.17
	31/05/89	10	1.3	48.2	252.7	5.24	33.00	6266	294	0	227.173	0.334	1.05
	02/06/89	10	1.5	24.4	71.8	2.94	11.30	3660	110	0	52.707	0.390	1.26
	07/06/89 *	10	1.4	32.2	54.8	1.70	8.99	4508	92	4	25.133	0.174	0.54
	09/06/89	10	1.5	42.0	88.0	2.10	14.65	6300	140	6	51.153	0.191	0.69
	12/06/89	10	1.6	15.6	41.3	2.65	6.67	2496	74	2	28.536	0.085	1.86
	16/06/89	10	2.0	74.0	334.0	4.51	46.23	14800	466	0	288.841	2.311	6.86
	19/06/89	10	2.1	92.2	616.8	6.69	73.30	19362	746	0	582.834	4.839	8.39
	21/06/89	10	2.1	74.2	194.0	2.61	31.42	15582	304	0	133.123	2.354	4.51
	26/06/89	10	2.2	28.6	172.9	6.05	21.41	6292	220	0	160.343	1.309	2.44
	30/06/89	10	2.0	31.4	265.1	8.44	28.55	6280	288	0	259.676	2.146	4.97

within a particular sampling date (ANOVA, $F_{17,288}=3.61$ $p<0.0001$). Within sampling dates, mean densities per m^2 ranged from none to 143 (11 February 1988, Calvert Hills). The maximum numbers caught in samples were 518 (11 February 1988, Calvert Hills) and 373 (19 June 1989, Mt Margaret). It is possible that at least part of the variation between dates was due to dispersal within or in and out of the transect from other habitats due to changes in water temperature, dissolved oxygen or the variation in water depth sampled. However, no significant correlations existed with any of these variables. Mean density ($\pm$ 95% CL) was highest for Mt Margaret (MM=40.0$\pm$16.21 CH=22.3$\pm$11.57), (ANOVA, $F_{1,319}=13.14$ $p=0.0003$). Estimates of population size per transect for each of the sampling dates are shown in Table 4.1. There was very high variance for these estimates during each of the three major sampling periods. Only one of the increases in population size could be explained by an observed recruitment event (Calvert Hills, 11 February 1988 - see Section 3.3.3, Table 3.13).

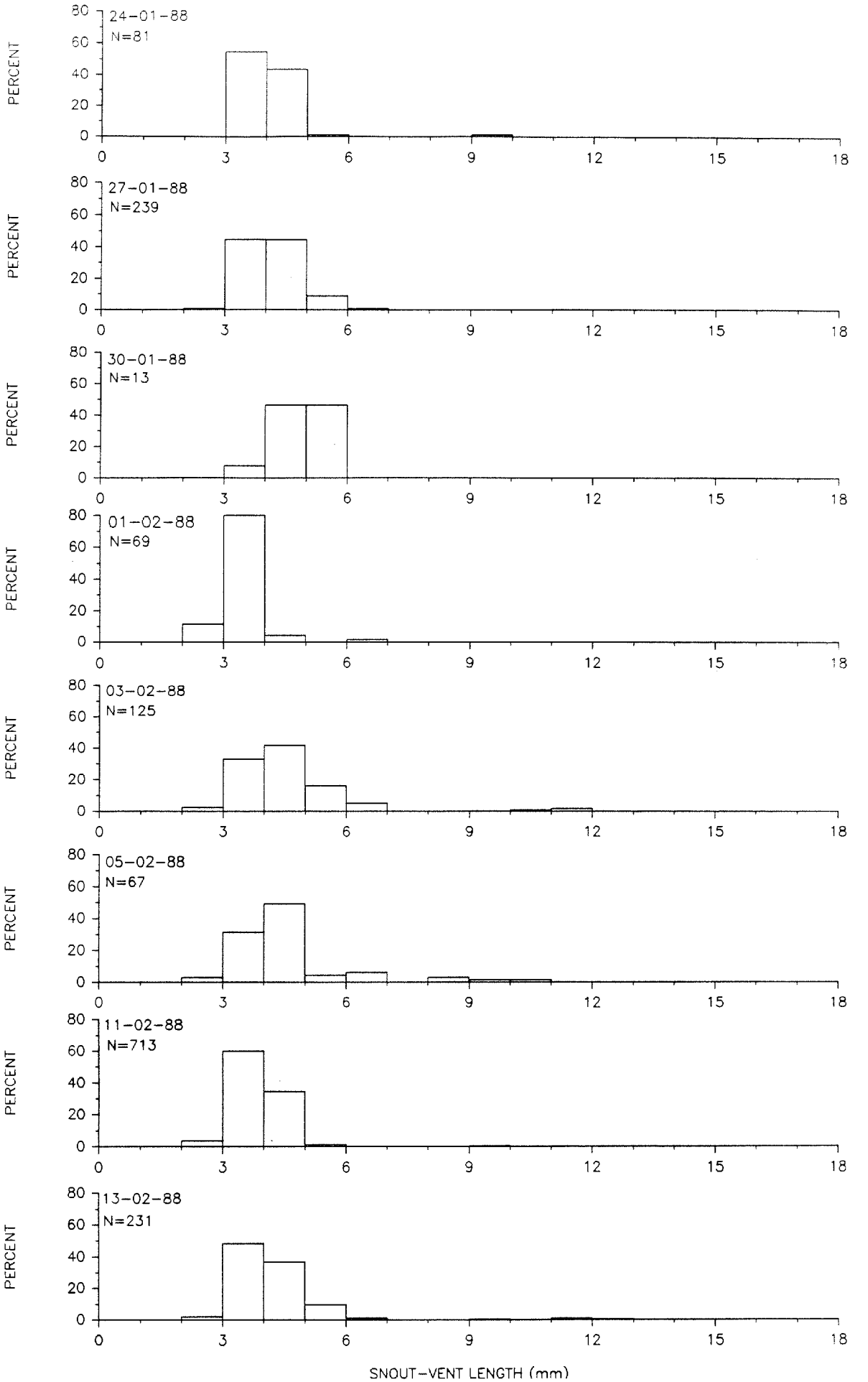
Within sampling dates, the variance-mean ratio for tadpoles caught each set of 10 box samples is always greater than one, indicating a high degree of aggregation. The high level of heterogeneity is also indicated by the ratio of Do to D_R. Values for Do return the highest estimates for effective density. Do would equal D_R only if all samples contained the same number of individuals. Aggregation of tadpoles poses difficulties in the interpretation of estimates such as population size as discussed below.

Size Frequency Distributions

The length frequency distributions for larvae sampled during the three sampling periods are presented in Figures 4.2A-C. For the wet season sampling period at Calvert Hills (January-February 1988, Figure 4.2A), the distributions show modal size classes predominantly in the 3 to 6 mm size-classes. This suggests input from multiple recruitment events observed over the sampling period (see section 3.3.3). Despite this, few tadpoles are represented in the larger size classes in any sample, indicating high mortality. The dry season sampling period at Calvert Hills (May 1988, Figure 4.2B) shows a bimodal size distribution suggesting the presence of two cohorts with larvae between 3 to 13mm on most sampling dates. A cohort of 3mm size class tadpoles appeared in the population on May 20. The dry season sampling period for Mt Margaret (May-June 1989, Figure 4.2C) shows a cohort present on May 23 progressing through to metamorphic size from May 31 to June 7. There is a second recruitment of 2-3mm tadpoles occurring on May 31 and progressing through to metamorphic size classes on June 19 through to June 30.

Separation of the modal size classes (using 1mm length intervals) was analysed using the ELEFAN system of programs after data were restructured to compensate for zero and low frequency values near peaks (see Brey and Pauly 1986, and Gayanilo *et al.* 1988 for complete descriptions of the method). This produced either a single mode or number of modes for each sampling date within each sampling period (Figure 4.3).

Figure 4.2A Length frequency distributions for larvae caught in box samples on each sampling occasion for Calvert Hills from 24 January 1988 to 17 February 1988. Date of sample and total number caught are noted in each case.


24-01-88
N=81
27-01-88
N=239
30-01-88
N=13
01-02-88
N=69
03-02-88
N=125
05-02-88
N=67
11-02-88
N=713
13-02-88
N=231
PERCENT
0
20
40
60
80
0
3
6
9
12
15
18
SNOUT-VENT LENGTH (mm)

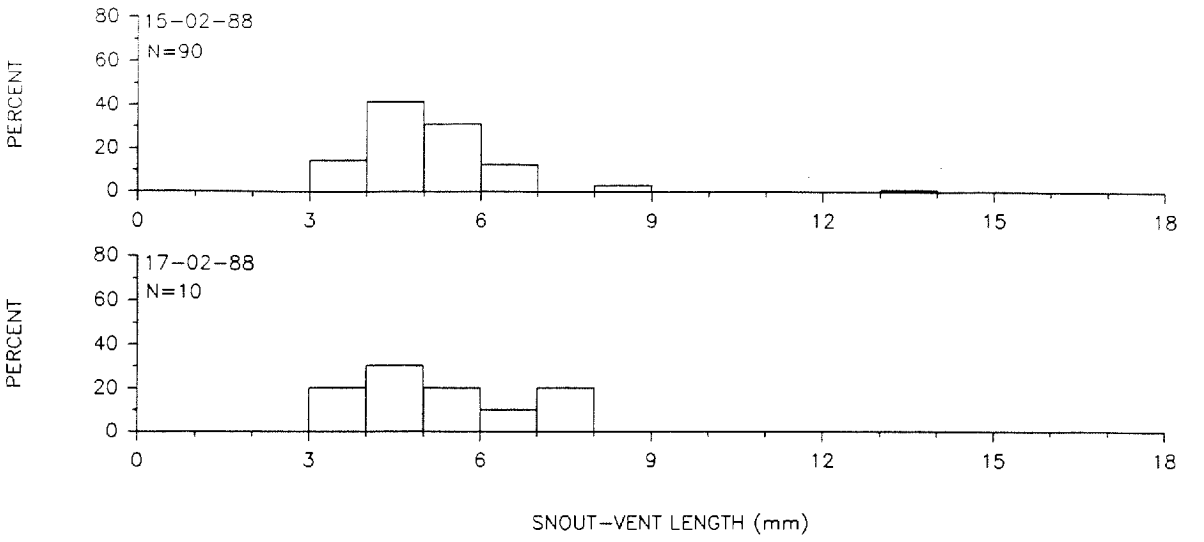

15-02-88
N=90
17-02-88
N=10
PERCENT
PERCENT
80
60
40
20
0
0
3
6
9
12
15
18
SNOUT-VENT LENGTH (mm)

Figure 4.2B Length frequency distributions for larvae caught in box samples on each sampling occasion for Calvert Hills from 6 May 1988 to 20 May 1988. Date of sample and total number caught are noted in each case.

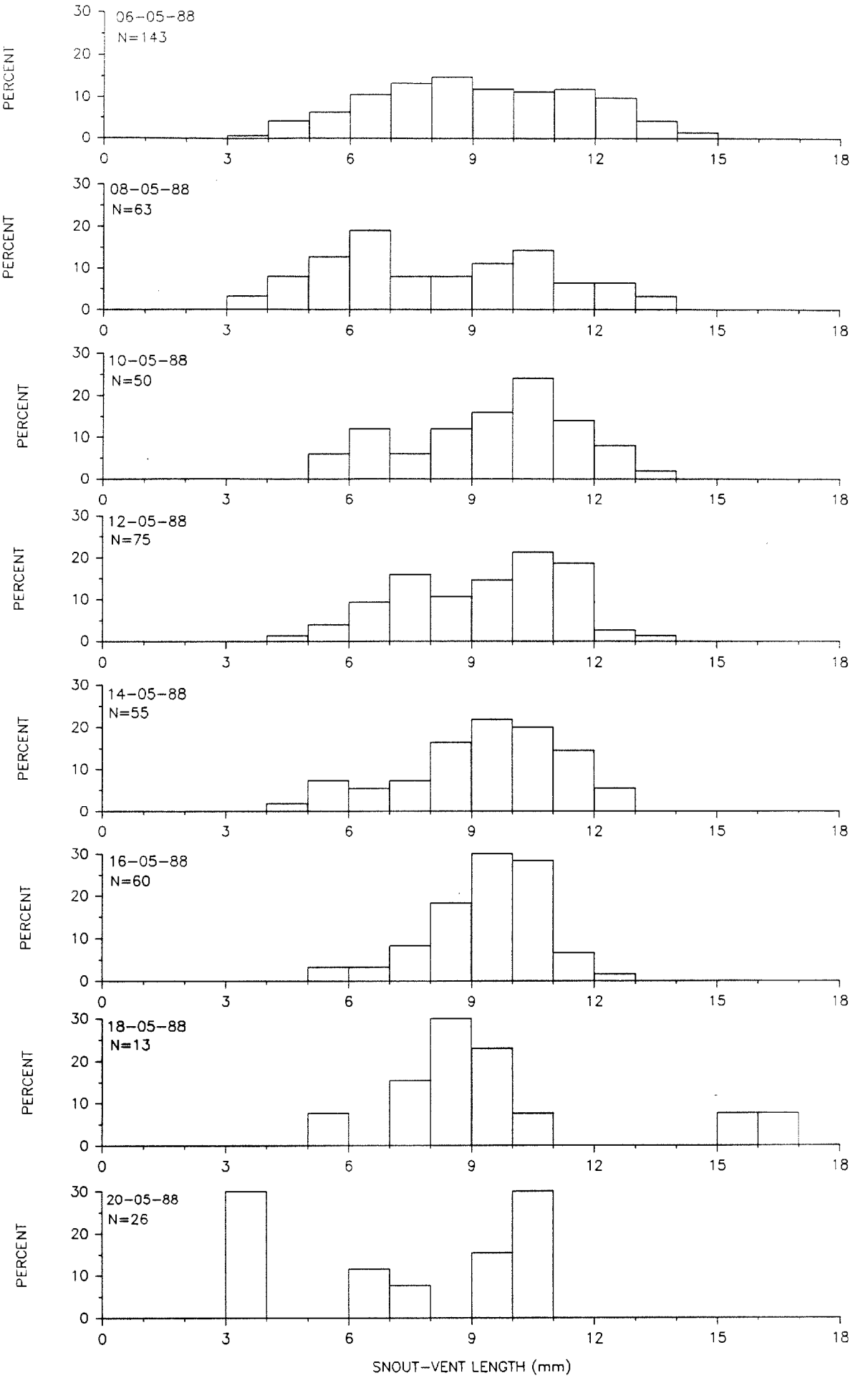
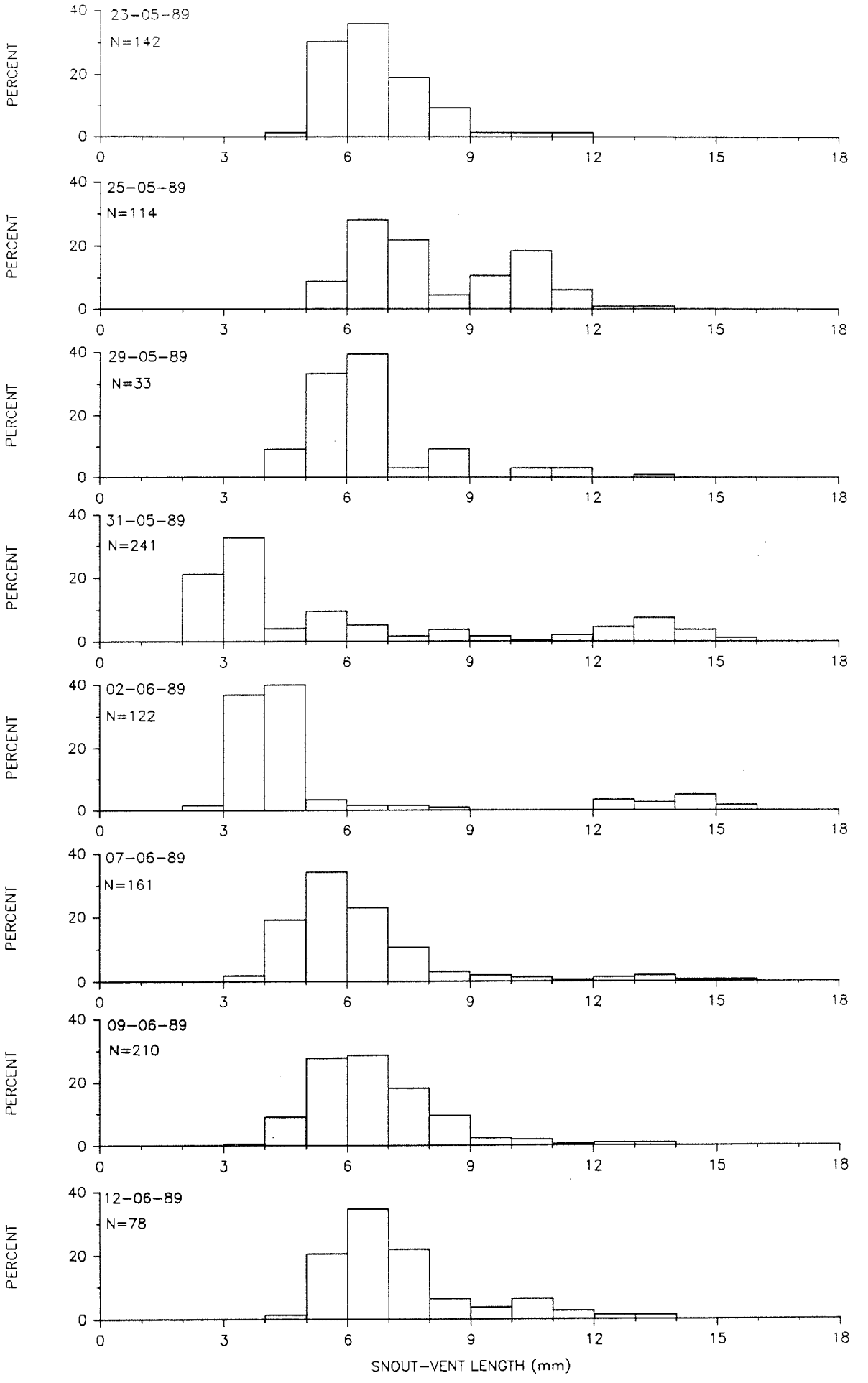

06-05-88
N=143
08-05-88
N=63
10-05-88
N=50
12-05-88
N=75
14-05-88
N=55
16-05-88
N=60
18-05-88
N=13
20-05-88
N=26
PERCENT
0
10
20
30
0
3
6
9
12
15
18
SNOUT-VENT LENGTH (mm)

Figure 4.2C Length frequency distributions for larvae caught in box samples on each sampling occasion for Mt Margaret from 23 May 1989 to 30 June 1989. Date of sample and total number caught are noted in each case.


23-05-89
N=142
25-05-89
N=114
29-05-89
N=33
31-05-89
N=241
02-06-89
N=122
07-06-89
N=161
09-06-89
N=210
12-06-89
N=78
PERCENT
0
20
40
0
3
6
9
12
15
18
SNOUT-VENT LENGTH (mm)

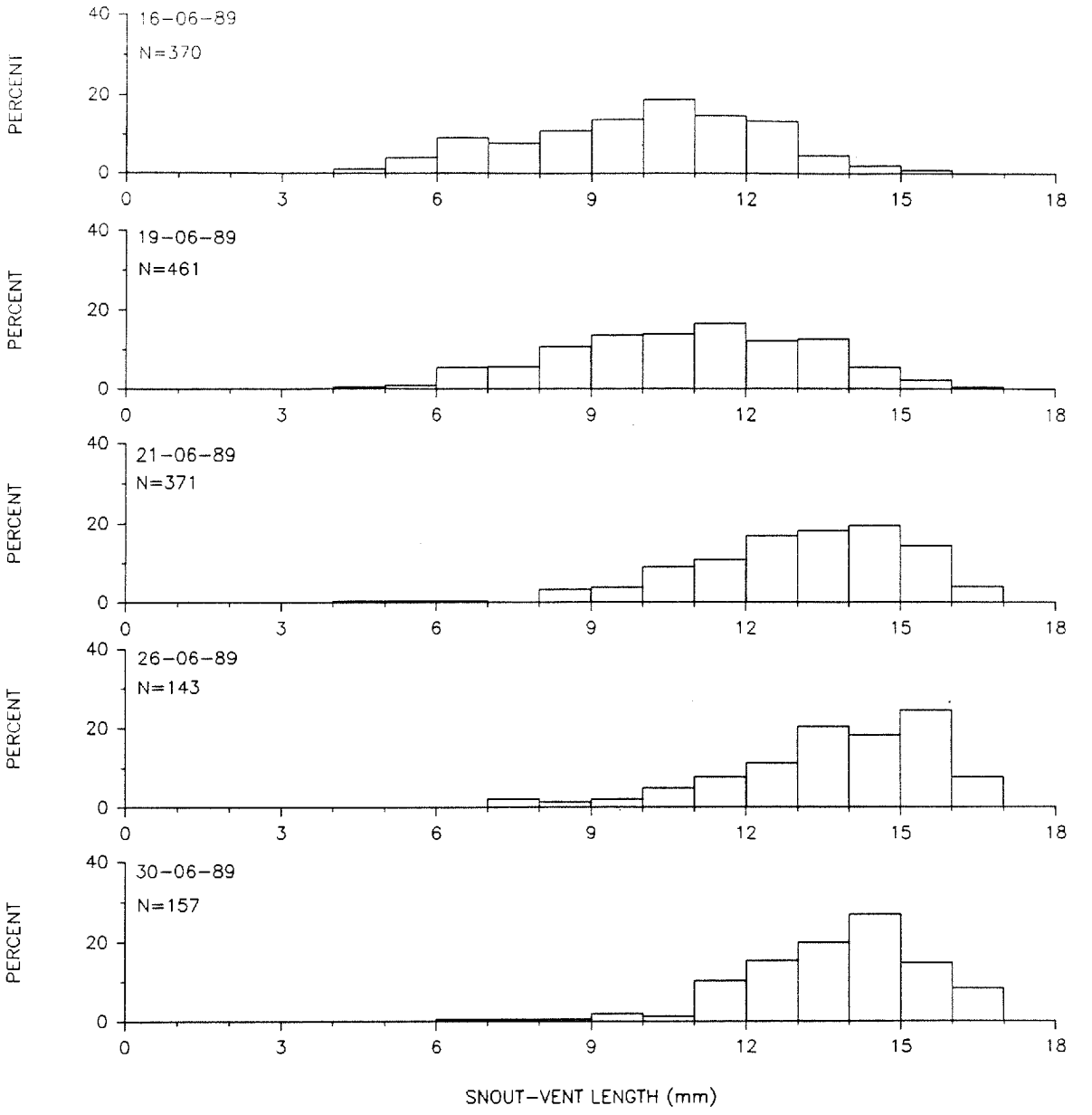
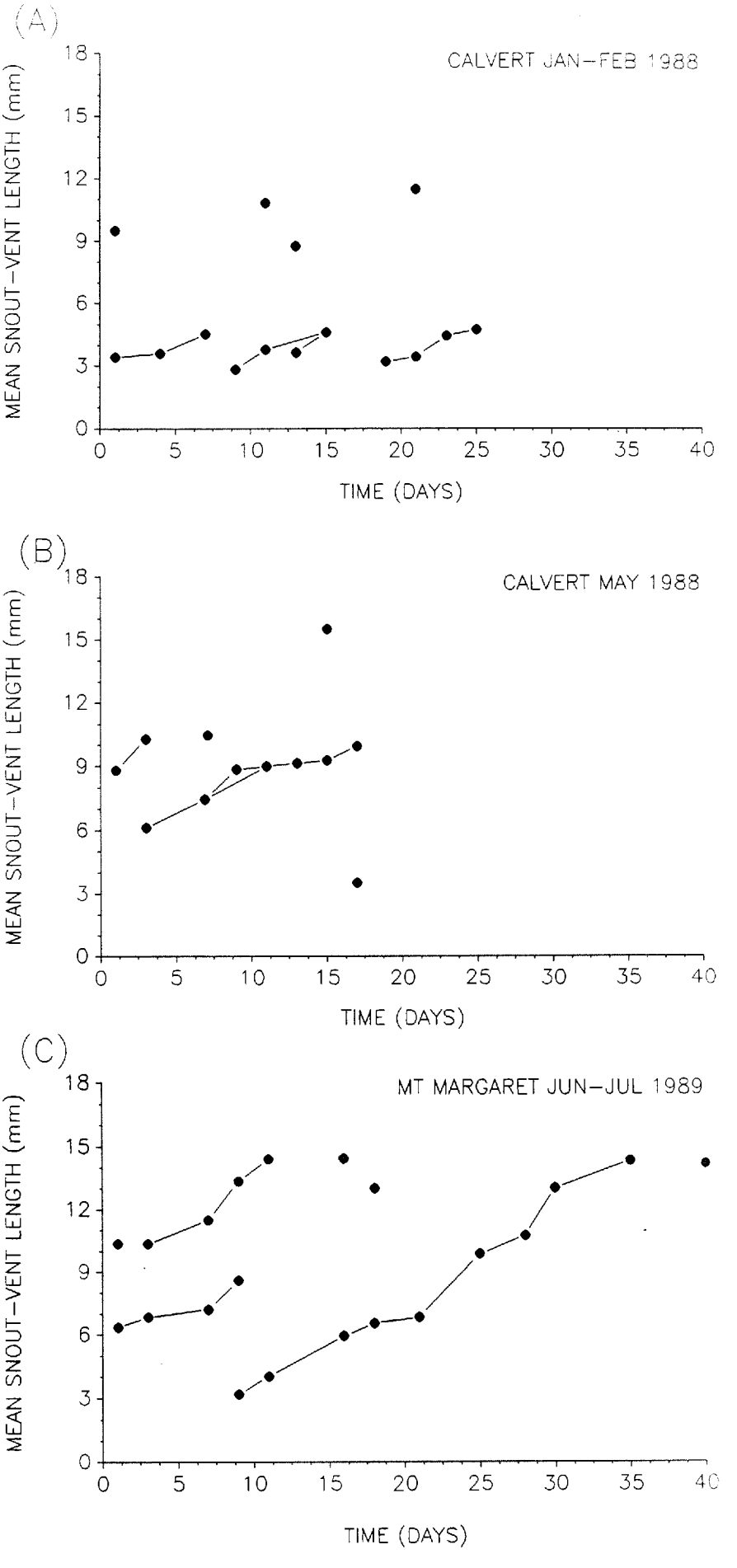

16-06-89
N=370
19-06-89
N=461
21-06-89
N=371
26-06-89
N=143
30-06-89
N=157
PERCENT
40
20
0
0
3
6
9
12
15
18
SNOUT-VENT LENGTH (mm)

Figure 4.3 Modes in the length-frequency distribution for each sample during each sampling period. Solid lines indicate the series of modes used in the analysis (see text).


(A)
CALVERT JAN–FEB 1988
MEAN SNOUT–VENT LENGTH (mm)
TIME (DAYS)
(B)
CALVERT MAY 1988
MEAN SNOUT–VENT LENGTH (mm)
TIME (DAYS)
(C)
MT MARGARET JUN–JUL 1989
MEAN SNOUT–VENT LENGTH (mm)
TIME (DAYS)

Based partially on results obtained from experimental analysis of growth, groups of modes were selected to represent progression of a cohort based on the following criteria:

(i) growth was positive and the increase in size for successive modes was not more than 0.5mm/day;

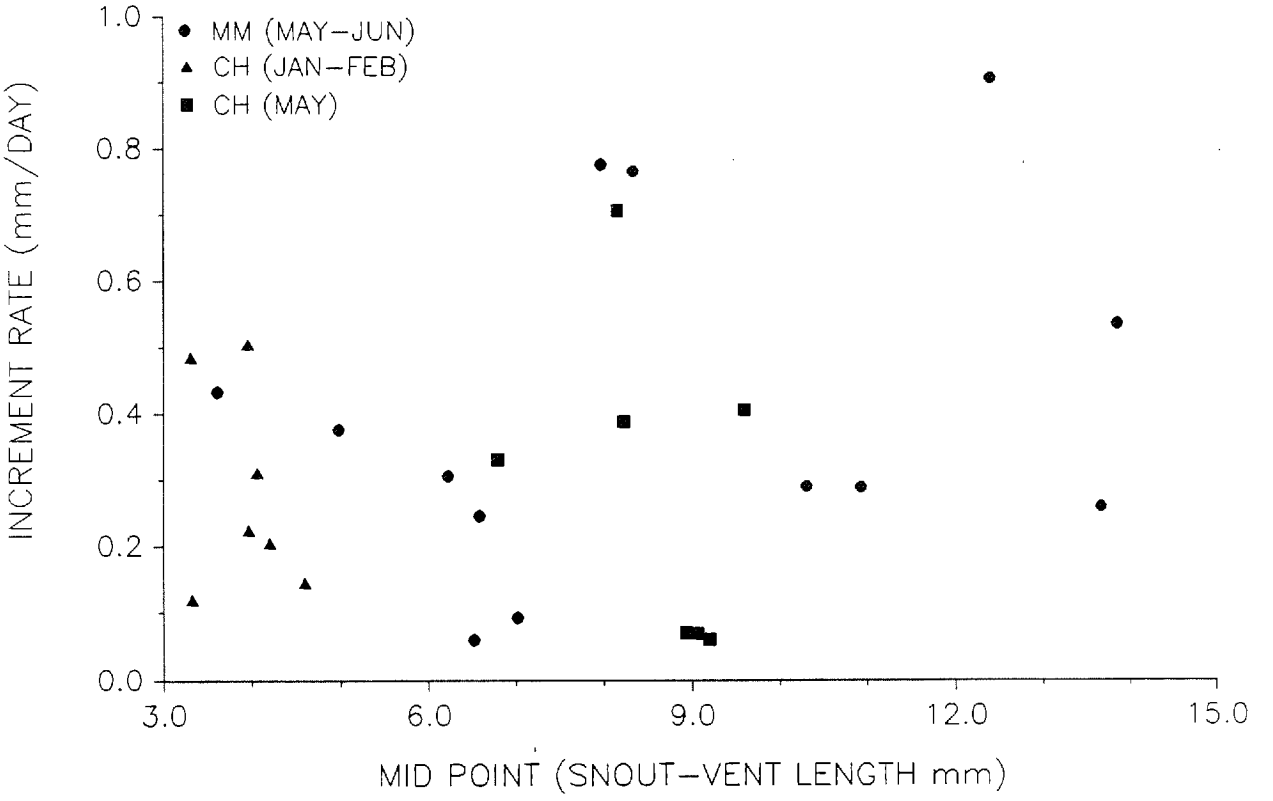
(ii) the time between two successive modes was between 1 and 5 days;

(iii) if it was known with certainty from observations while sampling that the individuals present were from a discrete cohort.

This restricted analysis to tadpoles below the size where growth can become negative prior to metamorphosis. With the time and rate restriction on growth, these modes are reasonably close to one another and are likely to represent real growth of a cohort rather than random points. The series selected from each sampling period are shown in Figure 4.3 as the modes connected by solid lines. The mean length and apparent growth increment in millimetres per day between each pair of successive nodes are plotted for all sites in Figure 4.4. The data for these increment rates do not suggest any type of growth curve. Satisfactory fits were not obtained for von Bertalanffy, Sigmoid or Gompertz growth curves and the increments are not well explained by a simple linear regression. There is a possible separation in growth rates in Figure 4.4 into faster (greater than 6.0mm per day) and slower (0.6mm or less per day) rates. However similar non-significant relationships were obtained by removing one or the other from the analysis.

The only growth relationship obvious from the data is a linear progression of the modes in Figure 4.3C starting from day 9 and continuing to day 35. In this case it can

Figure 4.4 Increment rate between pairs of modes for each series indicated in Figure 4.3.


MM (MAY–JUN)
CH (JAN–FEB)
CH (MAY)
INCREMENT RATE (mm/DAY)
1.0
0.8
0.6
0.4
0.2
0.0
3.0
6.0
9.0
12.0
15.0
MID POINT (SNOUT–VENT LENGTH mm)

assumed with a reasonable degree of certainty (from field observations and the degree of separation in the modes on any one date) that these modes came from a single egg-laying event and do not represent slow growing individuals from previous cohorts. For these points there is a significant linear regression (LENGTH=0.44*TIME-1.14, r^2=0.9748).

4.3.2 Growth and Survival of Eggs

The survival of eggs from Gosner stage 1 through to stage 24 is shown in Figure 4.5 for each date examined. The result of one experiment (JAN 88B) was inconsistent with the general trend and is not considered in the statistical comparison. The mean length of the developmental period was 70.6 hours (± 1.1 S.E.) ranging from 69 to 74 hours. Mean survival rate to stage 24 was 71.8 percent (± 1.29 S.E.), and ranged from 68.8 to 73.8 percent at a mean water temperature of 27.3°C. Survival was highest for the May 1988 sibship. Analysis of the survivorship curves for all of the experiments indicates that survival rate increased significantly over time (Profile Analysis MANOVA, Wilks'λ=0.0492, $F_{4,55}$=265.51, $p<0.0001$) though the resulting pattern of survival was similar for all experiments ($F_{3,58}$=0.06 p=0.9808).

The mean lengths of embryos from each experiment, measured at regular intervals throughout the developmental period are shown in Figure 4.6. Growth is represented for each experiment carried out in the laboratory but no embryos were collected from

Figure 4.5 Mean percent survival (log scale) of eggs (± 1 SE) raised in replicates of 100 from early embryos to Gosner stage 24 (hatchlings) during different experimental periods.

Legend:

open circles	- (JAN 88A) Calvert Hills, January 1988, experiment A
open diamonds	- (JAN 88B) Calvert Hills, January 1988, experiment B
solid circles	- (JAN 88 in situ) Calvert Hills, January 1988, experiment C
open triangles	- (MAY 88) Calvert Hills, May 1988
solid triangles	- (JAN 89) Mt Margaret, January 1989.

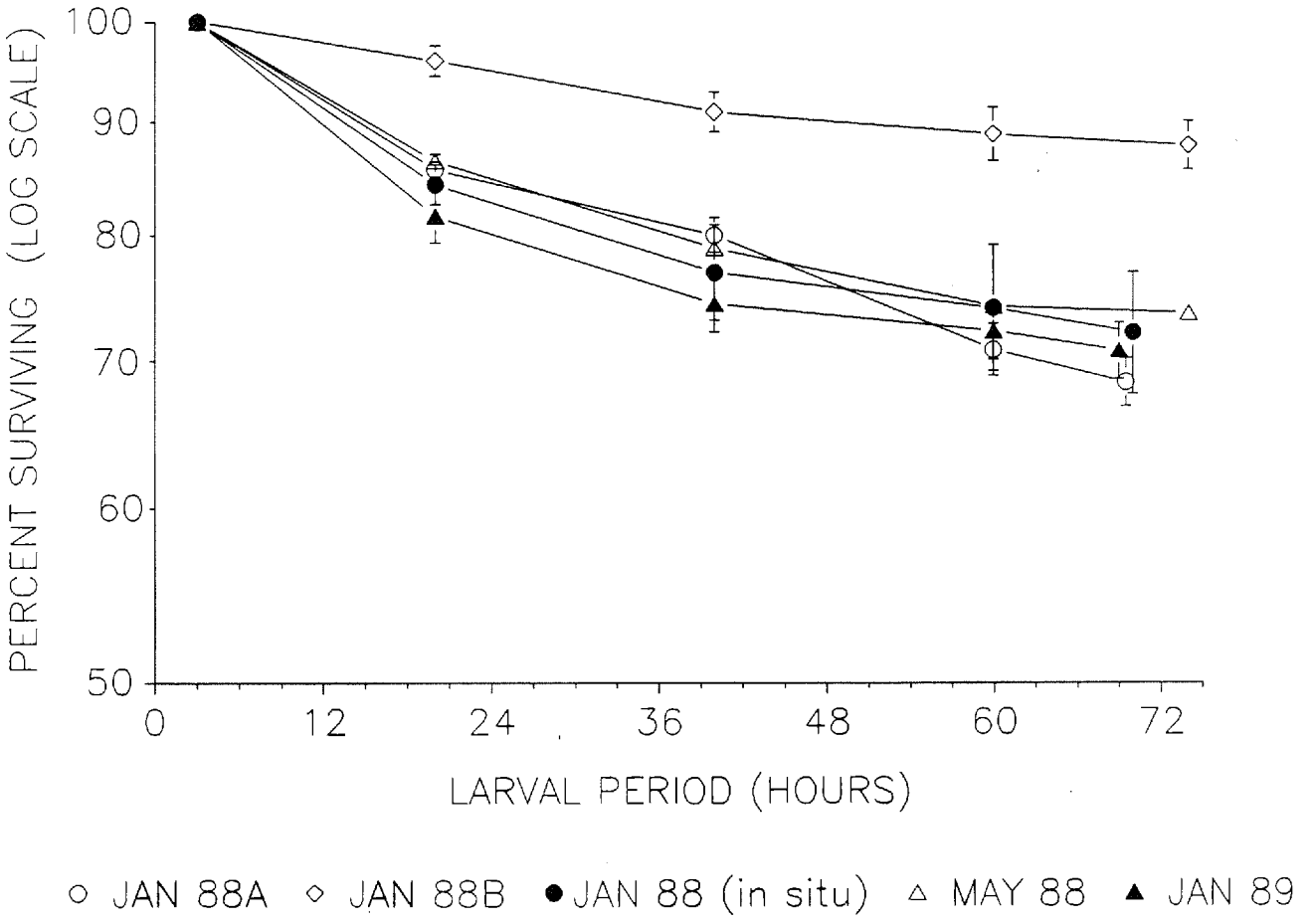
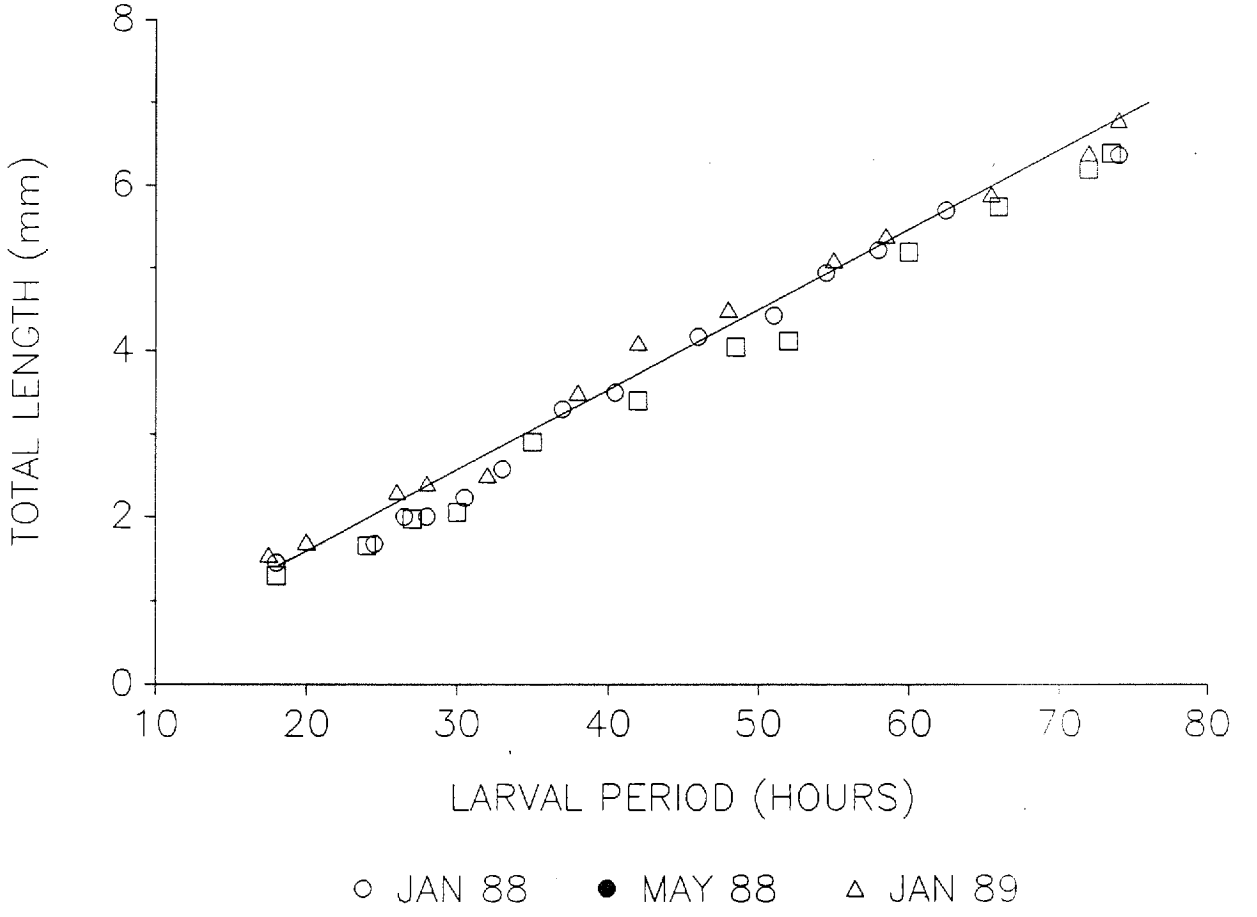

PERCENT SURVIVING (LOG SCALE)
100
90
80
70
60
50
0
12
24
36
48
60
72
LARVAL PERIOD (HOURS)
JAN 88A
JAN 88B
JAN 88 (in situ)
MAY 88
JAN 89

Figure 4.6 The relationship between total length (mm) and developmental period of larvae between Gosner stage 16 and stage 24. Different symbols represent measurements for separate experiments; circles, Calvert Hills - January 1988, squares, Calvert Hills - May 1988, triangles, Mt Margaret - January 1989.

The regression line fitted to the data is:

$$\text{TOTAL LENGTH} = 0.0976 * \text{TIME} - 0.5751 \quad (r^2 = 0.9843,\ p = 0.0001)$$


TOTAL LENGTH (mm)
0
2
4
6
8
10
20
30
40
50
60
70
80
LARVAL PERIOD (HOURS)
JAN 88
MAY 88
JAN 89

the *in situ* experiment. Measurements for the 0-18 hour period are excluded as this corresponds to the developmental phase where the embryos were still spherical, retaining the shape of the egg. This includes the period up to the late gastrula and neural fold formation of the embryo (stage 15). The mean rate of increase in diameter during this phase was 0.00694 mm/hour (SE=0.00321). After this period, corresponding to neural tube formation and tail budding, embryo shape changed to the elongated form of the tadpole. For stages 16 through to 24, growth was linear and analysis of the rate parameters for each of the three experiments indicate no difference in growth between the experiments (ANCOVA $F_{1,35}=3.45$ $p=0.3126$). The relationship between time and total length can be described by the equation:

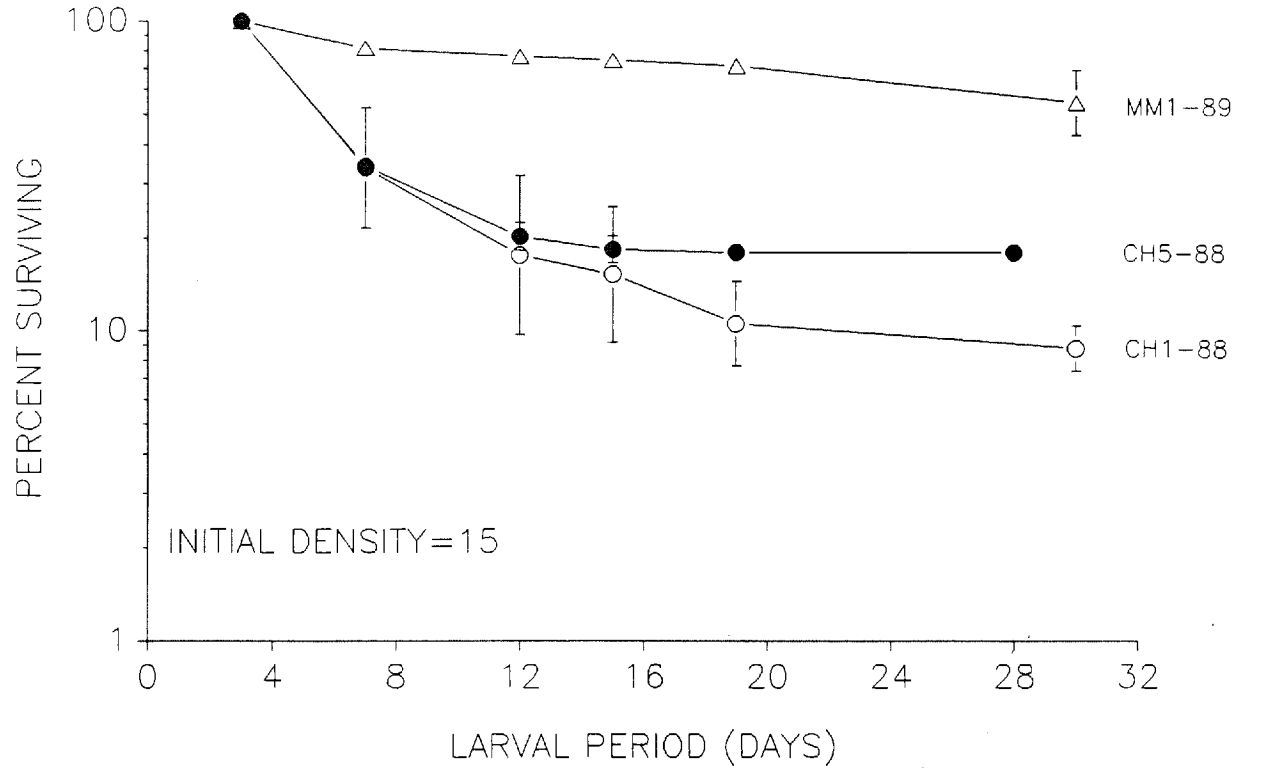
TOTAL LENGTH=0.0976*TIME-0.5751 ($r^2=0.9843$, $p=0.0001$)

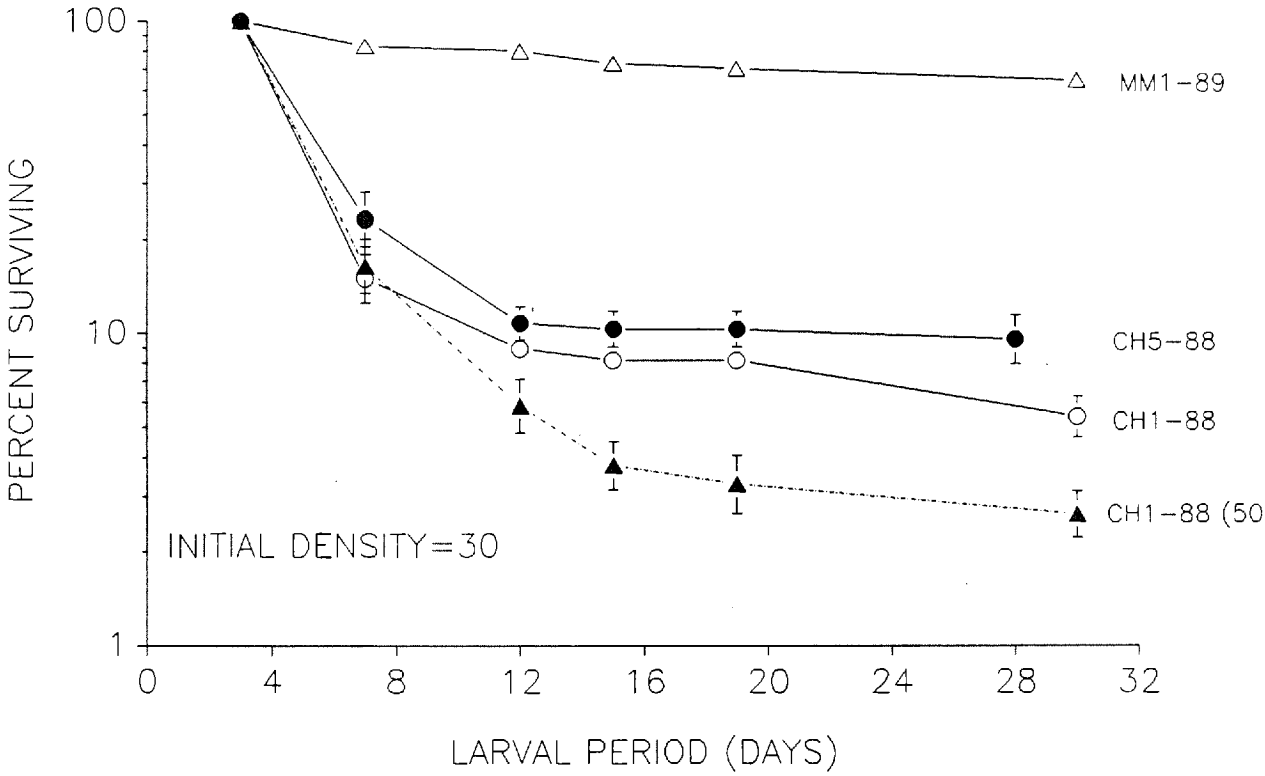
4.3.3 Larval Survival

In situ Enclosures

The survival of larvae (stage 25 to stage 42) reared at each initial density treatment of 15 (5 larvae/litre) and 30 (10 larvae/litre) for each experimental period is shown in Figure 4.7. Also shown are the results for larvae reared at an initial density of 50 (16.7 larvae/litre). Five larvae per litre represents the upper range of densities caught in field samples (see Table 4.1). Higher densities were used for additional treatments as this represents densities commonly found within shallow water aggregations. When

Figure 4.7 Percentage (± 1 SE.) of larvae reared in *in situ* enclosures surviving to metamorphosis in each treatment (initial density=15/30) during the wet season at Mt Margaret (MM1-89), and at Calvert Hills (CH) during the wet (CH1-88) and dry seasons (CH5-88). The response for the initial density=50 treatment (CH1-88) is shown on the lower figure with the dashed line.


100
10
1
PERCENT SURVIVING
INITIAL DENSITY=15
0
4
8
12
16
20
24
28
32
LARVAL PERIOD (DAYS)
MM1-89
CH5-88
CH1-88


100
10
1
PERCENT SURVIVING
INITIAL DENSITY=30
0
4
8
12
16
20
24
28
32
LARVAL PERIOD (DAYS)
MM1-89
CH5-88
CH1-88
CH1-88 (50)

sampling at a known depth and relating number of tadpoles to the area of the aggregation only, these densities can vary from 7 to 56 per litre within the aggregation (personal observations). Survival was highest for Mt Margaret during the wet season (MM1-89) but results appear to be inconsistent with the trends observed in all other experiments. At Mount Margaret, survival did not differ between the two initial density treatments (MANOVA, Wilks'λ=0.9111, $F_{5,46}$=0.89, p=0.4904), the overall mean and S.E. were 59.67 $\pm$ 3.846 percent.

MANOVA results for comparison of treatments or groups of survival curves are shown in Table 4.2. Models for these tests do not include the Mt Margaret treatments. Survival of larvae from the time of introduction to the enclosures (day 3) to day 19 (prior to metamorphosis) indicate that initial survival is dependent on initial density. Though significant variation existed between the density=15 treatments, contrasts between densities are all significant. The consistent trend is a rapid decrease in survival to about day 12 then a constant increase in survival rate to metamorphosis. The significant contrasts indicate that mortality rates increase with increasing initial density.

ANOVA results for final survival in each experiment are also presented in Table 4.2. Survival to metamorphosis was clearly density dependent. At an initial density of 15 per enclosure, survival varied between 18.0 and 8.8 percent. At 30 per enclosure, survival varied between 9.5 and 5.4 percent. At 50 per enclosure, survival decreased to a mean of 2.6 percent. Survival varied between experiments. Survival at each initial

Table 4.2 MANOVA results for analysis of survival over time and ANOVA results for analysis of final survival of larvae reared in 3 litre experimental enclosures at three initial densities (15 30 50). Contrast analyses test for differences between specific curves, eg, the D15 contrast tests for the differences between the initial density=15 curves only.

Key to abbreviations:

D15: Experiments with initial density=15: Calvert Hills wet season (1-88) and dry season (5-88)

D30: Experiments with initial density=30: Calvert Hills wet season (1-88) and dry season (5-88)

D50: Experiments with initial density=50: Calvert Hills wet season (1-88)

MANOVA of (arcsine-squareroot transformed) percent survival on days 3, 7, 12, 14 and 19.

Source of Variation	Wilks' λ	F	Degrees of Freedom (Num)	(Den)	p
Initial Density	0.0377	9.63	16	80.1	0.0001
Contrast					
D15	0.1100	52.58	4	26	0.0001
D30	0.8395	1.24	4	26	0.3175
D15-D30	0.1085	53.41	4	26	0.0001
D15-D50	0.1551	35.41	4	26	0.0001
D30-D50	0.6909	2.91	4	26	0.0410

ANOVA of (arcsine-squareroot transformed) percent final survival

Source of Variation	df	SS	MS	F	p
Initial Density	4	1245.0644	311.2661	9.68	0.0001
Residual	29	932.5932	32.1584		
Contrast					
D15-D30	1	210.5239	210.5239	6.55	0.0160
D15-D50	1	573.0398	573.0398	17.82	0.0002
D30-D50	1	218.8063	218.8063	6.80	0.0142

density was higher for the dry season experiment at Calvert Hills (ANOVA, $F_{1,26}=8.01$, $p<0.0001$), however this may have been in response to other factors (see section 4.3.4). Survival of larvae at an initial density of 50 was significantly lower than both of the lower densities (ANOVA, $F_{4,31}=12.67$, $p<0.0001$ and Tukey's HSD test).

Experimental Ponds

The survival to metamorphosis of larvae reared at the five initial densities (30, 60, 120, 240 and 480) is shown in Figure 4.8. The end-point of each line represents the mean length of the larval period for that initial density. Densities of larvae used fall within the range of densities encountered by individual larvae in field samples (Do, see Table 4.1). ANOVAs comparing the response of final survival and MANOVAs comparing the responses of mass at metamorphosis and larval period for each treatment are presented in Table 4.3. Also presented here are the results of analyses contrasting single treatments or groups of treatments. The results for the response of mass and larval period are examined further in section 4.3.4. Survival to metamorphosis depended on initial density. Though survivorship curves up to day 35 did not differ significantly between the treatments (MANOVA, Wilks'$\lambda=0.4163$, $F_{2,14}=1.92$, $p=0.0957$), mean survival to metamorphosis decreased with increasing density from 11.67% and 12.01% (for the 30 and 60 treatments respectively) to 2.92%, 1.15% and 0.57% for the 120, 240 and 480 treatments respectively. Mean survival was not

Figure 4.8 Percent survival (± 1 SE) of larvae reared from hatchlings to metamorphs at initial densities of 30, 60, 120, 240 and 480 per 1000 litres in experimental ponds.

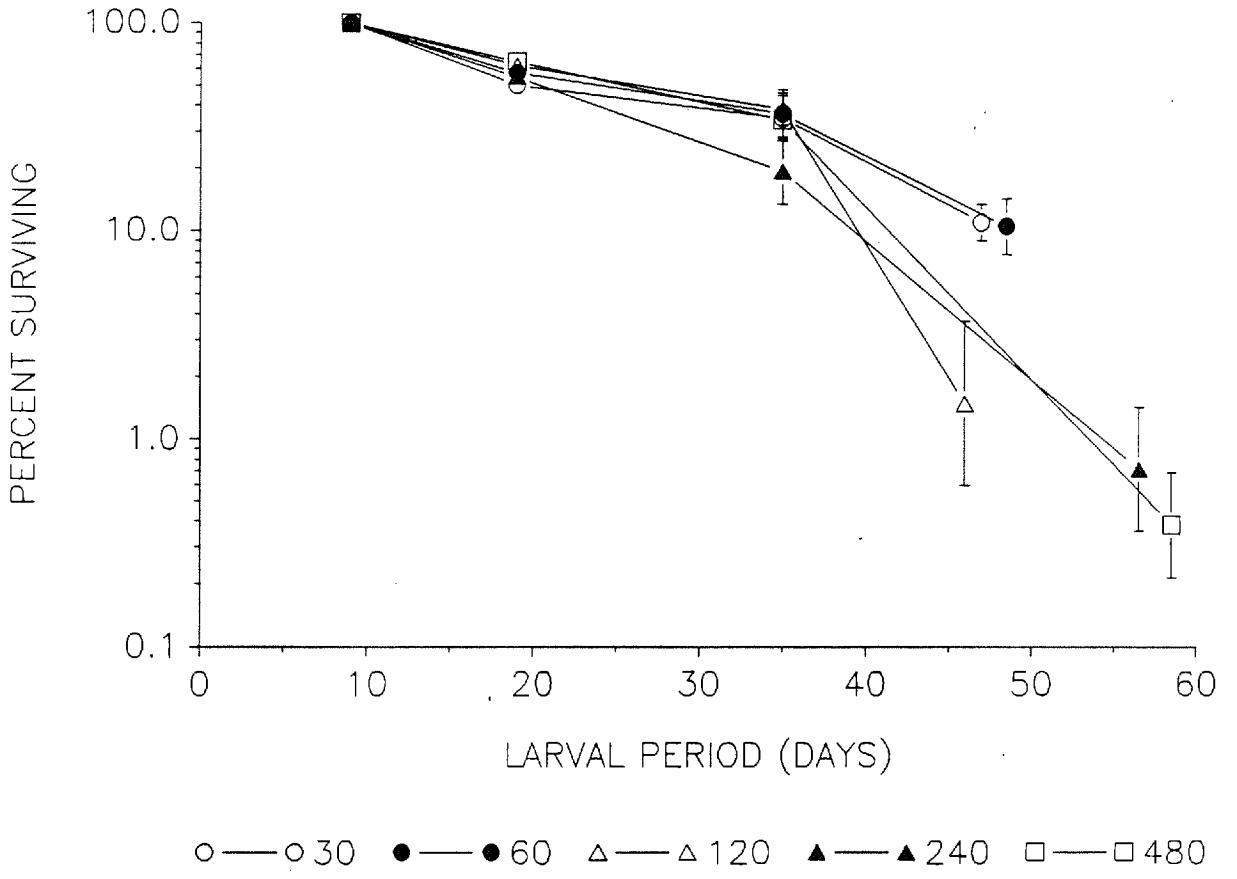

100.0
10.0
1.0
0.1
PERCENT SURVIVING
0
10
20
30
40
50
60
LARVAL PERIOD (DAYS)
30
60
120
240
480

Table 4.3 Final responses of mass, larval period and survival from larvae reared in experimental ponds at initial densities of 30 60 120 240 and 480 per pond. Mean final survival for treatments in the ANOVA are compared using Tukey's HSD test. Means with the same underline are not significantly different at the 0.05 level of significance. Contrast analyses for the MANOVA compare the responses for individual treatments or groups of treatments as specified.

TREATMENT Initial Tadpole Density	Mass at Metamorphosis (mg)			Larval Period (days)			Survival (percent)		
	Mean	SE	N	Mean	SE	N	Mean	SE	N
30	397.7	27.7	14	47.2	1.2	14	11.67	2.2	4
60	240.4	6.2	29	48.5	0.8	29	12.01	3.2	4
120	210.9	9.2	14	46.0	1.3	14	2.92	1.2	4
240	163.4	15.8	11	56.5	2.2	11	1.15	0.5	4
480	149.3	6.6	11	58.6	1.8	11	0.57	0.2	4

ANOVA of (arcsine-squareroot transformed) percent survival

Source of Variation	df	SS	MS	F	p
Initial Density	4	922.7382	248.1845	11.20	0.0002
Residual	15	332.3337	22.1556		

Tukey's HSD test for comparison of the means

30	60	120	240	480

MANOVA of mass at metamorphosis and larval period

Source of Variation	Wilks' λ	df	F	p
Initial Density	0.1901	8,146	23.61	0.0001
Contrasts				
60 - 120	0.9288	2,73	2.79	0.0674
30 - 60/120	0.4175	2,73	50.92	0.0001
30 - 240/480	0.2840	2,73	92.01	0.0001
240 - 480	0.9846	2,73	0.57	0.5666

significantly different between the 30 and 60 treatments and for the 120, 240 and 480 treatments (Tukey HSD Test).

4.3.4 Larval Growth

In situ Enclosures

Mean daily temperatures recorded during each experimental period are shown in Figure 4.9. Temperatures during May 1988 at Calvert Hills and January 1989 at Mt Margaret did not change significantly during the experiments while for January 1988, temperatures decreased (Table 4.4). Analysis of snout-vent length at metamorphosis and larval period for enclosure experiments (Table 4.5) show that initial density and experimental period affected both responses. Larval size decreased with higher initial densities in each experiment (Figure 4.10), though size was similar for the intermediate (30 per container) and highest density (50 per enclosure) for the January 1988 experiment. Size for each initial density also differed between experiments, however, the effect was consistent (ie, interaction not significant). There were no differences for larval period between densities within experiments (ANOVA, $F_{1,42}=0.05$, $p=0.8212$). Larval period ($\pm$ 1 SD) was shortest for the January 1988 experiment at Calvert Hills (22.1 $\pm$ 0.6 days), and longest for the January 1989 experiment at Mt Margaret (41.1 $\pm$ 2.7 days). Mean larval period for the May 1988 experiment at Calvert Hills was 31.1 $\pm$ 1.4 days. Larval periods for each experiment were significantly and inversely

Figure 4.9 Daily mean water temperatures recorded during each experimental period for larvae grown in *in situ* enclosures at Calvert Hills (January and May 1988) and Mt Margaret (January 1989).

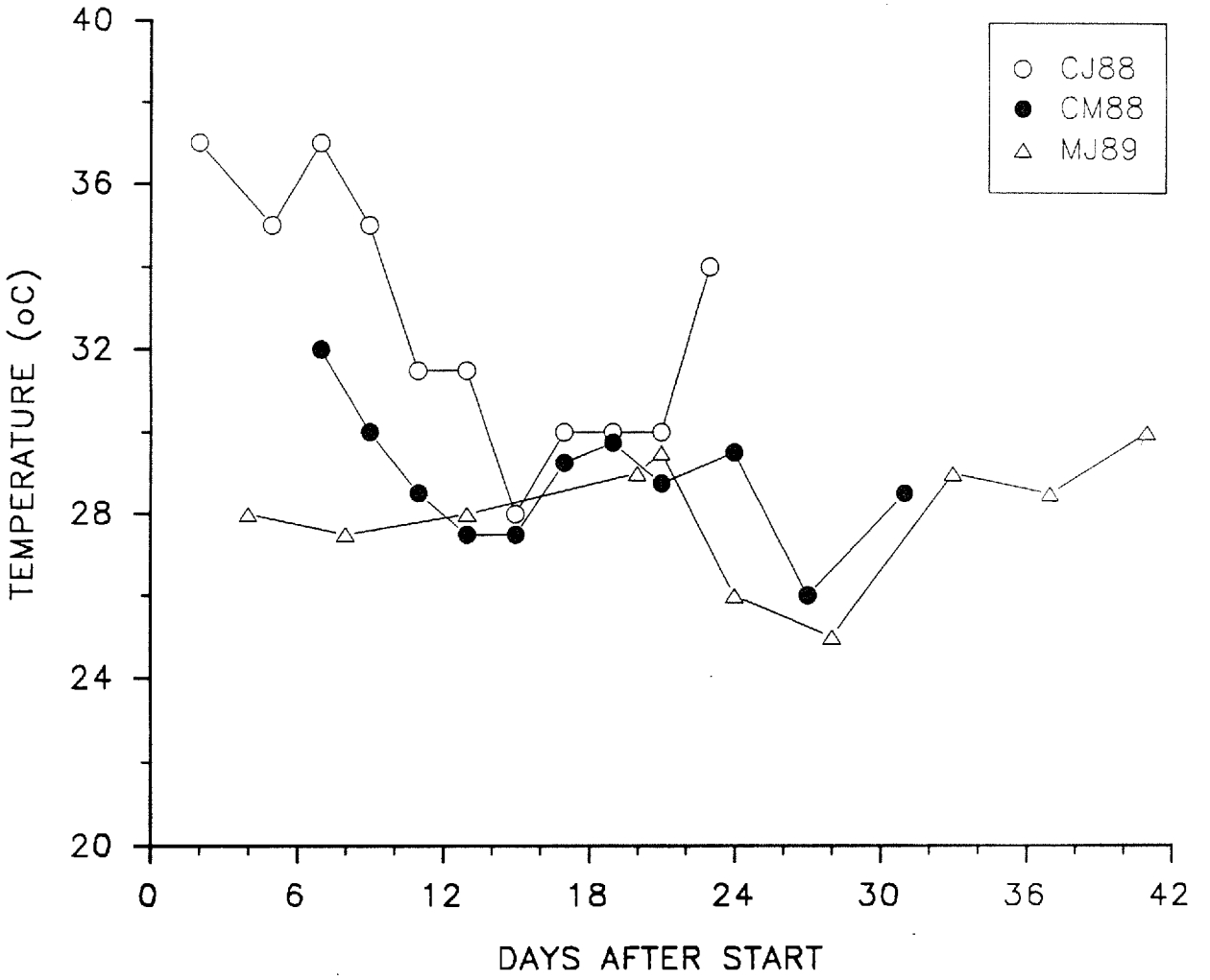

40
36
32
28
24
20
TEMPERATURE (oC)
0
6
12
18
24
30
36
42
DAYS AFTER START
CJ88
CM88
MJ89

Table 4.4 Regression analyses for daily mean water temperatures recorded during *in situ* larval growth experiments.

Experiment	*a*	SE(*a*)	*b*	SE(*b*)	N	p($b \neq 0$)
Calvert Hills						
January 1988	37.9	1.18	-0.454	0.088	8	0.0009
May 1988	30.1	1.74	-0.047	0.089	10	0.6056
Mt Margaret						
January 1989	27.6	1.07	0.018	0.043	8	0.6826

Table 4.5 Responses and MANOVA results for snout vent length at metamorphosis and larval period for tadpoles reared in 3 litre experimental enclosures at two initial densities (15 and 30) for three experiments (Calvert Hills January and May 1988 and Mt Margaret January 1989).

TREATMENT LEVEL		SVL at Metamorphosis (mm)			Larval Period (days)		
Initial Density	Experiment	Mean	SE	N	Mean	SE	N
15	JAN88 CH	11.7	0.1	2	22.0	0.0	2
	MAY88 CH	10.1	0.2	18	31.2	0.4	18
	JAN89 MM	8.7	0.1	86	41.2	0.3	86
30	JAN88 CH	10.2	0.2	8	22.2	0.2	8
	MAY88 CH	8.8	0.2	21	31.0	0.3	21
	JAN89 MM	7.5	0.1	186	41.0	0.2	186
50	JAN88 CH	10.1	0.0	2	22.0	1.0	2

MANOVA of snout vent length at metamorphosis and larval period (Density=50 excluded from the analysis)

Source of Variation	Wilks' λ	df	F	p
Initial Density	0.4413	2,40	25.31	0.0001
Experiment	0.0179	4,80	129.20	0.0001
Density*Experiment	0.9656	4,80	0.35	0.8413

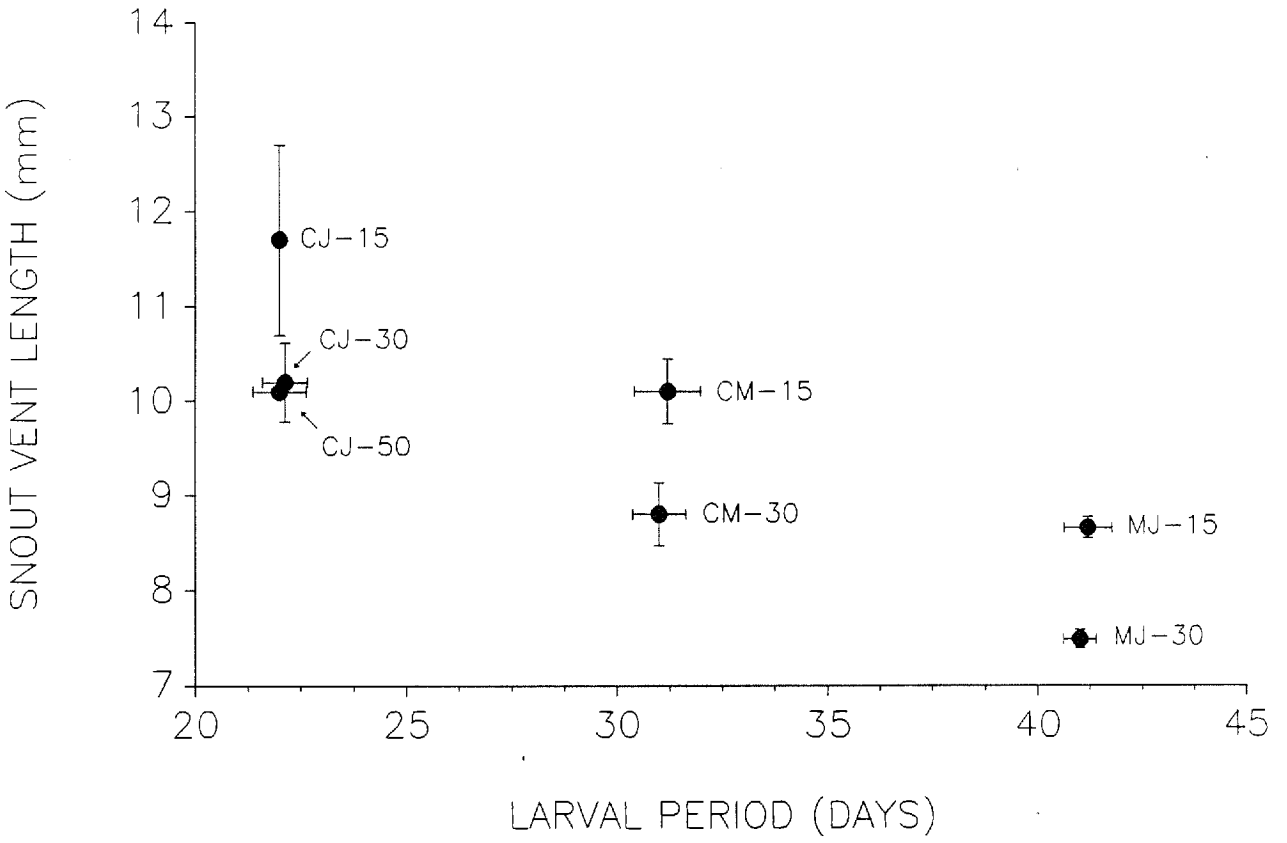
Figure 4.10 Mean snout vent length and larval period for each initial density from each enclosure experiment. Error bars represent 95 percent confidence limits for the mean. Experiment and density are indicated for each mean.

Key to abbreviations:

Experiments

CJ - Calvert Hills, January 1988 (wet season)
CM - Calvert Hills, May 1988 (dry season)
MJ - Mt Margaret, January 1989 (wet season)

Initial density is indicated by the number following experiment.


SNOUT VENT LENGTH (mm)
14
13
12
11
10
9
8
7
20
25
30
35
40
45
LARVAL PERIOD (DAYS)
CJ-15
CJ-30
CJ-50
CM-15
CM-30
MJ-15
MJ-30

correlated with mean daily maximum temperature recorded during each experiment (r^2=-0.9989, p=0.0296). Mean daily maximum water temperatures ($\pm$ 1 SD) for each experimental period were 35.85 $\pm$ 2.88°C for January 1988 at Calvert Hills, 31.2 $\pm$ 2.34°C for May 1988 at Calvert Hills, and 29.05 $\pm$ 2.08°C for January 1989 at Mt Margaret. Thus, initial density controlled mean size but not larval period. Differences in larval period between experiments appeared to be linked with mean daily maximum water temperatures.

The increase in snout vent length of larvae reared in *in situ* containers at each initial density is shown in Figures 4.11A-G. The solid lines represent fitted growth curves for the composite data (mean of all replicates) The points at each interval are means for snout vent length in individual replicates (pots). Larvae showed two distinct growth relationships in these experiments, explained by a two or a three parameter curve. For discussion, these will be called type A and type B relationships respectively. The type A curve can be represented by the exponential equation:

$$L_{ij}=\alpha_i \; e^{(\beta_i \, t_{ij})}$$

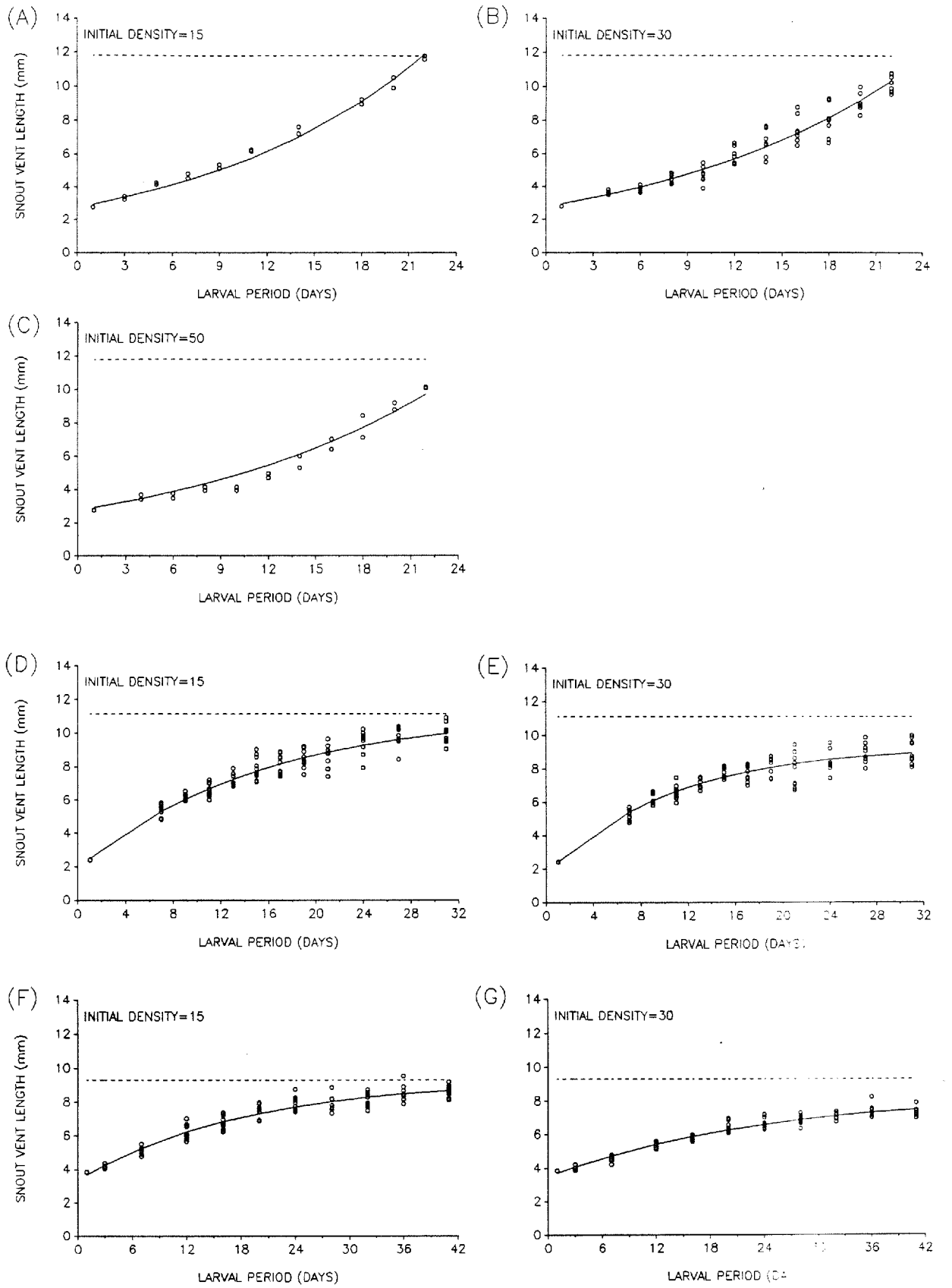
where subscripts i and j denote the ith replicate and the jth observation on that replicate; L_{ij} is the predicted snout vent length in millimetres at time t_{ij} (days), α_i represents size as a hatchling (at stage 23) and β_i represents growth rate as a proportion of the hatchling size. The Type B curve has the form of a decreasing exponential relationship described by the equation:

Figure 4.11 A-G Growth curves for larvae raised in experimental enclosures at differing initial densities. Symbols at each interval represent means for each replicate.

Figures A-C. Growth curves for mean snout-vent length of larvae raised at three initial densities (A-15, B-30, C-50) for Calvert Hills January 1988. Symbols at each interval represent means for each replicate. The dotted line on each figure represents the highest recorded mean from any enclosure (11.81 mm). Parameters for each equation are shown in Table 4.6.

Figures D-E. Growth curves for mean snout-vent length of larvae reared at two initial densities (D-15, E-30) for Calvert Hills, May 1988. The dotted line on each figure represents the estimated upper asymptotic length for the initial density=15 treatment (11.12 mm). Parameters for each equation are shown in Table 4.7.

Figures F-G. Growth curves for mean snout-vent length of larvae reared at two initial densities (F-15, G-30) for Mt Margaret, January 1989. The dotted line on each figure represents the estimated upper asymptotic length for the initial density=15 treatment (9.30 mm). Parameters for each equation are shown in Table 4.8.


(A)
INITIAL DENSITY=15
SNOUT VENT LENGTH (mm)
LARVAL PERIOD (DAYS)
(B)
INITIAL DENSITY=30
LARVAL PERIOD (DAYS)
(C)
INITIAL DENSITY=50
SNOUT VENT LENGTH (mm)
LARVAL PERIOD (DAYS)
(D)
INITIAL DENSITY=15
SNOUT VENT LENGTH (mm)
LARVAL PERIOD (DAYS)
(E)
INITIAL DENSITY=30
LARVAL PERIOD (DAYS)
(F)
INITIAL DENSITY=15
SNOUT VENT LENGTH (mm)
LARVAL PERIOD (DAYS)
(G)
INITIAL DENSITY=30
LARVAL PERIOD

$$L_{ij}=\alpha_i[1-e^{(\gamma_i+\beta_i t_{ij})}]$$

where subscripts i and j denote the ith replicate and the jth observation on that replicate; L_{ij} is the predicted snout vent length in millimetres at time t_{ij} (days), α_i is the upper asymptotic size of larvae (at stage 42), γ_i reflects the length as a hatchling and β_i represents growth rate as a proportion of the asymptotic size.

The type of growth curve exhibited by the larvae varied between different experimental periods but it is interesting that within experiments, the significant curve fits were consistent for all individual enclosures.

For larvae reared at Calvert Hills in the wet season, (January 1988, Figure 4.11A-C) type A relationships were obtained from enclosure means for each density (15, 30 and 50). Parameter estimates for individual and composite curves for each of the densities are shown in Table 4.6. Individual curves differ significantly from the composite curve for initial densities of 30 ($F_{12,63}=6.99$ $p<0.001$) and 50 ($F_{2,18}=8.55$ $p<0.005$) indicating growth differences between replicates. For composite curves, there was no significant difference in the growth rate parameter ($\chi=2.06$ df=2 $p=>0.05$), but differences were significant for hatchling size ($\chi=20.43$ df=2 $p<0.001$). However, the mean size of larvae used in each replicate for the three treatments was known and did not differ significantly from an overall mean of 2.75 mm. If the parameter for hatchling size (α) in the model is kept constant at 2.75, and the curves recalculated, then parameter estimates for growth rate become significantly different ($\chi=91.23$ df=2 $p<0.0001$), decreasing with increasing density.

Table 4.6 Parameter estimates of individual and composite growth curves for means of individual enclosures of larvae reared at three initial densities (15, 30 and 50) for Calvert Hills, January 1988. Growth rate (β_i) is shown for hatchling size (α_i) calculated by the two parameter model, and for hatchling size as a constant (α_i=2.75 ie single parameter model).

MSE, Mean Square Error, Number of observations per curve = 11, measurements in millimetres

Density	Replicate	Hatchling Size αi	SE	Growth Rate βi (αi calculated)	SE	MSE	Growth Rate βi (αi=2.75)	SE
15	1	2.8981	0.0881	0.0645	0.0018	0.0236	0.0673	0.0006
15	2	2.9551	0.1192	0.0622	0.0025	0.0420	0.0066	0.0010
15	Composite	2.9264	0.0723	0.0633	0.0015	0.0314	0.0667	0.0006
30	1	2.8289	0.1114	0.0567	0.0024	0.0332	0.0583	0.0008
30	2	2.7089	0.1188	0.0563	0.0027	0.0386	0.0554	0.0009
30	3	2.9630	0.2018	0.0615	0.0041	0.1123	0.0657	0.0014
30	4	2.5828	0.1036	0.0636	0.0024	0.0327	0.0600	0.0009
30	5	2.4340	0.1137	0.0659	0.0028	0.0418	0.0589	0.0012
30	6	2.9072	0.1516	0.0624	0.0032	0.0648	0.0655	0.0011
30	7	2.6062	0.2082	0.0602	0.0049	0.2843	0.0571	0.0016
30	Composite	2.7163	0.1192	0.0610	0.0018	0.1258	0.0603	0.0006
50	1	2.5043	0.1289	0.0613	0.0031	0.0510	0.0559	0.0012
50	2	2.2650	0.1560	0.0688	0.0041	0.0847	0.0578	0.0018
50	Composite	2.3821	0.1032	0.0651	0.0026	0.1191	0.0569	0.0011

Larvae reared at Calvert Hills in the dry season (May 1988, Figure 4.11D-E) and at Mt Margaret during the wet season (January 1989, Figure 4.11F-G) produced the type B growth curve. For Calvert Hills, parameter estimates for individual and composite curves are shown in Table 4.7. Individual curves for each density were significantly different from their composites (15: $F_{27,90}=4.52$, $p<0.001$; 30: $F_{24,81}=4.36$, $p<0.001$) indicating individual differences between replicates. For the composite curves, asymptotic size decreased significantly with higher density ($\chi=29.67$, $df=1$, $p<0.001$), and the growth rate as a proportion of the estimated asymptotic size was higher for the higher density, ($\chi=12.12$, $df=1$, $p<0.001$). The parameter for embryo size was similar for both densities ($\chi=0.76$ $df=1$ $p>0.95$).

For Mt Margaret (Figure 4.11F-G, Table 4.8), individual curves differed from the composite for both densities (for 15, $F_{24,72}=15.01$ $p<0.001$; for 30, $F_{24,83}=21.98$ $p<0.001$). Both size parameters differed significantly for the 15 and 30 composite curves (for asymptotic size, $\chi=8.34$ $df=1$ $p<0.005$; for embryo size, $\chi=16.06$ $df=1$ $p<0.001$). Both were larger for the 15 treatment. Growth rates however were not different between the two composite curves. Estimates for asymptotic lengths (α) for individual initial density=30 replicates differed from the composite curve estimate ($\chi=19.11$ $df=9$ $0.025<p<0.05$). All other individual curve parameter estimates were similar to the corresponding composite estimate.

In summary, all fits produced satisfactory residual plots, and in every case, the coefficient of determination (r^2) values for the regression were high (range 0.8818 to

Table 4.7 Parameter estimates of individual and composite growth curves for means of individual enclosures of larvae reared at two initial densities (15 and 30) for Calvert Hills, May 1988.

MSE, Mean Square Error, Number of observations per curve = 12, measurements in millimetres.

Density	Replicate	Asymptotic Size αi	SE	Growth Rate βi	SE	Hatchling Size γi	SE	MSE
15	1	12.5404	1.0914	-0.0489	0.0096	-0.1819	0.0243	0.0415
15	2	12.2720	1.0446	-0.0513	0.0101	-0.1836	0.0262	0.0484
15	3	11.5109	0.7992	-0.0641	0.0116	-0.1774	0.0325	0.0564
15	4	10.0741	0.6788	-0.0797	0.0167	-0.2051	0.0496	0.0843
15	5	12.4724	0.6464	-0.0565	0.0070	-0.1637	0.0186	0.0237
15	6	11.1938	0.9985	-0.0598	0.0139	-0.2114	0.0386	0.0704
15	7	8.5245	0.2969	-0.1274	0.0199	-0.2068	0.0547	0.0578
15	8	10.0730	0.6697	-0.0866	0.0184	-0.1787	0.0540	0.1043
15	9	13.4110	0.8711	-0.0529	0.0076	-0.1326	0.0193	0.0340
15	10	11.4997	0.8926	-0.0700	0.0145	-0.1515	0.0410	0.0936
15	Composite	11.1201	0.3002	-0.0672	0.0049	-0.1826	0.0139	0.0937
30	1	8.4134	0.3584	-0.1210	0.0224	-0.2162	0.0629	0.0757
30	2	11.6478	0.9662	-0.0605	0.0128	-0.1843	0.0351	0.0679
30	3	10.5051	0.3755	-0.0746	0.0077	-0.1807	0.0223	0.0209
30	4	9.0008	0.1495	-0.0984	0.0060	-0.2092	0.0176	0.0077
30	5	7.7843	0.2817	-0.1716	0.0368	-0.1943	0.0833	0.0951
30	6	8.7540	0.4819	-0.1075	0.0233	-0.2175	0.0681	0.1017
30	7	8.7550	0.4481	-0.1021	0.0199	-0.2238	0.0589	0.0772
30	8	9.8120	0.4211	-0.0931	0.0135	-0.1783	0.0394	0.0509
30	9	10.8155	0.4586	-0.0752	0.0091	-0.1679	0.0264	0.0315
30	Composite	9.3503	0.1932	-0.0950	0.0069	-0.2027	0.0204	0.1039

Table 4.8 Parameter estimates of individual and composite growth curves for means of individual enclosures of larvae reared at two initial densities (15 and 30) for Mt Margaret January 1989.

MSE, Mean Square Error, Number of observations per curve = 12, measurements in millimetres.

Density	Replicate	Asymptotic Size αi	SE	Growth Rate βi	SE	Hatchling Size γi	SE	MSE
15	1	9.0641	0.6079	-0.0573	0.0164	-0.4864	0.0568	0.0528
15	2	9.1403	0.4019	-0.0427	0.0065	-0.4809	0.0250	0.0095
15	3	9.2128	0.3054	-0.0727	0.1154	-0.4150	0.0402	0.0321
15	4	8.7824	0.2782	-0.0755	0.1214	-0.4413	0.0422	0.0300
15	5	10.2169	0.7821	-0.0388	0.0090	-0.4137	0.0349	0.0239
15	6	10.2831	0.6087	-0.0427	0.0080	-0.3952	0.0283	0.0213
15	7	9.5171	0.3587	-0.0425	0.0054	-0.4526	0.0201	0.0077
15	8	9.8245	0.4222	-0.0629	0.0113	-0.3976	0.0390	0.0343
15	9	8.9269	0.3510	-0.0558	0.0092	-0.4907	0.0320	0.0169
15	Composite	9.3515	0.2279	-0.0536	0.0050	-0.4492	0.0160	0.0557
30	1	8.1809	0.4416	-0.0553	0.0128	-0.5200	0.0451	0.0287
30	2	8.3140	0.3500	-0.0409	0.0063	-0.5692	0.0279	0.0062
30	3	8.8543	1.7847	-0.0336	0.0210	-0.5152	0.1130	0.0730
30	4	8.1871	0.7320	-0.0500	0.0176	-0.5095	0.0635	0.0569
30	5	10.5616	0.8452	-0.0234	0.0044	-0.4164	0.0354	0.0048
30	6	7.9070	0.2914	-0.0552	0.0093	-0.5894	0.0337	0.0120
30	7	8.6105	0.5711	-0.0365	0.0079	-0.5207	0.0380	0.0113
30	8	8.0581	0.2476	-0.0436	0.0051	-0.5719	0.0212	0.0046
30	9	8.8473	0.6643	-0.0430	0.0081	-0.5286	0.0436	0.0111
30	10	8.0157	0.2954	-0.0421	0.0059	-0.5816	0.0254	0.0053
30	Composite	8.4391	0.2190	-0.0406	0.0069	-0.5398	0.0160	0.0233

0.9962). The trend for growth in all the experiments suggests a density effect, as indicated by either smaller asymptotic lengths for larvae, lower growth rates, or both with increased initial density. In all experiments, some differences occurred between the individual and composite curves.

Experimental Ponds

The mass of larvae on days 9, 19, 35 and the mean date of metamorphosis is shown for each initial density in Figure 4.12. The forms of these growth curves (for mass) are similar to the type B curve shown by larvae (for snout-vent length) reared in *in situ* containers. The initial density=30 treatment, however, resembles the type A growth response. The response of mass from day 9 (when larvae were introduced into ponds) to day 35 for each treatment was evaluated using Profile Analysis MANOVA shown in Table 4.9. Up to day 35, shortly before metamorphosis started, growth depended on initial density. Growth in treatment 30, though similar to treatment 60, was significantly higher than treatment 120. Similarly, growth in treatment 60, while similar to 120, was significantly higher than treatment 240. Growth in treatment 480 was significantly lower than 240.

Mass at metamorphosis and larval period were analysed by MANOVA for all treatments and contrasts between treatments and are presented in Table 4.3. Larvae reared at an initial density of 30 became the largest metamorphs with a mean size of 397.714 mg

Figure 4.12 Mean larval mass of tadpoles reared at five initial densities in 1000 litre experimental ponds. The final point for each curve represents the mean mass of stage 46 metamorphs at in each treatment. Error bars represent 95% confidence limits for the mean.

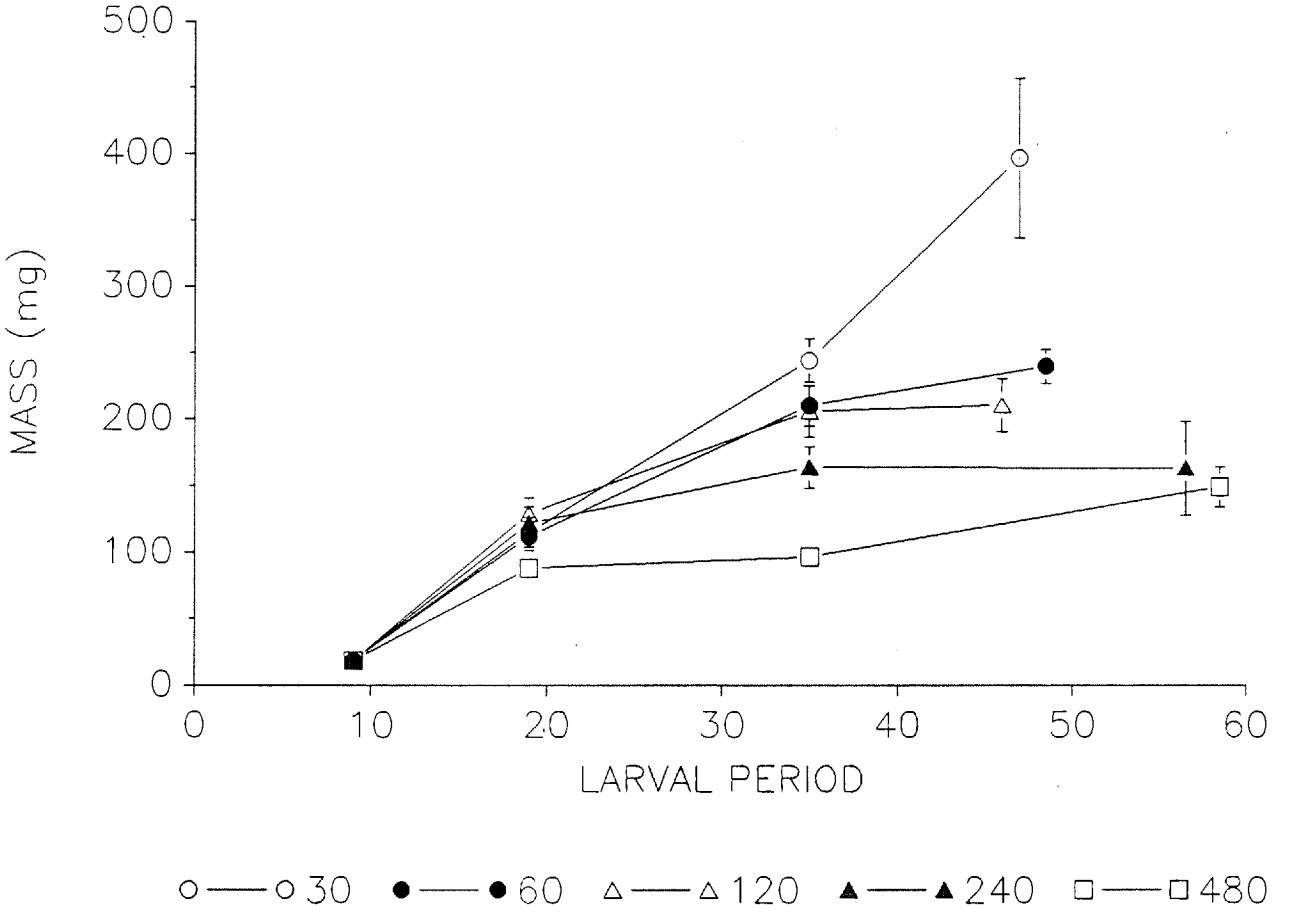

500
400
300
200
100
0
MASS (mg)
0
10
20
30
40
50
60
LARVAL PERIOD
30
60
120
240
480

Table 4.9. Profile Analysis MANOVA results for comparison of mean mass of larvae reared in 1000 litre experimental ponds at 5 different initial densities (30 60 120 240 480) from day 9 (day of introduction) to 36. Contrast analyses compare the responses for individual treatments as specified.

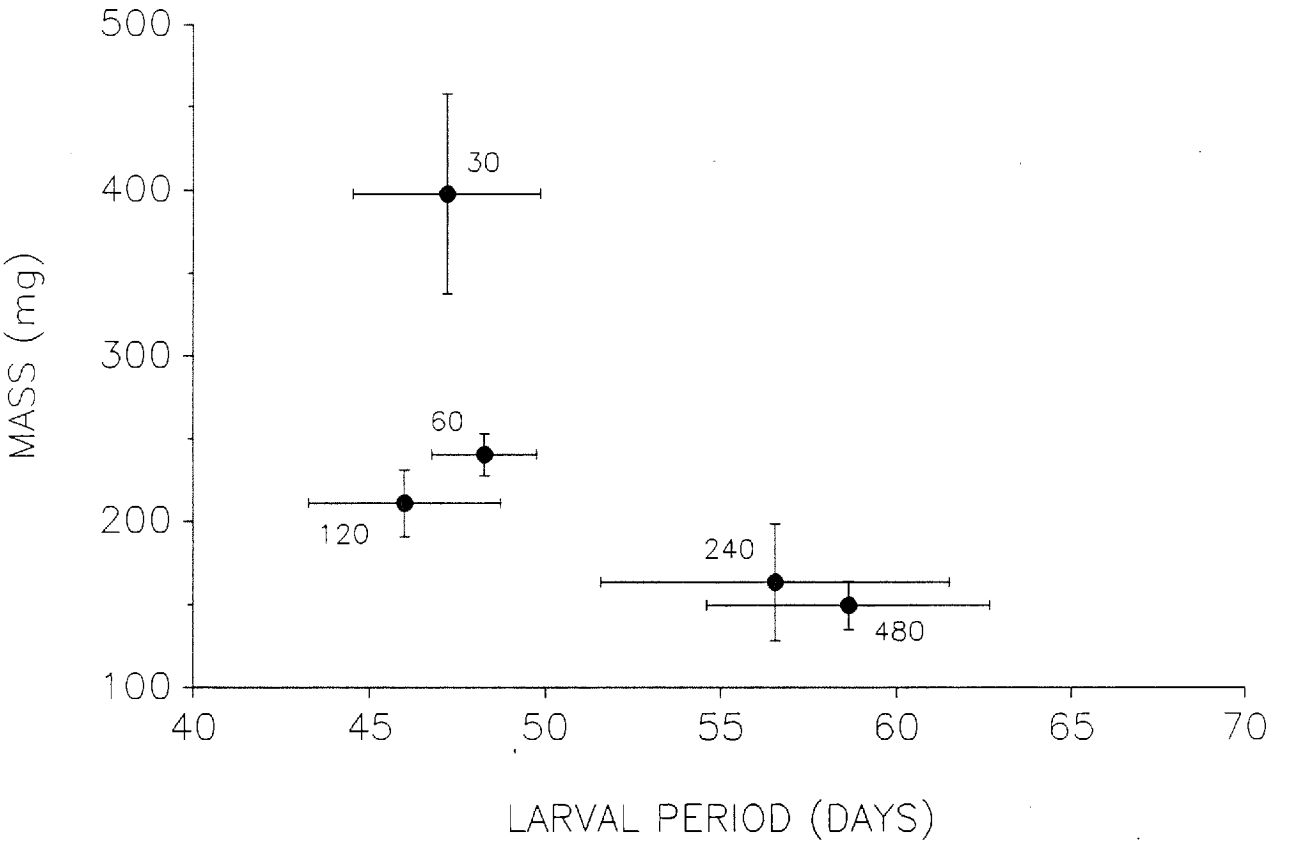
Source of Variation	Wilks' λ	F	Degrees of Freedom (Num)	(Den)	p
Initial Density	0.0875	8.33	8	28	0.0001
Contrast					
30 - 60	0.7350	2.52	2	14	0.1159
30 - 120	0.4649	8.06	2	14	0.0047
60 - 120	0.7383	2.48	2	14	0.1196
60 - 240	0.4884	7.33	2	14	0.0066
120 - 240	0.7465	2.38	2	14	0.1292
240 - 480	0.5515	5.69	2	14	0.0155

which was significantly greater than all other treatments. Larvae reared at 120 and 240 were similar in size (240.355 mg and 210.886 mg respectively) and were significantly larger than larvae reared at densities of 240 (163.436 mg) and 480 (149.282 mg). Mean metamorph mass and mean larval period were significantly affected by density. The treatment responses fall into three distinct groups (Figure 4.13). First, larvae raised at 30 per 1000 litres metamorphosed at the largest mass after a mean larval period of 47.2 days. Secondly, larvae reared at densities of 60 and 120 had larval periods of similar length with the 30 treatment, but emerged at a significantly smaller mass. Thirdly, when larvae were raised at initial densities of 240 and 480, metamorph size decreased and larval period increased.

4.4 Discussion

Data from larvae caught in quantitative box samples indicate the highly mobile and aggregated distribution of *Bufo marinus* tadpoles. This is reflected in fluctuations for estimated population size within the transect (N in Table 4.1) that cannot be explained by recruitment events. Heterogeneity is also indicated by D_O/D_R ratios greater than one (particularly the ratios for 6 May 1988 to 20 May 1988, Table 4.1) and the large estimates for variance-mean ratios. Sample numbers per m^2 on 11 February 1988 ranged from 0 to 1036. An aggregation from the single cohort of larvae sampled at Mt Margaret (June 1989) was observed in a position approximately 120 metres from its original location just 48 hours later. Thus the standard area-sampling techniques used

Figure 4.13. Mean mass at metamorphosis and mean larval period for each initial density treatment. Error bars represent 95% confidence limits for the mean.


500
400
300
200
100
MASS (mg)
30
60
120
240
480
40
45
50
55
60
65
70
LARVAL PERIOD (DAYS)

in this study may not be appropriate for estimates of total population size or mortality rates of cohorts. Perhaps more appropriate procedures in future studies may involve the separate sampling of aggregations and the peripheral populations and deriving population size from the means of both and the number of aggregations present in the sampling area.

The fragile nature of hatchlings also makes standard mark-recapture methods for population size and cohort survival analysis unsuitable. Despite the ability for kin recognition (Waldman 1982, O'Hara and Blaustein 1985), the aggregation behaviour of *Bufo* species may also tend to obscure results for analysis of growth if they are formed in part, or entirely on the basis of relative size (Breden et al. 1982, Caldwell 1989), particularly for premetamorphic tadpoles (Gosner stages 42-45, Beiswenger 1975). Size-based aggregation would tend to under-represent slower or faster growing individuals from the same cohort. As a result, modal size classes from size frequency distributions of tadpoles sampled during wet and dry season periods for this study were unsatisfactory for describing a growth relationship. Problems such as these tend to suggest that a greater emphasis be placed on field observations and experimental systems to resolve and complement these studies. Perhaps it is significant that few published studies modelling larval dynamics of anurans do so using field-based, quantitative sampling designs (see Calef 1973, Heyer 1979, Smith 1983, and Alford 1986b for anuran studies and Harris et al 1988 for urodeles).

The organism weighted ratio for density, Do, (Lewontin and Levins 1989) gives the highest estimates for effective density. From the perspective of the tadpole, small fluctuations in density measured by D_R may in fact be quite important when considering the density dependence of a particular process, such as growth. Density also becomes an increasingly important measure when tadpoles are influenced by interference competition, as has been demonstrated for *Bufo* (Banks and Beebee 1987, Petranka 1989b). Such factors require consideration when constructing hypothesis testing experiments where density is a factor. For example, investigation of the effect of density on growth and survival detailed in this chapter was set up with effective densities from 12 to 200 per m^2 in experimental ponds. Estimates for mean D_R from field data in this study show that this range extends far beyond what is experienced in natural habitats. The maximum mean D_R calculated is 143 per m^2, with the majority below 50 per m^2. This does not, however, account for the patchiness and crowding of tadpole distributions in these habitats. Estimates for Do demonstrate that 200 per m^2 is well within the range of mean densities experienced by individual tadpoles. Maximum estimates for Do include 616 and 781 per m^2 and the majority are greater than 50 per m^2. Thus if interference competition is exerting a significant controlling influence on growth, this alternative consideration of density, Do, may be crucial in the interpretation of experiments set up to investigate factors such as competition and food limitation in field situations.

Results for experimental and *in situ* survival of eggs indicate that with predators absent, approximately 70 percent survive to become tadpoles. At Calvert Hills , this did not

depend on site or season. However, a conflicting result for survival in a second experiment conducted in one season (Figure 4.5) suggests that significant variation can occur within seasons. These differences may be associated with parental and genetic effects (Wilbur 1976, Travis 1981, 1983, Travis et al. 1987). Similar variation was also displayed for tadpole survival (Figure 4.7). Without predators, egg mortality can be attributed to unsuccessful fertilisation or pathogen attack (Herreid and Kinney 1966, Calef 1973). It is possible that fungal infection and subsequent mortality, involves unfertilised eggs (Woodruff 1976). Hatching rates have been estimated for other bufonid species; 34 percent for *Bufo cognatus* (Bragg and Bresler 1951, in Turner 1962) and 95 percent for *Bufo americanus* (Voris and Bacon 1966). Estimates for other genera include 73 percent for *Ranidella signifera* (Williamson 1988) and for some *Rana* species; 70 to 90 percent (Licht 1974) and 96 percent (Seigel 1983). Such studies are rare in the literature.

Survival through tadpole stages (Gosner stages 25 to 44) in the absence of predation was low, ranging from 0.1 to 10 percent. Tadpole survival depended on the initial density in all experiments. Survival was highest at 5 per litre and decreased at higher densities of 10 per litre and 17 per litre. Initial density accounted for 57 percent of the total sums of squares for survival to metamorphosis for the *in situ* experiments and 74 percent in experimental ponds. Survival curves for embryo stages and for tadpole stages at all densities tested are in the form of Deevey's (1947) Type III curve. This depicts a low rate of survival during the initial phase of development and subsequent increases in rate during later stages. The periods during which lowest survival occured in

experimental enclosures were associated with late gastrulation and hatching. This corresponds to the first 12 to 36 hours of embryonic development and days three to twelve after laying. If survival is represented as a daily rate rather than by stage, then the rate of survival is lowest for the egg stages at approximately 90 percent/day based on a 72 hour egg phase, and higher for the tadpole stages at about 97 percent/day over a 31 day larval period. Few studies have looked at the survival of larvae in the controlled absence of predators (Wilbur 1977a, 1977b). Petranka (1985) reviewed a series of papers that studied larval amphibian survival including those that demonstrated Type III relationships (Herreid and Kinney 1966, Calef 1973, Cecil and Just 1979). He standardised the results by replotting survival on semilogarithmic scales used by Deevey (1947). He concluded that constant survival rate over time (Type II patterns) characterised most populations. Survival in these studies had been influenced by predation. It is possible that predation is age specific and dependent on characteristics of a species such as changes in microhabitat use (Alford 1986b) and palatability (Formanowicz and Brodie 1982). This would modify patterns from those observed without predation. Density may also have an age-specific influence on survival. Survival of larvae in experimental ponds was essentially constant at lower initial densities (Type II). However, at the highest densities, mortality rates increased towards the end of the larval period; the Type I pattern (Figure 4.8).

In a seven year study of *Rana sylvatica* populations, Berven (1990) found that 92 to 99 percent of the total mortality occurred in the premetamorphic stage and that this was caused primarily by density dependent factors. The number of successful metamorphs,

time to metamorphosis, and particularly size at metamorphosis were all strongly correlated to number of eggs deposited into the larval habitat. In addition, juvenile stages were also affected by these factors so that larger metamorphs had increased survival, faster growth rates and were larger as adults. Patterns of embryo and larval survival similar to this study have been reported for other *Rana* species (Herried and Kinney 1966, Licht 1974, Cecil and Just 1979), although Calef (1973) reported low mortality in the embryo stage (8 to 9 percent) with approximately 5 percent surviving to metamorphosis. Wilbur's (1977b) study of density dependence in *Bufo americanus* and Dash and Hota's (1980) similar study of *Rana tigrina* found that the proportion of each experimental population that metamorphosed was a negative exponential function of density. Wilbur suggested that density affects the growth rate of individuals such that in high density populations, a few individuals grow at the expense of the rest of the cohort, reducing the mean probability of metamorphosis.

Growth during the embryonic phase, from Gosner stages 16 to 24, was linear and did not vary between experiments. Prior to stage 16 the embryo is undergoing cellular differentiation and does not grow *per se*. It has been proposed that egg size influences larval fitness in amphibians as larval size is often positively correlated with egg size (Kaplan 1980, Crump 1984, Reading 1986). Increased size may lead to faster development (Kaplan 1980, 1985, Crump 1981, 1984, Berven 1982, 1988, Kaplan and Cooper 1985). However, a thorough study of a population of *Ranidella signifera* (Williamson and Bull 1989) revealed that no consistent relationship existed between egg size and development rate. Growth rates in this stage may be almost totally controlled

by temperature (Herreid and Kinney 1967, Beattie 1987). No comparable studies of growth patterns in the pre-hatchling stages exist for *Bufo marinus*. No difference in the size of eggs was shown for the clutches used in the separate experiments, though other work (see section 3.3.2, Figure 3.18) indicates that mean egg sizes differ between populations.

Growth of the swimming stages is non-linear and can follow either an exponential (Type A) or decreasing exponential (Type B) relationship. Growth models for individual replicates within all *in situ* experimental enclosures differed from the composite models. While this suggests that variation exists in growth, all composite models were significant and accounted for much of the variation in growth (Figure 4.11). Faster growth rates in the lower density treatments, as described by the composite growth models and, presumably, controlled by intraspecfic competition (Semlitsch 1987), resulted in larger larvae at metamorphosis. The mean snout-vent length of larvae at metamorphosis was larger for larvae reared at lower initial densities. Estimates for asymptotic length derived from Type B growth equations were also significantly larger at lower initial densities. These data show that both types of growth were density dependent.

Surprisingly, the range of densities used in experimental enclosures (5 and 10 per litre in all three experiments and 17 per litre as an extra treatment for the January 1988 experiment) did not influence duration of larval periods. There was however, a large amount of variation in mean larval period between the sets of enclosure experiments.

Mean larval period length for each experiment was negatively correlated with the mean daily maximum water temperatures. The duration of larval period (and metamorph size) of other anurans, (*Pseudacris ornata* Harkey and Semlitsch 1988, *Rana sylvatica*, *R.pipiens*, and *R.clamitans* Herreid and Kinney 1967, Smith-Gill and Berven 1979) has also been shown to decrease in response to higher temperatures during development. The differences in larval period demonstrated by larvae for each set of experiments could also be attributable to factors not controlled by the procedure. For any one set of experiments though, other physical conditions such as depth, distance from shoreline, container positioning and amount of sampling disturbance were consistent between individual replicates. Factors such as heterogeneity associated with small containers (Wilbur 1972, 1976) and qualities of the habitats used, such as water chemistry characteristics and turbidity may have influenced the amount of detritus entering the containers and the growth of algal turfs. Given that the influence of all these factors on such things as food resources were consistent for treatments within an experimental period, then larvae at the higher initial densities were responding to adverse conditions such as increased competition by metamorphosing at smaller mean sizes than those at lower initial densities.

Larvae displaying exponential growth were associated with the shortest mean larval period and the highest mean daily maximum water temperatures. Thus larvae may have been accelerating growth to metamorphosis (Wilbur 1980) as a response to favourable conditions. Although it is difficult to compare the magnitude of growth between *in situ* experiments, larvae with this growth pattern were of a larger size at metamorphosis.

Similarly, larvae grown at the lowest densities in experimental ponds also demonstrated this growth pattern in relation to mass. Presumably, larvae at the lowest densities should be growing under the most favourable conditions. These larvae also metamorphosed at the largest body size and after the shortest larval period (Figure 4.12). Analysis of daily mean water temperatures indicated that decreasing exponential growth was not a response to changing physiological influences such as decreasing temperature (Table 4.4). Mean temperatures were constant during these growth periods. The mean preferred temperature range for larval *Bufo marinus* has been calculated at 28.5°C, ranging from 26.7° to 30.4°C (Floyd 1984), though larvae can tolerate temperatures up to 42.5°C (Heatwole et al. 1968, Krakauer 1970). Mean daily maximum temperatures during the period when growth was exponential were 35.8°C but were decreasing to the preferred range (Figure 4.9). Larvae displaying decreasing-exponential growth were either reacting to lower, less favourable developmental temperatures (Harkey and Semlitsch 1988) or to other undetected conditions that influenced the growth pattern, or to both. Mean maximum water temperatures recorded during these experiments lay within, or very close to the preferred thermal range (Floyd 1984). This indicates that growth patterns were not responding to temperature alone and that some limiting factor may have been operating to decrease the growth rate below the maximum.

The results for growth and survival of tadpoles in experimental ponds corroborated data from enclosures, demonstrating that these characteristics were also strongly density dependent in larger experimental systems. With increased initial density, survival and

mass at metamorphosis are decreased. In contrast to enclosure results, the length of larval periods increased at higher initial densities. Other studies have shown corresponding trends. Semlitsch and Caldwell (1982) reared *Scaphiopus holbrooki* tadpoles at a range of densities and clearly showed a decrease in survival and growth with increasing density. Interestingly, they also found that above a certain abundance, increased density did not significantly lower growth. In this study, both tadpole growth (larval period and mass at metamorphosis) and survival were similar at the two highest densities (240 and 480 per pond). Results for *Bufo americanus* metamorphosis have shown that increased density of conspecifics led to greater variation in the number of days to metamorphosis (Breden and Kelly 1982). Other research has found that growth and survival of larval *Ambystoma opacum* under natural conditions was dependent on the density of individuals present in ponds (Petranka 1989a, Scott 1990). Scott's analysis highlighted intraspecific competition and habitat duration as important factors in influencing growth and survival of larvae. However, in Petranka's study, no reductions in biomass of zooplankton were detected in ponds with elevated densities of individuals (unfortunately, no benthic insect comparisons were done) that seemed to suggest intraspecific aggression was more important than exploitative competition. This seems an unlikely situation for *Bufo marinus* where intraspecific aggression is restricted to oophagy. No tail damage was observed in any experimental treatment during this study.

It is likely that food was a limiting factor controlling growth in experimental ponds. Densities of larvae used per experimental pond (basal area 2.4 m^2, densities of 12 to

200 per m^2) fall within the range of that sampled in field populations (2 to 781 per m^2 based on estimates for Do). Two remarkably similar studies, one on *Rana sylvatica* (Wilbur 1977a), the other on *Rana tigrina* (Hota and Dash 1981), demonstrated that in experimental populations, density and food supply had a significant effect on growth rate and body size at metamorphosis, however the two did not act independently. Wilbur suggested that at low food supply per individual, exploitation competition reduced growth. With increasing food supply per individual fewer individuals were able to compete successfully, while pollution effects occurred at highest supply-demand ratios. Steinwascher (1978) has shown that for *Rana utricularia*, interference mechanisms in the form of chemical exudates that decrease growth of conspecifics tend to supplant exploitative mechanisms as relative food levels decrease. In contrast, *Limnodynastes ornatus* has demonstrated that both exploitation and interference competition can affect growth similarly, with the effect increasing with density (R.Alford pers comm.)

It is interesting to compare the snout vent lengths of late growth stage tadpoles in field samples and experiments conducted concurrently during this study. Larvae caught in box samples and larvae raised in experimental enclosures simultaneously and in adjacent habitats are shown in Figures 4.2A-B and Figures 4.11A-E respectively. Larval size is 15 to 30 percent larger for uncaged populations of tadpoles. Sample data for January and February 1989 at Mt Margaret are not shown as these were interrupted when larval populations were greatly diluted by rains associated with cyclones. The data for these periods show that larvae sampled at Gosner stages 40 to 42 also fell within the higher size-range. This seems to suggest that enclosures of the type used do not allow typical

growth and are likely to adversely affect larval growth rates, rather than enhance them. This suggests that the effect of the design of enclosures on growth and survival should be evaluated before predictions being made for the population dynamics of the species under investigation.

No significant, non-linear relationships were derived for growth from field sampling of larvae. In addition, modal class analysis appears to be inappropriate for the sample data collected. Given the short larval period of tadpoles (20 to 40 days), it would be expected that sampling at intervals of two to four days should provide adequate resolution for analysis of the relationship between time and size. Factors such as size-sorted, premetamorphic aggregations (Beiswenger 1975) and reduction in snout-vent length prior to emergence may distort sample means. In addition, individuals in a population growing at different rates, thus skewing the size-frequency distribution may confuse the fit of appropriate models (Alford and Jackson 1991). Considering the range of factors that can modify and confound growth, perhaps the approach of Scott (1990) requires consideration. Large scale field enclosures (Scott used $23m^2$ and $41m^2$) would allow a close approximation to natural conditions. A cohort could then be introduced and all or most of the larvae sampled at appropriate intervals. This would allow for more precise measurements of cohort growth and survival than from the use of sample means.

A recent study of growth in cephalopod larvae (Alford and Jackson 1991) has shown that sample means from size-at-age data can lead to misinterpretations of growth

patterns. Computer simulated data for exponentially growing individuals were interpreted as having logistic or biphasic (exponential/power) growth patterns when sample means were analysed. Prior analysis of field data using sample mean data demonstrated logistic growth. Reanalysis on individual data instead of the sample means confirmed exponential growth. The principle cause of this anomaly was the transformation (or exit) of faster growing individuals from the sampled population early in the transformation period. This under-represented the larger individuals in the samples and simulated a power or asymptotic pattern to growth. This obscured the general fit of the data. This type of misinterpretation is certainly possible for anuran larvae that grow at constant size-specific rates. Asymptotic growth has been shown in several anuran species (Calef 1973, Wilbur 1977b, Dash and Hota 1980, Degani 1986). Exponential growth is apparent in other species (Herreid and Kinney 1967, Cecil and Just 1979). Given that growth of *Bufo marinus* in this study has been exponential under certain circumstances, and asymptotic under others, perhaps more refined experimentation is required to clarify the factor controlling switches in growth pattern.

In summary, densities of larvae in the field were extremely variable and larval aggregations were very mobile. This made estimates of population size, survival, and growth based on quantitative sampling alone very difficult. Best results were obtained from inferring natural densities from field samples and using these in appropriate experimental systems. It was shown that significant mortality occurred during the larval period of *Bufo marinus* larvae with 10 to 0.1 percent surviving to metamorphosis. Survival-rates were low initially, and increased as larvae approached metamorphosis.

While significant variation occurred due to density-independent factors such as temperature and habitat quality, density was an important and complex factor controlling survival and development. Larval size at metamorphosis, larval growth rate and survival were shown to decrease in response to increasing density. Asymptotic growth was demonstrated in most instances, similar to that described in other studies of larval anurans. However, growth can be exponential and this may be evidence for either improved growing conditions or physiologically controlled increases in growth rate.

Chapter 5. Predation and Competition.

5.1 Introduction.

One major concern of ecological studies is the role of predation and competition in structuring populations and communities of organisms (Connell 1983, Schoener 1983, Sih et al. 1985, Tilman 1987). In nearly all amphibian populations that have been studied, predation pressure on eggs and larvae is high (Duellman and Trueb 1986). Interspecific competition may occur between closely or distantly related taxa (Morin et al. 1988).

Intraspecific interference competition in the form of predation is a common occurrence in natural and experimental populations (Bragg 1964, Eickwort 1973, Fox 1975, Polis 1981, Kusano et al. 1985, Polis and Myers 1985). Among tadpoles, it is considered important to the ecology of many obligate carnivores and even within groups of herbivorous and detritivorous tadpoles (Crump 1983). For anurans, intraspecific predation is frequently recorded in species where development occurs in small, bounded bodies of water where food may be limiting. Opportunistic oophagy is a common form of this behaviour in anuran species (Polis and Myers 1985) and may facilitate rapid growth (Crump 1983). Nagai et al. (1971) demonstrated that *Bufo vulgaris* obtained the best conversion efficiency when fed ground conspecifics. However, egg predation is just as prevalent in sympatric species (Tejedo 1991).

Predators of anuran larvae include the larvae and adults of aquatic insect species, crustaceans and vertebrate predators such as urodeles and fish. Predation by dragonfly larvae may be important in regulating populations of larval anurans (Brockleman 1969, Calef 1973, Heyer et al. 1975, Caldwell et al. 1980, Smith 1983, Sheratt and Harvey 1989). Larval dragonflies are often conspicuous members of the predator assembledge of semi-permanent ponds that contain *Bufo marinus* larvae. These assembledges include members of the family Aeshnidae, which are large active predators with acute vision. They stalk and pursue prey using prey specific behaviours (Oakley and Palka 1967, Blois 1985, Rowe 1987). Their diet consists predominantly of insects and snails. The larvae and adults of predacious diving beetles (Dytiscidae) are important predators that may dominate fishless lentic communities (Holomuzki and Collins 1987, Banks and Beebee 1988). Crayfish of the genus *Cherax* are generalist detritivores and predators that have a very wide distribution in Australia; most species are adapted to warm waters (Riek 1969).

Striking differences are often found between communities of larval amphibians when fish are either present and absent (Duellman and Trueb 1986, Kats et al. 1988, Ireland 1989). This is often attributed to differences in the antipredator defences of larvae, such as palatability, toxicity, or behavioural responses. Few published studies of larval *Bufo marinus* record predation by fishes. None exist for tropical Australian species.

In this chapter, predation is examined in the context of intraspecific and interspecific interactions. The impact of predation by members of the native fauna and the consequences of intraspecific predation and competition are important for an understanding of the population biology of a species. First, what impact does egg predation by conspecifics have on the survival of younger larvae? This type of predation is commonly observed in field situations (personal observations). It was studied using a series of experiments to compare the survival of eggs in the presence and absence of older conspecific larvae. Secondly, what impact do native fishes and invertebrate predators common at the study sites, have on survival of eggs and larvae? This was examined using experiments. It aimed to establish baseline data on predation for more extensive studies of native freshwater communities. As an extension of the second aim: to what extent does predation by members of the native fauna or conspecifics influence larval growth and survival? To study this the interactive effects of larval density and the presence of a large odonate predator, *Hemianax papuensis*, on larval performance of *Bufo marinus* was studied in experimental ponds. The effects of intraspecific competition on survival and growth was also examined in experimental ponds for two cohorts of tadpoles of different ages.

5.2 Methods

5.2.1 Predation

Egg Predation by Conspecifics

Egg predation experiments were carried out during January 1988 and May 1988 at Calvert Hills. During each period, predation was examined concurrently with monitoring of egg survival, using eggs from the same egg masses collected from the field (see section 4.2.1). Egg predation by *Bufo marinus* tadpoles was monitored by placing larvae with egg string segments (100 eggs) in 400ml polypropylene containers. These were filled with stream water and the number of eggs surviving to hatching was monitored. Tadpoles were collected from the field, and were at Gosner stages 26 to 30 (rear limb buds developing but no toe differentiation), and between 8.0 and 9.5mm SVL. They were kept in clean water without food (faecal matter regularly removed by pipette) for four hours prior to introduction to eggs. Eggs were collected from the field soon after laying. Ten replicates were used; each consisted of five tadpoles in a container with one segment of eggs. Number of surviving eggs was noted at intervals until eggs reached the hatchling stage (Gosner stage 24). Predation usually ceased at this point as hatchlings became sufficiently active and were able to avoid capture.

The survival of eggs placed *in situ* in the stream was monitored using the method described in section 4.2.1. To restate briefly, single egg segments comprising 100 eggs were placed in twenty containers and randomly placed in a section of stream at Calvert Hills (January 1988). The control treatment (10 replicates) had 400μm mesh lids to exclude predators. Results for this treatment were presented in section 4.3.1. The second treatment (10 replicates) was unscreened, thus open to colonisation by predators. Number of surviving eggs was recorded at regular intervals until hatchlings dispersed from the containers.

Predation by *Hemianax papuensis*

This experiment compared the growth and mortality of larvae at low and high densities (60 and 120 per pond) in the presence and absence of a large odonate predator, *Hemianax papuensis* (Aeshnidae). It was conducted in 1000 litre artificial ponds with a two predator treatments (dragonfly larvae absent/present) and two density treatments (60/120 per pond). Each treatment was replicated three times. Ponds were set out in a 4X3 blocked array with no obvious environmental gradients. Ponds were prepared using the method described in section 4.2.2. Groups of five tadpoles were randomly assigned to each replicate of 60 or 120 larvae. Ten individuals from each replicate were weighed then replicates were assigned to blocks of ponds on day 9. Toad eggs were collected from the field near Townsville on 5 July 1989 (day 1). They were raised in the laboratory to stage 24 using the method

described in section 4.2.2. Dragonfly larvae were collected on 10 and 11 July 1989 from a dam near Townsville using a dip net and were introduced into the ponds on day 7. Five dragonfly larvae, all between 32mm and 40mm total length, were assigned to each predator replicate. On days 19 and 35, ponds were visually censused. At each census, ten larvae were collected from each pond, weighed and returned. Metamorphs, defined by the eruption of at least one forelimb, were removed as they emerged from each pond. They were kept in the laboratory until tail reabsorption was completed and were then weighed to the nearest 0.1 milligram.

The responses of mean mass at metamorphosis and length of larval period were analysed together using MANOVA as these effects are not considered independent (Wilbur and Collins 1973, Alford and Harris 1988). Mean percent survival (arcsine-squareroot transformed) from each treatment was analysed using a 2-way ANOVA as some replicates produced no survivors, leaving mean metamorph mass and larval period undefined. In addition, the responses of mean mass and percent surviving (arcsine-squareroot transformed) on days 9, 19, and 35 were analysed separately by MANOVA to contrast patterns of growth and survival.

Predation by Other Invertebrates

Predation rates by six invertebrate species were estimated at Calvert Hills in January 1988. One experiment independently examined predation by five species of

predators; larval *Cybister tripunctatus* (Dytiscidae), adult *Homeodytes scutellaris* (Dytiscidae), and larvae of three Libellulid dragonfly species; *Orthetrum caledonicum*, *Diplacodes bipunctata*, and *Hydrobasileus brevistylus*. All species were common in sweep samples taken from tadpole habitats. A toad egg mass was collected from the field and segments of 25 eggs were removed and placed in 400ml polypropylene containers with a single predator. Five tadpoles (stage 25) were also placed in each container. Four replicates were set up for each predator species. Number of tadpoles or eggs remaining was recorded at regular intervals over 48 hours. Rate was calculated as the number consumed over the experimental period.

A second experiment estimated rates of predation by the freshwater crayfish *Cherax quadricarinatus* on stage 25 larvae. Four adult males, carapace lengths 30.0, 31.1, 31.75, and 32.7mm, were placed individually in four 25 litre buckets filled to a depth of 300mm. Each bucket contained a 40mm layer of river sand as a substrate. Ten larvae were added to each bucket and the number remaining was recorded at intervals for 48 hours.

Predation by Vertebrates

Predation rates on eggs and larvae were determined for five species of fish. All species were recorded predators of invertebrates or small fish. These were *Melanotaenia splendida australis* (Rainbow Fish), *Amniataba percoides* (Banded

Grunter), *Leiopotherapon unicolor* (Spangled Perch), *Oxyeleotris lineolatus* (Sleepy Cod), and *Glossamia aprion* (Mouth Almighty). All fish were caught at Calvert Hills using handnets. These species are common at both study areas except *O.lineolatus*, which does not occur in the Townsville region. Four individuals of each species were placed individually in 25 litre buckets (set up as for the *Cherax quadricarinatus* experiment) and left unfed for a minimum of 12 hours. Ten stage 25 larvae (suitable for the mouth-size of each species) and a segment of 25 eggs were added to each bucket. Fish condition and behaviour and the number of remaining eggs and tadpoles were recorded at intervals for 24 hours.

5.2.2 Inter-Cohort Competition and Predation

This 24 pond experiment (six treatments, four replicates) compared the growth and survival of larvae of an early cohort (A) and a late cohort (B) when the late cohort is either introduced to cohort A as eggs, as swimming hatchlings or raised alone. The unit density of larvae used was 120 per pond (0.12 individuals/L). Three control treatments were used: one unit of cohort A (treatment 1; '120A'), cohort B (treatment 2; '120B') and cohort B introduced as eggs (treatment 3; '120BE'). Treatments with competing cohorts are referred to using two abbreviations, depending on which cohort's response is examined. The responding cohort is placed first, and the cohort which may have affected it is placed in brackets. The inter-cohort competition treatments were a unit of cohort B hatchlings introduced to a unit

of cohort A larvae on day 21 (treatment 4; cohorts '120A(B)' and '120B(A)') and a unit of cohort B eggs introduced to a unit of cohort A larvae on day 19 (treatment 5; cohorts '120A(BE)' and 120BE(A)'). An intra-cohort competition treatment of two units of cohort B (treatment 6; '240B') was used to compare with the inter-cohort competition treatments.

Prior to introduction of larvae, experimental ponds were prepared in the manner described in section 4.2.2. Ponds were arranged in a 6X4 blocked array with no obvious environmental gradients. Individuals from both cohorts were selected randomly from two separate egg masses collected from the field near Townsville and raised to hatchlings in aerated aquaria in the laboratory. Cohort A was collected on 16 December 1989 (day 1), and cohort B on 4 January 1990 (day 19).

On the chosen day of introduction for each cohort, groups of five hatchlings were counted from those in aquaria. For cohort B treatments introduced as eggs, segments of 5 eggs were used. These groups were then randomly assigned to replicates for each treatment. Ten individuals from each replicate were then weighed. The mean of these replicates for cohort B hatchlings was used for the initial weight of cohort B egg replicates. For cohort A, all treatment replicates were assigned to ponds on day 7 (22 December 1990), day 19 (4 January 1991) for cohort B eggs and day 24 (9 January 1991) for cohort B larvae. At intervals during the larval period, ten larvae from each replicate were weighed and returned. Cohort A tadpoles were weighed on day 13 and 22, cohort B tadpoles on day 30 and day 36.

Metamorphs, defined by the eruption of at least one forelimb, were collected and weighed as they emerged from each pond.

Growth of each cohort prior to the start of metamorphosis, measured as mean mass on days 6, 12, and 19, was analysed using MANOVA. The responses of mean mass at metamorphosis and length of larval period were analysed together using MANOVA (see section 5.2.1). For both these analyses, secondary contrast tests were used to compare groups of cohorts within the model to determine which treatments differed significantly. Mean percent survival (arcsine-squareroot transformed) for each cohort was analysed using ANOVA as some replicates produced no survivors, leaving mean metamorph mass and larval period undefined.

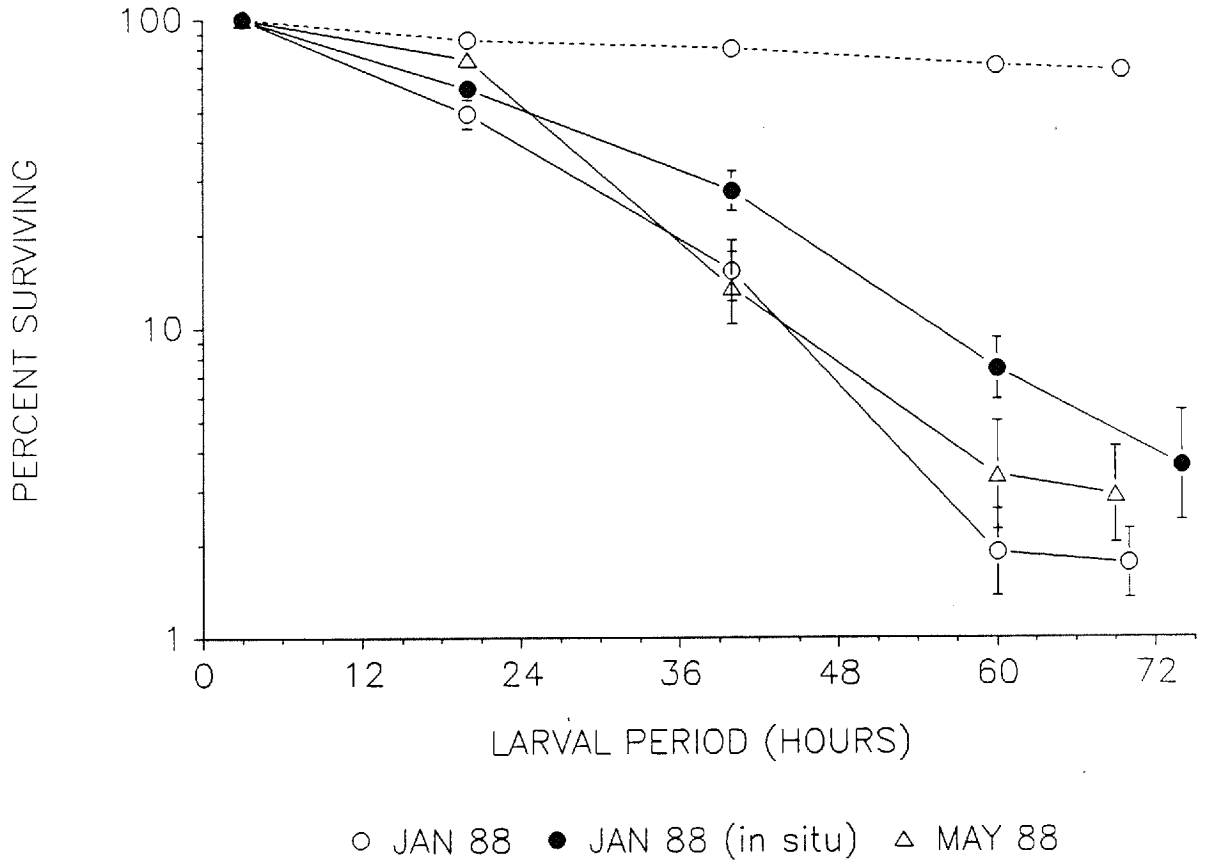
5.3 Results

5.3.1 Predation

Egg Predation by Conspecifics

Figure 5.1 shows the survival of eggs to hatchlings while being preyed on by older conspecifics. Survival profiles differed significantly among the three experiments (MANOVA, Wilks'λ=0.0909, $F_{8,22}$=6.37, P=0.0003). However, final survival at

Figure 5.1. Mean percent of larvae surviving from egg stage to stage 24 exposed to predation by older *Bufo marinus* larvae. Error bars represent 95% confidence limits for the mean. For comparison, the dotted line contrasts the mean survival for the non-predator treatment of the JAN88 experiment (see Figure 4.5).


100
10
1
PERCENT SURVIVING
0
12
24
36
48
60
72
LARVAL PERIOD (HOURS)
JAN 88
JAN 88 (in situ)
MAY 88

Gosner stage 24 was not significantly different (ANOVA, $F_{2,14}=1.04$, $P=0.3784$). Mean survival was 2.88 $\pm$ 0.93 percent (SE, N=30 replicates). Mean survival varied between 0.0 and 15.0 percent. Survival in all experiments was significantly lower than for control experiments with no predators (section 4.3.1), (ANOVA, $F_{6,72}=662.17$, $P<0.0001$) where mean survival was shown to be 71.8 $\pm$ 1.29 percent (N=5 experiments).

Predation by *Hemianax papuensis*

The percentage of larvae surviving to metamorphosis in each treatment is shown in Figure 5.2. The results of the MANOVA on percent survival over time prior to metamorphosis and the ANOVA on final percent survival are presented in Table 5.1. Both final survival and survival over time were significantly higher in the lower density treatments and in treatments without predators. Mean survival in predation treatments for both densities (60P and 120P) is clearly lower throughout the larval period and at metamorphosis. Interactions between the treatments were not significant in either case although the ANOVA and the data suggest that the effect of density on survival rate was greater in the absence of predators (Figure 5.2).

The mean mass for each treatment on days 9, 19, 35 and at the mean length of the larval period is presented in Figure 5.3. The MANOVA for mean mass up to day 35 of the larval period (Table 5.2) show that increases in mass differed significantly

Figure 5.2. Mean percent of larvae surviving in each treatment on days 9, 19, 35 and mean final day for larvae reared each initial density (60 and 120 per pond) with and without predators (*H.papuensis*). Error bars represent 95% confidence intervals for the mean.

Key to abbreviations: numbers refer to the initial density of larvae (60 or 120), C = predators absent, P = predators present.

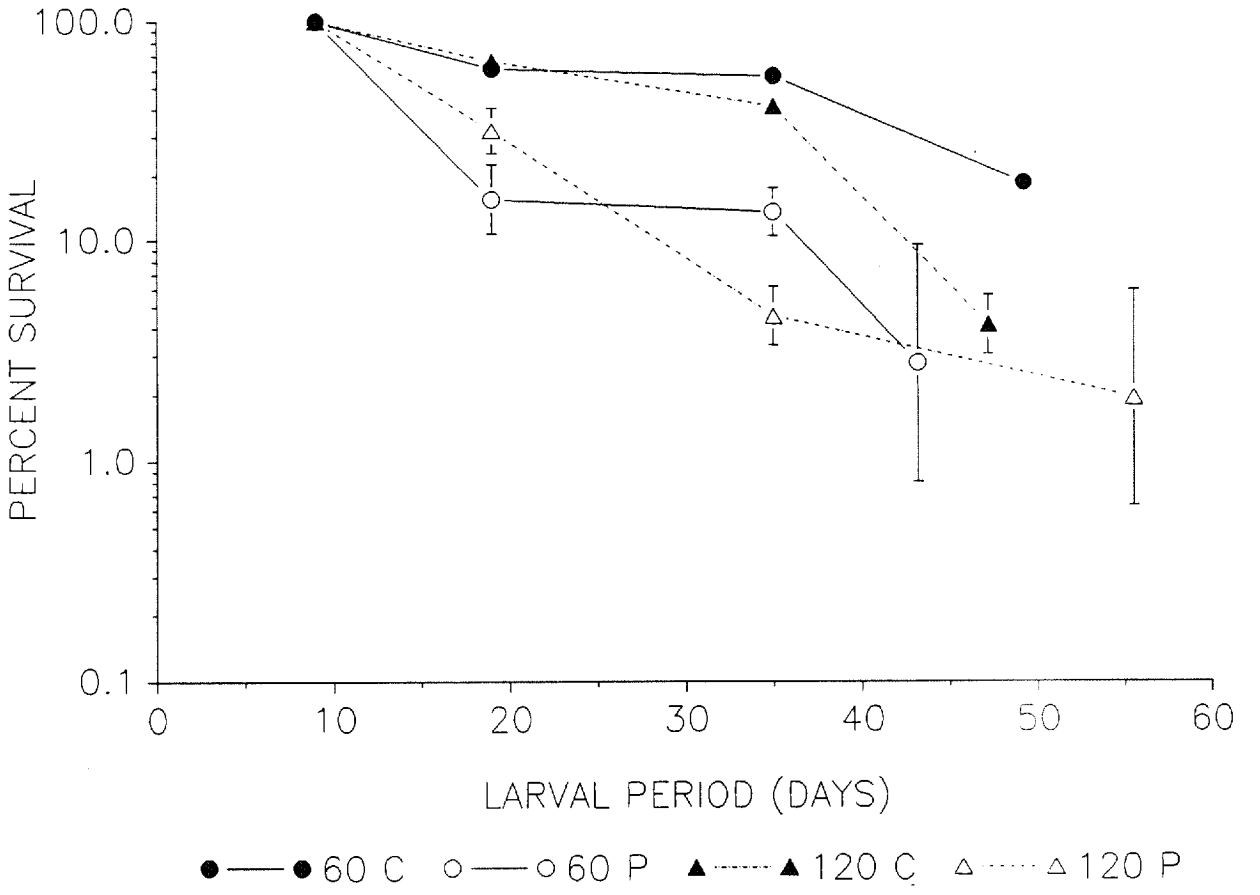

100.0
10.0
1.0
0.1
PERCENT SURVIVAL
0
10
20
30
40
50
60
LARVAL PERIOD (DAYS)
60 C
60 P
120 C
120 P

Table 5.1. Treatment responses, MANOVA results for analysis of survival over time (days 9, 19 and 35), and ANOVA results for analysis of final survival of larvae reared in experimental ponds at two initial densities (60 and 120 per pond), with and without predators (*H.papuensis*).

Predation Treatment	Initial Tadpole Density	Percent Survival			Overall Means	Percent Survival		
		Mean	SE	N		Mean	SE	N
					Density			
Absent	60	18.9	2.0	3	60	10.8	3.8	3
Present	60	2.8	2.0	3	120	3.1	0.9	3
					Predator			
Absent	120	4.2	1.0	3	Absent	11.5	3.4	3
Present	120	1.9	1.2	3	Present	2.4	1.1	3

MANOVA of (arcsine-squareroot transformed) percent survival on days 9, 19, and 35.

Source of Variation	Wilks' λ	F	df	P
Predator regime (P)	0.0723	44.90	2,7	0.0001
Initial tadpole density (D)	0.2139	12.86	2,7	0.0045
P X D	0.5292	3.11	2,7	0.1078

ANOVA of (arcsine-squareroot transformed) percent final survival

Source of Variation	df	SS	MS	F	P
Predator regime (P)	1	412.6385	413.6385	15.85	0.0041
Initial tadpole density (D)	1	171.9553	171.9553	6.61	0.0331
P X D	1	127.1103	127.1103	4.88	0.0581
Residual	8	208.2159	26.0270		

Figure 5.3. Mean mass for larvae in each treatment on days 9, 19, 35 and mean final day for larvae reared each initial density (60 and 120 per pond) with and without predators (*H.papuensis*). Error bars represent 95% confidence intervals for the mean.

Key to abbreviations: numbers refer to the initial density of larvae (60 or 120), C = control treatments, P = predator treatments.

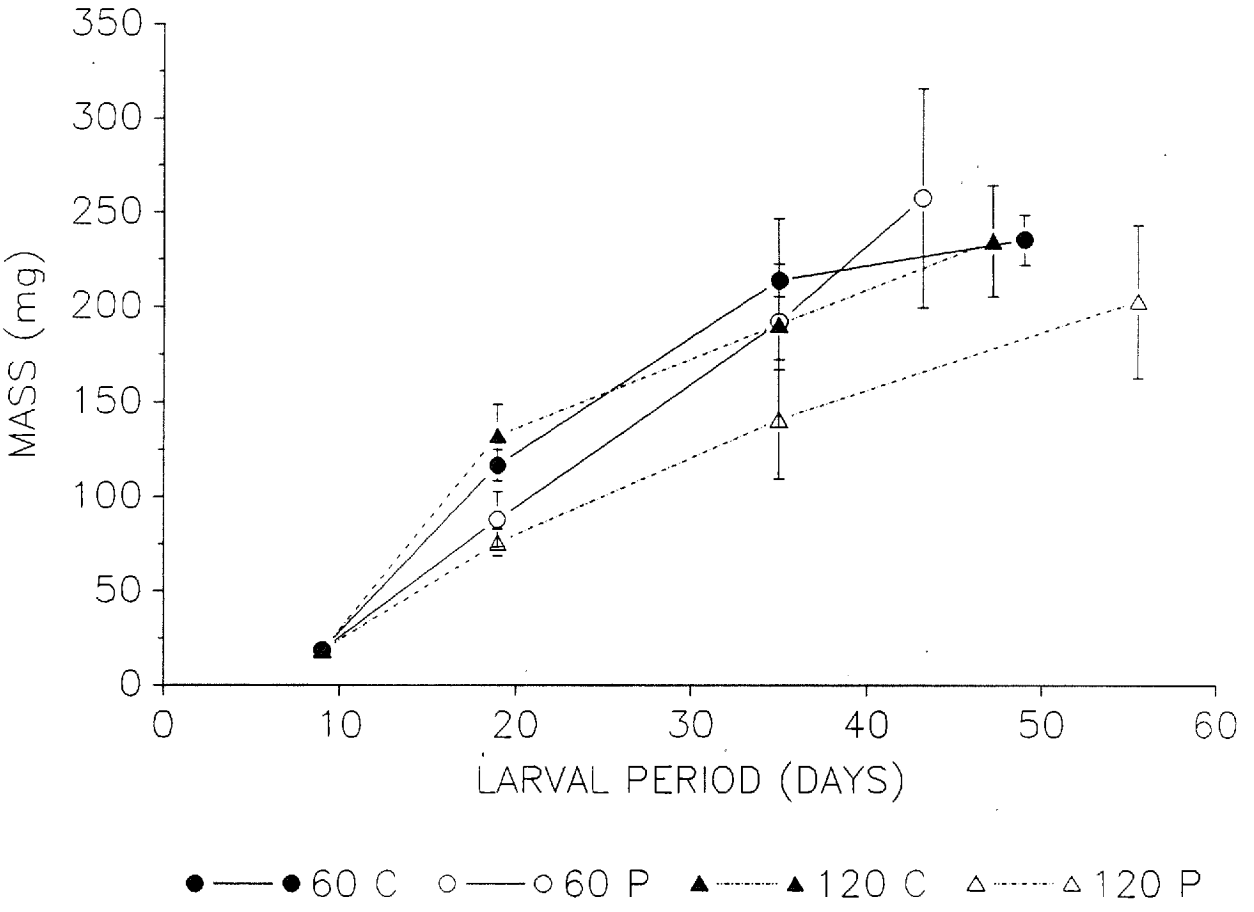

350
300
250
200
150
100
50
0
MASS (mg)
0
10
20
30
40
50
60
LARVAL PERIOD (DAYS)
60 C
60 P
120 C
120 P

Table 5.2. Final responses of mass and larval period from larvae reared in experimental ponds at two initial densities (60 and 120 per pond), with and without predators (*H.papuensis*). Tests shown are MANOVAs for mean tadpole mass over time (days 9, 19 and 35), and for mean mass at metamorphosis with mean larval period.

Predation Treatment	Initial Tadpole Density	Mass at Metamorphosis (mg)			Larval Period (days)		
		Mean	SE	N	Mean	SE	N
Absent	60	235.8	6.6	34	49.2	1.1	34
Present	60	257.8	21.0	5	43.2	1.3	5
Absent	120	235.3	13.8	15	47.2	1.3	15
Present	120	203.3	15.7	6	55.5	1.2	6

MANOVA of tadpole mass on days 9, 19 and 35

Source of Variation	Wilks' λ	F	df	P
Predator regime (P)	0.1658	12.58	2,5	0.0112
Initial tadpole density (D)	0.2281	8.46	2,5	0.0248
P X D	0.6497	1.35	2,5	0.3402

MANOVA of mass at metamorphosis and larval period

Source of Variation	Wilks' λ	F	df	P
Predator regime (P)	0.9932	0.19	2,55	0.8287
Initial tadpole density (D)	0.8694	4.13	2,55	0.0213
P X D	0.7887	7.37	2,55	0.0015

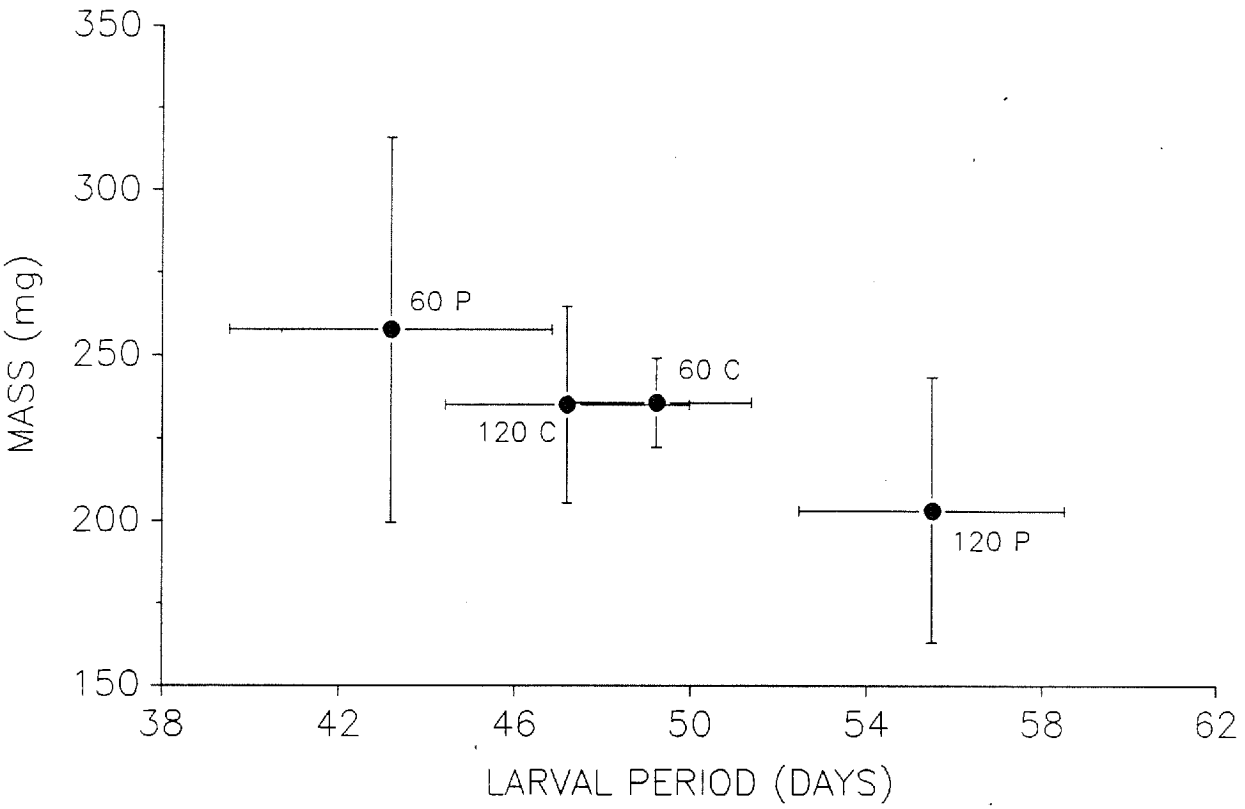
for predator regime and initial tadpole density. The overall mean mass of larvae at the initial density of 120 per pond was significantly smaller than for larvae at 60 per pond. Similarly, larvae in the control treatments were larger than those in predator treatments.

The results of the MANOVA on mass at metamorphosis and larval period for each treatment (Table 5.2, Figure 5.4) show that predator regime and initial density affected these responses interactively; initial density had an additional effect that did not depend on predator regime. Larvae from the higher density control (120C) and predator (120P) treatments metamorphosed at an overall smaller mass and a longer larval period. However, the simultaneous effect of predator regime and initial density had a more pronounced effect. Density controlled metamorph size and the larval period but only if predators were present. Larvae reared in the higher density, predator treatment (120P) emerged at a smaller mass after a longer larval period. This contrasted with the response of larvae reared in the lower density, predator treatment (60P). Larvae in this treatment had the shortest larval periods and were larger at metamorphosis (Figure 5.4). Metamorphs in the control treatments for both densities (60C and 120C) were equivalent in mass and had similar larval periods.

Qualitative observations of tadpole behaviour were made on random examinations of the ponds. In ponds without *Hemianax*, larvae were periodically observed to form small aggregations. Foraging was conspicuous, on top of the substrate and on algae growing on the sides of the ponds. In predator treatments, larvae appeared to reduce

Figure 5.4. Mean larval period and mean mass for metamorphs in each treatment for larvae reared two initial densities (60 and 120 per pond) with and without predators (*H.papuensis*). Error bars represent 95% confidence intervals for the mean.

Key to abbreviations: numbers refer to the initial density of larvae (60 or 120), C = control treatments, P = predator treatments.


350
300
250
200
150
MASS (mg)
60 P
60 C
120 C
120 P
38
42
46
50
54
58
62
LARVAL PERIOD (DAYS)

their visibility. Swimming speeds were noticeably quicker. They were almost exclusively positioned singly, or less commonly in groups of up to three larvae, underneath leaves and sticks among the substrate. When disturbed, activity was reduced to short bursts to suitable cover. No attacks by *Hemianax* on tadpoles were observed but carcasses with the tail removed and the abdomen torn open were occasionally observed among the substrate.

Predation by Other Invertebrates

The mean total length and recorded diet for all invertebrate species used in experiments is shown in Table 5.3. Of the insect species investigated, only the adult dytiscid species had any impact on *Bufo marinus* survival (Figure 5.5). The dytiscid larvae, (*C.tripunctatus*) were observed repeatedly attacking the tadpoles and egg segments but rarely persisted. One dytiscid larva died at 39 hours after consuming a tadpole at 8 hours. The recorded mean predation rate was 0.026 larvae/hour. The adult species, *H.scutellaris*, had a more significant impact, reducing survival of tadpoles to 47 percent over the 48 hour period at a rate of 0.276 larvae/hour. Neither dytiscid species consumed eggs.

The three dragonfly species did not feed readily on either eggs or tadpoles although tail damage on tadpoles indicated that predation was attempted. At the end of 48 hours, one *O.caledonicum*, one *H.brevistylus* and three *D.bipunctata* were dead.

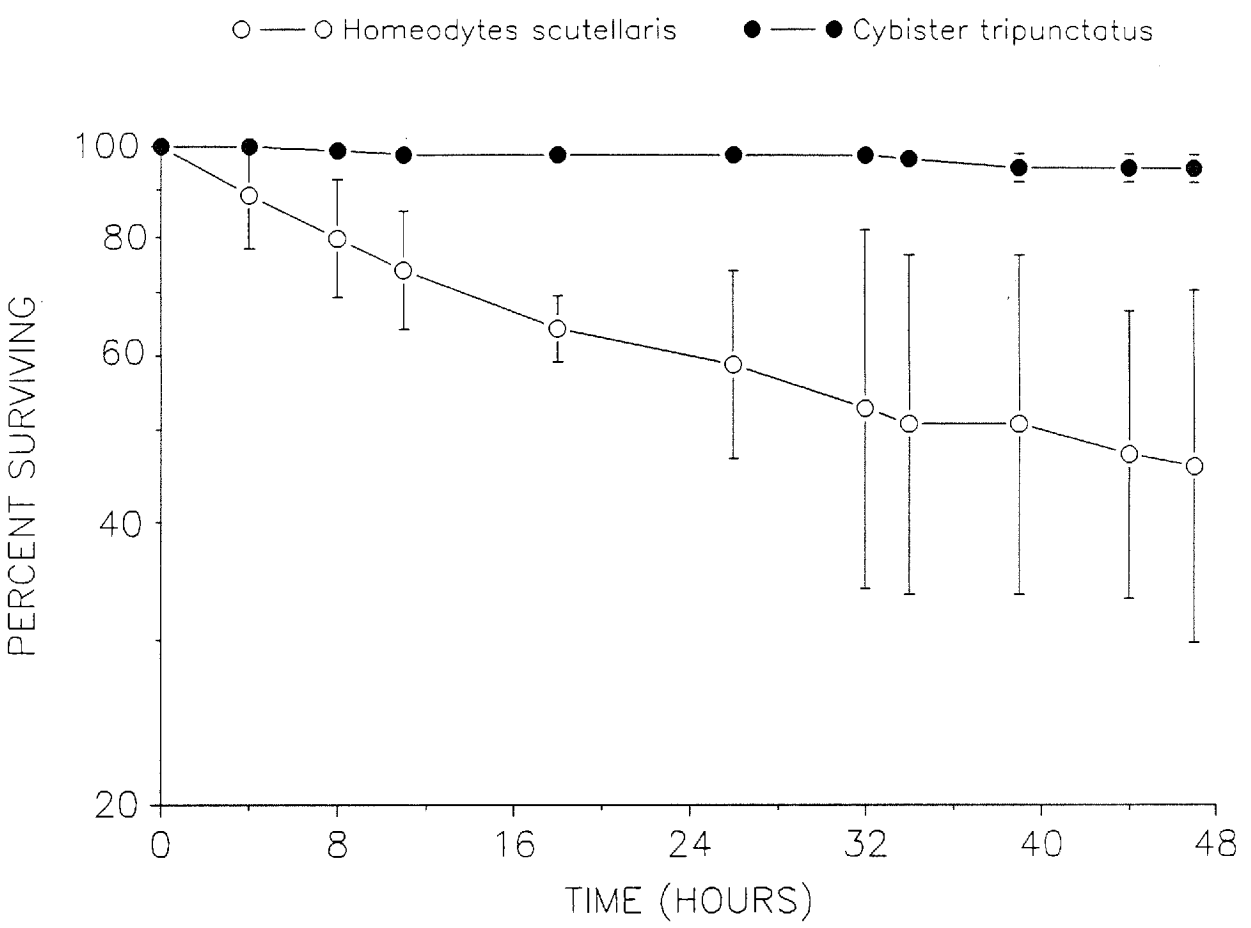
Table 5.3 Mean total length (mm), standard deviation and recorded diet for invertebrate and vertebrate species used in predation rate experiments on *Bufo marinus* eggs and stage 25 larvae.

FAMILY / Species	Mean TL	SD	Recorded Diet
DYTISCIDAE			
Cybister tripunctatus	31.9	5.2	molluscs/small fish (1)
Homeodytes scutellaris	25.7	3.2	molluscs/small fish (1)
LIBELLUDAE			
Orthetrum caledonicum	16.6	1.5	insect larvae/small crustaceans (2)
Diplacodes bipunctata	15.2	0.8	insect larvae/small crustaceans (2)
Hydrobasileus brevistylus	18.2	1.6	insect larvae/small crustaceans (2)
PARASTACIDAE			
Cherax quadricarinatus	31.4	2.0	plants/molluscs/detritus (3)
MELANOTAENIIDAE			
Melanotaenia s. australis	80.7	3.6	algae/crustaceans/insects (4)
TERAPONIDAE			
Amniataba percoides	55.8	4.5	plants/algae/crustaceans/insects (4)
Leiopotherapon unicolor	86.1	3.1	plants/crustaceans/molluscs/insects(4)
ELEOTRIDAE			
Oxyeleotris lineolatus *	57.7	2.9	crustaceans/insects/small fish (4)
APOGONIDAE			
Glossamia aprion	83.5	5.2	crustaceans/insects/fish (4)

* size range used represents immature individuals for this species

1 CSIRO, 1970
2 R.Rowe, personal communication
3 Lake and Sokol, 1986
4 Merrick and Schmida, 1984

Figure 5.5. Percentage of stage 25 tadpoles surviving predation by *Homoedytes scutellaris* adults and *Cybister tripunctatus* larvae (Dytiscidae) in experimental aquaria over 48 hours. Error bars represent 95% confidence intervals of the mean.


O — O Homeodytes scutellaris
● — ● Cybister tripunctatus
PERCENT SURVIVING
100
80
60
40
20
0
8
16
24
32
40
48
TIME (HOURS)

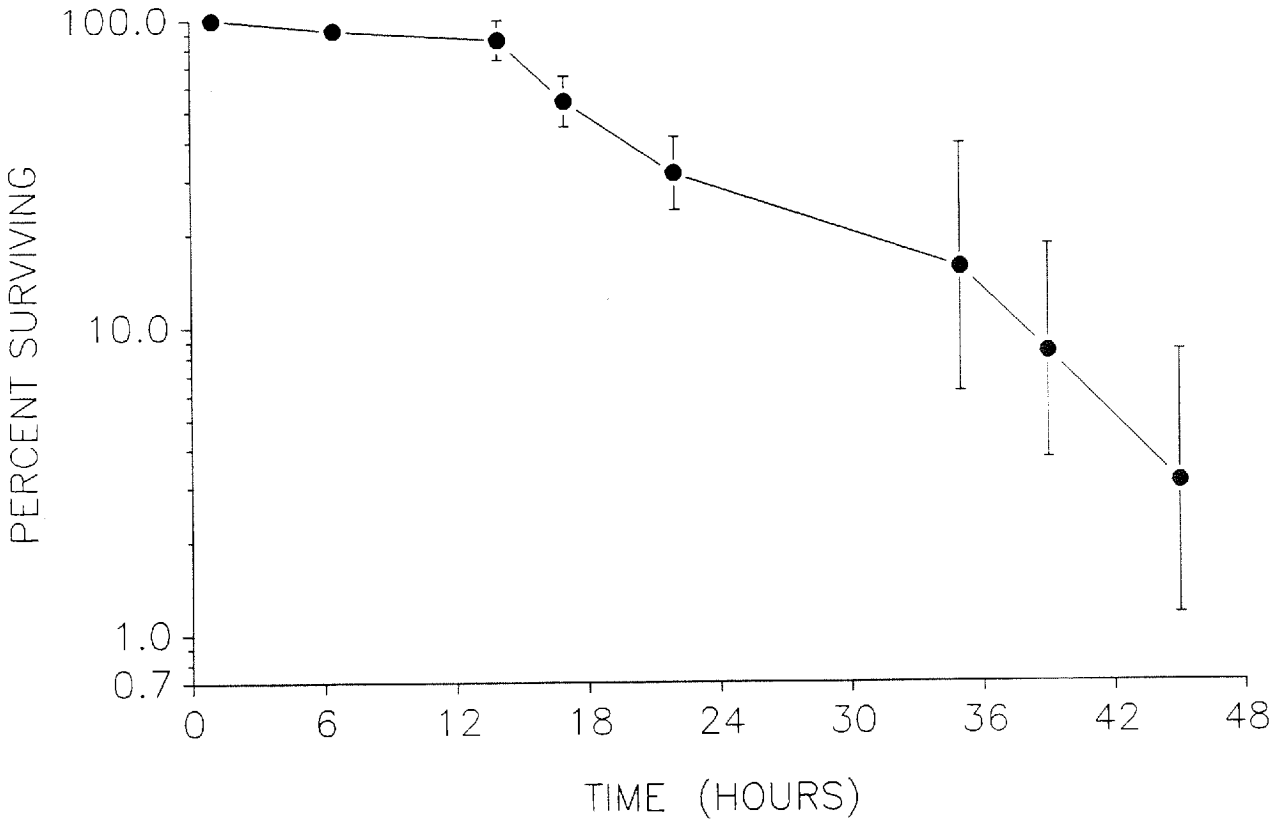
Tail damage occurred on tadpoles in all replicates except in single replicates for *O.caledonicum* and *H.brevistylus*, both of which survived. No deaths of tadpoles were recorded. Tail damage occurred as rips in the peripheral fin tissue and in one replicate, about 25 percent of the tail had been removed. No dragonfly species consumed eggs.

Cherax quadricarinatus readily consumed stage 25 tadpoles over the 48 hour period at an average rate of 0.483 per hour. All individuals used in the experiment were kept up to 4 days after the experiment. No mortality occurred. Survival of the tadpoles over the experimental period is shown in Figure 5.6.

Predation by Vertebrates

The mean total length and recorded diet for all fish species used in experiments is shown in Table 5.3. No species successfully consumed eggs or larvae. In all but three cases, at the end of the 24 hour period, all tadpoles were alive and all eggs remained intact. Two *L.unicolor* were recorded as unhealthy at 2 hours, the first lying on its side, the second swimming upside down and erratically. Both were dead at 12 hours. One *A.percoides* was observed lying on its side at 2 hours and was dead at 14 hours. In each case, a tadpole was recorded as dead or injured. Each fish had attempted to ingest a tadpole and had rejected it. No tadpoles in the remaining treatments were found dead or injured. All fish species except

Figure 5.6. Percentage of stage 25 tadpoles surviving predation by *Cherax quadricarinatus* in experimental aquaria over 48 hours. Error bars represent 95% confidence intervals of the mean.


100.0
10.0
1.0
0.7
PERCENT SURVIVING
0
6
12
18
24
30
36
42
48
TIME (HOURS)

M.splendida australis readily consumed *Litoria inermis* larvae or small fish fry (*Melanotaenia* sp.) while being kept prior to the introduction of *Bufo marinus* eggs and larvae. *M.splendida australis* fed on fish flakes, ground-up house flies and adult mosquitoes.

5.3.2. Inter-Cohort Competition and Predation.

Survival to Metamorphosis

The percent surviving in each cohort for each treatment is presented in Figure 5.7. Analysis of survival and a comparison of cohort means is presented in Table 5.4. Survival differed significantly between the cohorts. Mean survival was highest and similar for the control cohorts; 120A, 120B and 120BE. Larvae in cohorts 120A(BE), 120A(B) and 120B(A) survived at lower but not significantly different rates. Survival decreased significantly for larvae of cohort 240B and the cohort 120BE(A), which was heavily preyed upon by 120A(BE) tadpoles before eggs hatched. Thus the late cohort only suffered a significant decrease in survival when it was encountered by older conspecifics during the embryonic stages. Survival of cohort 240B, similar aged individuals at a higher density, was not significantly different from any of the competing cohorts (except 120BE(A)) or two of the three control cohorts except that larval period were almost doubled. Despite these contrast results, clear trends in survival are obvious (Figure 5.7). Survival is clearly affected

Figure 5.7. Mean percent of larvae surviving to metamorphosis and mean larval period for each cohort in the experiment. Error bars represent 95% confidence intervals for the mean.

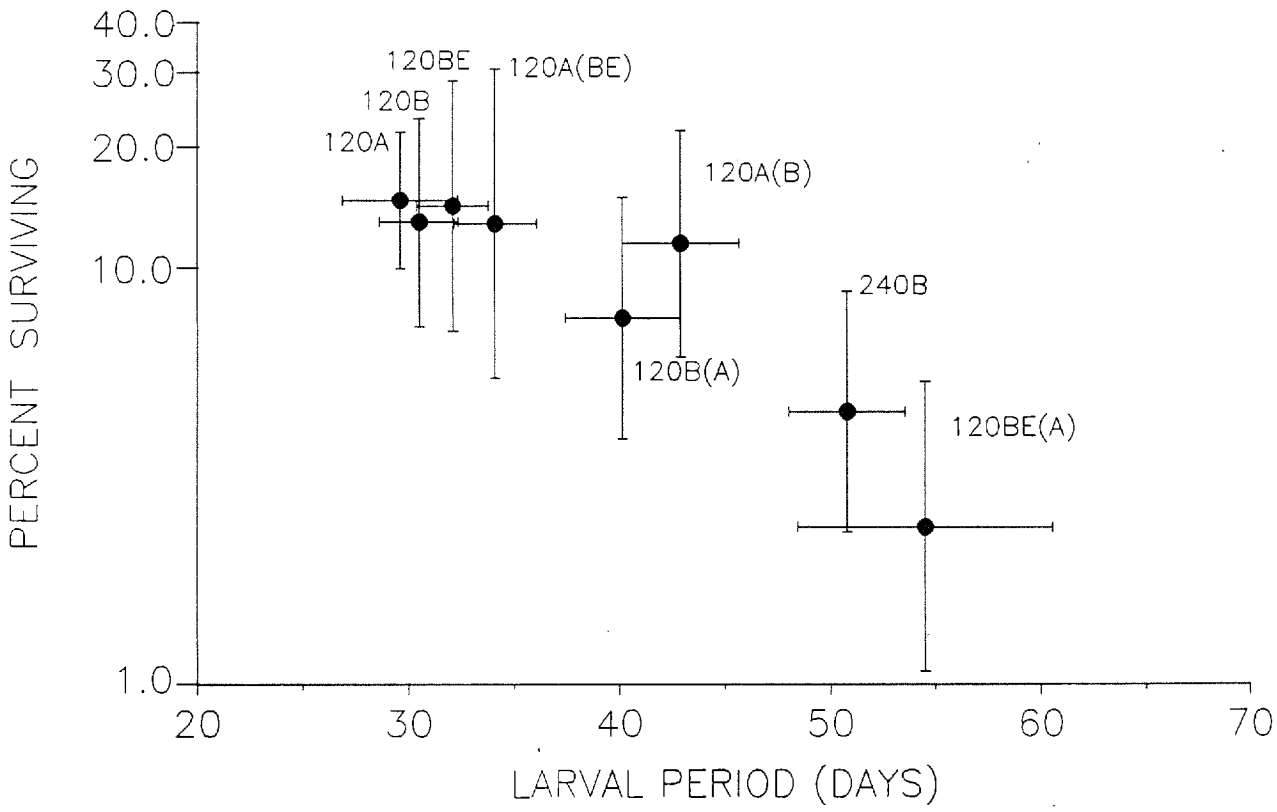

40.0
30.0
20.0
10.0
1.0
PERCENT SURVIVING
120BE
120A(BE)
120B
120A
120A(B)
240B
120B(A)
120BE(A)
20
30
40
50
60
70
LARVAL PERIOD (DAYS)

Table 5.4 Final responses of mass, larval period and survival for two cohorts of larvae reared in experimental ponds. Cohort survival means for the ANOVA are compared using Tukey's HSD test. Means with the same underline are not significantly different at the 0.05 level of significance. Contrast analyses for the MANOVA compare the responses for pairs or groups of cohorts as specified.

TREATMENT	Mass at Metamorphosis (mg)			Larval Period (days)			Survival %		
Cohort	Mean	SE	N	Mean	SE	N	Mean	SE	N
120A	128.3	2.4	69	32.1	0.9	72	14.71	1.2	4
120B	125.4	2.3	60	30.5	0.9	62	13.05	1.2	4
120BE	123.9	2.3	65	29.6	1.4	66	14.25	1.1	4
120A(B)	120.5	2.5	52	40.1	1.4	55	11.58	1.2	4
120B(A)	100.1	1.8	33	42.9	1.4	35	7.66	1.2	4
120A(BE)	130.3	2.9	61	34.1	1.0	64	12.93	1.3	4
120BE(A)	152.6	4.3	6	54.5	2.3	6	2.40	1.2	4
240B	95.4	1.8	42	50.8	1.4	43	4.55	1.2	4

ANOVA of (arcsine-squareroot transformed) percent survival

Source of Variation	df	SS	MS	F	p
All Cohorts	7	990.6080	141.5154	7.71	0.0001
Residual	24	440.4642	18.3527		

Tukey's HSD Test for comparison of means

120A 120BE 120A(BE) 120B 120A(B) 120B(A) 240B 120BE(A)

MANOVA of mass at metamorphosis and larval period

Source of Variation	Wilks' λ	df	F	p
All Cohorts	0.4487	14,788	27.74	0.0001
Contrasts				
120A-120B-120BE	0.9885	2,394	2.82	0.1030
120A-120A(BE)	0.9942	2,394	1.15	0.3169
120A(B)-120B(A)	0.9356	2,394	13.56	0.0001
240(B)-120B(A)	0.9599	2,394	8.24	0.0003
120A(BE)-120A(B)	0.9232	2,394	16.38	0.0001

when cannibalism occurs, though no advantage is accrued by the older cohort. Competiton between individuals of similar ages results in longer larval periods and decreased survival when compared with tadpoles of disparate ages.

Mass at Metamorphosis and Length of Larval Period

The mean mass of metamorphosing individuals from cohorts in each treatment is presented with the mean larval period in Figure 5.8. MANOVAs comparing all cohort responses and secondary analyses contrasting pairs or groups of cohorts are presented in Table 5.4. The responses of the cohorts were significantly different. Contrasts showed that all of the control cohorts (treatments 1 to 3; 120A, 120B, and 120BE respectively) were similar.

For competing cohorts there were two types of response. Larval periods always increased and mean mass was similar or less than the controls for each cohort for all of the treatments except for 120BE(A), in which mass was greatly increased. For the competing cohorts in treatment 4 - 120A(B) and 120B(A), mass decreased and larval period increased for both cohorts but more significantly so for 120B(A), the late cohort. For treatment 5, 120A(BE) and 120BE(A), mean size and larval period were also affected for both cohorts though in a manner different from treatment 4. Larvae of the early cohort, 120A(BE), attained a slightly larger size over a longer larval period when compared to the controls, but larval period was shorter than for

when cannibalism occurs, though no advantage is accrued by the older cohort. Competiton between individuals of similar ages results in longer larval periods and decreased survival when compared with tadpoles of disparate ages.

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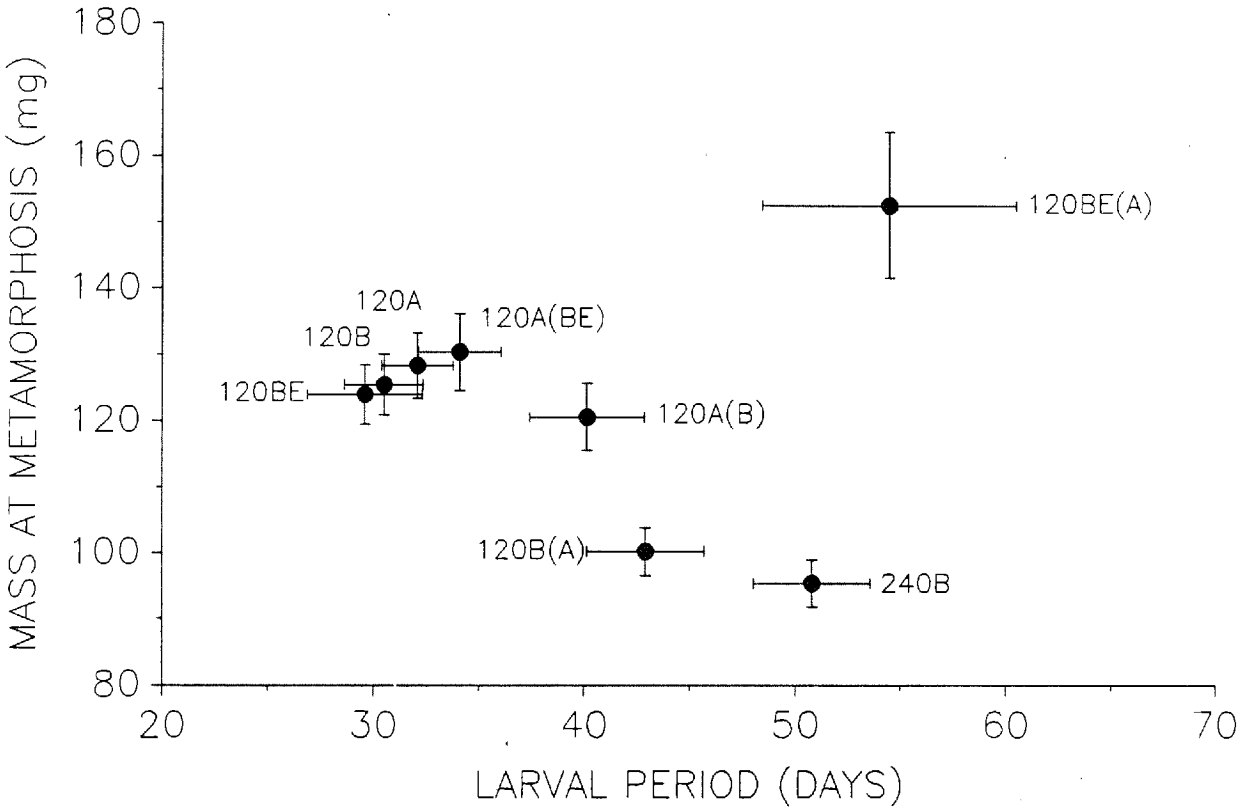
Figure 5.8. Mean mass at metamorphosis and mean larval period for each cohort in the experiment. Error bars represent 95% confidence intervals for the mean.


MASS AT METAMORPHOSIS (mg)
180
160
140
120
100
80
20
30
40
50
60
70
LARVAL PERIOD (DAYS)
120BE(A)
120A
120A(BE)
120B
120BE
120A(B)
120B(A)
240B

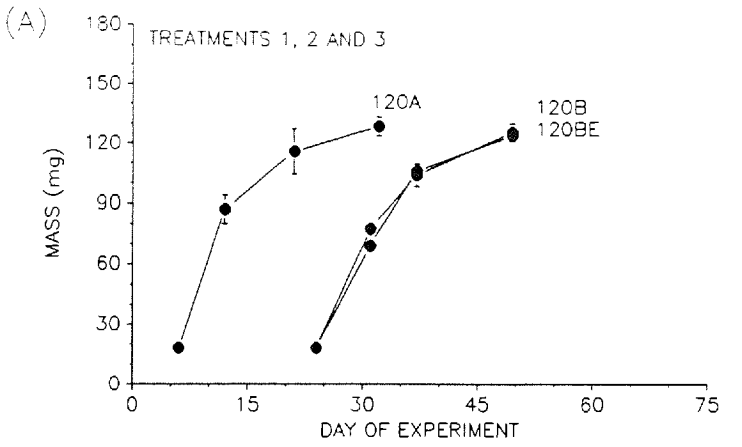
cohort A raised with cohort B tadpoles, ie 120A(B), in treatment 4. Larvae from 120BE(A) attained the highest mean mass as metamorphs with the longest mean larval period.

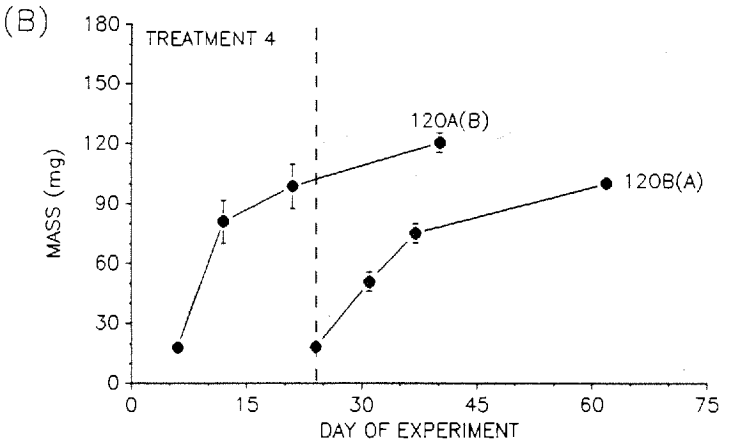
Larvae in treatment 6, (240B), showed the inverse response to 120BE(A) with respect to mass, metamorphosing at the smallest size with the longest mean larval period. Thus, increases in initial density appear to have a variable effect. Both the late cohorts in the competition treatments, 120B(A) in treatment 4 and 120BE(A) in treatment 5, were initially at twice the density of the controls, similar to 240B. For cohorts 120B(A) and 240(B), the result was a decrease in mass. However, for cohort 120BE(A), where cannibalism decreased numbers before any density effects could occur, the result was a considerable increase in mass at metamorphosis.

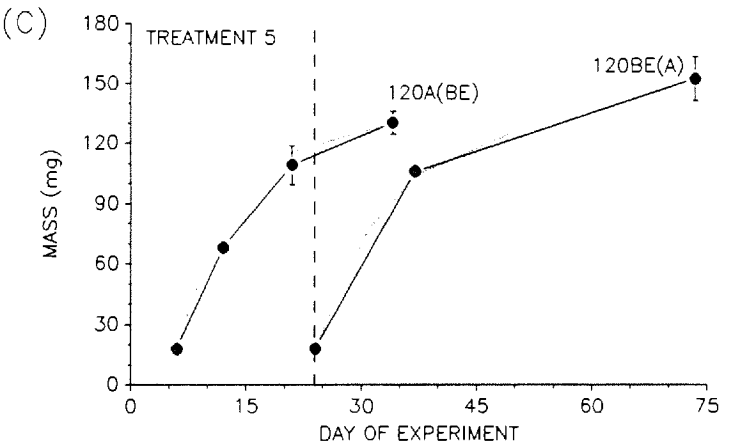
Growth

Growth to metamorphosis for each cohort in each treatment is shown in Figure 5.9. MANOVAs comparing growth for each cohort to day 19 of the larval period (up to the time of appearance of metamorphs) are presented in Table 5.5. Analysis of all cohorts show that patterns of growth during this period were significantly different. Growth curves for each on the control treatments (120A, 120B, and 120BE) are shown in Figure 5.9A. The growth patterns of these cohorts did not differ

Figure 5.9 (A) to (D). Growth (mean body mass) for each cohort in each treatment relative to the absolute time scale of the experiment, so that day 7 corresponds to the introductions of cohort A and day 24 for cohort B. The responses for the control cohorts (treatments 1 to 3) in 5.9A are shown as dotted lines for comparison to the treatment responses in Figures 5.9B to 5.9D


(A)
TREATMENTS 1, 2 AND 3
120A
120B
120BE
MASS (mg)
DAY OF EXPERIMENT
0
30
60
90
120
150
180
0
15
30
45
60
75


(B)
TREATMENT 4
120A(B)
120B(A)
MASS (mg)
DAY OF EXPERIMENT
0
30
60
90
120
150
180
0
15
30
45
60
75


(C)
TREATMENT 5
120A(BE)
120BE(A)
MASS (mg)
DAY OF EXPERIMENT
0
30
60
90
120
150
180
0
15
30
45
60
75

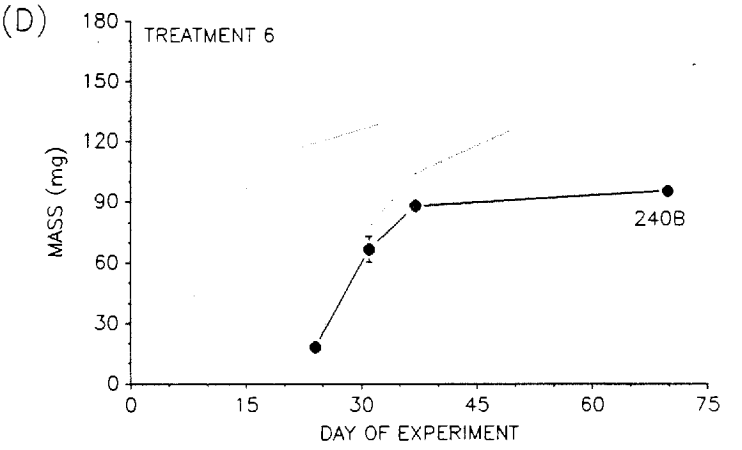

(D)
TREATMENT 6
240B
MASS (mg)
DAY OF EXPERIMENT
0
30
60
90
120
150
180
0
15
30
45
60
75

Table 5.5 MANOVAs of mean tadpole mass over time (days 6, 12 and 19) comparing growth prior to metamorphosis. Contrast analyses compare pairs or groups of cohorts as specified. The treatment number to which the contrast analyses refer (as referred to in the text) is shown to the left of the contrast group.

MANOVA of tadpole mass on days 6, 12 and 19

Source of Variation	Wilks' λ	df	F	P
All Cohorts	0.2463	14,44	3.19	0.0016
Treatment/Contrasts				
1-3. 120A-120B-120BE	0.8669	2,22	1.69	0.2078
4. 120A(B)-120A	0.7739	2,22	3.21	0.0596
120B(A)-120B	0.6659	2,22	5.77	0.0097
120B(A)-120A(B)	0.6939	2,22	4.85	0.0180
5. 120A(BE)-120A(B)	0.7156	2,22	4.37	0.0252
120A(BE)-120A-120B-120BE	0.9039	2,22	1.17	0.3291
120BE(A)-120BE	0.9741	2,22	0.29	0.7494
120BE(A)-120B(A)	0.6401	2,22	6.18	0.0074
6. 240B-120(B)	0.8627	2,22	1.75	0.1969
240B-120B(A)	0.8875	2,22	1.39	0.2692

significantly. For comparison, these curves appear as dotted lines in Figures 5.9B to 5.9D.

In treatment 4 (Figure 5.9B), the effect of competition decreased the growth of larvae in the late cohort relative to the control. Analysis for growth of 120A(B) did not include a period where it was competing with 120B(A) so no difference in growth was expected. For 120A(B), the 95 percent confidence limits of the means overlap with the control's (120A) until the introduction of 120B(A) on day 21, after which growth rate and final size decreased significantly (see Table 5.4). The mean size of tadpoles in 120B(A) was significantly smaller than for the control, 120B, at all intervals. The overall effect, however, was that growth was affected more for the late cohort, 120B(A), being significantly decreased relative to 120A(B) (Table 5.5).

For treatment 5 (Figure 5.9C), the growth of cohort 120A(BE) was not different to that of the control, even when final mass and larval period was considered (see Table 5.4). Thus, it was not affected at all by competition with cohort 120BE(A). In contrast to cohort 120B(A), the growth of larvae from cohort 120BE(A), after 19 days of their larval period, was similar to growth of the control, 120BE. However, after this time, growth was modified so that tadpoles remained for longer larval periods and metamorphosed at larger masses.

For larvae of cohort 240B (Treatment 6, Figure 5.9D), growth after 19 days was not affected relative to the control, 120B. It was, however, significantly modified after

this time so that larvae were smaller in mass and took considerably longer to metamorphose. Notably, growth was not significantly different from that of cohort 120B(A) competing with older conspecifics in cohort 120A(B). This suggests that the effect of competition on growth was independent of the age of the competitors except where predation by the older cohort occurred in the early stages of the larval period of the second cohort.

In summary, where competition occurred the cohort most affected in terms of growth, final mass and length of larval period, was always the late cohort. The effect of predation on these responses and also survival was clearly demonstrated in treatment 5. However, there was no apparent advantage in growth, final mass, or survival for the older cohort.

5.4 Discussion

Intraspecific predation clearly affects egg survival, both in isolated experimental systems and in controlled field situations. Mortality is increased from approximately 30 percent when older cohorts are absent (see section 4.3.1) to greater than 95 percent when older conspecifics are present. Eickwort (1973) proposed that where age-specific mortality is high, intraspecific predation would be selectively favoured. This high mortality is usually the case for the larval phase of *Bufo marinus* where survival to metamorphosis typically ranges from 0.1 to 10 percent in the absence of

predators (this study). The outcome of this behaviour has implications for both the cohorts involved in the relationship.

The purpose of the *Hemianax papuensis* experiment was to study the effects of predation on larval performance and any interactive effect that tadpole density may have on the outcomes. Larval *Bufo americanus* have been shown to metamorphose at smaller sizes in the presence of larval *Anax junius* (Skelly and Werner 1990, Wilbur and Fauth 1990). Members of the genus *Anax* have predatory behaviour very similar to *H.papuensis* (Rowe 1987). In addition to the growth response, Skelly and Werner demonstrated that *B.americanus* tadpoles responded behaviourally to *Anax*. Tadpoles reduced the amount of time spent foraging and moved away from the predator cage to the opposite side of the 2.4 litre container. They suggested that this behavioural modification led to reduced growth rates of the larvae. Wilbur and Fauth's (1990) data also suggest that the cue for accelerated metamorphosis in *B.americanus* at a smaller size was the presence of *Anax* rather than a perceived predation intensity alone. Observations made during this experiment did indicate that larvae may have been reducing their visibility in the presence of *Hemianax*. To avoid predation risk by seeking refuge areas (Wilbur and Fauth 1990), larvae may be foraging in sub-optimal resource patches that result in slower growth (Gilliam and Fraser 1988). The consequences include smaller sizes at metamorphosis, greater periods at risk to predation, or increased risk of mortality by not obtaining the minimum size necessary for metamorphosis before larval habitat deteriorates (Wilbur 1988). Similar responses by *Hyla gratiosa* tadpoles to *Anax junius* have been

reported; Caldwell et al. (1980) suggested that this behaviour is most likely due to the visual prey detection of *Anax*. Studies with larval salamanders have shown that visually orienting predators (*Lepomis macrochirus*) can be major factors influencing their diel vertical migrations (Stangel and Semlitsch 1987). The chemical cues given off by injured prey or by the predator itself may also be important in modifying behaviour. Increased dispersal in reaction to the simulated presence of predators has been demonstrated for *B.americanus* (Petranka 1989c). Larvae rapidly dispersed from food-rich sources when exposed to extracts prepared from conspecifics. Another species, *Hyla chrysocelis*, spent increased time in refuges and significantly less time foraging when exposed to water conditioned by the predator *Lepomis cyanellus* (Petranka et al. 1987).

In contrast to Skelly and Werner's (1990) study, performance increased for larvae in the lower density treatment relative to non-predator controls. Larvae were larger at metamorphosis and had significantly shorter larval periods. Other studies by Morin (1983a, 1986), Wilbur et al. (1983), Wilbur (1987), Van Buskirk (1988) and Fauth (1990) have also found similar responses by anuran larvae subject to predation. This effect is presumably due to surviving larvae growing faster than those at the higher density where increased competition for food was occurring (Wilbur 1980). Larvae in the lower densities may also have reached a size-limited refuge to predation from *Hemianax* faster than those at higher densities (Caldwell et al. 1980, Travis et al. 1985, Semlitsch and Gibbons 1988). Alternatively, all tadpoles remained susceptible to predation but at low density, encounter rates between predator and prey were

lowered. Due to predation, larval numbers were then at a level where food was far less limiting and growth to metamorphosis was enhanced. Deaths of some larval dragonflies in these ponds by the time metamorphosis had begun seemed to indicate that starvation may have been occurring. At the higher density, growth in predator treatments was affected early in the larval period. Thus the change in behaviour to lower the risk of predation (sensu Skelley and Werner 1990) coupled with the increased demand for resources was such that mass at metamorphosis was smaller and larval periods were longer. Thus it appears that *B.marinus* has the ability to assess both mortality risk and growth benefits in timing their metamorphosis as reported for other *Bufo* species (Werner 1986, Wilbur and Fauth 1990).

The results of feeding experiments with invertebrate and fish species common at one or both the study sites indicate that relatively few predators are capable of consuming *Bufo marinus* eggs and larvae. Only three of the twelve species examined, *Hemianax papuensis* (as discussed above), *Homoedytes scutellaris*, and *Cherax quadricarinatus* consumed larvae with any measurable degree of impact. The toxicity and unpalatability of all stages of the life history for *Bufo* species have been noted in many studies (eg, Voris and Bacon 1966, Licht 1967, 1968, Wassersug 1971, 1973, Brodie et al. 1987). Previous studies of predation by dytiscid species have found they can strongly influence freshwater community structure (Holomuzki and Collins 1987), and the mortality (Herreid and Kinney 1966, Brodie and Formanowicz 1983, Crump 1984, Formanowicz 1986) and spatial distribution of amphibian larvae (Holomuzki 1986, Roth and Jackson 1987, Formanowicz and

Bobka 1989). All stages of *B.americanus* larvae except premetamorphic individuals are palatable to larvae of *Dytiscus verticalis* (Formanowicz and Brodie 1982). Larvae of *Cybister tripunctatus* in this study demonstrated no propensity to *Bufo* predation and may be harmed when attempting it. Adult *Homeodytes scutellaris* present a greater threat to larval populations. Numbers of these beetles in temporary and permanent ponds in the Townsville region can reach densities of up to $15/m^2$ in sweep net samples of margins (unpublished data).

Predation rates estimated for *Cherax quadricarinatus* indicate its importance as a predator of *Bufo* tadpoles, though eggs were not offered. It has the highest consumption rate of the non-*Bufo* predators examined. In association with the dytiscid species investigated, *Cherax* have the potential to contribute most to the mortality of swimming tadpoles. Habitat use by *Cherax* species include streams and ponds that dry out during part of the year (Riek 1969). It has an adaptation for this event that would impact heavily on colonising *Bufo* populations where habitat use overlaps. Rivers and streams that dry intermittently usually do so as a series of ponds after flow ceases and water levels diminish. The time between drying and refilling can be longer but some are either dry only for a few weeks or retain a small quantity of water. If all standing water evaporates, it is common to find *Cherax quadricarinatus* (up to 50mm CL) burrowed in the moist or saturated substrate beneath rocks, boulders and log debris (personal observations). Some species survive considerable dry periods by excavating a deep burrow that ends in a large water-containing chamber (Reik 1969). When ponds partially fill in the warmer,

early wet period before flow recommences, as often happens, they are used by *Bufo* for breeding (personal observations). The presence of *Cherax* in these instances where ponds are refilling or as they diminish, could act as an important population control on larval populations, especially in periods prior to recolonisation by aquatic insect predators. However, both the Calvert River and Mt Margaret study sites have sections that do not fully dry out. These would act as an important source of recruitment for *Cherax* recolonisation after refilling occurs, although to my knowledge, members of this genus have not yet colonised the Mt Margaret site or the creeks that flow into it. Few studies of *Cherax* or other crayfish species as predators of anuran larvae exist in the literature. Predation by *Cambarus bartonii* on *Hyla chrysocelis* reduced the mean size and survival of tadpole cohorts over time (Fauth 1990). However, the size effect was due to depressed growth rates rather than size-specific foraging by *Cambarus*. This suggests that tadpoles may have been acting in a fashion similar to those in the *Hemianax* experiment by reducing foraging activity to avoid predation. Studies of predation by *Oronectes* and other crayfish species usually refer to competition and impact on fish and periphyton-grazing snails (eg Crowl and Snell 1990, Weber and Lodge 1990). Incidental observations have confirmed that *Cherax* are predators of larval and even adult toads (Hutchings 1979) though no size or species details were given.

None of the six fish species examined in feeding experiments successfully consumed *Bufo* eggs or larvae. Indeed, only three of the twenty individuals examined even attempted consumption. All three died within twelve hours. Similar experiments

with similar results have been obtained for *Ambassis* sp, *Hypseleotris* sp, and *Melanotaenia* sp (RA Alford pers comm) and for the Purple-spotted Gudgeon, *Mogurnda mogurnda*, and Firetail Gudgeon, *Hypseleotris galii* (Pearse 1980). Assuming the toxins in the larvae are the same as those analysed from adults (Chen and Kavarikova 1967, Ingram 1988), it would appear that most vertebrate species that swallow prey whole (as most fish do) will be susceptible to the cardio-toxic substances present.

Of interest however, is the small proportion of the predators examined that actually attempted predation and died as a direct result. This would suggest that any detrimental effects to the predator assemblege may be small for the habitats sampled. However, given the extent of the predator community still to be examined, much research is required before this premise can be asserted with any degree of confidence. As shown in other studies, larval *B.marinus* are preferred over other tadpoles by predatory anuran larvae and dragonfly larvae in some tropical habitats (Heyer et al. 1975). In addition, their palatability can change with developmental stage (Brodie et al. 1987), becoming more palatable at premetamorphic stages.

Results from the experiment investigating inter-cohort interactions suggest that cohorts of tadpoles can have a variable effect on the growth and survival of younger cohorts. Intercohort competition affected older and younger cohorts differently. Older tadpoles operated as size-limited predators when the younger cohort appeared as eggs. Predation ceased when hatchlings grew, were able to swim and avoided

contact with older conspecifics. Survival of the younger cohort was low so that growth of the older tadpoles was not affected by competition. Older tadpoles did not appear to gain enough nutritional benefit by consuming eggs to result in increased body size (Crump 1990) though larger numbers of eggs representing an entire egg mass (10,000 to 40,000) may have produced this effect. However, growth of the younger cohort was profoundly modified. Predation removed competitors permitting the subsequent rapid growth of survivors (Wilbur 1987). This situation is most likely in anuran systems dominated by scramble competition (Nicholson 1954, Wilbur 1980) and has been clearly demonstrated in *Bufo* species (Alford and Harris 1988). Growth rate *per se* of the younger cohort did not appear to increase after individuals of the older cohort metamorphosed. However, larvae in these treatments appeared to be maximising fitness in the context of the ecological model proposed by Wilbur and Collins (1973), by increasing larval period to attain a maximum body size before metamorphosis. They increased the time spent growing in a habitat where food was most likely not limiting, as few larvae had survived after initial egg predation and the risk from predation was negligible after larvae were capable of swimming. Also, there was probably no perception of threat from habitat desiccation (Werner 1986, 1988, Wilbur 1987) or diminishing water levels (Crump 1989, Newman 1988a, 1989). However, caution in interpretation must be applied. Alford and Harris (1988) pointed out that complex difficulties in interpretation do arise when experiments are not set up to test specifically the assumptions of proposed models. Further recent advances in the evaluation and proposal of complex models

that predict size and timing of metamorphosis (Rowe and Ludwig 1991) have made this even more pertinent.

When the younger cohort appeared as mobile hatchlings, predation did not occur. However, growth rate, larval period and size at metamorphosis were affected for both cohorts, with the younger cohort being most affected. Thus, larvae in this treatment are presumably initiating metamorphosis at a smaller size due to the increased level of competition for resources exploited by more survivors (Smith 1983). In contrast to the treatments where egg predation occurred, the higher survival of the younger cohort and the overlap in generations between the two cohorts was enough to reduce the growth of the older cohort. The growth rate of the older cohort was reduced only with the introduction of the younger cohort. Growth rates of the second cohort was depressed relative to controls at all stages of the larval period (see Figure 5.9B). Again, the results here support the proposals of the Wilbur-Collins model.

Parentage of larval cohorts has been shown to affect vulnerability to predation (Alford 1986a), and the growth and competitive ability of anuran larvae (Alford 1989). Genotypic variation in survival and growth (mass and larval period) was not manifested in the control treatments for the two cohorts used in this experiment.

The benefits of cannibalism in anurans have been shown to include increased body size at metamorphosis, though little effect on the duration of the larval period was

evident (Crump 1990). Increased body size at metamorphosis may be expected to improve the fitness of individuals. It allows access to a greater diversity of prey items, reduces the number of potential predators and may accelerate growth to reproductive maturity (Wilbur 1980, Werner 1986, John-Alder and Morin 1990) or lead to increased size at reproductive maturity (Smith 1987). However, as stated earlier, this effect was not apparent in the results. Alternatively, cannibalism and other forms of predation (incidental ingestion or mutilation) on the earlier stages may lead to a reduction in population number resulting in competitive release (Polis 1988). This would lead to more rapid growth, enabling escape from desiccating habitats or predation risks, or to larger sizes at metamorphosis (see section 4.3.3), and increased fitness (Pough and Kamel 1984, Taigen and Pough 1985). The results of this study indicate that this is occurring in cohorts of *Bufo marinus* tadpoles. This reduction of density dependent stresses on larvae has also been looked at by Semlitsch and Caldwell (1982), and Travis (1984). Both studies simulated the departure of a cohort of competitors from another using different methods. Semlitsch and Caldwell demonstrated that *Scaphiopus holbrooki* tadpoles were able to take advantage of release from high density stresses and capitalise on favourable growth conditions. Alternatively, Travis showed that the effect of a 'competitive release', rather than increasing the mean size of *Hyla gratiosa* larvae, increased the variation in size at metamorphosis, producing more larger individuals.

In addition to the potential for decimation of a cohort through opportunistic oophagy, there are other selective disadvantages in late breeding by females. These include

reduced growth rates and survival of offspring in habitats containing increased numbers of intraspecific competitors (Woodward 1987), interspecific competitors and predators, and reduced standing crops of periphyton (Wilbur and Alford 1985, Morin et al. 1990).

In systems where interactions exist between age-classes, one cohort of larvae suppressing younger cohorts commonly results in oscillation of adult population numbers (Hastings and Costantino 1991). To understand the impact of variable recruitment on population size and how this changes due to variation in these interactions more detailed study and modelling of discrete populations is required.

In summary, survival during the early larval phase is greatly reduced by conspecific predation. Predation ceases when larvae become mobile and are able to avoid capture. Successful predation by members of the native fauna was demonstrated for only three of the twelve species examined. This suggests that the presence of older cohorts may be a more significant influence on the regulation of tadpole populations. However, native species may be important regulators of *Bufo* colonisation during periods of high abundance or in the absence of older conspecifics. This can depend heavily on predator population dynamics and potential predator density as well as individual predator functional response to *Bufo* larvae. Complex interactions exist between predators (invertebrate species and older conspecifics), their phenology and the density of *Bufo* cohorts. Outcomes of these interactions are similar to those predicted by the model of Wilbur and Collins (1973). Competition between cohorts

leads to increased larval periods and smaller sizes at metamorphosis. Interspecific and intraspecific predation increases mortality but results in 'competitive release' at lower initial densities of tadpoles, leading to greater sizes at metamorphosis at the expense of longer larval periods.

Chapter 6. General Discussion.

6.1 Review of Findings

6.1.1 Reproductive Ecology

Patterns of reproduction demonstrated in this study were similar for populations at Calvert Hills and Mt Margaret. Population sizes of adults estimated from mark-recapture studies were different for the two sites, though this could be a function of factors not considered in this study such as differences in both the amount and type of habitat or shelter available at each site. Other influences, such as predation by native water rats (*Hydromys chrysogaster*) may have been important in regulating observed numbers at Mt Margaret. However, intrinsic features of the populations such as variation in size relative to season, operational sex ratios, proportions of active individuals, the size-specific fecundity of females were similar. Egg size, however, was not. Females at Mt Margaret, with larger egg sizes, were investing a larger amount of energy in ovary production such that despite the disparity in egg size, size specific fecundity was similar for both populations. For either sex, only a small percentage of the total population were active in transects. The highest proportions of the male population that were active occurred during the reproductive seasons, generally December to June. During these periods, this proportion was 10 to 30 percent of the estimated male population. Males made up 90 percent of all individuals active in the transects during the wet and early dry seasons. In most cases, less than 20 percent of these were involved in calling behaviour. Others were peripheral, non-calling males (Krupa 1989, Sullivan 1989b). The active proportion

of the female population increased in the late dry season periods, representing approximately 50 percent of individuals in the transects. This proportion represents approximately 15 percent of total female numbers, compared with less than 5 percent being active in wet and early dry season periods. Similarly, the number of females involved in amplexus is low when compared to the total number active. Despite the considerable effort applied to monitoring breeding aggregations, only a small percentage of active individuals were observed in amplexus. Despite this, levels of egg recruitment averaged between 3000 and 6000 per day over all breeding periods. Only longer-term studies, supplemented with data on juvenile and adult survival (ie. the derivation of life-tables and estimates for R_o) will determine whether the rates of recruitment seen are sufficient to sustain or increase population numbers or whether populations rely on juvenile recruitment through immigration from adjacent habitats (Pulliam 1988).

Bufo marinus exhibit a range of behaviours that are characteristic of both prolonged and explosive breeders. Marked spatial clumping at oviposition sites, variable chorus participation, and alternative mate-seeking behaviours (chorusing and silent-searching males) are characteristic of explosive breeding populations. However, the continual presence of a percentage of females with mature ova and very high percentages of males with mature spermatozoa in the population indicate that breeding is extended over a protracted period when reproduction is concentrated but opportunistic. The alternative strategy of non-calling mate-searching behaviour in

smaller males may be a way of increasing their relative reproductive success in systems where large males have an advantage.

The importance of these studies however is that observed recruitment at both sites is sporadic and is spread over a prolonged breeding period (up to seven months). It is unclear what factors control patterns of recruitment as very little association was evident between frequency of amplexus and the environmental cues of temperature, rainfall or ambient illumination. Amplexus in the Mt Margaret populations was frequently correlated to male density. However, extremely low observed numbers of females in this population, and the absence of this trend in populations at Calvert Hills where females were in higher numbers, necessitate caution in accepting this interpretation.

A more comprehensive analysis of size-specific fecundity has shown that previous studies have underestimated egg/body-size relationships for smaller females and overestimated for large females. Egg size was larger for Mt Margaret females, conferring a possible fitness advantage to the survival and growth of hatchlings. However, larval studies did not indicate this was occurring.

6.1.2 Larval Dynamics and Predation

Estimates for tadpole population size within transects were highly variable and showed no distinct trends. High densities of larvae occur in all seasons but patterns

appear to be inconsistent with observed recruitment. *Bufo* larvae aggregate (Wassersug 1973), so that densities of larvae sampled at either site were extremely variable on both spatial and temporal scales. The difficulties encountered in modelling growth and survival from field sample data was due to this distribution characteristic. This made sampling using established techniques inappropriate, even when modified to account for the mobility of tadpole aggregations. Aggregation behaviour involving mixed cohorts and any tendency for size selective sorting, as described in other *Bufo* species (Breden et al. 1982), would further confuse efforts to determine growth of particular cohorts in the field.

A more refined approach to sampling larval populations appears to be required. A method that allows separate calculation of mean densities for tadpole aggregations and peripheral individuals may be a more accurate procedure for estimating population size. The behaviour of tadpoles can greatly influence their benthic distributions and densities (Beiswenger 1975, Caldwell 1989). Therefore, the method should also involve sampling restricted to when tadpoles are exhibiting a commonly occurring, specific behaviour such as a feeding aggregation. Furthermore, improved estimates of growth and survival data for field cohorts may be obtained through the use of large-scale field enclosures (Scott 1990) and known starting densities of eggs.

Measures of organism-weighted density, Do (Lewontin and Levins 1989), are better indicators of the effective density in a species that has an aggregated distribution.

Therefore, this measure of density was deemed appropriate for determining upper limits for experimental studies.

Experimental results indicated that egg survival and hatchling growth did not vary with either site or season, but were profoundly affected by predation from older conspecifics. Oophagous tadpoles were the largest source of mortality in pre-hatching larvae as none of the other predators examined consumed eggs. Pre-hatching survival was reduced from approximately 70 percent to less than 5 percent when exposed to cannibalistic conspecifics. This can, however confer an advantage on the surviving larvae. Predation in experiments ceased when hatchlings became mobile and commenced swimming. The older cohort was close to metamorphosis and overlap of generations was short. The remaining young larvae were at a low density which allowed for rapid growth rates and resulted in larger metamorphs (Figure 5.9C). Where predation did not occur, competition between the cohorts decreased growth in both cohorts, and competition within the younger cohort reduced reduced its growth.

Embryos grew linearly to hatchling stages then assumed either exponential or decreasing exponential growth patterns. 'Best' growth for tadpoles, indicated by shortest larval periods and largest metamorph size was shown by larvae growing exponentially. Factors influencing the type of larval growth observed in experimental enclosures were most likely abiotic controls on physiology, such as temperature, and indirect effects such as the quality of periphyton resources growing

inside enclosures. This growth pattern was demonstrated separately by larvae reared at the highest daily maximum temperatures in enclosures and by larvae at lowest initial density in experimental ponds. These influences represent conditions under which larval fitness is maximised.

Body mass or snout-vent length at metamorphosis, larval period duration, and survival rates of larvae all depended on density. Increased density negatively affected the growth rates of larvae resulting in either exponential or declining exponential growth patterns. Asymptotic size of larvae was also negatively affected in those larvae with decreasing exponential growth.

Predation on toad larvae was limited to a small number of species. This component of the study was intended as a baseline for future work. Few of the invertebrate predators examined would consume eggs or tadpoles. Predation pressure from the fish community appears to be negligible. Invertebrate predators capable of significant impact on tadpole numbers were a crayfish species, a larval dragonfly species, and the adult of a species of dytiscid. These species have the potential to greatly influence tadpole population sizes. As burrowers, crayfish are well adapted for persisting in seasonally wet-dry habitats that are colonised by *Bufo marinus* at the beginning of the wet. Thus, after refilling, they are present before other predatory species colonise. In the absence of effective fish predators, larval dragonflies and adult dytiscids are a significant source of mortality. In combination, all three species

represent the only documented predators of *Bufo marinus* tadpoles present in Australian freshwater communities.

Predation also affected tadpole growth in subtle and indirect ways. The presence of large, effective predators, rather than perceived reductions in density alone can alter activity, habitat use, and growth patterns (Wilbur and Fauth 1990, Werner 1991). In the presence of the predator, *Hemianax*, *Bufo marinus* larvae appeared to reduce their visibility by remaining concealed under components of the substrate (leaves & sticks). Swimming speeds were noticeably quicker and distances travelled were shorter. Alteration of their behaviour in this fashion may have affected foraging efficiency. Initially, larvae suffered high mortality. Subsequently, the density of remaining larvae determined growth rate to metamorphosis. Density interacted with food availability to affect foraging efficiency to reduce growth for high initial densities and increase growth for low densities.

6.2 Conclusions

The results of this study indicate that factors controlling reproduction and the growth and survival of larvae at either site are similar. Allowing for seasonal variation, few differences between the populations were observed for any of these characters. Clearly, there is a great deal of scope for further research on the factors that affect larval populations. More detail is needed on factors controlling rates of recruitment

and factors causing different larval growth patterns. Similarly, studies of the impact on the native anuran and aquatic predatory fauna are required. However, this study provides enough information to begin predicting the dynamics of larval *Bufo* populations.

Studies that model the complete aquatic/terrestrial cycle of *Bufo marinus* are required for a clearer definition of population trends. In general, studies and modelling of size-classified populations that include iteroparity (more than one reproducing age/size class) suggest that increased plasticity in the growth of larval individuals will have a stabilising effect at the level of the population (Ebenman 1988a, 1988b). Given the high degree of determinism that exists in the majority of tadpole populations and assemblages (Alford 1991), studies need to search for mechanisms that control growth and survival. The mechanisms that operate for larval *Bufo marinus*, demonstrated in this study, are predation and intraspecific competition within and between age classes. The magnitude of the control these have on growth and survival is regulated primarily by density.

Bufo marinus is currently thought to be a major pest in Australian ecosystems (Freeland 1984). The effect of *Bufo* eggs and larvae on assemblages of aquatic vertebrate and invertebrate predators is still uncertain. Their impact on native anuran larvae is even less clear. It is clear that consumption of *Bufo* tadpoles is detrimental to sections of the invertebrate assemblage (eg libellulids) but is certainly beneficial to others (eg parastacid crayfish and some dytiscids). Fish generally ignore *Bufo* as

potential prey; of the twenty fish from five species examined, three (two *Leiopotherapon unicolor* and one *Amniataba percoides*) attacked *Bufo* and died.

This study demonstrated that growth and mortality rates of *Bufo* larvae are likely to be strongly density-dependent at densities often encountered in the field. Cannibalistic predation of *Bufo* eggs by older larvae is also a major source of mortality. These facts indicate that control measures directed at eggs and larvae are unlikely to be successful; any control measure that reduced numbers would be compensated for by decreases in density-dependent mortality. Moderate reductions in numbers of eggs or larvae might even increase the rate of survival to and through the early terrestrial stages. The cohort competition experiment (section 5.2.2) showed that significant predation by older larvae enhanced the growth of surviving individuals. Growth to metamorphosis was either increased or larvae attained a considerably larger size before metamorphosis. As a consequence of significant but incomplete removal, metamorphs would emerge sooner, or at a larger size. This would be likely to increase rates of survival to metamorphosis in habitats that dry up, and larger metamorphs would probably experience greater terrestrial survival rates. Mechanical removal or trapping would be difficult and inefficient given the wide range and isolation of habitats colonised by *Bufo*. As larvae tend to aggregate at very high densities, the introduction of viral or bacterial pathogens is a method that may remain a promising option for control.

It is clear that larval fitness is also influenced by adult reproductive phenology. At the beginning of breeding seasons, a large number of spawnings may occur over one or two nights (personal observations, WJ Freeland pers comm). This elevated input of eggs can result in high mortality and the increased density will reduce the fitness of the surviving metamorphs. If recruitment over the breeding period is sporadic, as demonstrated during this study, then survival rates, and consequently population size, will be greatest inthe early cohorts and possibly in very late cohorts (but see Wilbur and Alford 1985 about historical effects on late breeders). However, most breeding populations observed undergo an initial burst of reproduction followed by sporadic spawnings by lower numbers of females.

During recruitment of larvae, many predators (larval insects) are also colonising. If predation of larvae is size-limited, then the initial tadpole cohorts have the opportunity to outgrow predators, thus reducing predation pressure further. Subsequent larval cohorts are then exposed to significantly increased mortality, especially in the pre-hatchling stages when predation by older tadpoles may occur. After hatching, both survival and growth of the tadpole stages are affected by competition with older conspecifics for food resources and by predation from crustacean and insect species. This reduction in fitness will be less severe for survivors when their densities are low and generation overlap is minimal. However some cohorts enter late in the season while habitats persist. If these represent additional clutches deposited by females that have already reproduced earlier in the

season, then any surviving metamorphs increase fitness for those females (Morin et al. 1990).

Control efforts may be amplified by reducing the number of breeding adults at these sites during periods where breeding is sporadic, such as middle and late sections of the breeding period. During these times reproductive activity and numbers of successful spawnings are reduced; this lowers the level of competition between cohorts and reduces the chance of cannibalistic interactions. If survival of metamorphs is also density-dependent, then decreasing the number of reproducing pairs during the period where metamorph fitness would normally be enhanced may have the effect of increasing the proportion of individuals in the surviving metamorph population with reduced fitness. However, as mentioned above, reductions in density would be compensated for by an increased tadpole survival rate.

In view of the information presented in this study, sound predictions can now be made with respect to phenological modelling of the dynamics of larval *Bufo marinus* populations, and the likley influence of co-occurring, aquatic predators. However, more detailed information is required on the predation rates, phenology and density of these predators, and the extent of competitive interactions with native anuran larvae before fully mechanistic models incorporating all species interactions are achieved.

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Appendix 1. Jolly-Seber and Triple-catch Population Parameter Estimates

Table A1. Jolly-Seber and Triple-Catch population parameter estimates from mark-recapture models. Petersen Estimates are presented in Table 3.2.

Site: Calvert Hills - Males

			Jolly-Seber (* = Triple Catch)					
			Ni		Phi		bi	
	Date	Day	est	SE	est	SE	est	SE
	20/01/88	1			1.25	0.24		
	21/01/88	2	191.95	74.27	1.35	0.33	237.32	159.67
	22/01/88	3	497.14	162.62	0.65	0.15	266.16	197.96
	23/01/88	4	588.65	203.18	0.80	0.22	-222.91	163.30
	24/01/88	5	247.57	84.89	NON EST	NON EST	NON EST	NON EST
	25/01/88	6	NON EST	NON EST	82.50	NON EST	144.38	NON EST
	26/01/88	7	144.38	71.10	3.82	3.65	20.08	319.10
	27/01/88	8	572.25	571.41	0.66	0.83	166.20	513.31
	28/01/88	9	542.50	664.25	0.83	0.77	342.81	513.68
	30/01/88	10	795.48	409.84	0.82	0.35	-306.64	282.71
	31/01/88	11	345.93	123.43	1.52	0.91	131.38	228.11
	01/02/88	12	657.88	397.90	0.34	0.18	56.25	79.97
	09/02/88	13	278.03	66.31	0.95	0.37	-30.87	70.82
	10/02/88	14	232.89	100.44	0.85	0.49	153.11	228.23
	11/02/88	15	350.00	266.66	5.39	5.76	-13.17	1329.54
	12/02/88	16	1872.83	1947.46	0.40	0.44	-167.06	320.47
	13/02/88	17	583.56	342.31	2.00	2.22	532.26	819.33
	14/02/88	18	1698.00	1762.43	0.25	0.27	46.59	208.24
	15/02/88	19	469.63	266.06	0.73	0.50	-38.02	148.87
	16/02/88	20	306.86	190.42	0.66	0.47	-59.15	76.41
	17/02/88	21	143.33	77.96				
	18/02/88	22						
	06/05/88	1			0.63	0.10		
	07/05/88	2	262.29	121.56	1.08	0.20	-64.86	135.95
	08/05/88	3	219.64	58.23	0.90	0.19	2.60	50.60
	09/05/88	4	199.64	43.89	0.66	0.14	19.01	30.52
	10/05/88	5	150.59	32.80	1.19	0.32	52.17	48.91
	12/05/88	6	231.27	66.06	1.27	0.53	298.29	237.74
	13/05/88	7	592.86	298.13	0.46	0.18	-142.53	103.45
	14/05/88	8	129.41	34.51	1.41	0.52	184.29	110.58
	15/05/88	9	366.36	145.77	0.90	0.41	-10.64	112.08
	16/05/88	10	319.09	137.25	0.48	0.20	-33.47	43.26
	18/05/88	11	121.11	35.77	1.67	0.96	23.58	43.82
	19/05/88	12	225.27	123.32				
	20/05/88	13						
*	10/08/88	1	129.20	.	1.22	2.50	-0.90	.
	11/08/88	2	82.67	48.76	1.22	.	-0.90	.
	12/08/88	3	52.89	.	.	.	.	.
*	01/11/88	1	26.49	.	0.75	1.12	0.48	.
	02/11/88	2	38.57	17.13	0.75	.	0.48	.
	03/11/88	3	56.16	.	.	.	.	.

Site: Mt Margaret - Males

		Jolly-Seber (* = Triple Catch)					
		Ni		Phi		bi	
Date	Day	est	SE	est	SE	est	SE
05/03/89	1			1.29	0.49		
06/03/89	2	216.89	136.39	0.86	0.38	-20.69	117.30
07/03/89	3	166.25	81.94	1.29	1.25	-40.28	108.09
08/03/89	4	174.00	177.73	NON EST	NON EST	NON EST	NON EST
09/03/89	5	NON EST	NON EST	NON EST	NON EST	NON EST	NON EST
10/03/89	6	NON EST	NON EST	NON EST	NON EST	NON EST	NON EST
12/03/89	7	NON EST	NON EST	23.00	NON EST	34.50	NON EST
13/03/89	8	34.50	33.85	0.50	0.34	-5.25	12.10
14/03/89	9	12.00	NON EST	1.00	NON EST	NON EST	NON EST
15/03/89	10	12.00	NON EST	3.04	1.45	79.08	55.25
16/03/89	11	115.58	67.85	0.79	0.51	69.81	70.40
17/03/89	12	160.87	98.78	0.94	0.99	29.18	97.47
18/03/89	13	180.00	194.18	NON EST	NON EST	NON EST	NON EST
19/03/89	14	NON EST	NON EST				
21/03/89	15						
21/06/89	1			1.75	0.77		
22/06/89	2	70.00	NON EST	0.57	0.14	-26.50	NON EST
25/06/89	3	13.50	8.81	1.27	0.36	4.01	14.61
26/06/89	4	21.11	11.19	1.06	0.49	-1.64	12.75
27/06/89	5	20.67	11.21	1.03	0.49	7.85	10.51
28/06/89	6	29.14	10.71	1.00	0.60	14.86	25.06
30/06/89	7	44.00	33.16	0.94	0.71	29.14	66.70
01/07/89	8	70.50	72.91	0.84	0.53	0.18	54.22
02/07/89	9	59.14	28.09	1.18	1.16	5.98	27.76
03/07/89	10	75.56	73.10				
04/07/89	11						
* 01/11/89	1	200.57	.	0.13	0.12	-0.86	.
02/11/89	2	13.50	NON EST	0.13	.	-0.86	.
03/11/89	3	0.91	.	.	.	.	.

Site: Calvert Hills - Females

Jolly-Seber (* = Triple Catch)

	Date	Day	Ni est	Ni SE	Phi est	Phi SE	bi est	bi SE
	20/01/88	1	NO ESTIMATES					
	21/01/88	2						
	27/01/88	3						
	28/01/88	4						
	30/01/88	5						
	31/01/88	6						
	01/02/88	7						
	09/02/88	8						
	11/02/88	9						
	14/02/88	10						
	15/02/88	11	NON EST	NON EST	0.56	NON EST	45.00	NON EST
	16/02/88	12	45.00	NON EST	NON EST	NON EST	NON EST	NON EST
	17/02/88	13	NON EST	NON EST				
	18/02/88	14						
	06/05/88	1			0.00	0.00		
	07/05/88	2	NON EST	NON EST	1.38	NON EST	11.00	NON EST
	08/05/88	3	11.00	7.26	NON EST	NON EST	NON EST	NON EST
	09/05/88	4	NON EST	NON EST	1.63	NON EST	7.80	NON EST
	10/05/88	5	7.80	2.94	NON EST	NON EST	NON EST	NON EST
	12/05/88	6	NON EST	NON EST	NON EST	NON EST	NON EST	NON EST
	13/05/88	7	NON EST	NON EST	NON EST	NON EST	NON EST	NON EST
	14/05/88	8	NON EST	NON EST	NON EST	NON EST	NON EST	NON EST
	15/05/88	9	NON EST	NON EST	1.00	NON EST	1.00	NON EST
	16/05/88	10	1.00	NON EST	NON EST	NON EST	NON EST	NON EST
	18/05/88	11	NON EST	NON EST	NON EST	NON EST	NON EST	NON EST
	19/05/88	12	NON EST	NON EST				
	20/05/88	13						
*	10/08/88	4	40.00	.	1.50	4.84	-1.00	.
	11/08/88	9	30.00	43.21	1.50	.	-1.00	.
	12/08/88	8	22.50	.	.	.	.	.
*	01/11/88	51	65.63	.	0.65	0.95	0.56	.
	02/11/88	46	97.16	28.58	0.65	.	0.56	.
	03/11/88	41	143.84	.	.	.	.	.

Site: Mt Margaret - Females

		Jolly-Seber (* = Triple Catch)					
Date	Day	Ni est	SE	Phi est	SE	bi est	SE
07/03/89	1	NO ESTIMATES					
11/03/89	2						
16/03/89	3						
17/03/89	4						
18/03/89	5						
20/03/89	6						
21/06/89	1	NO ESTIMATES					
22/06/89	2						
25/06/89	3						
26/06/89	4						
27/06/89	5						
28/06/89	6						
30/06/89	7						
01/07/89	8						
02/07/89	9						
03/07/89	10						
04/07/89	11						
* 01/11/89	8	NO ESTIMATES					
02/11/89	26						
03/11/89	13						

Appendix 2. Correlation Results for Transect Data

Table A2. Correlation coefficients for all number/number and number/environmental-factor pairings. Significant correlations are shown in bold with the associated p-value (α=0.05).

Total = total male and females caught per night
Amplex = amplexing pairs
Call = number of calling males
Eggs = gravid
NoEggs = not gravid
Temp = air temperature
Tdiff = difference in air temperature from previous night
Rain n = rain in last n hours
Moon illumn = percent total illumination for current phase

Site : Calvert Hills Season : Wet Season

	Total	Number Male	Number Female	Male Recaps	Female Recaps	Total Recap	Amplex	Call	NoEggs	Eggs
Total	.	0.9766 **0.0001**	0.1493	**0.7049** **0.0002**	0.0543	**0.6981** **0.0003**	0.2975	**0.7814** **0.0001**	0.1275	0.1772
Number Male		.	-0.0668	**0.6596** **0.0008**	-0.0724	**0.6387** **0.0014**	0.2249	**0.8633** **0.0001**	-0.0733	-0.0186
Number Female			.	0.2375	**0.5845** **0.0043**	0.3027	0.3465	-0.34433	**0.9289** **0.0001**	**0.5941** **0.0036**
Male Recaps				.	0.0976	**0.9929** **0.0001**	**0.4420** **0.0394**	0.2945	0.2889	0.1909
Female Recaps					.	0.2150	-0.1792	-0.2226	**0.5941** **0.0036**	0.3839
Total Recap						.	0.4123 0.0565	0.2625	0.3544	0.2331
Amplex							.	-0.0567	0.1856	**0.5706** **0.0056**
Call								.	-0.3302	-0.2916
NoEggs									.	**0.7070** **0.0002**
Eggs										.
Temp	0.0549	0.0428	0.0576	0.0790	0.0011	-0.0775	0.1588	0.1639	0.0437	0.1079
Tdiff	0.0031	-0.0344	0.1547	-0.0356	0.0537	-0.0251	0.2544	-0.0297	0.1023	0.1945
Rain24	0.0081	0.0866	-0.3607	-0.0397	-0.2031	-0.0631	-0.1789	0.3150	-0.3011	-0.3710
Rain48	-0.3661	-0.2945	-0.3448	-0.1654	-0.1803	-0.1839	-0.1787	-0.1015	-0.2743	-0.3755
Rain72	-0.2217	-0.1538	-0.3213	0.0053	-0.2036	-0.0191	0.1031	-0.1781	-0.3125	-0.2793
Moon Illumn	-0.0580	-0.1219	0.2918	0.0870	-0.2522	0.0553	**0.8565** **0.0001**	-0.2227	0.1670	**0.4865** **0.0217**

Site : Calvert Hills Season : Early Dry Season

	Total	Number Male	Number Female	Male Recaps	Female Recaps	Total Recap	Amplex	Call	NoEggs	Eggs
Total	.	**0.9911** **0.0001**	**0.7108** **0.0065**	-0.0221	-0.0724	-0.0724	0.2136	-0.2204	**0.5961** **0.0315**	0.5383
Number Male		.	0.6107	-0.0360	-0.1488	-0.0586	0.2124	-0.2036	-0.4994	0.4875
Number Female			.	0.0585	0.3548	0.1135	0.1483	-0.23493	**0.9056** **0.0001**	**0.6250** **0.0224**
Male Recaps				.	0.1617	**0.9873** **0.0001**	0.2472	0.3204	-0.2093	0.4976
Female Recaps					.	0.3166	-0.2433	-0.2906	0.4033	0.0361
Total Recap						.	0.1984	0.2611	-0.1361	0.4841
Amplex							.	**0.7507** **0.0031**	-0.0044	0.4402
Call								.	-0.3614	0.2723
NoEggs									.	0.2794
Eggs										.
Temp	0.4099	0.3549	**0.5625** **0.0224**	**-0.6618** **0.0137**	-0.0157	**-0.6387** **0.0188**	-0.2444	-0.4544	**0.6838** **0.0001**	-0.0344
Tdiff	-0.5575 0.0596	**-0.6322** **0.0274**	0.0443	-0.2516	0.3754	-0.1727	-0.2699	-0.1077	0.1110	-0.1610
Rain24	-0.5443 0.0545	**-0.5565** **0.0482**	-0.2967	-0.0012	-0.1736	-0.0291	-0.1267	0.1052	-0.1891	-0.3079
Rain48	0.0790	0.0821	0.0361	0.4404	-0.1875	0.3931	**0.9463** **0.0001**	**0.8303** **0.0004**	-0.1370	0.4330
Rain72	-0.5004	-0.5109	-0.2767	-0.0140	-0.1875	-0.0437	-0.1082	0.1107	-0.1370	-0.3175
Moon Illumn	**0.7191** **0.0056**	**0.7138** **0.0061**	0.5049	**-0.5971** **0.0312**	-0.2148	**-0.6086** **0.0273**	0.0840	-0.3630	**0.5905** **0.0336**	0.0818

Site : Calvert Hills Season : Late Dry Season

	Total	Number Male	Number Female	Male Recaps	Female Recaps	Total Recap	NoEggs	Eggs
Total	.	0.6281	**0.9959** **0.0001**	-0.3889	0.5618	0.4115	**0.9096** **0.0119**	**0.9850** **0.0003**
Number Male		.	0.5548	-0.2848	0.2901	0.1204	-0.1571	0.0377
Number Female			.	-0.0083	0.5668	0.4252	**0.9056** **0.0129**	**0.9915** **0.0001**
Male Recaps				.	0.5818	0.7883	-0.1571	0.0377
Female Recaps					.	**0.9591** **0.0025**	0.2153	0.6535
Total Recap						.	0.1082	0.5076
NoEggs							.	**0.8427** **0.0352**
Eggs								.
Temp	**0.9465** **0.0042**	0.5881	**0.9433** **0.0047**	0.0615	0.6112	0.4834	**0.8449** **0.0342**	**0.9382** **0.0056**
Tdiff	-0.0726 0.0596	0.3177	-0.1245	**-0.9983** **0.0017**	-0.2578	-0.4675	-0.0401	-0.1376
Moon Illumn	**0.8824** **0.0199**	0.4428	**0.8917** **0.0169**	-0.3467	0.1444	-0.0114	**0.9435** **0.0047**	**0.8424** **0.0353**

Site : Mt Margaret Season : Wet Season

	Total	Number Male	Number Female	Male Recaps	Female Recaps	Total Recap	Amplex	Call	NoEggs	Eggs
Total	.	0.9980 0.0001	0.5581 0.0199	0.7843 0.0002	**	0.7843 0.0002	0.5784 0.0150	0.9345 0.0001	0.5220 0.0316	0.5038 0.0392
Number Male		.	0.5047 0.0388	0.7647 0.0003		0.7843 0.0002	0.5784 0.0150	0.9345 0.0001	0.5220 0.0316	0.5038 0.0392
Number Female			.	0.6748 0.0030		0.6748 0.0030	0.8322 0.0001	0.38247	0.7280 0.0009	0.9728 0.0001
Male Recaps				.		1.0000 0.0000	0.7846 0.0005	0.6899 0.0022	0.5767 0.0154	0.6275 0.0070
Female Recaps					.					
Total Recap						.	0.7486 0.0005	0.6899 0.0022	0.5767 0.0154	0.6275 0.0070
Amplex							.	0.4306	0.5746 0.0158	0.8211 0.0001
Call								.	0.2961	0.3661
NoEggs									.	0.5494 0.0224
Eggs										.
Temp	-0.0819	-0.1171	0.4201	0.2581		0.2851	0.3062	-0.1881	0.3079	0.4081
Tdiff	0.1738	0.1794	0.0299	0.1325		0.1325	-0.1205	0.1577	0.1067	0.0000
Rain24	0.	0.	0.	0.		0.	0.	0.	0.	0.
Rain48	0.	0.	0.	0.		0.	0.	0.	0.	0.
Rain72	0.	0.	0.	0.		0.	0.	0.	0.	0.
Moon Illumn	-0.0990	-0.1192	0.2136	0.1014		0.1014	0.3051	-0.1681	0.2160	0.2159

** No recorded female recaptures

Site : Mt Margaret Season : Early Dry Season

	Total	Number Male	Number Female	Male Recaps	Female Recaps	Total Recap	Amplex	Call	NoEggs	Eggs
Total	.	0.9874 0.0001	0.7787 0.0010	0.9548 0.0001	0.5862 0.0276	0.9595 0.0001	0.5862 0.0276	0.8511 0.0001	0.3652	0.7838 0.0009
Number Male		.	0.6695 0.0088	0.9323 0.0001	0.5678 0.0342	0.9365 0.0001	0.5678 0.0342	0.8333 0.0002	0.2533	0.7251 0.0033
Number Female			.	0.7847 0.0009	0.4998	0.7902 0.0008	0.4998	0.6905 0.0063	0.7093 0.0045	0.8037 0.0005
Male Recaps				.	0.5257	0.9972 0.0001	0.5257	0.8039 0.0005	0.3654	0.7921 0.0007
Female Recaps					.	0.5878 0.0271	0.8592 0.0001	0.8592 0.0001	0.4640	0.3092
Total Recap						.	0.5878 0.0271	0.8400 0.0002	0.3882	0.7804 0.0010
Amplex							.	0.8592 0.0001	0.4640	0.3092
Call								.	0.4206	0.6133 0.0197
NoEggs									.	0.1505
Eggs										.
Temp	0.3810	0.3451	0.4195	0.4845	0.4084	0.4966	0.4084	0.5489 0.0421	0.1316	0.4771
Tdiff	-0.1659	-0.2154	0.0761	-0.1336	0.2253	-0.1064	0.2253	-0.0551	0.5439	-0.3420
Rain24	0.	0.	0.	0.		0.	0.	0.	0.	0.
Rain48	0.	0.	0.	0.		0.	0.	0.	0.	0.
Rain72	0.	0.	0.	0.		0.	0.	0.	0.	0.
Moon Illumn	-0.2411	-0.1874	-0.3884	-0.4043	-0.2037	-0.4025	-0.2037	-0.3782	-0.1687	-0.4024

Site : Mt Margaret Season : Late Dry Season

	Total	Number Male	Number Female	Male Recaps	Female Recaps	Total Recap	NoEggs	Eggs
Total	.	0.9958 0.0585	0.9781	0.4369	0.7431	0.8491	0.8491	0.5168
Number Male		.	0.9549	0.5175	0.6786	0.8939	0.8939	0.5931
Number Female			.	0.9781	0.8660	0.7206	0.7206	0.3273
Male Recaps				.	0.6786	0.2774	0.2774	-0.1890
Female Recaps					.	0.2774	0.2774	-0.1890
Total Recap						.	**1.0000** **0.0000**	0.8910
NoEggs							.	0.8910
Eggs								.
Temp	-0.0329	0.0588	-0.2401	0.8846	-0.6934	0.5000	0.5000	0.8386
* Tdiff								
Moon Illumn	-0.7174	-0.6506	-0.8467	0.3135	**-0.9993** **0.0239**	-0.2412	-0.2412	0.2256

* Recorded temperature difference for the three day period was zero

Appendix 3. Placement of Drop Box Sampler

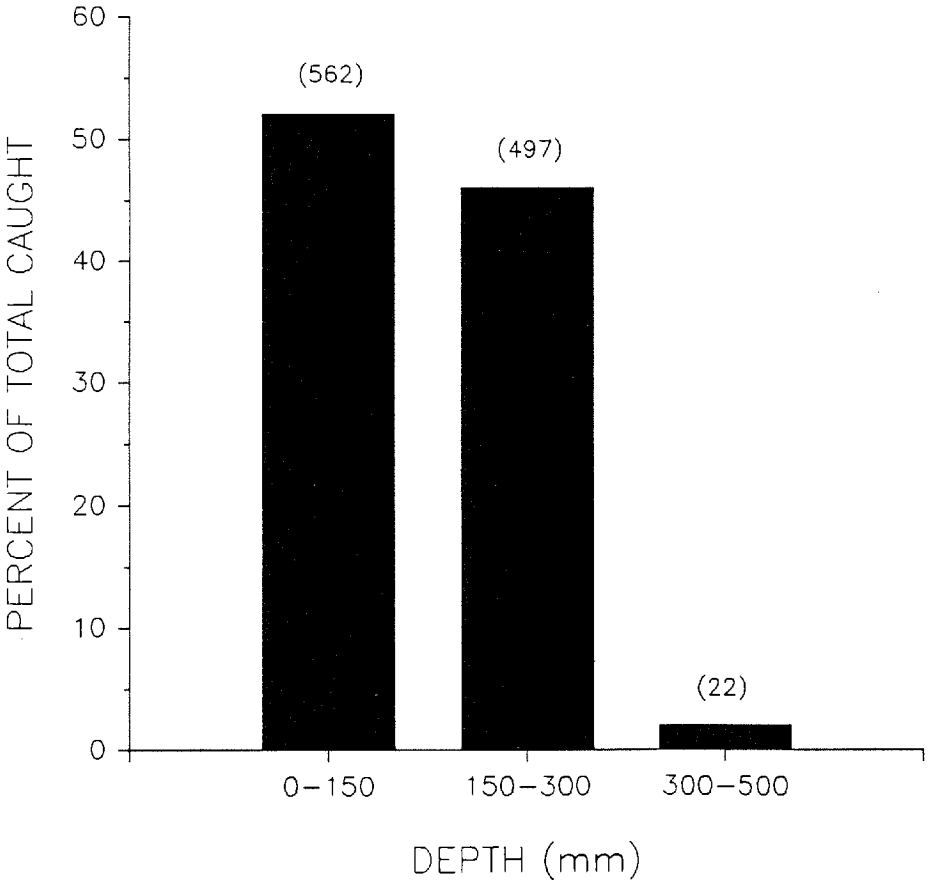
Personal observations and previous studies on *Bufo marinus* have shown that this species utilises the shallow margins of water bodies. Consistently placing the sampler at a depth where tadpoles do not occur would produce an underestimate for density. To avoid this bias, a decision about where to place the sampler (in relation to depth) was required.

A preliminary sampling effort was conducted in September 1987 at the Big Calvert River to determine the limits of tadpole distribution. 60 box samples (box dimensions: base 0.5m by 1.0m by height 0.5m) were taken over a two day period, 20 at each of three depth ranges. For convenience, these were ankle depth (0-150mm), shin depth (150-300mm) and knee depth (300-500mm). As the maximum height of the sampler was 500mm, a series of additional sweeps were taken in depths up to two metres. This involved sweeping a 0.5m wide net through the water column from surface to bottom over approximately 1 metre of substrate. This was conducted for 10 sweeps.

Results from the sampling are shown in Figure A1. Significantly higher numbers (98 percent of the tadpoles caught) occurred within the 0-300 mm range ($\chi^2=482.38$, 2 df, $p<0.0001$). No tadpoles were caught in the sweep samples although personal observations have shown that occasionally, tadpoles do occur swimming at the surface in this depth range. It was concluded that all subsequent sampling programs

conducted in this study should target the 0-300 mm depth range in order to accurately assess the density of tadpoles present.

Figure A1. Percent of total tadpoles caught within three depth ranges for 60 box samples at the Calvert River, September 1987. (Numbers for each depth range are indicated in parentheses, total caught = 1081.)


60
50
40
30
20
10
0
PERCENT OF TOTAL CAUGHT
(562)
(497)
(22)
0–150
150–300
300–500
DEPTH (mm)