The Environmental Physiology of *Bufo bufo* L. and *Bufo calamita* Laur. Tadpoles.

by

Philip David Nicolle B.Sc. Hons.

A thesis submitted in part fulfilment
of the degree of Doctor of Philosophy
of the
Council for National Academic Awards

School of Natural Sciences,
Liverpool Polytechnic,
September 1989

THE FOLLOWING HAVE NOT BEEN COPIED ON INSTRUCTION FROM THE UNIVERSITY

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The Environmental Physiology of Bufo bufo L. and Bufo calamita Laur. Tadpoles by P. D. Nicolle BSc. Hons.

ABSTRACT

Using spawn collected from the north Merseyside sand dune system, the effects of temperature on growth, development, metabolism and metamorphosis in B. bufo and B. calamita tadpoles were investigated, together with a limited study on the effects of L-thyroxine. The effects of density on growth, development and metamorphosis were examined. Physiological and behavioural effects of B. bufo tadpoles on B. calamita tadpoles were also studied.

B. calamita spawn and tadpoles survive at higher temperatures than B. bufo. Below 20°C B. bufo spawn, and below 15°C their tadpoles, develop at a greater rate than B. calamita. B. calamita spawn and tadpoles' growth and development is faster at higher temperatures.

B. calamita tadpoles have higher metabolic rates than B. bufo across the temperature range 15-30°C. Faster rates of metabolism and development in B. calamita result in smaller tadpoles and toadlets than B. bufo. B. calamita may compensate for this with increased metamorphic efficiency in terms of energy. It is speculated that differences in tadpole thyroid physiology could account for observed differences. Metamorphic efficiency was greatest, and rates of development and growth maximised, at the tadpoles' preferred body temperature.

Increased density reduced growth and development of tadpoles, but influenced different stages in the two species. Metamorphosis in all B. bufo tadpoles was delayed, whereas in B. calamita a proportion of the population metamorphosed apparently unaffected by increased density.

The responses of tadpoles to temperature and density were related to the species' prefered spawning environments.

In the presence of B. bufo tadpoles, B. calamita growth, development and metabolism was suppressed. The pattern of development and timing of metamorphosis in B. calamita became similar to that of B. bufo. Inhibition was not relieved by L-thyroxine, and did not effect tadpole behaviour. Mass specific food consumption was increased. It is speculated that the inhibitor is a parasite or a substance which affects assimilation.

ACKNOWLEDGEMENTS

I would like to thank Dr. P.E. Wheeler, my supervisor, for his advice and support throughout this project. Also Dr. P.H. Smith for his valuable help. Many other people have assisted me during the course of my research. I would like to thank the following, who have helped me both with academic input and moral support: Kevin Ayre, Pete Carty, Dave Casson, Debra Scott, Dave LeQuesne, Chris McCarty, Sarah Molyneaux, Steve Muddiman, Roy Sanderson, Claire Sandys-Winch, Pat Thompson, Amory Thomas and Dave Wheeler. I would also like to acknowledge the help of both ecology and physiology technicians in Liverpool Polytechnic. Finally I would like to thank my father, mother and Denise for providing me with the support and encouragement that has allowed me to complete this thesis.

This work was done whilst in receipt of a Science and Engineering Research Council studentship. Work with the natterjack toad was carried out under licence from the Nature Conservancy Council.

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CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Distribution of B. bufo and B. calamita

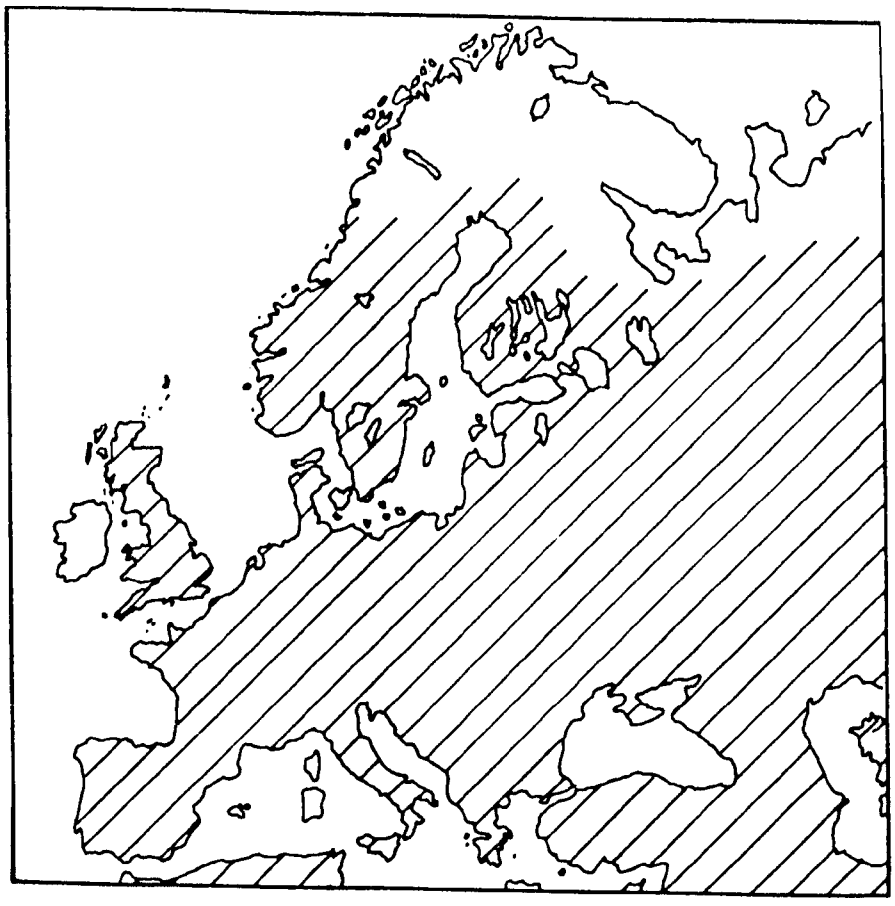
The British Isles have three native anurans, the common frog Rana temporaria L., the common toad Bufo bufo L. and the natterjack toad Bufo calamita Laur. (Smith, 1951). The two native bufonids, B. bufo and B. calamita are similar morphologically and are closely related (Boulenger, 1897). The tadpoles of both species are almost identical, being distinguished primarily by differences in dentition (Mathias, 1971). In terms of habitat selection, behaviour and physiology there are marked differences between the species (Davis, 1985; Mathias, 1971).

B. bufo has a widespread distribution, throughout mainland Britain (Fig. 1.1) although not in Ireland. In Europe B. bufo is also widespread being found as far north as latitude 65° North. (Douglas, 1948) and reaching North Africa in the south (34° N, Douglas, 1948).

B. calamita has a more restricted distribution (Fig. 1.2). In Europe, B. calamita is found as far as latitude 55°N in west Sweden (Mathias, 1971); it is widely distributed in Spain and Portugal but no further south. It is also found in France, the Benelux countries, Switzerland and West Germany with some colonies occurring in western soviet Russia (Beebee, 1979a).

Figure. 1.1. (A) European distribution (Frazer, 1983) and (B) British distribution (Biological Records Centre) of B. bufo.

A



B

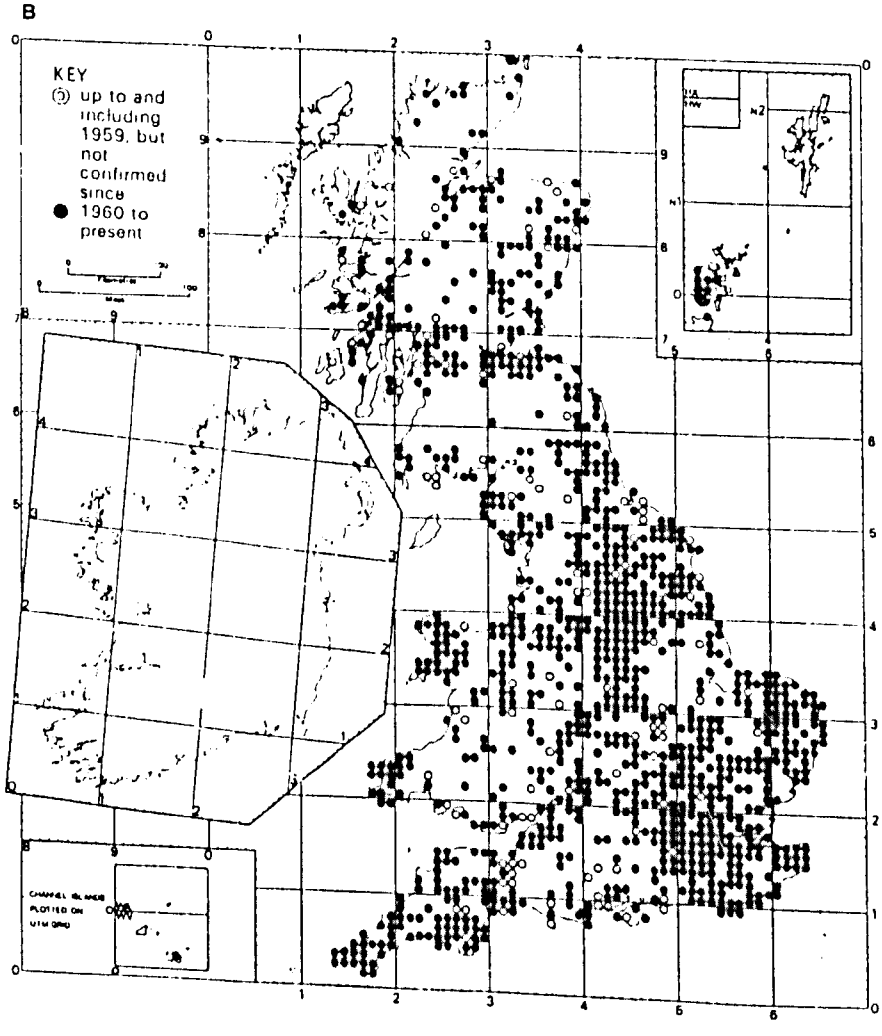
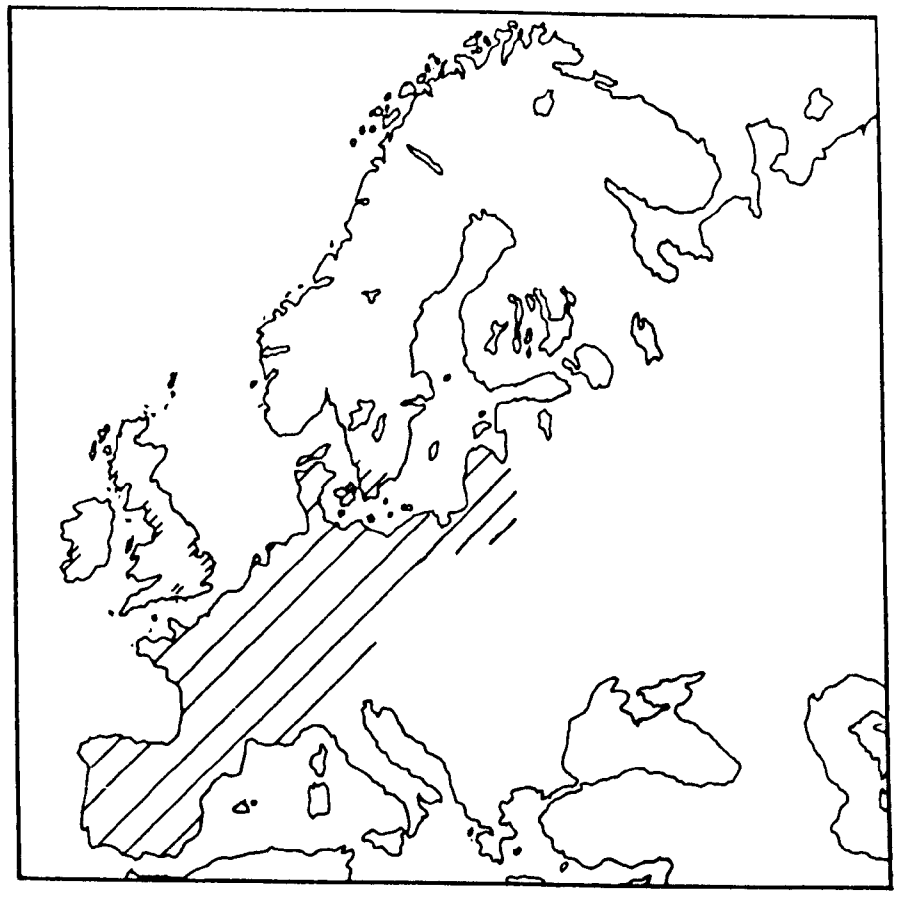

KEY
up to and including 1959, but not confirmed since
1960 to present

Figure. 1.2. (A) European distribution (Beebee, 1979a) and (B) British distribution (Biological Records Centre) of B. calamita.

A



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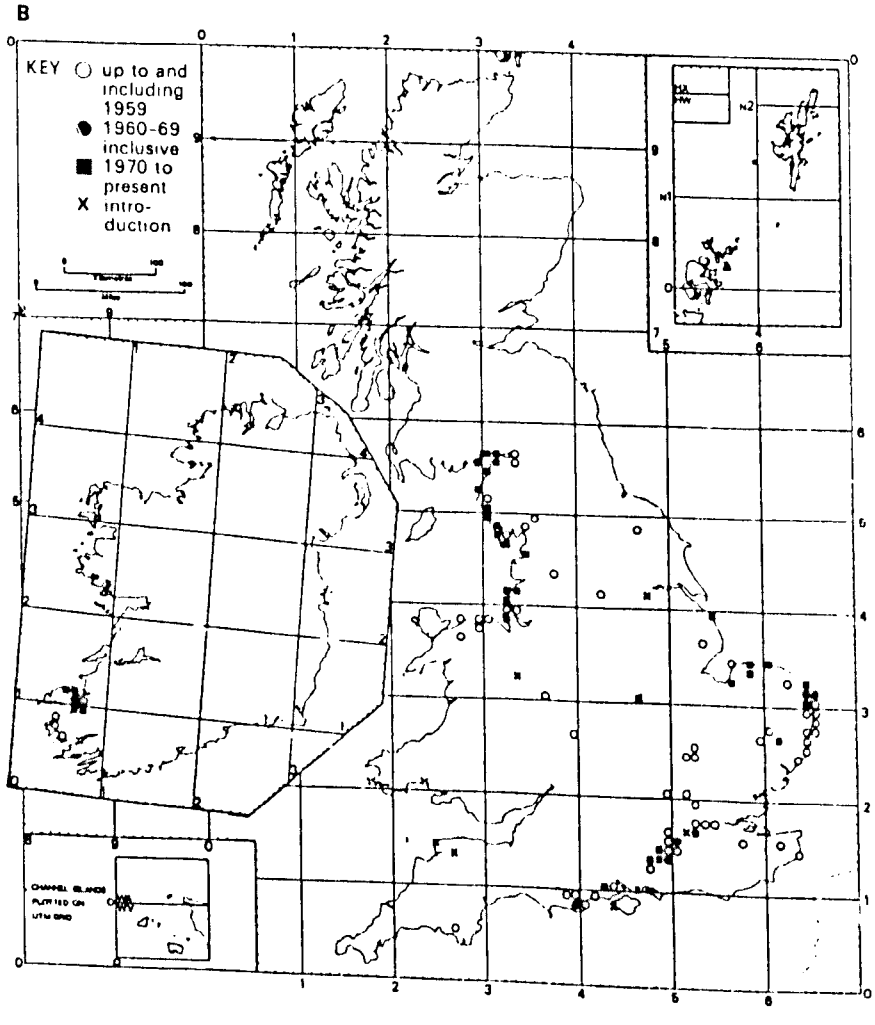

KEY
up to and including 1959
1960-69 inclusive
1970 to present
X introduction

In Britain, B. calamita is now found only on the coastal sand dune systems and landward edges of saltmarshes in Merseyside, Cumbria, East Anglia, Lincolnshire and south west Scotland, with inland distribution restricted to two sites in south east England (Beebee, 1987). B. calamita is therefore Britain's rarest native anuran. Decline of B. calamita numbers (Beebee, 1973; 1975; Prestt et al, 1974) led to its protection under the Conservation of Wild Creatures and Wild Plants Act 1975 and subsequently under the Wildlife and Countryside Act 1981.

1.2 Habitat

In general the habitat requirements of B. bufo and B. calamita are distinct. B. bufo occupies a large range of terrestrial habitats including woodlands, scrub, grassland and also gardens, hibernating in holes or beneath stones (Frazer, 1983).

B. calamita has a far more restricted habitat preference. In Britain it is found mainly in coastal sand dune systems and saltmarshes. B. calamita was formerly widespread on the heathlands of southern England although altered patterns of land use in these areas have resulted in extinction of most colonies (Beebee, 1987). B. calamita adults hibernate in burrows dug into sandy substrates (Beebee, 1983).

The favoured aquatic habitats of the two species are also different. B. bufo prefer to spawn in deep permanent and vegetated pools (Harrison, 1985; Frazer, 1983). B. calamita spawns in shallow

ephemeral pools associated with the terrestrial habitat in which it is found (Frazer, 1983; Smith and Payne, 1981).

Despite the differences in the preferred habitats of the two species some overlap does occur. Both species can be found occupying the same terrestrial and aquatic habitats. In these situations competition occurs and it appears that B. bufo outcompetes B. calamita (Banks and Beebee, 1987; Beebee, 1987; 1979).

1.3 Interaction between B. bufo and B. calamita.

The north Merseyside sand dune system contains one of the largest populations of B. calamita in Britain (Davis, 1985; Smith and Payne, 1981). It also supports large numbers of B. bufo (Mathias, 1971). There is a continuum of breeding pond types from shallow, non vegetated dune slacks to deeper, permanent and well vegetated ponds. In many of these both B. bufo and B. calamita breed, and B. bufo appears to be more successful, competitively superior (P.H. Smith, in Lit.).

During the breeding season, several factors maintain the separation of B. bufo and B. calamita. Generally, B. bufo adults emerge from hibernation and spawn in March before the emergence of B. calamita in April (Smith, 1951). B. bufo is an explosive breeder, spawning in one season being usually completed over a two week period (Mathias, 1971). B. calamita spawn at a later date, when environmental temperatures are higher. This may be 9-25 days after B. bufo spawning (Mathias, 1971). Overlap of breeding period occurs in some seasons.

When the two species are found together in the breeding pools, species separation of adults is maintained by several mechanisms. B. bufo spawn more actively during the day, B. calamita tend to mate and spawn nocturnally. B. bufo mate and spawn preferentially in deeper parts of pools whereas B. calamita prefer the shallow pool margins (Mathias, 1971). Mathias (1971) considered that these factors resulted in very little interspecific competition between the adults of the two species. Smith (1974) suggests that some interspecific pairing may occur between B. bufo and B. calamita in the north Merseyside sand dune system resulting in hybrid progeny. The viability of such progeny is unknown.

Beebee (1977) recognised the encroachment of scrub, and subsequent colonisation of heathland B. calamita breeding pools by B. bufo, as a cause of B. calamita decline. In the north Merseyside sand dune system it has also been noted that little B. calamita recruitment occurs in ponds containing B. bufo and B. calamita when compared with ponds containing only B. calamita (P.H. Smith in Lit.). Increased mortality and reduction of metamorphic success, as a result of the presence of B. bufo tadpoles, has also been demonstrated by Banks and Beebee (1988).

1.4 Mortality of B. bufo and B. calamita tadpoles.

The key mortality factors for B. calamita tadpoles are invertebrate predation and desiccation as a result of the drying up of breeding pools (Banks and Beebee, 1988; Davis, 1985). Kadel (1975) estimated

that approximately 93% of viable tadpoles are predated by invertebrates and 6.7% perish as a result of desiccation. Only 0.3% of eggs produced by a pair of toads reached metamorphosis.

Davis (1985) studied B. calamita tadpole survival in the north Merseyside sand dune system. He estimated that over a three year period (1981-1983) predation was responsible for 95.7, 87.2 and 97.1% of tadpole mortality. Desiccation accounted for 4.3, 12.8 and 2.9% of mortality respectively. Banks and Beebee (1988) investigated B. calamita mortality in a dune system on the Cumbrian coast. Breeding sites included mainly shallow slacks but also some deeper pools and shallow depressions in upper saltmarsh habitat. They estimated that 49·7% of tadpoles died as a result of desiccation, 47·6% were predated and 2·65% reached metamorphosis.

In B. bufo tadpoles, desiccation does not appear to be a major mortality factor. Harrison (1985) studied B. bufo tadpole mortality in Mid Wales: The drying up of ponds did not pose a serious threat to B. bufo tadpoles as the adults invariably spawned in deeper, permanent areas of ponds. He concluded that the key mortality factor was invertebrate predation.

Predation is a key mortality factor for both B. calamita and B bufo tadpoles although B. calamita tadpoles also are subject to desiccation (Banks and Beebee, 1988; Davis, 1985; Harrison, 1985; Kadel, 1975). When the two species occur together B. bufo tadpoles appear to be more successful than B. calamita (Banks and Beebee, 1987).

Differential mortality of B. bufo and B. calamita tadpoles, where they are found together, could be a result of differences in the species' palatability to predators. B. bufo tadpoles are less palatable to fish than B. calamita tadpoles (Nicolle, 1985). This may not explain differences in levels of predation when fish are not present. Beebee (1983) suggests that toxic chemicals in the skin of a tadpole are no defence against invertebrate predators with piercing mouthparts. It is possible that other factors result in the apparent reduction in metamorphic success of B. calamita tadpoles in the presence of B. bufo tadpoles.

1.5 Interactions of B. bufo and B. calamita tadpoles.

Much work has been carried out on the effect of tadpole density and growth, and also inhibition of growth by interspecific and intraspecific interactions (Alford and Wilbur, 1983; Berger, 1968; Hota and Dash, 1977; Lynn and Edelman, 1936; Wilbur, 1987). Heusser (1972a, 1972b) has shown that growth of B. calamita tadpoles is retarded when they are reared in water that has previously contained B. bufo tadpoles (conditioned water). This may produce an advantage to one of the sympatric species. Thus, Morin (1987) found that competition between tree frog tadpoles resulted in an inhibited species being exposed to predators for longer periods of time. This reduced their chances of survival and also increased resources for the species causing the inhibition.

Another factor that could assist B. bufo's apparent ability to outcompete B. calamita is the relative time of spawning. B. bufo is able to breed earlier in the year than B. calamita, probably as a result of its greater acclimatory ability (Davis, 1985). B. bufo spawns approximately two weeks before B. calamita (Mathias, 1971). Thus the B. bufo tadpoles are larger than those of B. calamita during most of the developmental period. Larger tadpoles of many anuran species inhibit smaller ones intraspecifically (Gromko et al, 1973; Guyetant, 1970; Licht, 1967). The greater size of B. bufo tadpoles in relation to B. calamita tadpoles may enhance interspecific inhibition. B. bufo tadpoles are also known to predate B. calamita spawn and larvae (Beebee, 1983).

B. bufo may be able to outcompete B. calamita tadpoles where they are sympatric. The pools which are recognised as typical breeding sites for B. calamita in Great Britain, shallow, ephemeral dune slacks, are often devoid of B. bufo which therefore removes potential competition. These pools also have reduced numbers of predators (Mathias, 1971). However, the pools are short lived and tadpoles developing in them are vulnerable to desiccation. B. calamita tadpoles have the fastest developmental rates found in any of the European anurans (Beebee, 1983). This could give them an increased chance of survival in an ephemeral environment.

1.6 Aims of the project.

Great Britain has two native bufonids, B. bufo and B. calamita. The

latter is a native endangered species protected under the Wildlife and Countryside Act 1981. Both species are found in the north Merseyside sand dune systems. Here they breed in a range of pool types from permanent well vegetated ponds to shallow dune slacks. Each species can be found in ponds where the other is absent. There are also ponds in which both species breed. Spawn collected from this region will therefore be representative of B. bufo and B. calamita populations that can be found spawning in the same or distinct breeding breeding ponds.

While information is available concerning the environmental requirements, general ecology and population dynamics of both species, there has been little comparative work. Information on both species' physiology is limited.

Some of the differences previously observed between the two species' tadpoles (e.g. developmental rates) may be a result of inherent differences in their physiologies. The tadpoles' physiology will be affected by environmental conditions. These may in turn affect the outcome of any competition between species and also determine the optimum conditions for metamorphic success where the two species do not compete.

The aim of this project is to:

1. Investigate the effect of temperature on B. bufo and B. calamita spawn development and tadpole growth and development.

2. Determine metabolic rates for B. bufo and B. calamita tadpoles and relate them to their growth and development.

3. Investigate differences in the species' response to the growth hormone, L-thyroxine, and relate this to their growth and development.

4. Investigate the effect of temperature on the timing and efficiency of metamorphosis in B. bufo and B. calamita.

5. Investigate the effect of tadpole density on growth, development and metamorphosis in B. bufo and B. calamita.

6. Investigate the mechanism of any competition between the species, comparing intraspecific density effects in both species with the effect of B. bufo tadpoles on those of B. calamita.

All observations made in this project will be related to environments in which B. bufo and B. calamita breed. This study should provide information on the species' physiology that could help to explain observed differences in their ecology. It is hoped that any information gained may also assist in the conservation of B. calamita.

CHAPTER 2

2.0 The effect of temperature on development and growth in B. bufo and B. calamita spawn and tadpoles.

2.1. INTRODUCTION

It is well documented that an increase in environmental temperature results in faster anuran tadpole development at the expense of the tadpoles' size (Moore, 1939; Marian & Pandian, 1985). This is a result of increased metabolic rates at higher temperatures requiring increasing amounts of energy. Thus, energy that would go towards growth at lower temperatures is utilised by increased metabolism at higher temperatures. As metabolic rate rises, in general, growth decreases.

Work on North American anurans has shown that species breeding earlier in the year, when environmental temperatures are lower, can remain unharmed at lower temperatures in the egg and larval stages. They do not survive at higher temperatures, which species with a more southerly distribution can tolerate. Species with a northern distribution often compensate for the lower environmental temperatures with relatively high developmental rates at the same temperature (Moore, 1939; Zawadowsky & Sidorov, 1928). This allows them to complete their development at temperatures low enough to retard the development of more southerly distributed species. In general, species adapted to northern and therefore cooler conditions have a more rapid rate of development than southern forms.

B. bufo spawns approximately two weeks before B. calamita at a time when environmental temperatures are lower (Smith, 1954). Mathias (1971) suggests this is because it is adapted to northern and hence cooler conditions.

B. bufo and B. calamita have distributions that do not allow either species to be categorised as a northern or southern species. In Europe B. calamita is limited to a relatively narrow latitudinal zone (36°-55° N; Smith, 1954) whilst B. bufo extends both north and south of its range (34°-65° N; Douglas, 1948).

Mathias (1971) studied the temperature tolerances of B. bufo and B. calamita spawn. He followed spawn development until the resulting tadpoles had reached stage 25 of development (see Appendix II). The lower tolerance levels for the two species were different. At 5°C the spawn of both species failed to develop beyond gastrulation (stage 11, Gosner, 1960). At 10°C B. bufo spawn developed slowly to stage 25. B. calamita developed at a slower rate and failed to reach stage 25. B. bufo appears to be able to tolerate lower environmental temperatures.

The upper temperature tolerance level for B. calamita tadpoles was between 29°C (100% survival to stage 25) and 33°C (no development past stage 11). The upper tolerance level for B. bufo was between 20°C (>50% survival to stage 25) and 25°C (most embryos stopped development at stage 12). Mathias (1971) came to the conclusion that B. calamita

has higher thermal tolerance levels and this is why it has a restricted distribution. This does not account for the more southerly distribution of B. bufo.

Davis (1985) suggested that B. bufo is physiologically better adapted to compensate for temperature changes in field conditions. From measurements of metabolic rates of the two species, he concluded that B. calamita tadpoles are less able to acclimate to temperature changes than B. bufo and this could be a reason for the former's more restricted distribution.

B. calamita is renowned as having the fastest developmental rates of any European amphibian (Nature Conservancy Council, 1983). Mathias (1971) however, suggests that the developmental rates of B. bufo and B. calamita are similar over a range of temperatures. He looked only at spawn development, and did not follow the resulting tadpoles' development through to metamorphosis.

In this chapter, the effect of temperature on development of spawn and growth and development of tadpoles of B. bufo and B. calamita under otherwise similar rearing regimes will be investigated. Results should establish the thermal tolerance limits of spawn and tadpoles for both species through to metamorphosis and, within the tolerance limits, rates of both growth and development.

2.2. MATERIALS AND METHODS

2.2.1 Effect of temperature on spawn development

To ensure that it was freshly fertilised, spawn was collected from pairs of amplexant toads (B. bufo and B. calamita). B. calamita spawn was collected under NCC licence (for collection dates of both species' spawn see Appendix I). Approximately 30-ova were taken from each of 5 pairs of both species. The spawn was transported back to the laboratory in plastic buckets containing pond water, then transferred to plastic trays (35 x 24 x 4 cm) containing water at 15 ± 1.5°C overnight. Each 30 ova portion of spawn was then subdivided into 3 sections containing 10 ova, these were all placed into a beaker containing water at 15°C (a total of fifteen 10-ova sections for each species) and gently mixed.

For each species three 10-ova sections were then removed to each of five 2 l beakers containing water at 15°C, these were then placed in water baths at 10, 15, 20, 25 and 30°C (± 1°C). The temperature of the water in the beakers equilibrated to that of the water bath at approximately 1°C per minute.

The stage of development (Gosner, 1960 see Appendix II) of 20 ova in each temperature treatment was recorded every 24 hours. Recording continued for 10 days or until the mean stage of development was greater than 24. For observation, spawn was removed from the treatments using a wide-bore Pasteur pipette and placed in a petri dish on the stage of a binocular

microscope at x40 magnification.

2.2.2 Spawn collection and initial rearing for tadpole experiments

For the majority of the experimental work on tadpoles, spawn collection and initial rearing was similar (any changes from the method described here will be discussed in the relevant chapter). Spawn strings from B. bufo and B. calamita were removed from dune slacks in the Formby Point area of the north Merseyside sand dune system (For collection dates see Appendix I). The spawn was removed within twelve hours of deposition or preferably collected from a pair of spawning toads to ensure freshness. Six sections of spawn were taken from separate spawn strings of each species, each containing approximately 200 ova.

The spawn string sections were removed from six separate ponds to ensure that each section came from different parents. This meant that the collected spawn, as far as possible, represented a random sample of the B. bufo and B. calamita populations. B. calamita spawn was collected under NCC licence.

Spawn was transported back to the laboratory in plastic buckets containing pond water. In the laboratory the individual spawn strands were placed in shallow plastic trays (35 x 24 x 4 cm) containing 2 l of dechlorinated tap water. All water required for tadpole rearing and other experimental work was dechlorinated prior to use by standing it overnight in large polythene tanks.

The trays were maintained at 15 ± 1.5°C. Spawn was examined daily and any dead or fungal-infected spawn removed to prevent the spread of any infection. The water in the trays was replenished daily.

The spawn was allowed to develop through stages 1-25 (Gosner, 1960). When approximately 90% of the tadpoles were free swimming (stage 26), they were transferred to the appropriate experimental conditions.

2.2.3 Effect of temperature on growth and development of tadpoles

After initial rearing (section 2.2.2), tadpoles of both species were transferred to temperature acclimation tanks. The tadpoles were placed in plastic beakers containing 2 l of water at 15°C and the water temperature raised or lowered to the designated rearing temperature at a rate of approximately 1°C every minute.

For both species, tadpoles were reared at 5, 10, 15, 20, 25 and 30°C. At each temperature 20 individual tadpoles were maintained in each of six 2 l beakers which were continually aerated. This rearing density was chosen to minimise intraspecific density effects (Chapter 6). The experiments in chapter 6 demonstate that at a density of 10 tadpoles l^{-1} mortality is low and growth and development proceed normally. The water temperatures were maintained at the correct values by placing the beakers in water baths that held the appropriate temperature ± 0.5°C. The temperature in the beakers was continually monitored by thermometer.

Tadpoles were fed ad libitum with washed lettuce and ground rat pellets as a protein supplement. The rearing water was changed and the beakers cleaned every four days.

2.2.4 Collection and recording of results obtained from tadpole rearing experiments

Throughout the rearing period, a sample of tadpoles was weighed and stage of development determined at four day intervals. In all the experimental treatments involving the weighing and staging of tadpoles, the following methodology was used.

Samples consisting of 20 tadpoles were removed from a particular experimental treatment randomly and placed in a beaker of water (for temperature treatments the water was at an equivalent temperature). Tadpoles were removed individually from the beaker using a wide-bored Pasteur pipette, and placed in a nylon mesh sieve (0.4 mm). This was lowered onto absorbent cotton matting, which drew surplus surface moisture from the tadpole. The tadpole was then immediately transferred to a tared beaker of water on a top pan balance. The tadpole mass was recorded to the nearest 0.005 g.

The tadpole was then removed from the beaker on the balance with the Pasteur pipette to a water filled petri dish. The stage of development was noted using a binocular microscope at 10x magnification. Stages of development recorded were those described by Gosner, (1960) with

modifications that made scoring of B. bufo and B. calamita tadpoles more accurate (Appendix II). After measurement tadpoles were returned to the appropriate rearing treatments.

Measurements of the tadpoles continued through the development stages until stage 41, forelimb emergence. The number attaining this stage, at each treatment, was recorded every day. The metamorphosing tadpoles were then transferred to small plastic boxes (19 x 11 x 7 cm) containing moist filter paper until stage 46 (metamorphosis complete) was reached, when they were weighed. Before weighing, the toadlets were surface dried with absorbent cotton. They were then weighed using the technique described for the tadpoles.

As the metamorphosing tadpoles were removed from the treatments the correct experimental densities were maintained by transferring tadpoles from other replicate treatments. It was necessary to maintain tadpoles at the same rearing density throughout the course of the experiment, since a reduction in density could result in increased rates of development and growth (Chapter 6). Measurements of tadpoles continued for each experimental treatment until the appropriate rearing density could not be maintained.

After weighing, the toadlets were released close to the ponds from which the spawn had been taken.

2.3. RESULTS

2.3.1 Effect of temperature on development of spawn of B. bufo and B. calamita

B. bufo spawn development occurred initially across the range 10-30°C, although after 2 days all the spawn at 30°C had died (Fig. 2.1). Development continued rapidly at 25°C, stage 24 being reached after 3.5 days. This stage was reached after 5 days at 20°C and 9 days at 15°C. Spawn at 10°C attained stage 16 at 4 days, but did not develop beyond stage 18 during the 10 days of the experiment.

In B. calamita, spawn development also occurred over the range 10-30°C (Fig. 2.2). Unlike the B. bufo spawn, which was destroyed at 30°C, B. calamita attained stage 24 after 2.5 days at this temperature. The spawn at 25°C reached this stage at 3 days and spawn at 20°C after 6 days. B. calamita spawn at 15°C developed at a slower rate than B. bufo spawn at the same temperature, after 10 days, the B. calamita spawn reached stage 20 compared to B. bufo which had reached stage 24. At 10°C, B. calamita attained stage 16 after 10 days but development did not proceed beyond this.

2.3.2 Effect of temperature on development of B. bufo and B. calamita tadpoles.

B. bufo development was arrested at 5°C and after 48 days no tadpoles survived (Fig. 2.3). At 10°C development of B. bufo did proceed,

Figure. 2.1. Effect of temperature on rate of development of B. bufo spawn. (●) 10°C, (△) 15°C, (□) 20°C, (▲) 25°C, (○) 30°C. Means ± 95% confidence intervals, n=20 for each point. A point without a confidence interval has no variance about the mean.

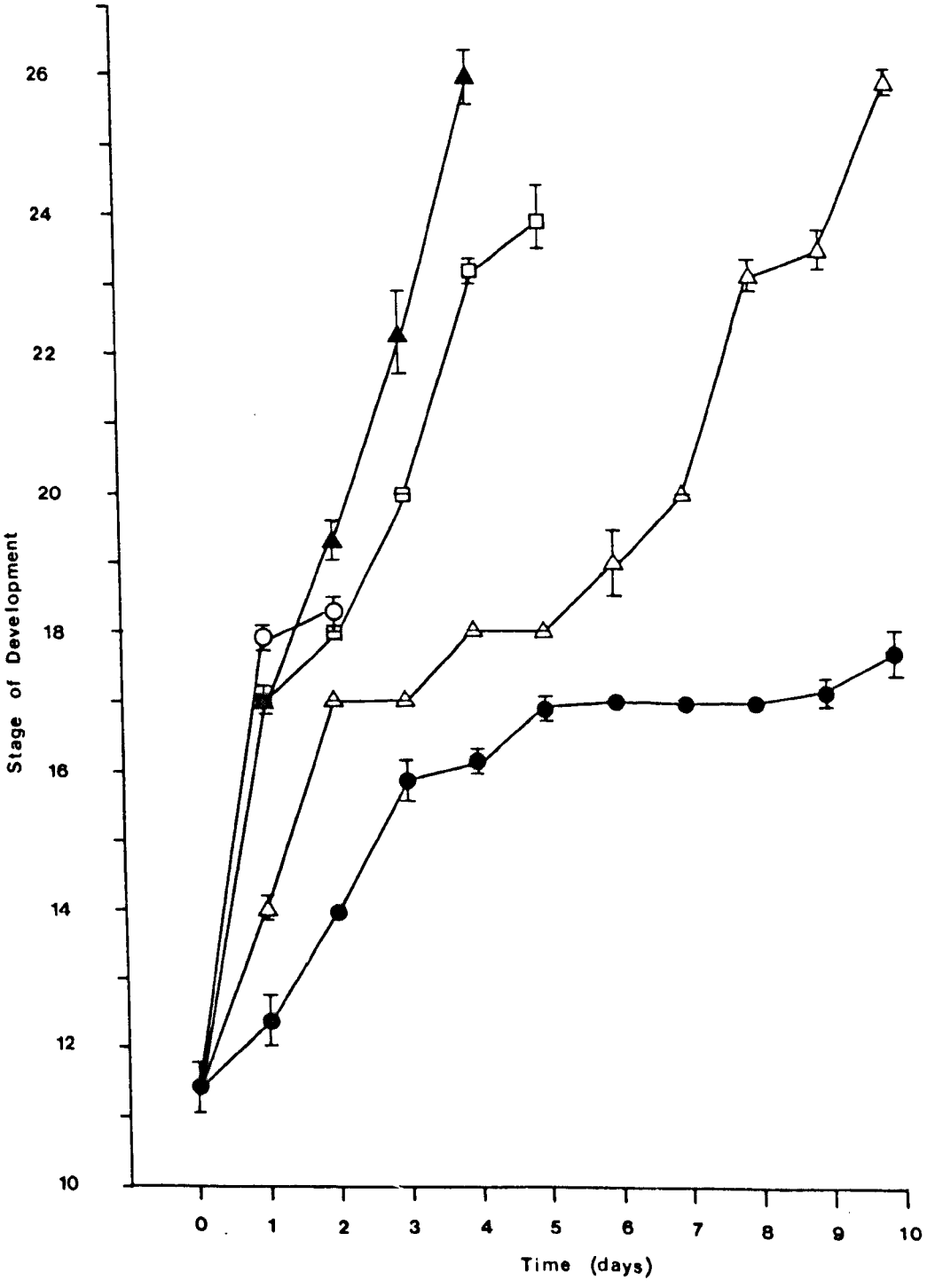

Stage of Development
26
24
22
20
18
16
14
12
10
0
1
2
3
4
5
6
7
8
9
10
Time (days)

Figure. 2.2. Effect of temperature on rate of development of B. calamita spawn. (●) 10°C, (△) 15°C, (□) 20°C, (▲) 25°C, (○) 30°C. Means ± 95% confidence intervals, n=20 for each point. A point without a confidence interval has no variance about the mean.

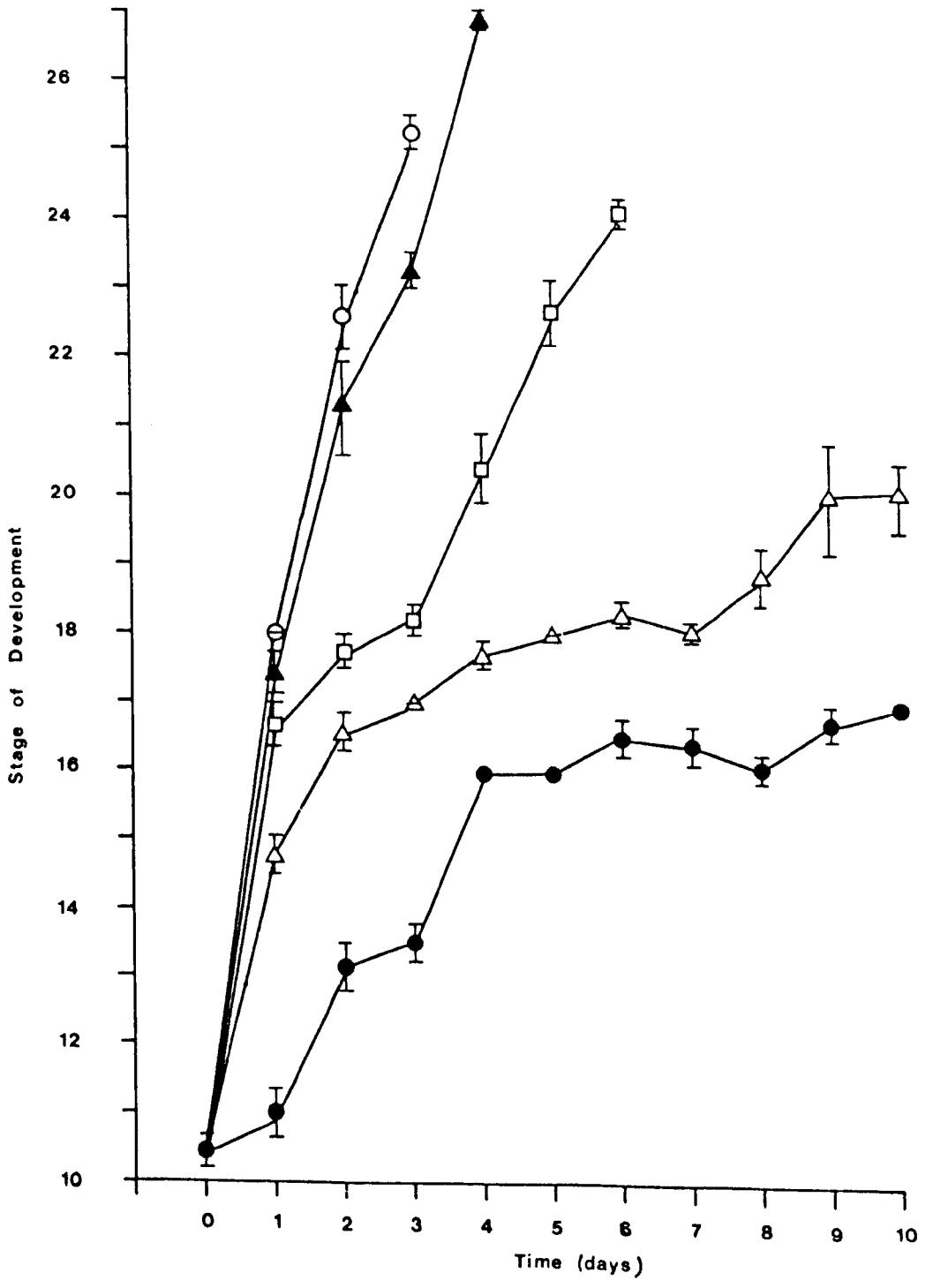
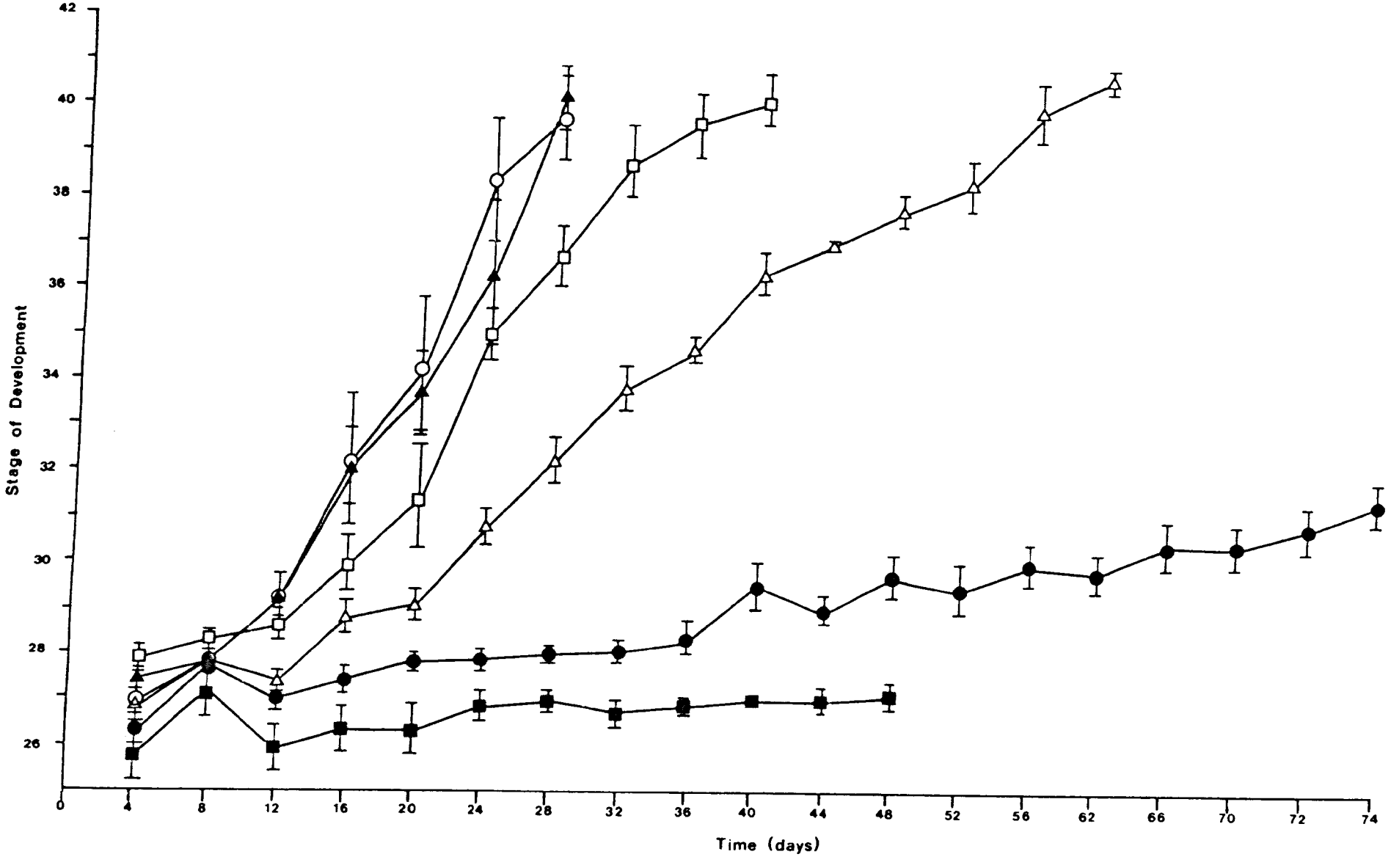

Stage of Development
26
24
22
20
18
16
14
12
10
0
1
2
3
4
5
6
7
8
9
10
Time (days)

Figure. 2.3. Effect of temperature on rate of development of B. bufo tadpoles. (■) 5°C, (●) 10°C, (△) 15°C, (□) 20°C, (▲) 25°C, (○) 30°C. Means ± 95% confidenence intervals, n=20 for each point.


Stage of Development
42
40
38
36
34
32
30
28
26
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
62
66
70
72
74
Time (days)

tadpoles reaching stage 31 after 76 days, although development did not continue past that stage. At 15°C, development proceeded through to metamorphosis in 64 days. The rate was increased at 20°C, taking only 40 days. Rates of development were greatest at the two highest temperatures (25 and 30°C), metamorphosis being reached in only 28 days. The rates of development through to metamorphosis at these two temperatures were not significantly different ($P > 0.05$).

A similar pattern was observed in B. calamita (Fig. 2.4). At 5°C, there was no development and no tadpoles survived beyond 8 days. Tadpoles at 10°C survived for 16 days but again no development occurred. At 15°C, development progressed slowly through the initial stages. After stage 34, development slowed and after 72 days, all tadpoles had died. At 20°C development was rapid, metamorphosis being achieved after 28 days. Development to metamorphosis took only 16 days at 25 and 30°C. Again the rates of development at these two temperatures were not significantly different ($P > 0.05$).

Comparing rates of development for the two species (Fig. 2.5), the time taken to reach stage 32 was shorter in B. calamita than in B. bufo at 15°C. By stage 39, B. calamita development had slowed, B. bufo reached this stage more rapidly (in 54 days compared to 58). At 20-30°C, B. calamita tadpoles were approximately 12 days faster at reaching stage 32 than B. bufo tadpoles. This difference was maintained through to stage 39.

From figures 2.3 and 2.4, the duration of each developmental stage can

Figure. 2.4. Effect of temperature on rate of development of B. calamita tadpoles. (■) 5°C, (●) 10°C, (△) 15°C, (□) 20°C, (▲) 25°C, (○) 30°C. Means ± 95% confidence intervals, n=20 for each point.

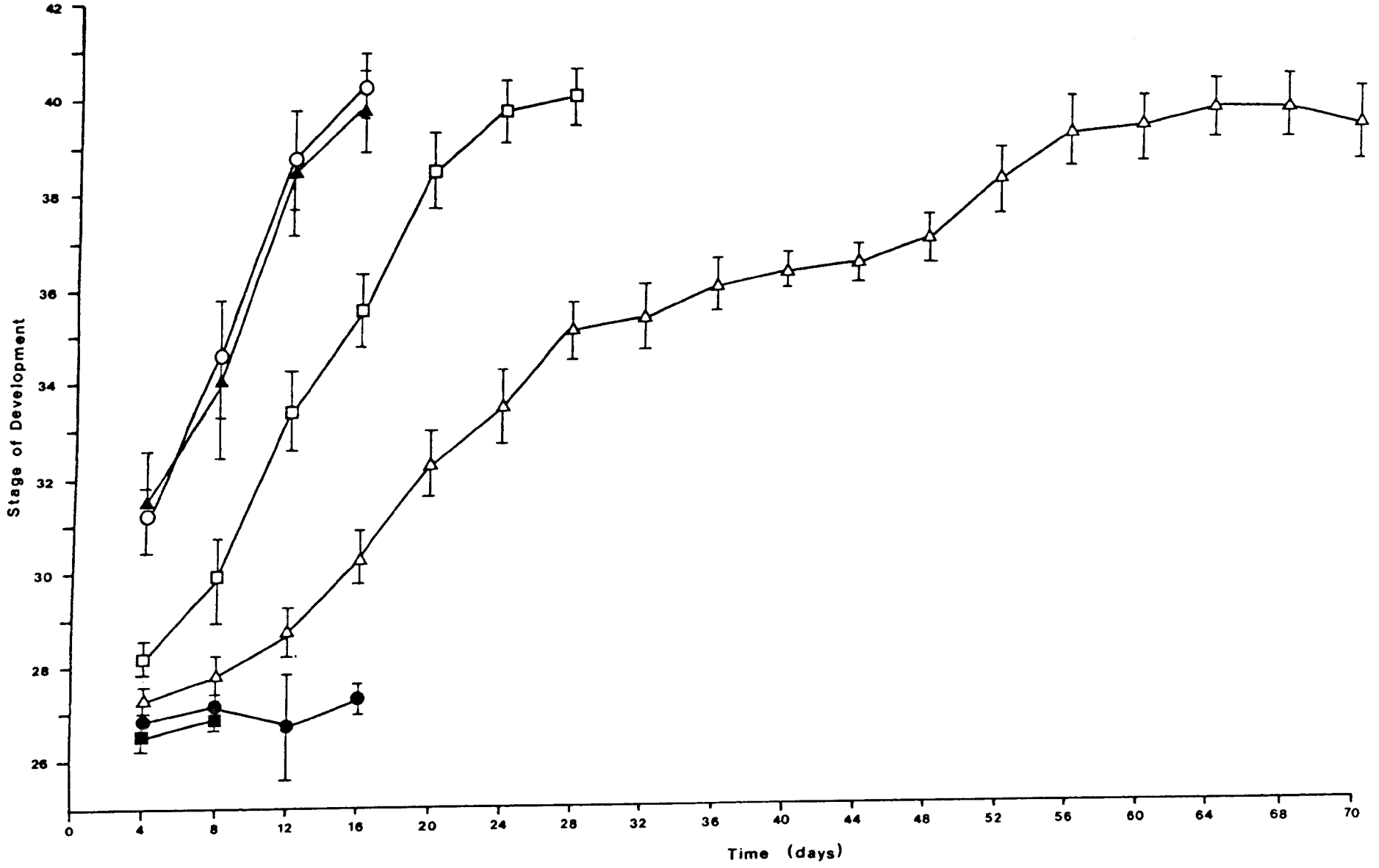
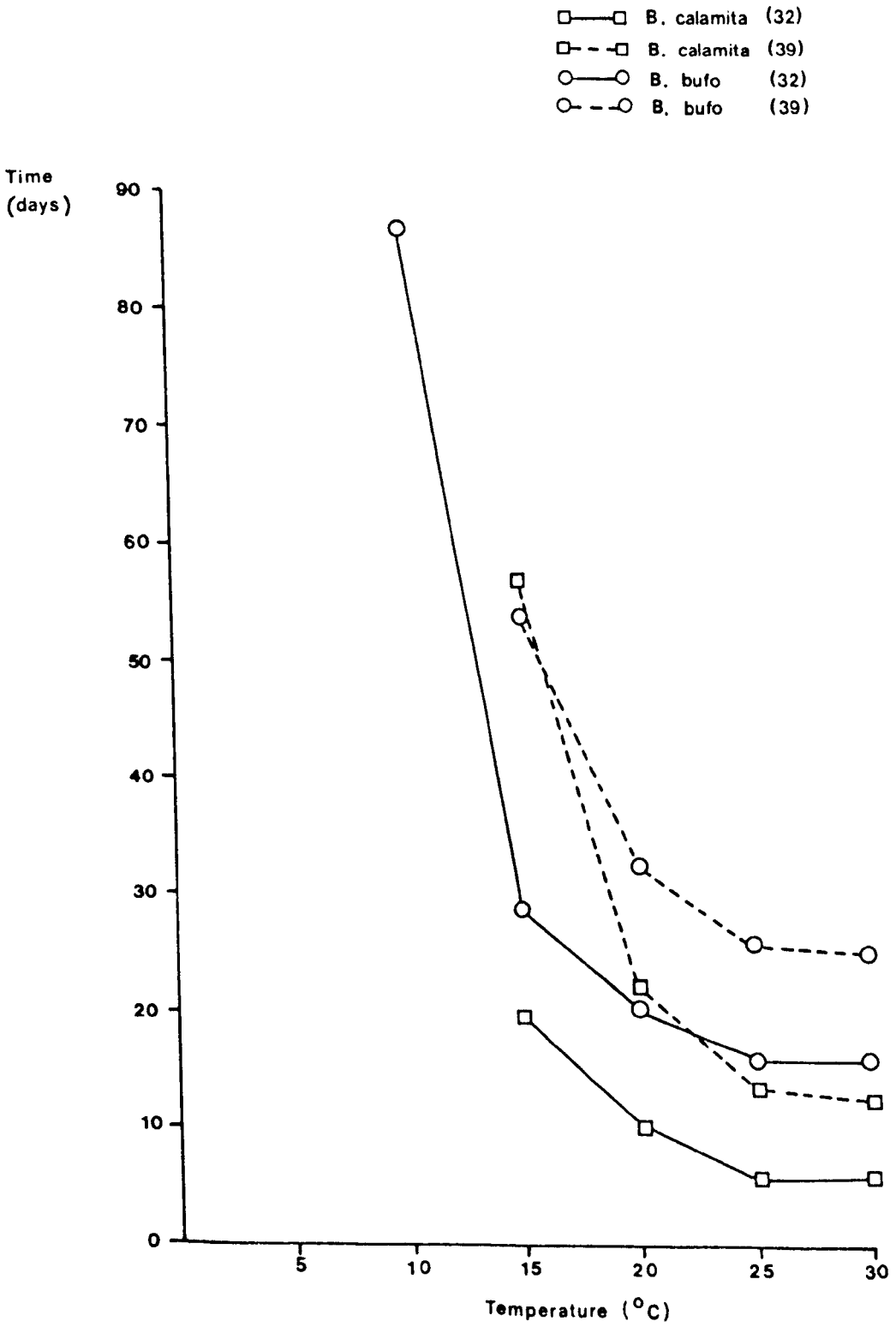

Stage of Development
42
40
38
36
34
32
30
28
26
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
70
Time (days)

Figure. 2.5. Time taken for B. bufo and B. calamita tadpoles to reach the stages of development 32 and 39, at different rearing temperatures. Values obtained from figures 2.3 and 2.4.


B. calamita (32)
B. calamita (39)
B. bufo (32)
B. bufo (39)
Time
(days)
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
Temperature (°C)

be calculated. This is shown in figure 2.6. In B. bufo, there was an overall decrease in duration of each of the stages as temperature increased. Stages 33-35 were passed through rapidly at 20°C when compared to the other temperatures. At 25 and 30°C the stages that were shortened most were 36-38.

The developmental rates of B. calamita at 25 and 30°C were similar, and faster than at 20°C. Most of the increase in rate was due to faster development during the early stages (28-32).

The effect temperature has on developmental stage duration in relation to the total developmental time is shown in figure 2.7. Values were obtained using data from figure 2.6.

As acclimation temperature increased, there was not a clear pattern in the changing proportion of time spent at each developmental stage in B. bufo. Comparing B. bufo tadpoles at 15 and 20°C to those reared at 25 and 30°C, it was seen that generally a lower proportion of total developmental time was spent in the later stages of development at higher acclimation temperatures. B. calamita development exhibited no obvious pattern as acclimation temperature increased from 20-30°C.

Comparing the two species, B. bufo generally spent a greater proportion of its developmental time in the early stages (28-33) and this increased with temperature (54% at 15°C, 62% at 30°C). B. calamita spent less than half of its developmental time at stages 28-33, (46% at 20°C) and this proportion was reduced with increasing temperature (35%

Figure. 2.6. Duration of tadpole developmental stages (stage number shown in boxes) for (A) B. bufo and (B) B. calamita, at different rearing temperatures.

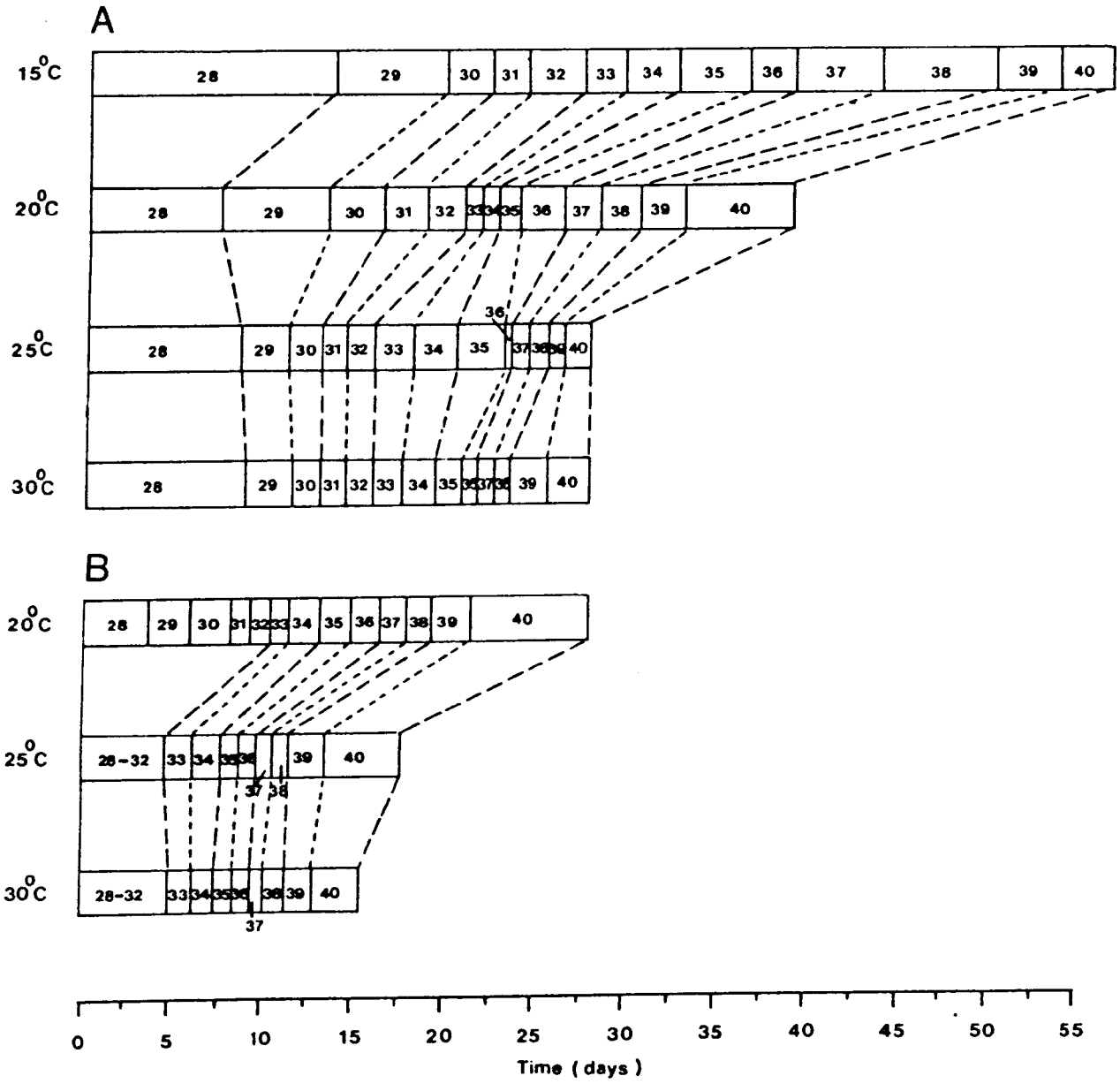
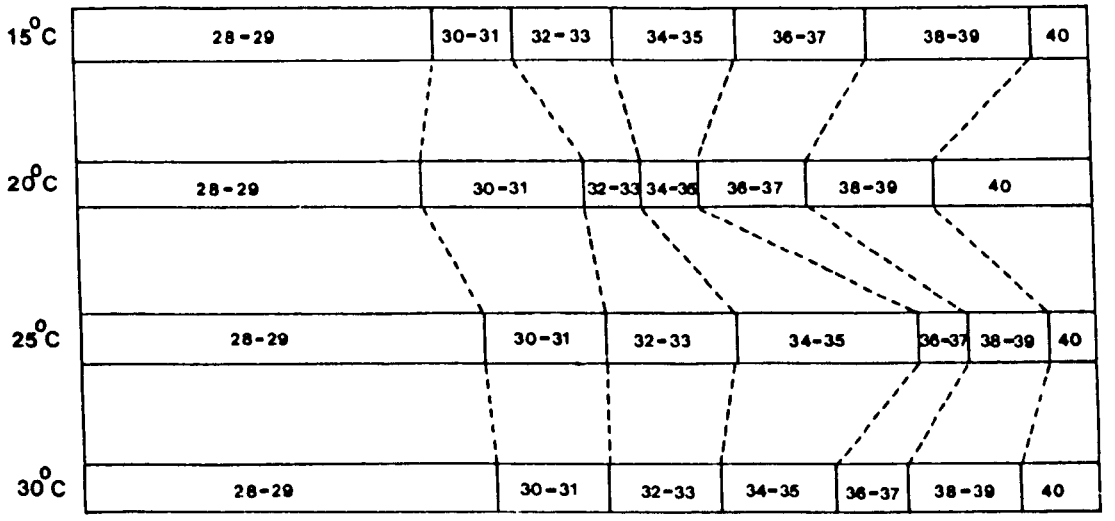
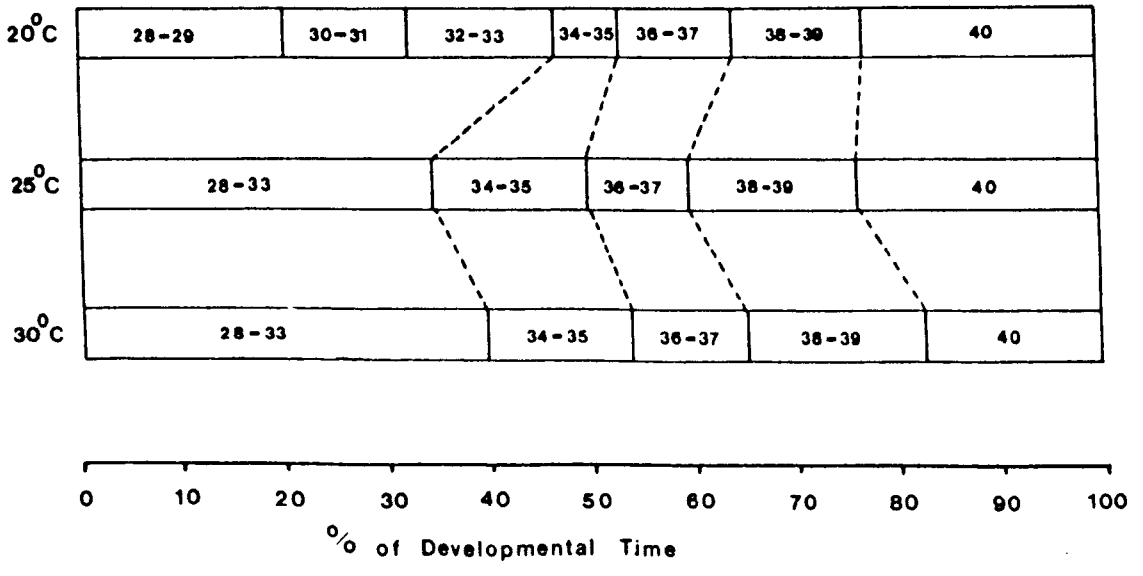

A
15°C
28
29
30
31
32
33
34
35
36
37
38
39
40
20°C
28
29
30
31
32
33
34
35
36
37
38
39
40
25°C
28
29
30
31
32
33
34
35
36
37
38
39
40
30°C
28
29
30
31
32
33
34
35
36
37
38
39
40
B
20°C
28
29
30
31
32
33
34
35
36
37
38
39
40
25°C
28-32
33
34
35
36
37
38
39
40
30°C
28-32
33
34
35
36
37
38
39
40
0
5
10
15
20
25
30
35
40
45
50
55
Time (days)

Figure. 2.7. Proportion of time spent at different stages of development (stage numbers shown in boxes) as a percentage of the total developmental time for tadpoles of (A) B. bufo and (B) B. calamita at a range of rearing temperatures.

A


15°C
28-29
30-31
32-33
34-35
36-37
38-39
40
20°C
28-29
30-31
32-33
34-35
36-37
38-39
40
25°C
28-29
30-31
32-33
34-35
36-37
38-39
40
30°C
28-29
30-31
32-33
34-35
36-37
38-39
40

B


20°C
28-29
30-31
32-33
34-35
36-37
38-39
40
25°C
28-33
34-35
36-37
38-39
40
30°C
28-33
34-35
36-37
38-39
40
0
10
20
30
40
50
60
70
80
90
100
% of Developmental Time

at 25°C and 40% at 30°C). Thus at all acclimation temperatures investigated, B. bufo spent a greater proportion of its developmental time in the early stages of development (28-33). B. calamita appeared to spend the majority of its developmental time in the later stages of development.

2.3.3 Effect of temperature on growth of B. bufo and B. calamita tadpoles.

There was no significant growth of B. bufo at 5°C (Fig. 2.8). At 10°C, B. bufo tadpoles increased in mass until they had all died after 76 days. In B. bufo growth rates at 15-30°C were similar. At temperatures that allow development to metamorphosis the duration of growth was inversely related to temperature. Thus the maximum mass attained was at 15°C.

B. calamita tadpoles did not increase in mass significantly at 5 or 10°C (Fig. 2.9). Growth rates were similar at 20-30°C, the rate at 15°C being significantly lower ($p > 0.05$).

Looking at the time taken to reach a designated mass, the rates of growth can be compared for the two species (Fig. 2.10). The time taken for B. calamita tadpoles to reach 0.08 g was less than B. bufo at the temperatures above 15°C. At 15°C, B. bufo tadpoles took 15 days and B. calamita 16 days. B. calamita took 48 days to reach 0.08 g, at 10°C, 18 days more than B. bufo. At 5°C, B. bufo reached 0.08 g after 70 days, B. calamita tadpoles died before reaching this size.

Figure. 2.8. Effect of temperature on rates of growth in B. bufo tadpoles. (■) 5°C, (●) 10°C, (△) 15°C, (□) 20°C, (▲) 25°C, (○) 30°C. Means ± 95% confidence intervals, n=20 for each point. A point without confidence intervals has no variance about the mean.

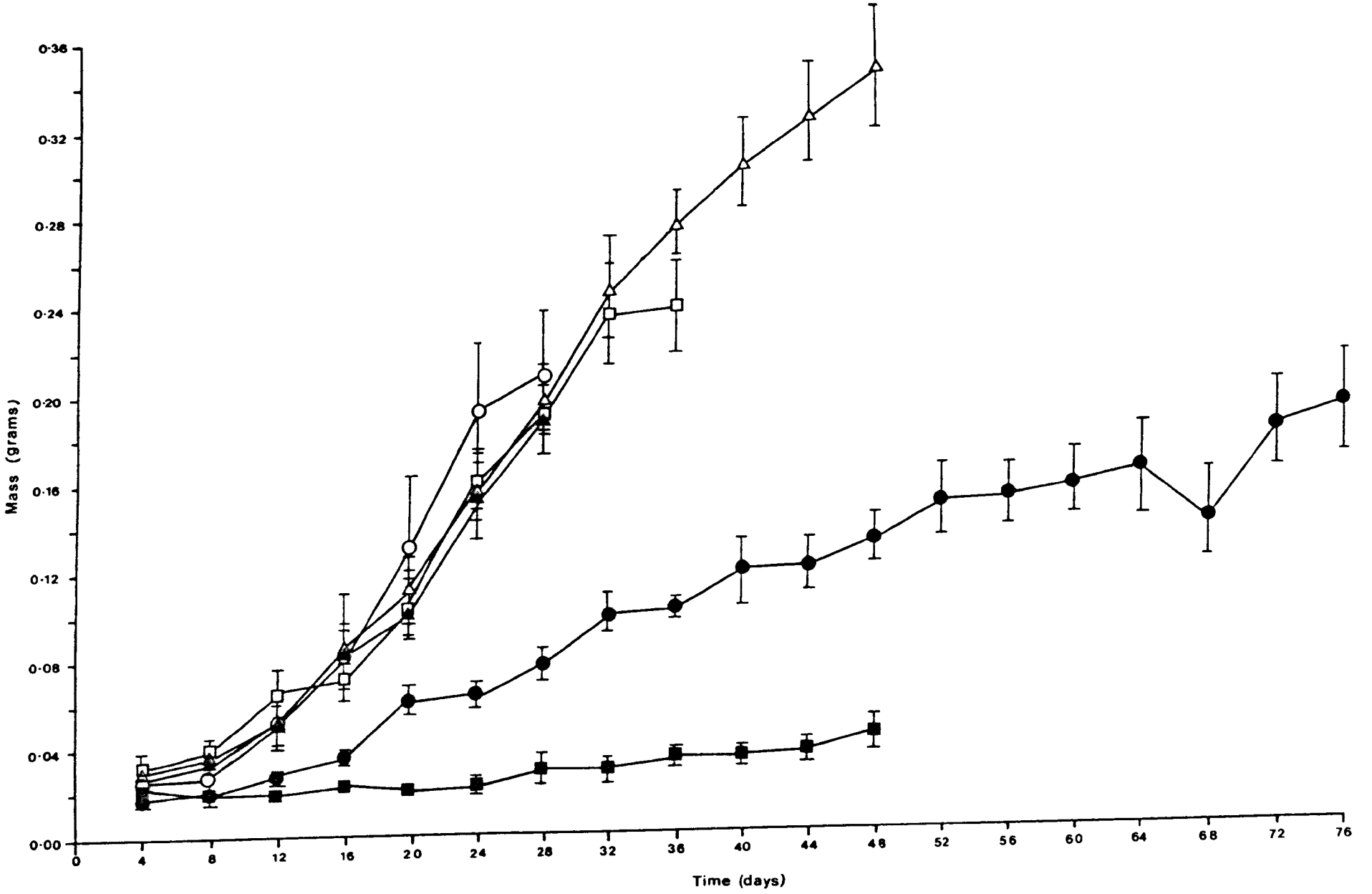

Mass (grams)
0·00
0·04
0·08
0·12
0·16
0·20
0·24
0·28
0·32
0·36
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
76
Time (days)

Figure. 2.9. Effect of temperature on rates of growth in B. calamita tadpoles. (■) 5°C, (●) 10°C, (△) 15°C, (□) 20°C, (▲) 25°C, (○) 30°C. Means ± 95% confidence intervals, n=20 for each point.

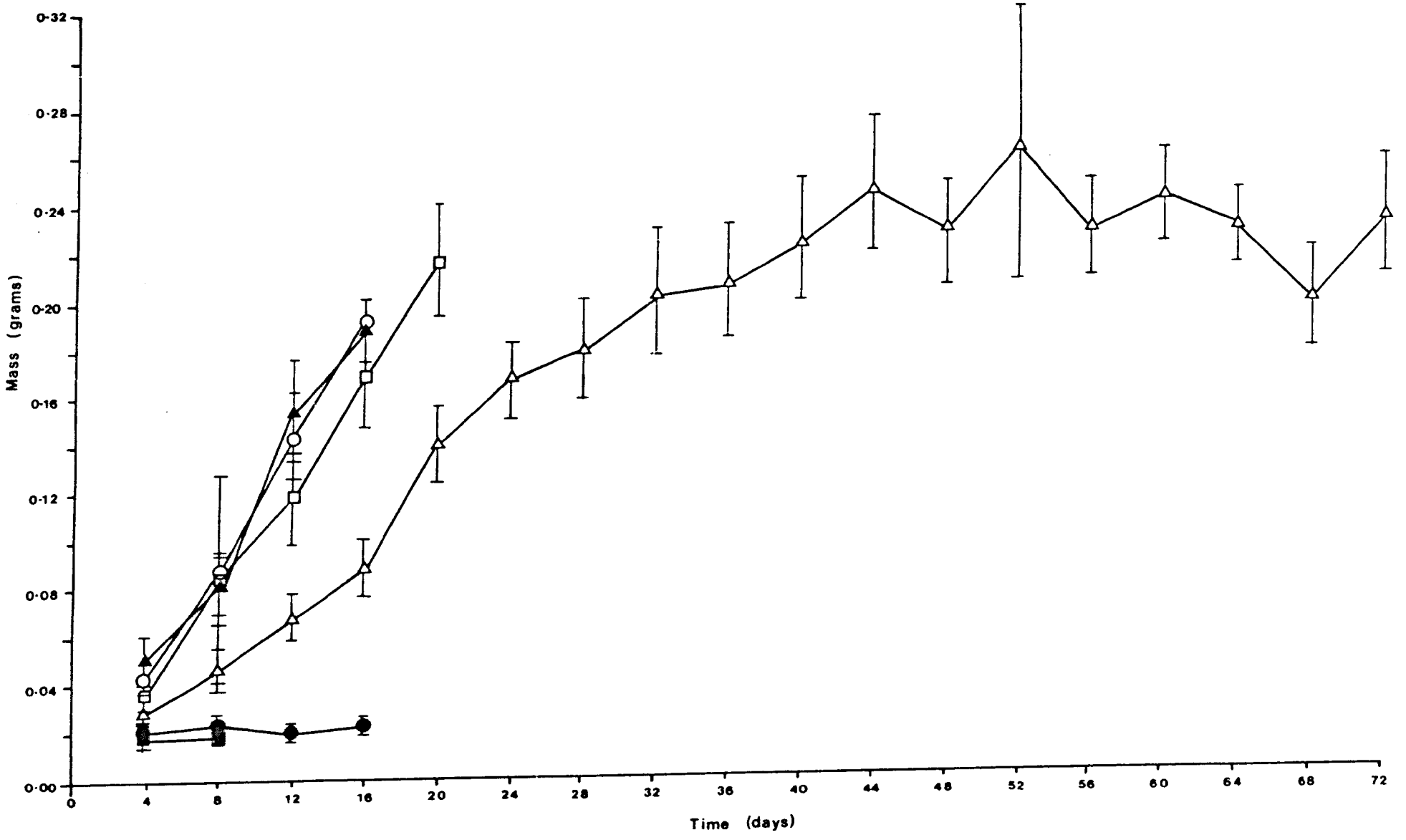
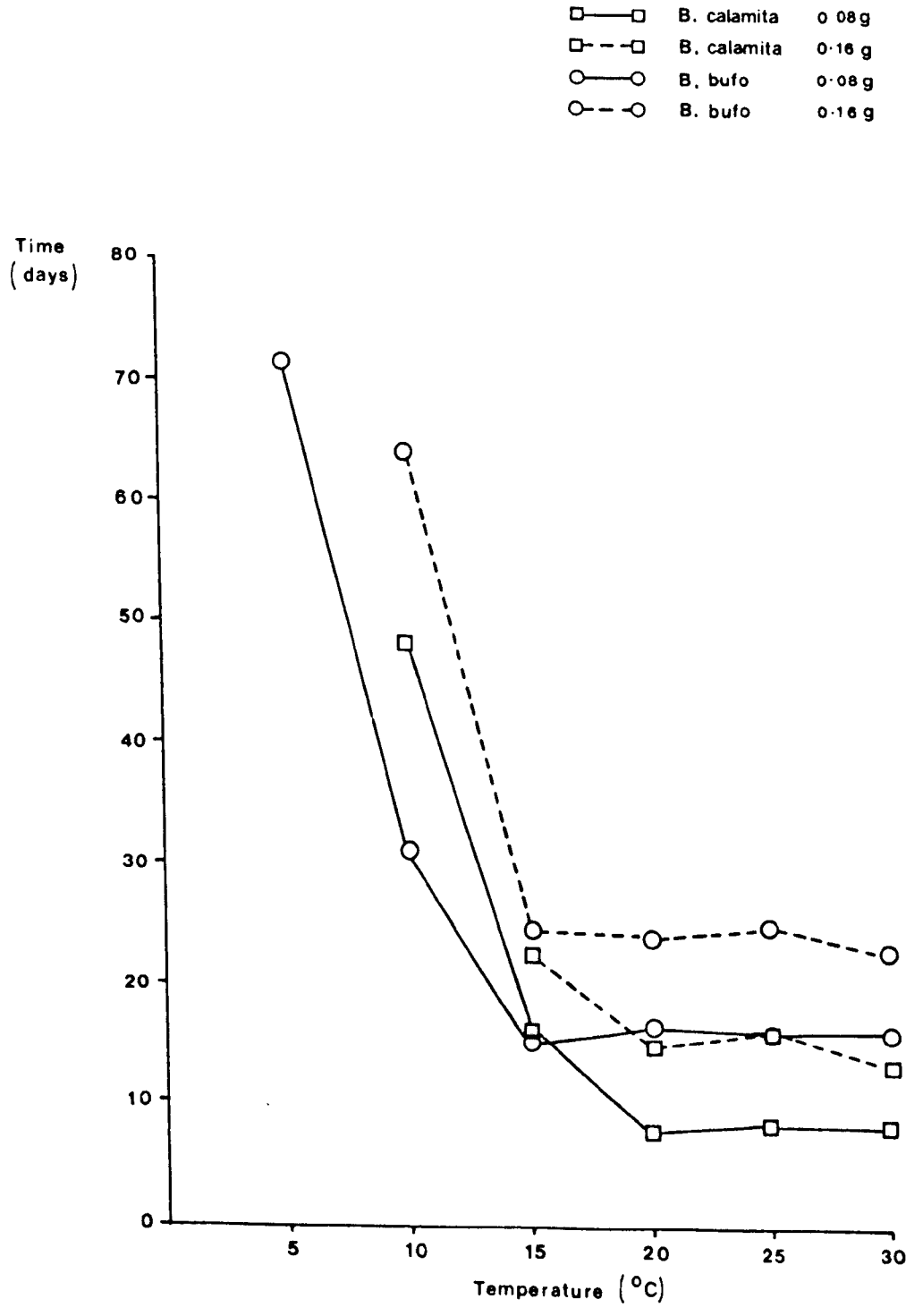

0·32
0·28
0·24
0·20
0·16
0·12
0·08
0·04
0·00
Mass (grams)
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
Time (days)

Figure. 2.10. Time taken for B. bufo and B. calamita tadpoles to attain a mass of 0.08 g and 0.16 g at different rearing temperatures. Values obtained from figures 2.8 and 2.9.


B. calamita 0 08g
B. calamita 0·16 g
B. bufo 0·08 g
B. bufo 0·16 g
Time (days)
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
Temperature (°C)

The time taken to reach 0.16 g was lower for B. calamita at 15-30°C, than for B. bufo. B. bufo attained this mass after 64 days at 10°C. No B. calamita tadpoles reached 0.16 g at 10°C. At 15°C, only two days separated the two species but in the 20-30°C range this difference was extended to approximately 10 days.

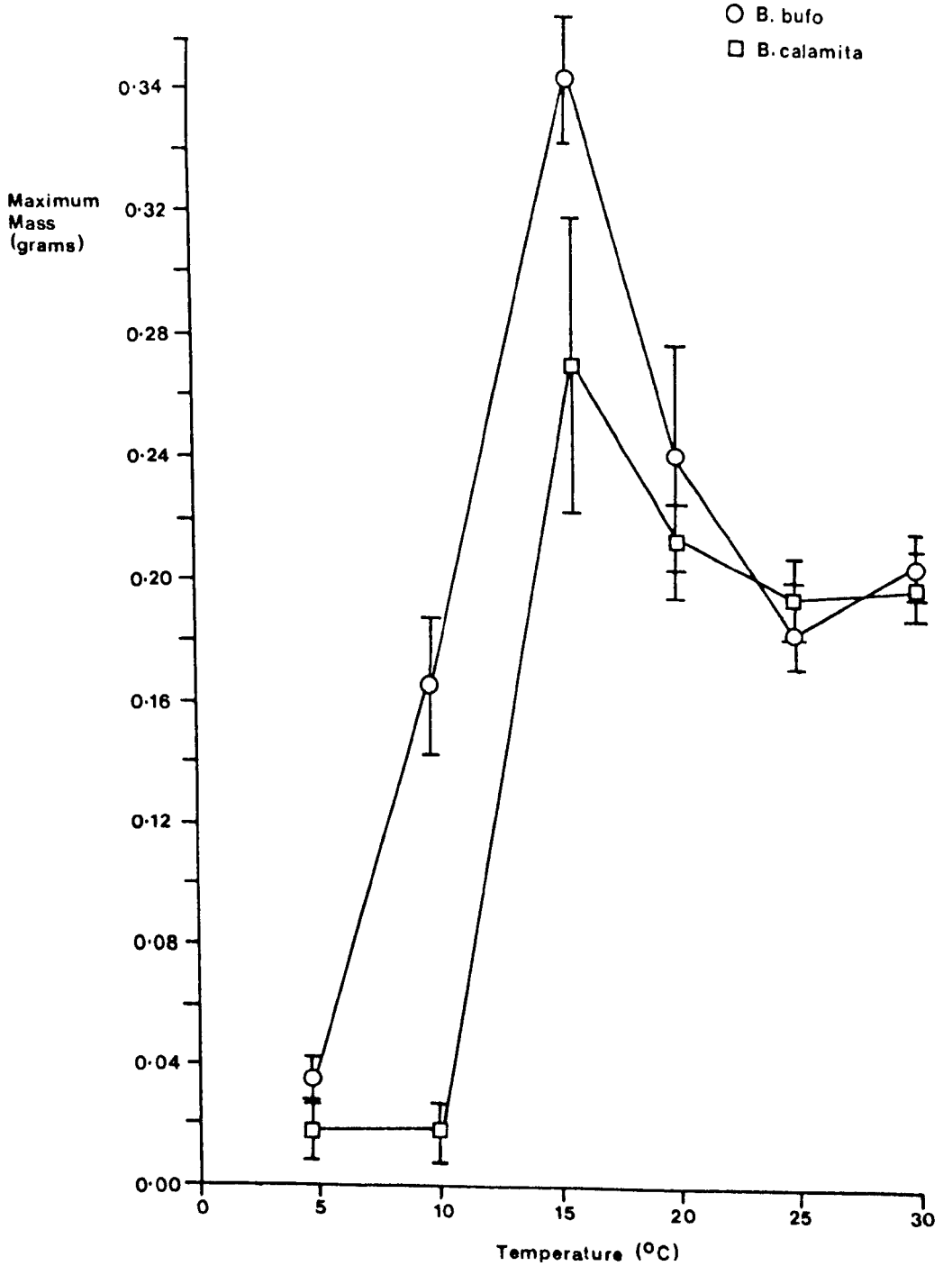
The time taken for B. bufo tadpoles to reach the designated masses (0.08 and 0.16 g) decreased with an increase in temperature from 5-15°C. However, after 15°C, it remained constant up to 30°C. The B. calamita tadpoles showed a similar trend but the time taken to reach 0.08 and 0.16 g continued decreasing until 20°C. Increase in temperatures above 20°C did not further reduce the time taken to attain the designated masses.

The maximum masses obtained by tadpoles at each temperature are shown in figure 2.11. At all temperatures, with the exception of 25°C, B. bufo tadpoles reached greater maximum masses, the highest being attained at 15°C. At the higher temperatures (20-30°C) the maximum masses attained by the two species converged. There was no significant difference between the species at 25 and 30°C ($p < 0.05$). At 25°C B. calamita tadpoles reached a greater mass than B. bufo.

2.3.4 Effect of temperature on tadpole mass at different developmental stages

The mass of B. bufo tadpoles remained unchanged as they developed at 5

Figure. 2.11. Maximum mean mass attained by B. bufo and B. calamita tadpoles at reared at different temperatures. Means ± 95% confidence intervals, n=20 for each point.


O B. bufo
□ B. calamita
Maximum Mass (grams)
0·34
0·32
0·28
0·24
0·20
0·16
0·12
0·08
0·04
0·00
0
5
10
15
20
25
30
Temperature (°C)

and 10°C (Fig. 2.12). Tadpoles at 15°C achieved a greater mass through stages 29-41 than those at 20-30°C. In general, as rearing temperature increased the mass of a tadpole at a particular stage decreased.

The greatest masses attained in B. calamita during early development were by tadpoles at 10°C (Fig. 2.13). At this temperature, development ceased at stage 30. The greatest mass attained was again at 15°C, 0.25 g at stage 40. This compared with B. bufo at the same stage and temperature weighing 0.32 g. At the lower temperatures (15 and 20°C), the mass of tadpoles decreased from stage 38-40. At 25 and 30°C, mass increased with stage.

2.3.5 Effect of temperature on time taken for tadpoles to reach metamorphosis.

In B. bufo tadpoles, (Fig. 2.14) the period across which all tadpoles reached metamorphosis at each temperature was similar, being 13, 14, 16 and 15 days at 15, 20, 25 and 30°C respectively. Although the duration of the period in which metamorphosis was achieved was similar at each temperature the mean time taken to reach metamorphosis varied considerably with temperature.

At higher temperatures metamorphosis was achieved after a shorter time period with means of 29.8 days at 30°C, 32.9 days at 20°C, 40.3 days at 20°C and 71.3 days at 15°C.

Figure. 2.12. Mean mass of individual developmental stages of B. bufo reared at (■) 5°, (●) 10°C, (△) 15°C, (□) 20°C, (▲) 25°C, (○) 30°C. Sample size = 20 for each point.

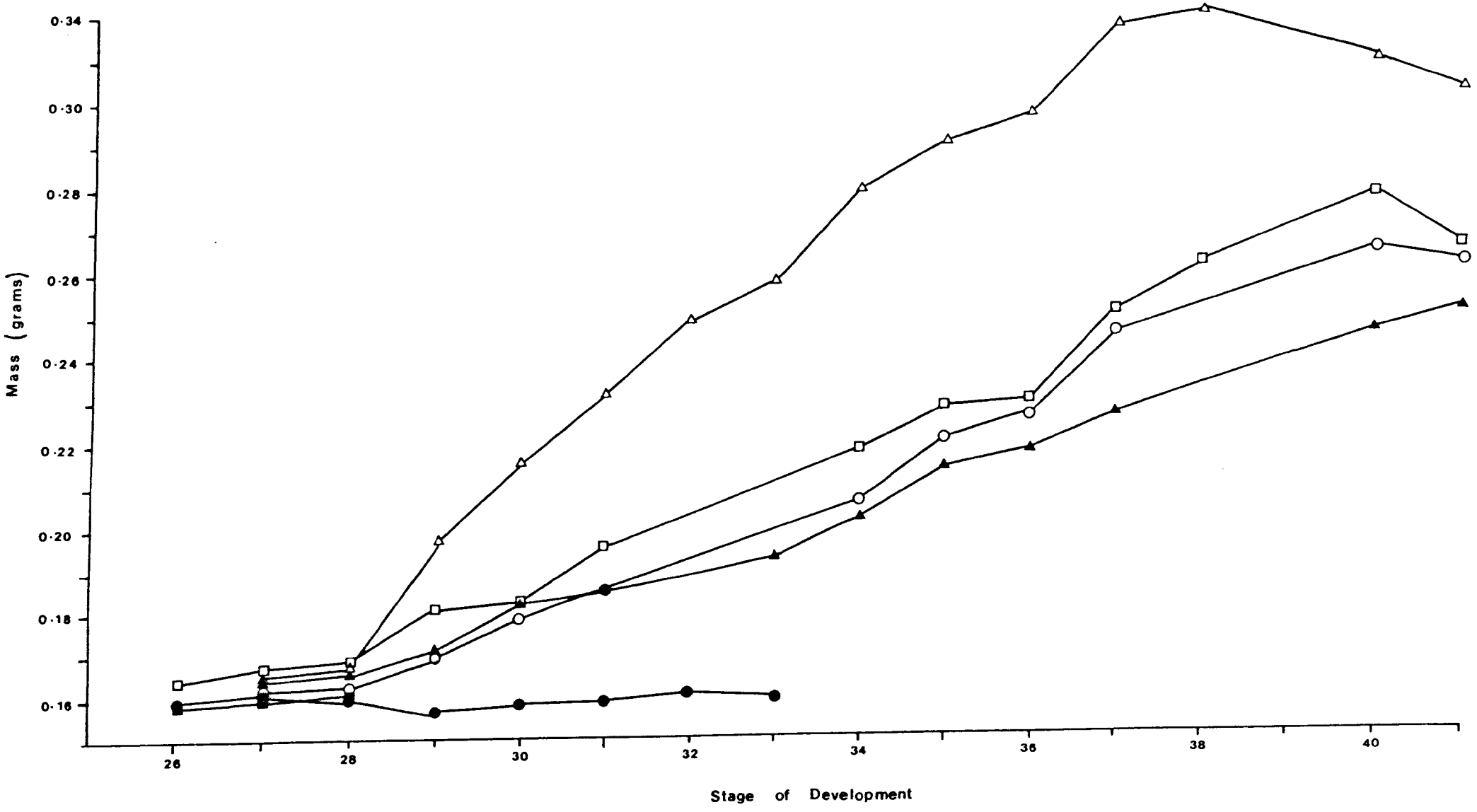

0·34
0·30
0·28
0·26
0·24
0·22
0·20
0·18
0·16
Mass (grams)
26
28
30
32
34
36
38
40
Stage of Development

Figure. 2.13. Mean mass of individual developmental stages of B. calamita reared at (■) 5°C, (●) 10°C, (△) 15°C, (□) 20°C, (▲) 25°C, (○) 30°C. Sample size = 20 for each point.

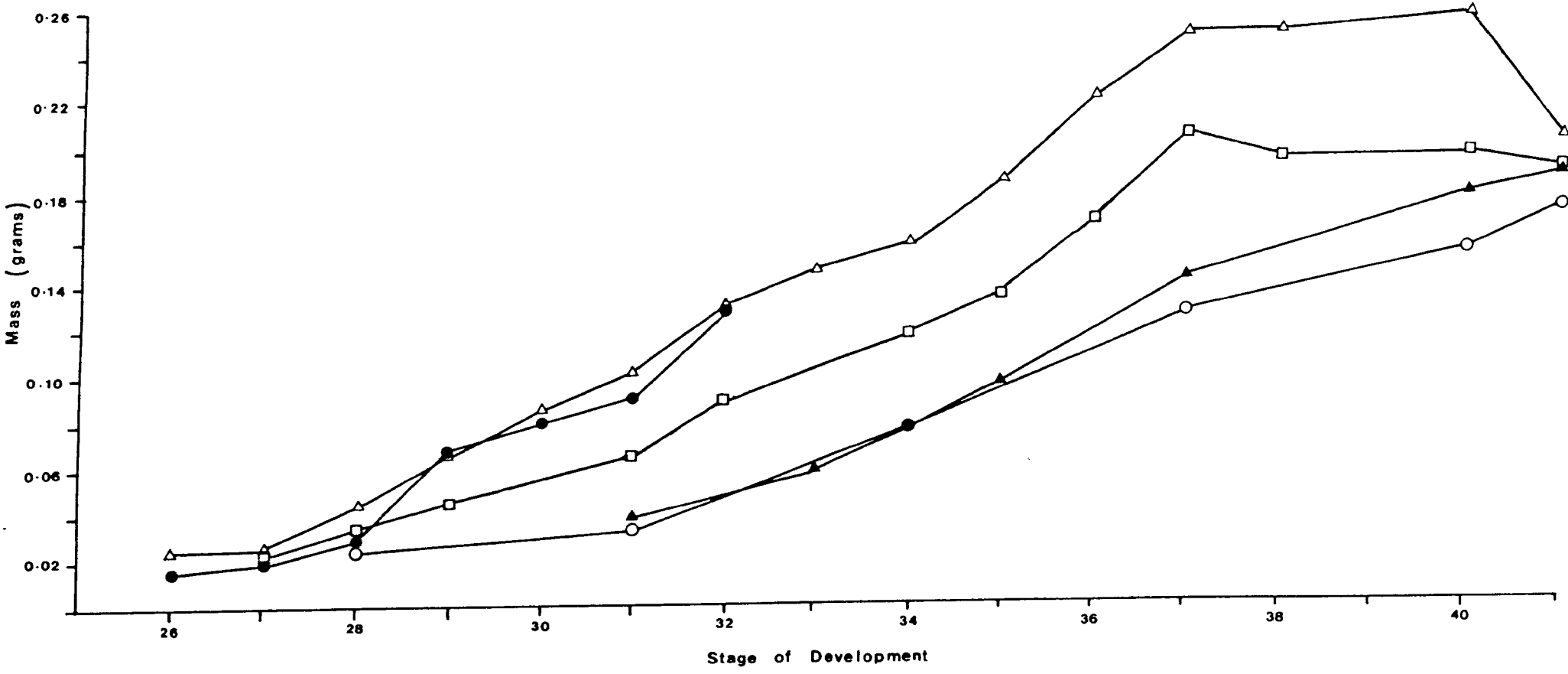
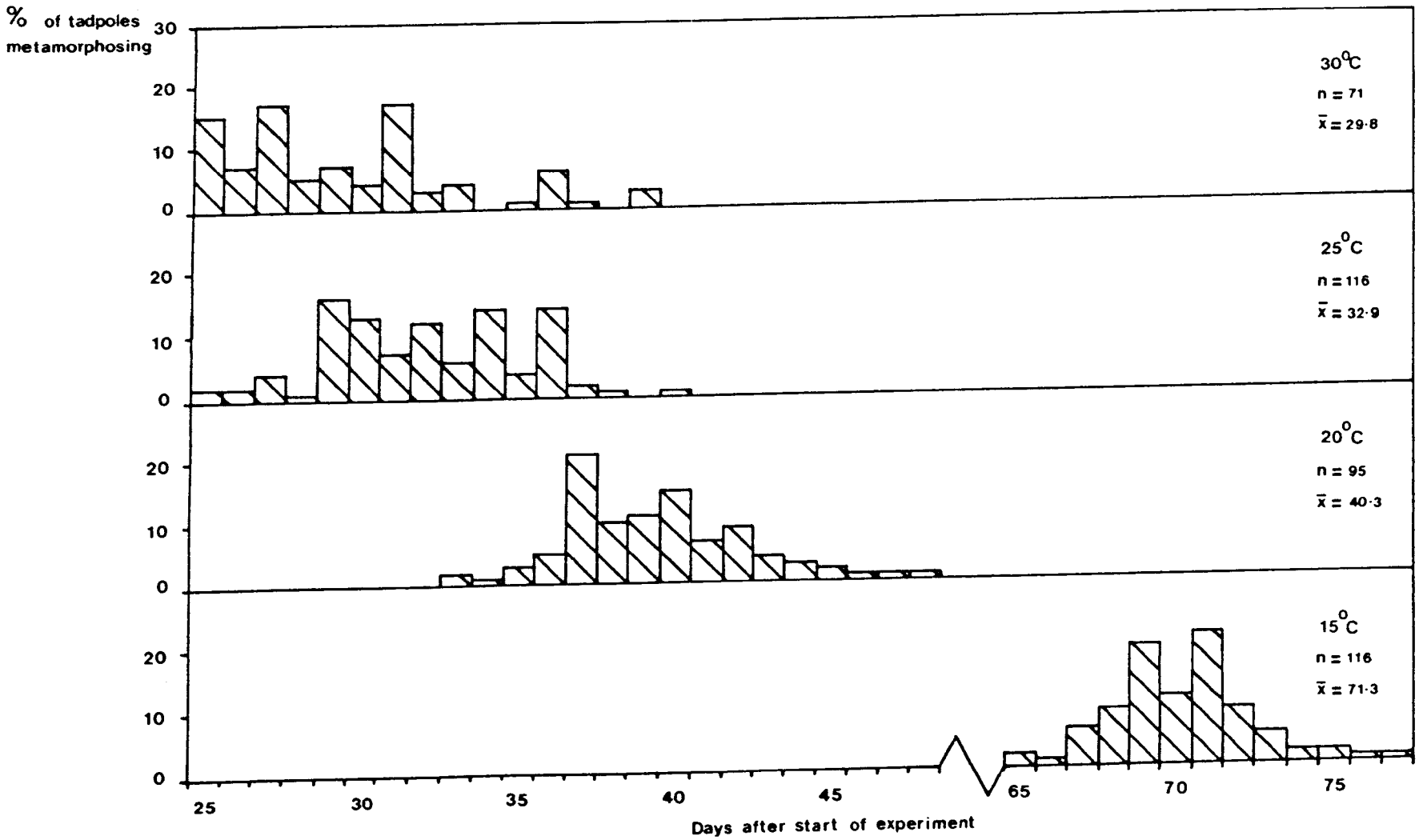

0·26
0·22
0·18
0·14
0·10
0·06
0·02
Mass (grams)
26
28
30
32
34
36
38
40
Stage of Development

Figure. 2.14. Time taken for B. bufo tadpoles to reach metamorphosis when reared at 15-30°C. % metamorphosis represents the proportion of total number of tadpoles successfully metamorphosing at each temperature.


% of tadpoles metamorphosing
30
20
10
0
30°C
n = 71
x̄ = 29·8
25°C
n = 116
x̄ = 32·9
20°C
n = 95
x̄ = 40·3
15°C
n = 116
x̄ = 71·3
25
30
35
40
45
65
70
75
Days after start of experiment

The time period across which all B. calamita tadpoles reached metamorphosis at 30 and 25°C was 16 and 14 days respectively (Fig. 2.15). At 20°C the period was 20 days. No successful metamorphosis took place at 15°C. The mean time to reach metamorphosis at 20-30°C was much less than in B. bufo: 15.9, 17.8 and 29.8 days at 30, 25 and 20°C. This is 13.9, 15.1 and 10.8 days faster respectively. At 25 and 30°C the duration of the population's metamorphosis was similar for both species. At 20°C, B. calamita metamorphosis was spread over 20 days compared to 16 days in B. bufo.

2.3.6 Effect of temperature on survival of B.bufo and B. calamita tadpoles to metamorphosis.

Survival to metamorphosis at 30°C, was more successful in B. calamita (Table 2.1), 79.2% (n=120) of tadpoles metamorphosing compared to 59.2% (n=120) in B. bufo. B. bufo was more successful at 25°C than B. calamita, 96.7% (n=120) compared to 78.3% (n=120). Both species had similar success at 20°C, 79.2% (n=120). At 15°C, 96.7% (n=120) of B. bufo tadpoles reached metamorphosis. The B. calamita tadpoles reared at 15°C resulted in only 2 individuals (1.7% n=120) reaching metamorphosis. These did not survive to the toadlet stage (stage 46).

Figure. 2.15. Time taken for B. calamita tadpoles to reach metamorphosis when reared at 15-30°C. % metamorphosis represents the proportion of total number of tadpoles successfully metamorphosing at each temperature.

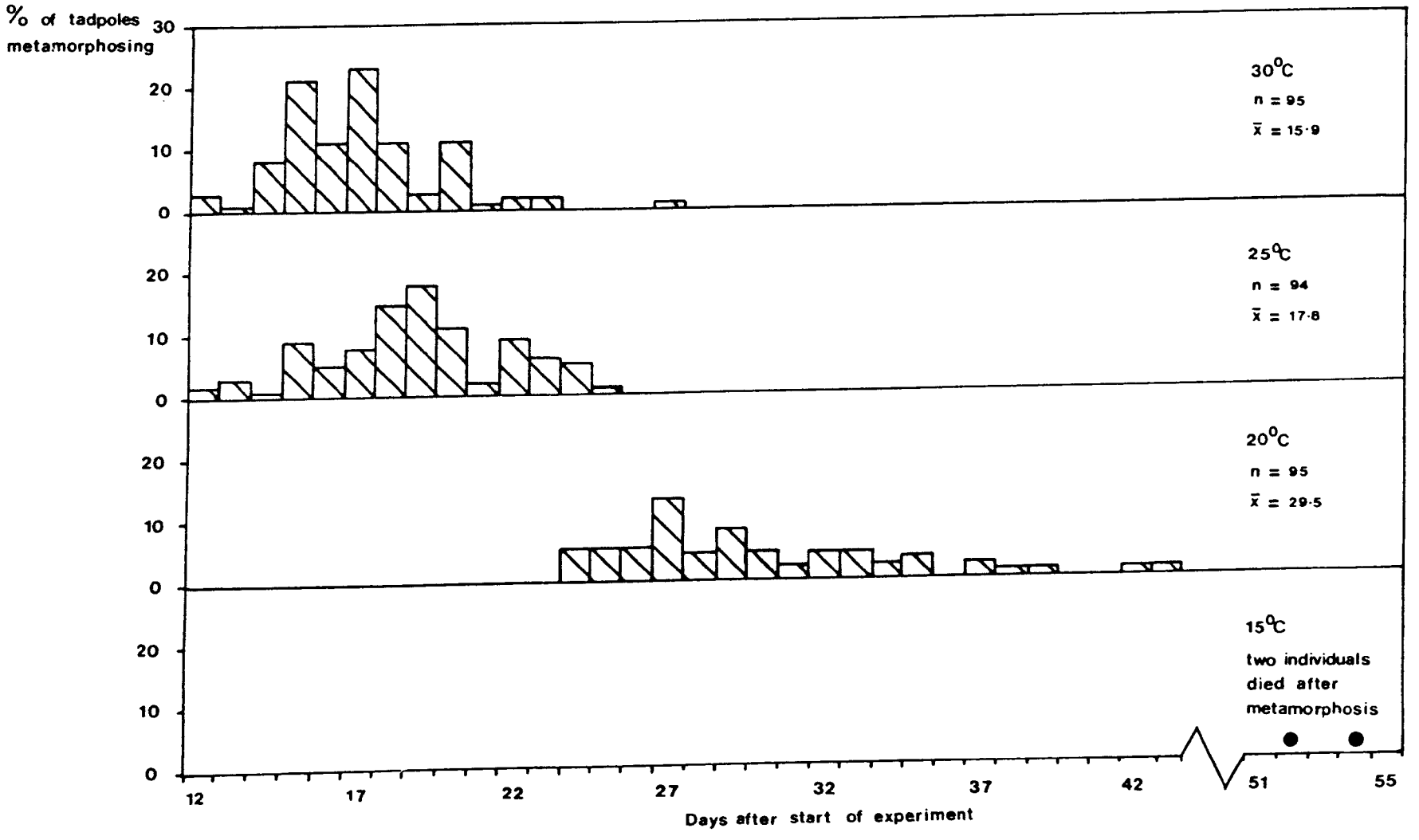

% of tadpoles metamorphosing
30
20
10
0
30°C
n = 95
x̄ = 15·9
25°C
n = 94
x̄ = 17·8
20°C
n = 95
x̄ = 29·5
15°C
two individuals died after metamorphosis
12
17
22
27
32
37
42
51
55
Days after start of experiment

Table 2.1. The effect of temperature on % of B. bufo and B. calamita tadpoles reaching metamorphosis (n= 120 at each temperature).

Temperature °C	5	10	15	20	25	30
Bufo bufo	0.0	0.0	96.7	79.2	96.7	59.2
Bufo calamita	0.0	0.0	*1.7	79.2	78.3	79.2

* Toadlets of B. calamita reared at 15°C died shortly after reaching metamorphosis.

2.4. DISCUSSION

2.4.1 The effect of temperature on development and growth in B. bufo and B. calamita spawn and tadpoles.

Previous investigations into temperature effects on tadpole development have shown that interspecific differences are simple e.g. one species develops faster than another across a wide temperature range (Moore, 1939). This is not the case with B. bufo and B. calamita. There appears to be a 'cross-over' point between the two species at 15-20°C. B. bufo spawn develops faster below 20°C and tadpoles below 15°C, B. calamita having faster rates above these temperatures.

B. calamita is unable to metamorphose at temperatures of 15°C and below. At temperatures above this, metamorphosis is achieved earlier than in B. bufo tadpoles reared under the same conditions. In both the species, a higher rearing temperature decreases the mean time to metamorphosis without apparently changing the total population's metamorphic spread. Thus, the whole population metamorphoses faster or slower at reduced temperatures. B. calamita reaches metamorphosis earlier than B. bufo at temperatures greater than or equal to 20°C.

Amphibians that spawn in temporary water bodies must, of necessity, have short larval periods if they are to metamorphose before the water disappears (Newman, 1987; Nicholson, 1954; Trowbridge and Trowbridge, 1937). The increased rates of development at higher temperatures seen in B. calamita spawn and tadpoles is probably an adaptation to the

length of time that its ephemeral breeding pools are available. Davis (1985) recorded the mean temperatures of water in a typical B. calamita breeding pool, a dune slack in the north Merseyside sand dune system. He recorded the May, June and July, 1982 temperatures (at 10 cm depth) as 18.4 ± 8.2, 18.5 ± 6.6 and 19.1 ± 5.6°C respectively. It is during these months that B. calamita is spawning and tadpoles are developing in the pools (Smith, 1951). The mean temperatures did not drop below 18°C. At this temperature B. calamita tadpoles would have a faster rate of development than B. bufo and would reach metamorphosis more quickly.

The north Merseyside sand dune system is towards the northern limit and therefore coolest part of B. calamita's range. Temperatures in more southerly areas inhabited by B. calamita would probably be greater. Davis (1985) recorded the temperatures at 10 cm. Temperatures in the shallow pond margins may be up to 30°C (Beebee, 1979). B. calamita tadpoles tend to aggregate in shallow areas of breeding pools (Beebee, 1983). Thus their developmental rates will be increased, affording them a greater chance of survival.

B. bufo breeding ponds are typically deeper and not ephemeral (Chapter 1). The mean water temperatures would therefore be lower than those found in shallow pools. The tadpoles can develop at lower temperatures than B. calamita and, as they do not have to contend with a desiccating water body, they can afford to have longer developmental times.

B. bufo spawn and tadpoles can develop at lower temperatures than

B. calamita. This could explain why B. bufo are able to breed earlier in the year than B. calamita. An earlier spawning date should allow longer developmental period prior to metamorphosis and hence larger toadlets at emergence (Morin, 1987). Spawning early in a season could also confer a competitive advantage over species that subsequently spawn in the same pond (Morin, 1987).

It is important to consider the effect of temperature on spawn and tadpoles separately. The breeding adults select the site of spawn deposition and hence the environmental conditions to which the spawn will be subjected during development. B. calamita spawn is usually laid on open, sandy pool bottoms 8-15 cm deep, with no attempt to intertwine it or conceal it (Frazer, 1983). B. bufo toads in the same area typically use deeper water, their spawn being entangled around aquatic plants at an average depth of 21-22 cm (Beebee, 1983).

By spawning in shallow areas B. calamita enhances the spawn developmental rates in comparison with B. bufo. Dark coloured pigments in the ova of the spawn may also act as a heat absorber (Douglas, 1948). Thus spawn deposited in the open (B. calamita) would experience greater temperatures than spawn within and therefore shaded by pond vegetation.

B. calamita spawn develops at a greater rate at temperatures above 20°C than B. bufo. Again, this is probably an adaptation to an ephemeral environment. The higher the temperature of the water surrounding the spawn string the greater the chance of spawn desiccation due to pond

drying. A faster developing embryo in these conditions will have a greater chance of survival. The time that the spawn will be exposed to predators is also reduced at greater temperatures.

Once the spawn has hatched, the tadpoles can move away from the areas of the pool that are drying. Tadpoles can select the areas in the pond which afford them the best conditions for growth.

In both species, as rate of development increased with temperature there was a corresponding reduction in tadpole size (mass). This is probably a result of conflicting demands between maintenance metabolism and growth. As temperature increases so does metabolic rate (Chapter 6). The energy required to maintain metabolic rates therefore increases with temperature and thus proportionately less energy is available for growth.

Temperatures above 25°C appear to offer no advantage in increased developmental rates in B. bufo and B. calamita but the energetic cost of maintenance metabolism will be greater (see Chapter 4). In B. calamita, this results in a smaller metamorphic size at 30°C, after the same period of development as tadpoles reared at 25°C. Thus, it appears that there is no advantage for the tadpole to develop at 30°C. It would appear that the optimum temperature for development and growth is 25°C.

Anuran tadpoles show preference for certain temperatures when placed in a thermal gradient. The temperature selected is known as the preferred

body temperature (PBT) and has been demonstrated in many anurans (DeVlaming and Bary, 1970; Lucas and Reynolds, 1967). Workers studying other lower vertebrates have found that PBTs are similar within a genus (Bogert, 1949; Huey and Slatkin, 1976; Licht et al, 1966; Spellerberg, 1972). Beiswenger (1978) found that B. boreas and B. americanus aggregated in a temperature range of 28-34°C and 27.5-37°C respectively. Davis (1985) determined PBT for B. bufo and B. calamita, they were 25 and 24°C respectively (tadpoles acclimated at 20°C). It would appear that the tadpoles can select a temperature in a thermally varying environment which will produce the fastest developmental rates with least expense of tadpole mass. This is of importance in metamorphosis and will be discussed in chapter 3.

Differences between laboratory gradients of temperature and natural conditions are substantial. Other factors may override temperature choice in a natural environment e.g. foraging or escape behaviour (Davis, 1985) and aggregative behaviour, raising temperature above that of its surroundings (Bragg, 1965; Brattstrom and Warren, 1955). This means that even with similar PBTs, B. bufo and B. calamita might not be able to maintain that temperature due to the interaction of many environmental variables.

CHAPTER 3

3.0 The effect of rearing temperature on mass and energy changes during B. bufo and B. calamita metamorphosis.

3.1 INTRODUCTION

During metamorphosis, major changes occur in both structure and physiology of tadpoles. These have been categorised by Dodd and Dodd (1976): a) Regression of structures and functions that are significant only to tadpoles. b) Transformation of larval structures into those suitable for adult life. c) Development of *de novo* structures. In order to proceed through these stages of metamorphosis, energy is required.

Tadpoles do not feed during metamorphosis. Therefore the only energy available to sustain the tadpole through this period will be that found in the premetamorphic tadpole. An environmental factor that affects the premetamorphic tadpole body size will probably affect the energy content of that tadpole and hence the energy content of the resulting toadlet.

Pandian and Marian (1985a) investigated energy costs of metamorphosis in *Rana tigrina* and concluded that a greater accumulation of body size (and hence energy) in a terminal tadpole is advantageous as it enhances metamorphic efficiency. Temperature has an effect on growth and developmental rates of tadpoles. It therefore influences the size of a

tadpole at metamorphosis (see chapter 2). In general, mass at metamorphic climax is inversely related to rearing temperature and it therefore follows that energy content is inversely proportional to temperature of rearing (Collins, 1979; Pandian and Marian, 1985a; 1985b).

Wilbur and Collins (1973) suggest that the size of a tadpole at metamorphosis has upper and lower limits set by the uncertainty of the larval environment, risk of predation and survivorship of the juvenile on land. B. calamita is a species that normally spawns in a temporary environment (Beebee, 1983) and has faster larval development than B. bufo (see chapter 2). If the Wilbur and Collins hypothesis is true for these species, the size of B. bufo and B. calamita at metamorphosis will probably differ. Wilbur and Collins (1973) also suggest that energy accumulation may be independent of growth and differentiation. Thus differences seen in growth and developmental rates might not truly reflect differences in energy accumulation in the tadpoles.

In this chapter the affect of temperature on mass and energy accumulation at metamorphosis will be examined. The affect this has on the resulting toadlets mass and energy content will also be investigated.

3.2 MATERIALS AND METHODS

3.2.1 Spawn collection, rearing and weighing.

Spawn collection and initial rearing was as described in section 2.2.2. Tadpoles were reared as in section 2.2.3, tadpoles and toadlets being weighed as in section 2.2.4.

3.2.2 Calorimetry

Ten tadpoles were removed from each of the rearing treatments on reaching stage 40 (premetamorphic tadpoles). Ten toadlets were removed from their rearing treatments at stage 46 (fully metamorphosed). Tadpoles and toadlets of B. bufo were taken from the rearing temperatures 15-30°C and those of B. calamita from 20-30°C, these being the temperature ranges at which metamorphosis was reached in both species (see chapter 2).

The wet masses of individuals were measured as in section 1.4. The tadpoles and toadlets were then dried individually in universal tubes in a standard oven at 80°C (Paine, 1971) for at least 48 hours until constant mass. The dried individuals were then placed in foil envelopes and stored in a desiccator over silica gel.

For determination of calorific value the dry, individual tadpole/toadlet remains were weighed to the nearest 0.0001 g on a top pan balance. They were then transferred to a tissue homogeniser

(GALLENKAMP TKW-300-03T) with an equivalent mass (± 0.0005 g) of thermochemical standard benzoic acid. The tadpole/toadlet remains and the benzoic acid were then ground to a fine powder and transferred to a pellet former. The pellet was weighed (pellet masses were in the range 0.010 ± 0.004 g) and the calorific value obtained using a Phillipson microbomb calorimeter (Phillipson, 1964) connected to a chart recorder. The operation of the calorimeter, calibration and subsequent calculation of energy values are described in the Gentry instruments operation manual, Gentry instruments inc., 1007 Owens Street, Aiken, S.C., USA.

Percentage ash content was determined by burning 4 B. bufo tadpoles and 4 toadlets and 3 B. calamita tadpoles and 3 toadlets, individuals being from each of the rearing regimes. The burning took place in a muffle furnace at 550°C for 4 hours (Reiners and Reiners, 1972; Crump, 1981). The mean value obtained was 12.987% (± 0.7%, 95% confidence interval), this was used to convert KJ g^{-1} to ash-free mass specific energy content (KJ g^{-1} ash-free).

3.3. RESULTS

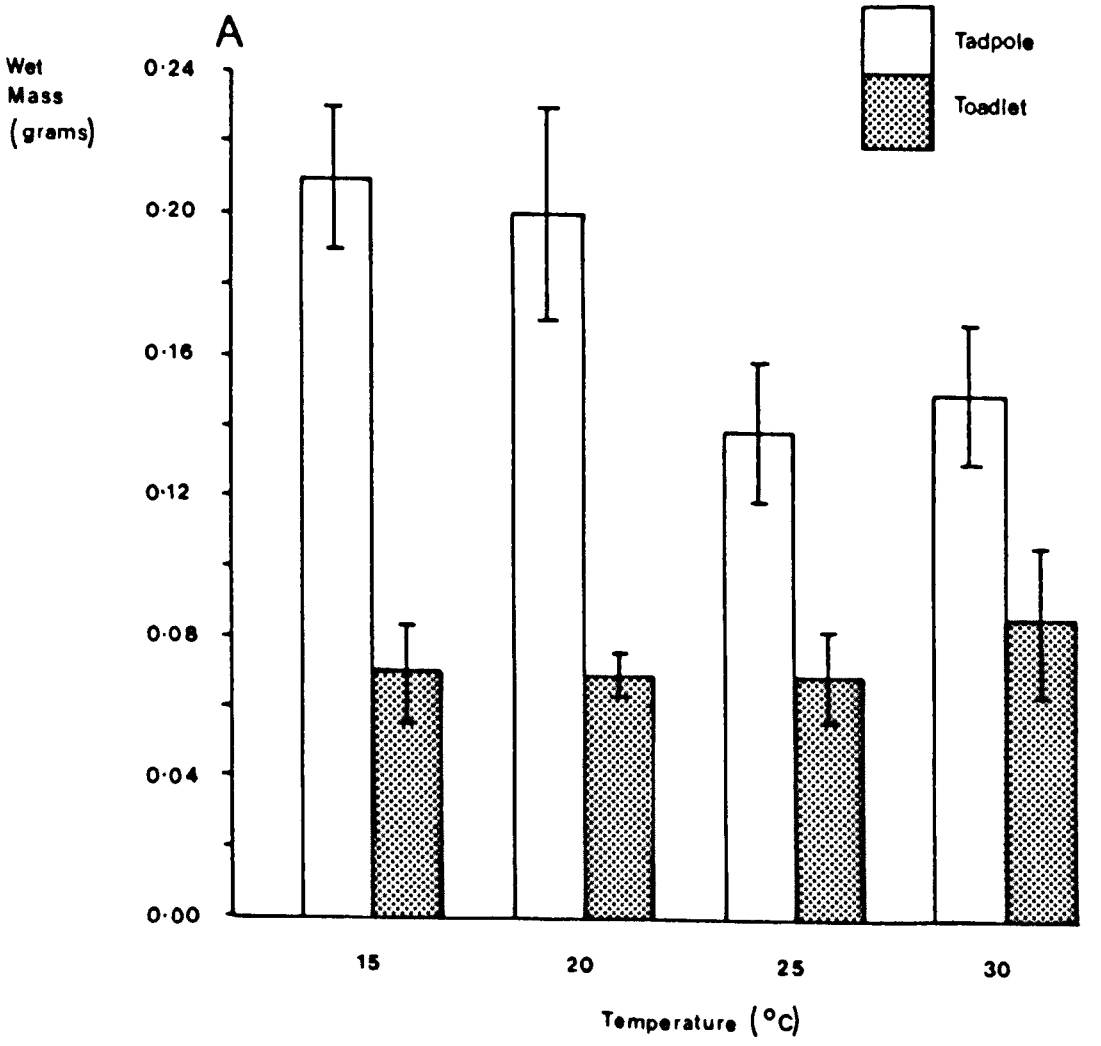
3.3.1. Wet masses of tadpoles and toadlets.

The highest premetamorphic wet mass was attained by B. bufo at 15°C (Fig. 3.1) , 0.21 g. The values for temperatures 20-30°C were 0.20, 0.138 and 0.15 g respectively. Masses at 15 and 20°C were significantly different from those at 25 and 30°C ($p > 0.05$). The wet masses of B. bufo toadlets were not significantly different at the four rearing temperatures ($p > 0.05$). The mean B. bufo toadlet mass was significantly lower than the mean mass of the corresponding premetamorphic tadpoles ($p > 0.05$) for each rearing temperature.

There was no successful metamorphosis at 15°C in B. calamita. The greatest premetamorphic mass was 0.157 g attained at 20°C. This was significantly larger than the 0.113 and 0.100 g achieved at 25 and 30°C respectively ($p > 0.05$). The wet masses of B. calamita toadlets decreased with an increase in temperature. At 20°C the wet mass was 0.068 g. This was higher than at 25°C (0.051 g) and significantly higher than the value obtained at 30°C , 0.039 g ($p > 0.05$).

In the temperature range investigated the wet masses of B. bufo tadpoles were all significantly greater ($p > 0.05$) than the corresponding B. calamita tadpoles (Table 3.1). There was no significant difference between the two species' toadlet wet mass at 20°C ($p > 0.05$) but wet masses at 25 and 30°C were significantly different ($p > 0.05$).

Figure. 3.1. Wet mass of premetamorphic tadpoles and postmetamorphic toadlets of (A) B. bufo and (B) B. calamita reared at 15-30°C. Means ± 95 % confidence intervals, n=10.


A
Wet
Mass
(grams)
0·24
0·20
0·16
0·12
0·08
0·04
0·00
Tadpole
Toadlet
15
20
25
30
Temperature (°C)

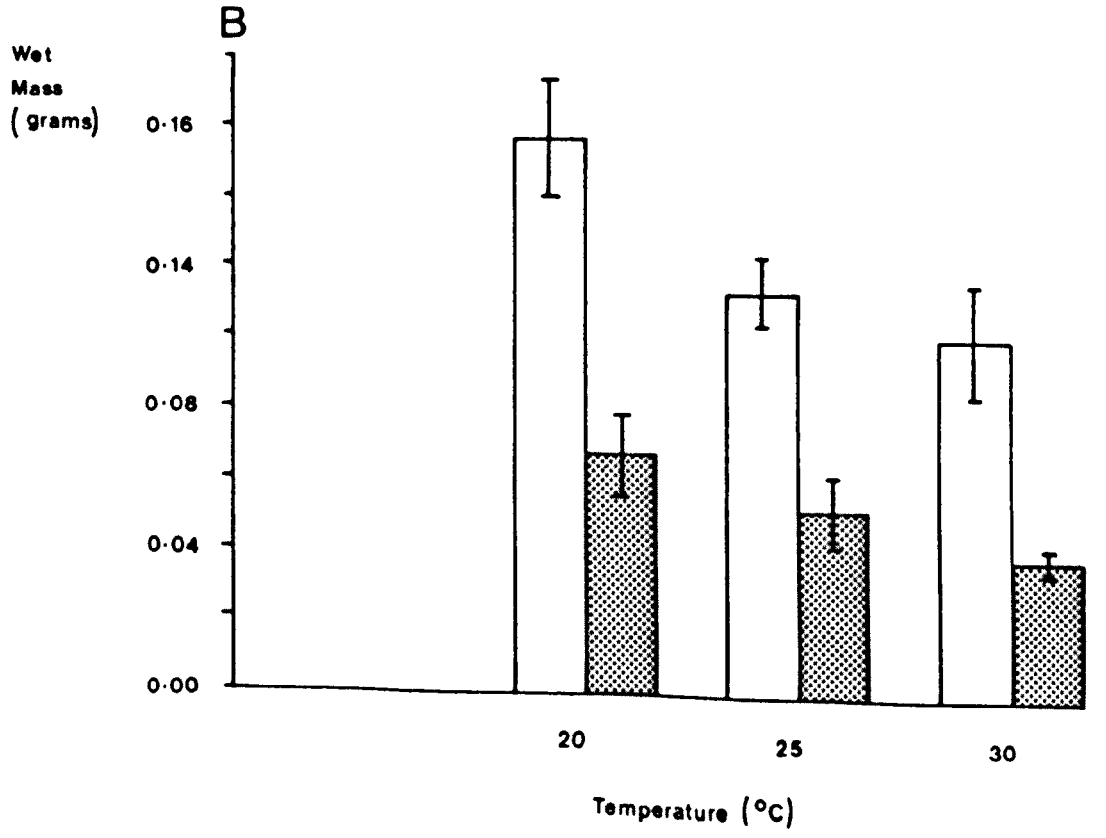

B
Wet
Mass
(grams)
0·16
0·14
0·08
0·04
0·00
20
25
30
Temperature (°C)

Table 3.1. T-tests comparing wet masses of B. bufo and B. calamita tadpoles (i) and toadlets (ii).

Wet masses (g) : B. bufo = B. calamita

(i) Tadpoles (n=10)

Temperature	B.b. $\bar{x}$	B.c. $\bar{x}$	DF	T	P
20°C	0.200	0.157	16.9	4.43	0.001
25°C	0.138	0.113	13.3	2.61	0.022
30°C	0.150	0.100	16.9	4.34	0.001

(ii) Toadlets (n=10)

Temperature	B.b. $\bar{x}$	B.c. $\bar{x}$	DF	T	P
20°C	0.069	0.068	17.7	-0.40	0.690
25°C	0.069	0.051	14.5	3.27	0.006
30°C	0.082	0.039	4.4	5.06	0.007

3.3.2. Dry masses of tadpoles and toadlets

The dry mass of both species was highest at the lowest temperatures (Fig. 3.2). B. bufo at 15 and 20°C had a premetamorphic dry mass significantly higher ($p > 0.05$) than that of the toadlets. At 25 and 30°C the premetamorphic mass was greater than that of the toadlets, but this was not significant at the 95% level. The dry masses of the B bufo toadlets at 15-30°C were not significantly different ($p > 0.05$).

The B. calamita dry masses of both tadpoles and toadlets showed a decrease with temperature. The dry mass of tadpoles at 20°C (0.0089 g) was significantly higher than at 25 and 30°C (0.0062 and 0.0052 g respectively) ($p > 0.05$). The dry mass of the toadlets was significantly lower than that of the corresponding tadpole ($p > 0.05$) at 25 and 30°C, at 20°C the dry mass of the toadlet was lower but not significant at the 95% level.

Comparing the dry masses of the two species (Table 3.2), the tadpoles and toadlets had similar masses at 20°C ($p > 0.05$) but they were significantly different at 25 and 30°C ($p > 0.05$).

3.3.3. Wet mass to dry mass ratios.

Wet mass to dry mass ratios (Fig. 3.3) were higher in the premetamorphic tadpoles than in the toadlets. There was no significant difference between the species at 25 and 30°C ($p > 0.05$). At 20°C the

Figure. 3.2. Dry mass of premetamorphic tadpoles and postmetamorphic toadlets of (A) B. bufo and (B) B. calamita reared at 15-30°C. Means ± 95% confidence intervals, n=10.

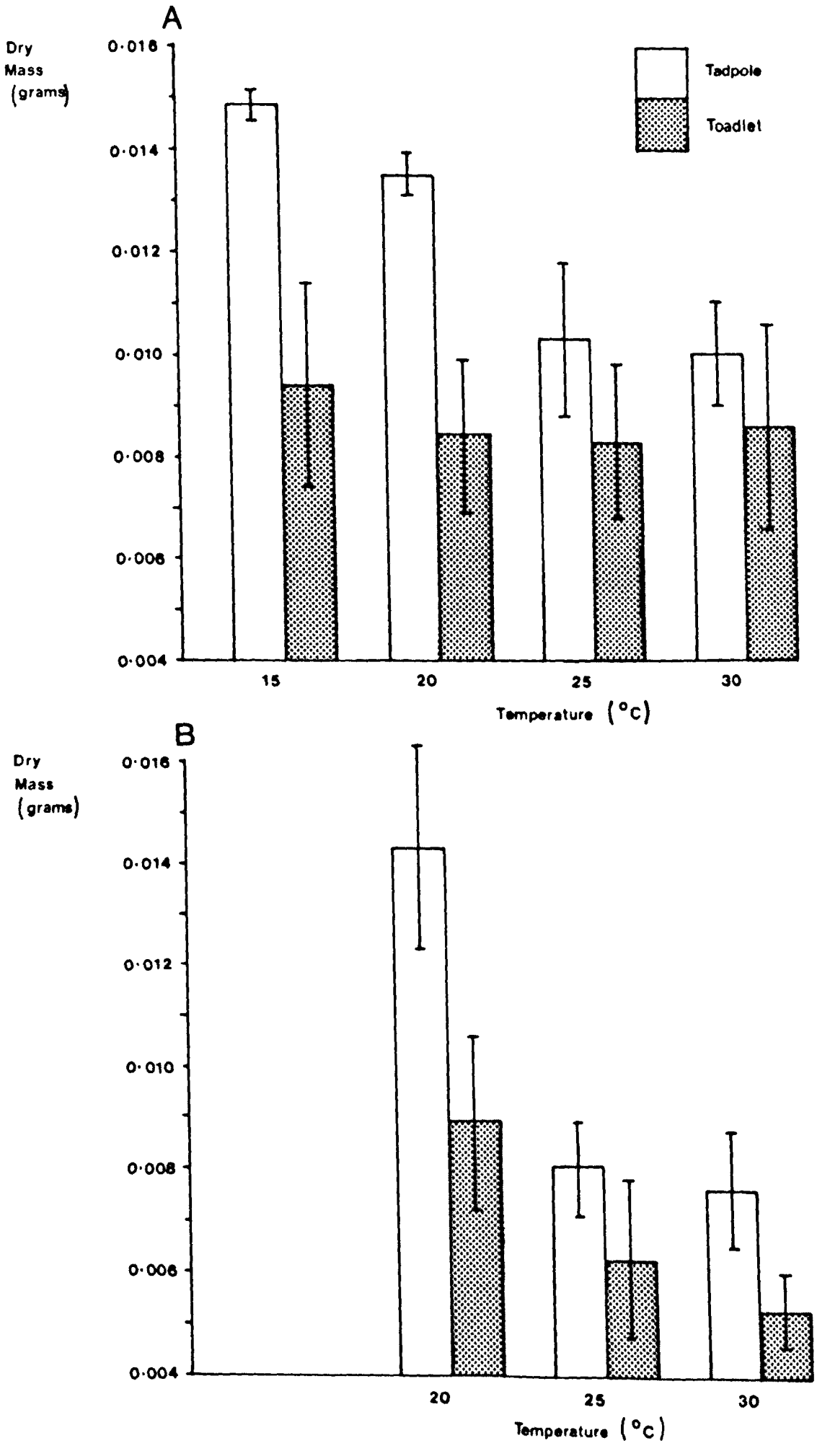

A
Dry Mass (grams)
0·016
0·014
0·012
0·010
0·008
0·006
0·004
Tadpole
Toadlet
15
20
25
30
Temperature (°C)
B
Dry Mass (grams)
0·016
0·014
0·012
0·010
0·008
0·006
0·004
20
25
30
Temperature (°C)

Table 3.2. T-tests comparing dry masses of B. bufo and B. calamita tadpoles (i) and Toadlets (ii)

Dry masses (g): B. bufo = B. calamita

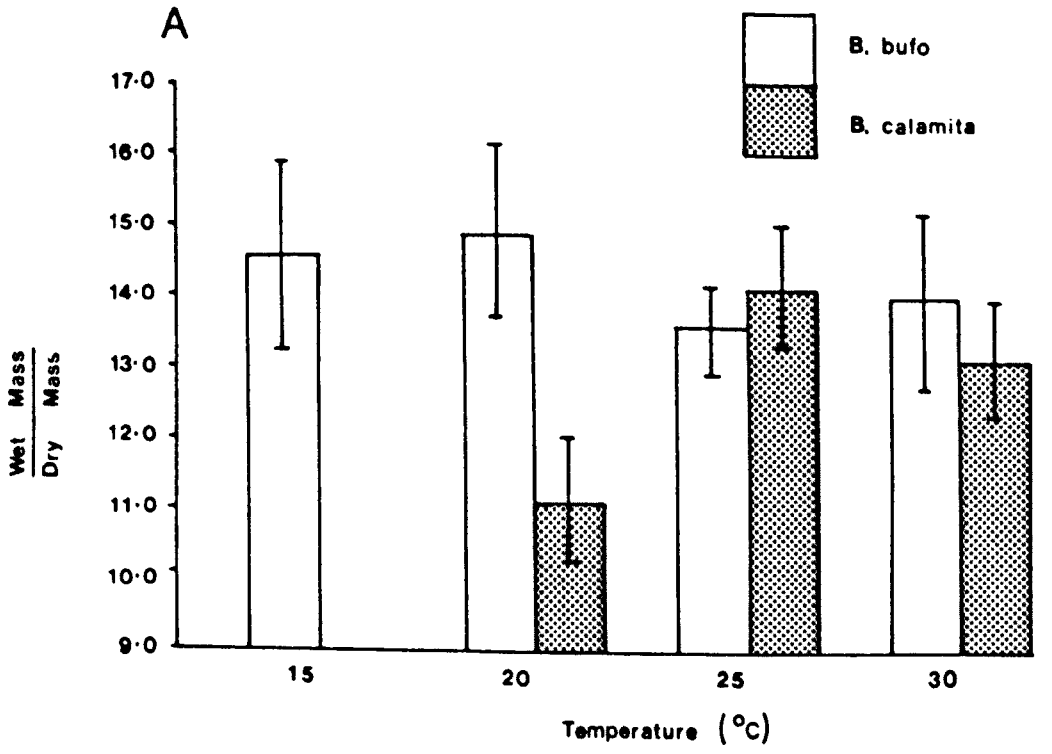
(i) Tadpoles (n=10)

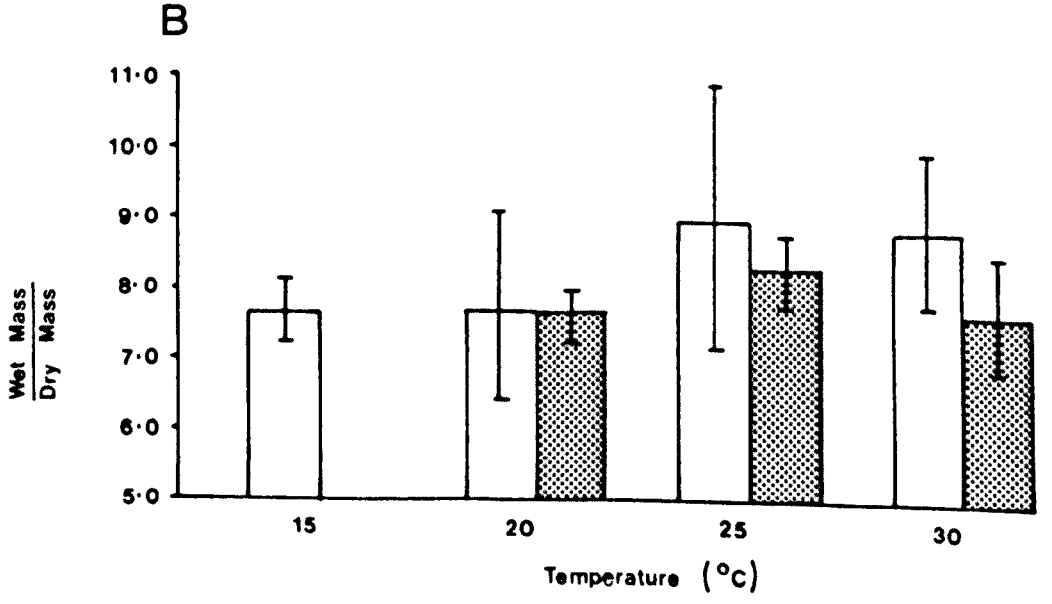
Temperature	B.b. $\bar{x}$	B.c. $\bar{x}$	DF	T	P
20°C	0.0135	0.0143	11.6	-0.74	0.470
25°C	0.0102	0.0080	15.1	2.91	0.011
30°C	0.0099	0.0075	17.4	4.97	0.000

(ii) Toadlets (n=10)

Temperature	B.b. $\bar{x}$	B.c. $\bar{x}$	DF	T	P
20°C	0.0084	0.0089	11.8	-0.72	0.470
25°C	0.0082	0.0062	18.0	2.15	0.046
30°C	0.0085	0.0052	5.7	4.28	0.008

Figure. 3.3. Wet mass : Dry mass ratios of B. bufo and B. calamita tadpoles (A) and toadlets (B) reared at 15-30°C. Means ± 95% confidence intervals, n=10.


A
B. bufo
B. calamita
17·0
16·0
15·0
14·0
13·0
12·0
11·0
10·0
9·0
Wet Mass / Dry Mass
15
20
25
30
Temperature (°C)


B
11·0
10·0
9·0
8·0
7·0
6·0
5·0
Wet Mass / Dry Mass
15
20
25
30
Temperature (°C)

B. calamita tadpoles had a wet mass to dry mass ratio that was significantly lower than that of B. bufo ($p > 0.05$), the toadlets at 20°C having similar ratios. The toadlets of B. bufo had higher wet mass to dry mass ratios than those of B. calamita but this was not significant at the 95% level.

3.3.4. Mass specific energy values of tadpoles and toadlets.

Mass specific ash-free dry mass energy content (KJ g^{-1}) of B. bufo tadpoles and toadlets was similar across the temperature range 15-30°C (Table 3.3). Overall toadlets had higher mean mass specific energy content than tadpoles although this was not significant at the 95% level. Values ranged from 22.8 - 24.4 KJ g^{-1} ash-free dry mass.

B. calamita tadpoles and toadlets at 20°C had similar ash-free dry mass energy contents of 25.06 and 25.02 KJ g^{-1} respectively. At 25 and 30°C the mean mass specific energy content of B. calamita toadlets was greater than those of the corresponding tadpoles. The difference between these two stages were 1.78 KJ g^{-1} at 25°C and 2.93 KJ g^{-1} at 30°C.

The percentage change in mass specific energy content is shown in figure 3.4. B. bufo showed an increase in energy content at 15-25°C but a small (0.2%) decrease at 30°C. B. calamita decreased 0.2% at 20°C but at 25°C increased 7.3% and at 30°C increased by 13.2%.

Table 3.3. Mass specific energy content of B. bufo and B. calamita premetamorphic tadpoles and toadlets.

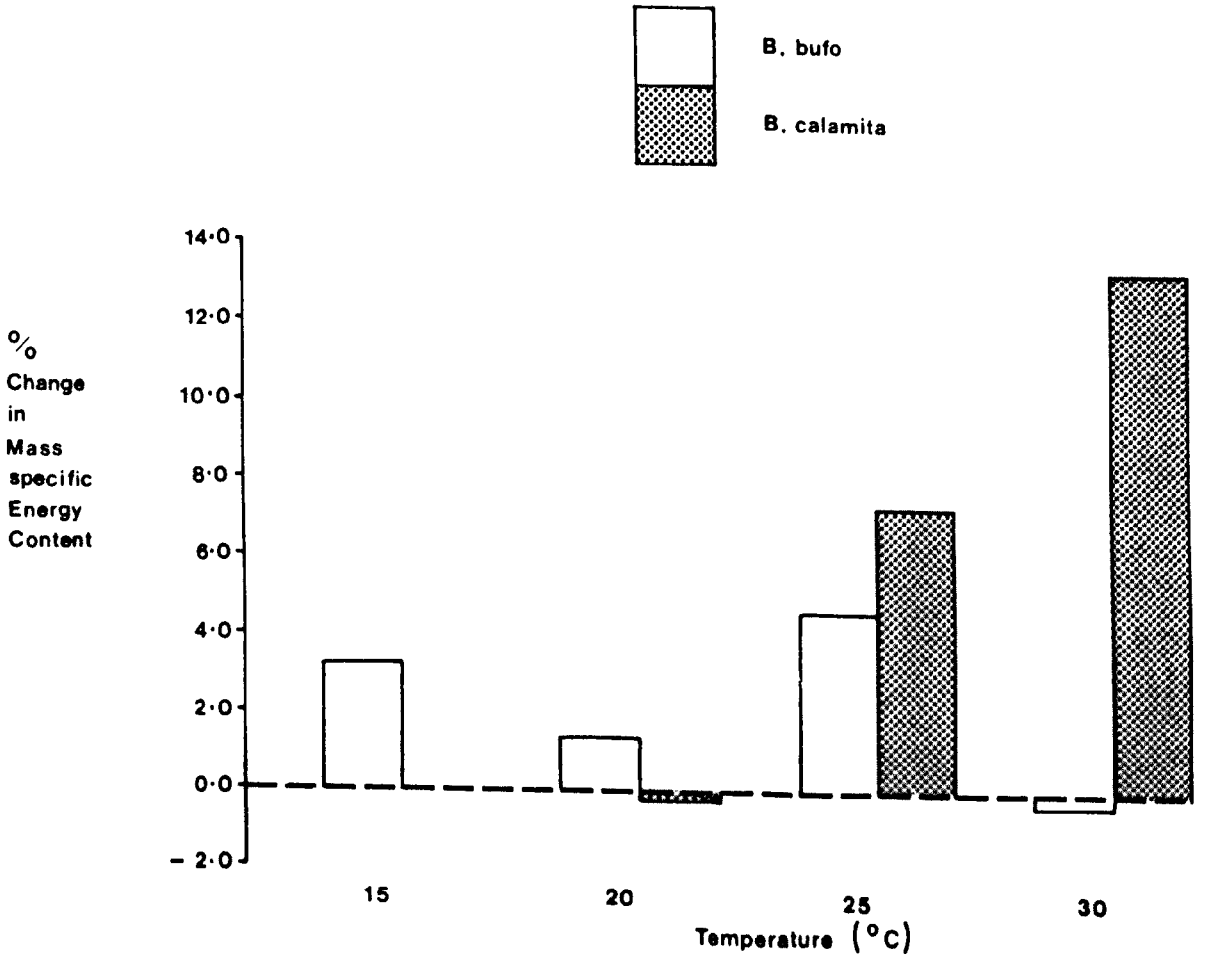
(i) Bufo bufo ($n = 10$)

Stage	Temperature	Ash free dry mass energy content KJ g^{-1}	95% confidence interval
Tadpole	15°C	23.58	± 1.74
Tadpole	20°C	22.95	± 1.66
Tadpole	25°C	22.80	± 1.25
Tadpole	30°C	24.00	± 1.67
Toadlet	15°C	24.40	± 1.81
Toadlet	20°C	23.25	± 1.28
Toadlet	25°C	23.82	± 2.02
Toadlet	30°C	23.92	± 3.67

(ii) Bufo calamita ($n = 10$)

Stage	Temperature	Ash free dry mass energy content KJ g^{-1}	95% confidence interval
Tadpole	20°C	25.06	± 0.84
Tadpole	25°C	24.59	± 0.58
Tadpole	30°C	23.44	± 1.87
Toadlet	20°C	25.02	± 1.21
Toadlet	25°C	26.37	± 2.35
Toadlet	30°C	26.55	± 2.83

Figure. 3.4. Effect of temperature on percentage change in mass specific energy content in the tadpole to toadlet transformation during metamorphosis. Values obtained from table 3.2.


B. bufo
B. calamita
% Change in Mass specific Energy Content
14·0
12·0
10·0
8·0
6·0
4·0
2·0
0·0
- 2·0
15
20
25
30
Temperature (°C)

3.3.5. Total energy content of tadpoles and toadlets.

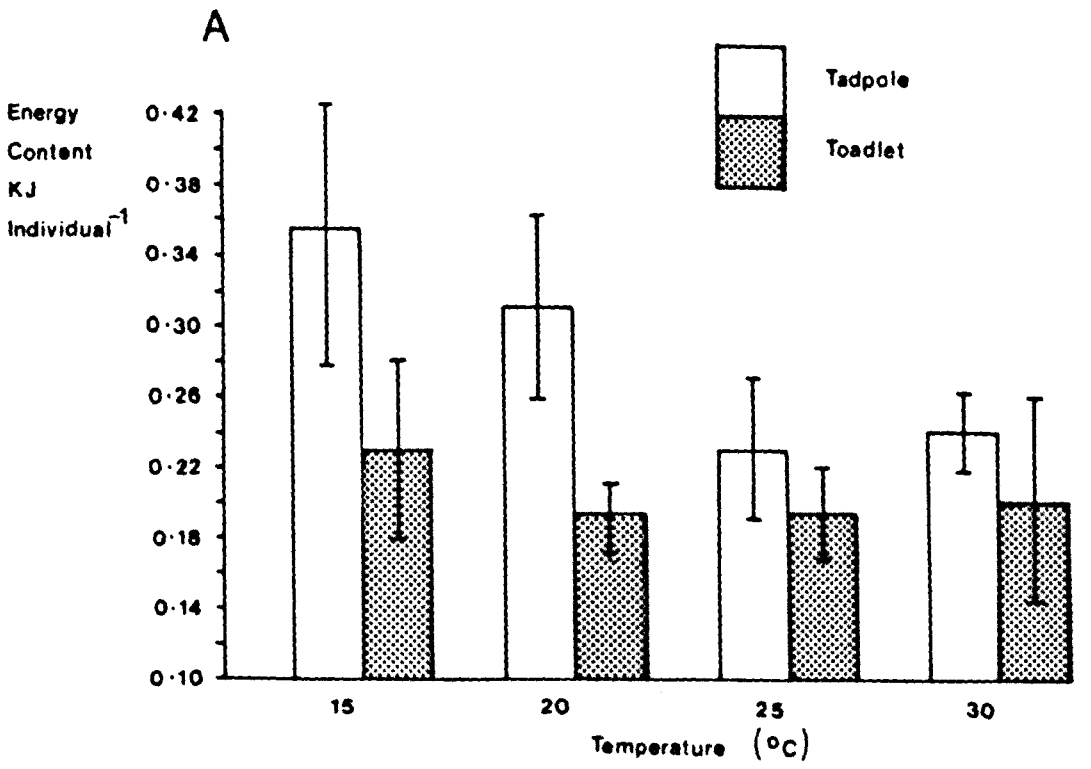
The total energy content of tadpoles and toadlets was obtained by multiplying their mass specific ash-free dry mass energy content (Table 3.3) by their dry masses (Fig. 3.2). Results are shown in figure 3.5.

The mean energy content of the two species' tadpoles decreased as temperature increased, with the exception of B. bufo at 30°C which had higher values than at 25°C. In B. bufo the energy content of the toadlets did not show the marked decrease with temperature seen in the tadpoles. There was a small increase in energy content from 0.175 KJ individual^{-1} at 20°C to 0.205 KJ individual^{-1} at 30°C however this was not significant at the 95% level. Unlike B. bufo, B. calamita toadlets decreased in total energy content as temperature increased; from 0.220 KJ individual^{-1} at 20°C to 0.140 KJ individual^{-1} at 30°C.

Comparing B. bufo and B. calamita (Table 3.4), tadpoles at 20°C had similar energy content ($p > 0.05$). At 25 and 30°C B. bufo tadpoles had a significantly higher energy content per individual ($p > 0.05$). The energy content of the two species' toadlets at 20-30°C was not significantly different ($p > 0.05$).

In both species there was no significant difference ($p > 0.05$) between the total energy content of the premetamorphic tadpole and that of the toadlet at 25 and 30°C (Table 3.5). At temperatures below 25°C, in both species, the total energy in the toadlets was significantly less

Figure. 3.5. Energy content of tadpoles and toadlets of (A) B. bufo and (B) B. calamita reared at different temperatures. Means ± 95% confidence intervals, n=10.


A
Energy
Content
KJ
Individual^{-1}
0·42
0·38
0·34
0·30
0·26
0·22
0·18
0·14
0·10
Tadpole
Toadlet
15
20
25
30
Temperature (°C)

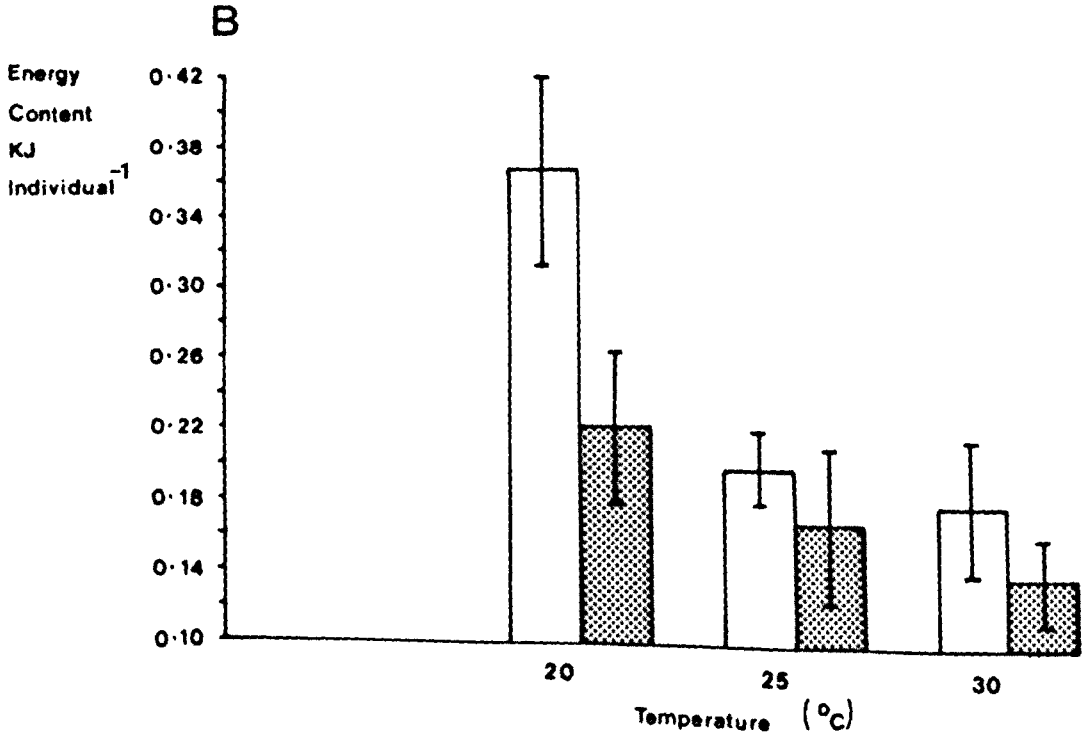

B
Energy
Content
KJ
Individual^{-1}
0·42
0·38
0·34
0·30
0·26
0·22
0·18
0·14
0·10
20
25
30
Temperature (°C)

Table 3.4. T-tests comparing the energy content of B. bufo tadpoles and toadlets with that of B. calamita tadpoles and toadlets.

(i) Tadpoles: B. bufo = B. calamita
Energy content, KJ individual^{-1}, n=10.

Temperature	B.b. $\bar{x}$	B.c. $\bar{x}$	DF	T	P
20°C	0.310	0.368	14.8	1.84	0.088
25°C	0.223	0.198	14.3	3.69	0.002
30°C	0.240	0.179	15.7	2.70	0.016

(ii) Toadlets: B. bufo = B. calamita
Energy content, KJ individual^{-1}, n=10.

Temperature	B.b. $\bar{x}$	B.c. $\bar{x}$	DF	T	P
20°C	0.191	0.220	10.9	-1.36	0.200
25°C	0.191	0.166	14.0	1.28	0.220
30°C	0.196	0.138	5.6	2.13	0.086

Table 3.5. T-tests comparing energy content of premetamorphic tadpoles with energy content of toadlets in B. bufo and B. calamita.

(i) B. bufo: Tadpole = Toadlet
Energy content, KJ individual^{-1}, n=10.

Temperature	Tadpole $\bar{x}$	Toadlet $\bar{x}$	DF	T	P
15°C	0.352	0.229	15.7	2.70	0.016
20°C	0.310	0.191	14.6	7.73	0.000
25°C	0.223	0.191	16.9	1.42	0.180
30°C	0.240	0.196	5.6	1.69	0.150

(ii) B. calamita: Tadpole = Toadlet
Energy content, KJ individual^{-1}, n=10.

Temperature	Tadpole $\bar{x}$	Toadlet $\bar{x}$	DF	T	P
20°C	0.368	0.229	18.0	4.17	0.001
25°C	0.198	0.166	11.4	1.20	0.250
30°C	0.166	0.138	13.4	1.66	0.120

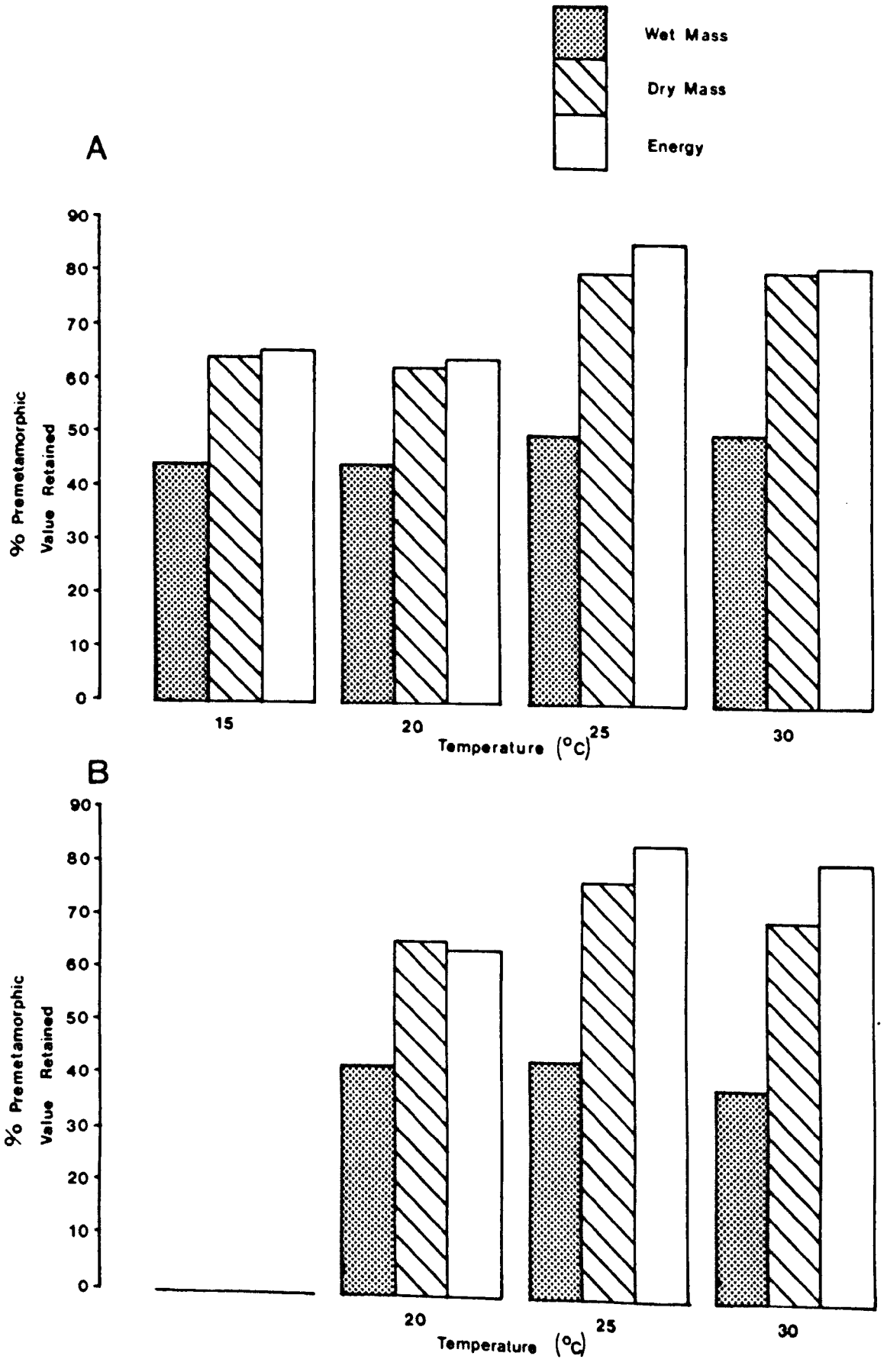
than that in the tadpoles ($p > 0.05$).

Metamorphic efficiency is the proportion of the original mass or energy remaining after a premetamorphic tadpole has undergone metamorphosis. In both B. bufo and B. calamita, the metamorphic efficiency (wet mass) was low, B. bufo retained only 45-50% of its wet mass (in the temperature range 15-30°C) after metamorphosis (Fig. 3.6). B. calamita lost proportionately more wet mass with a metamorphic efficiency of 40-45%.

With increasing temperature, the efficiency of dry mass conservation increased in B. bufo; 64% of dry mass remained at 15°C rising to 81% at 30°C (Fig. 3.6). This was paralleled by an increase in energy retention (KJ individual^{-1}) rising from 65% efficient (15°C) to 83% at 30°C. B. bufo had a maximum metamorphic efficiency in terms of energy retention at 25°C, when 86% of the energy found in the premetamorphic tadpole was found in the toadlet.

B. calamita also had maximum metamorphic efficiency at 25°C in terms of dry mass (77%) and energy (84%). These values were similar to those of B. bufo. At 30°C dry mass metamorphic efficiency in B. calamita was 70% however the comparable energy efficiency was 81% (a difference of 11%). At 20°C the values of wet mass, dry mass and energy metamorphic efficiency were similar for both species.

Figure. 3.6. Efficiency of metamorphosis: % of premetamorphic energy/mass remaining in toadlet after metamorphosis in (A) B. bufo and (B) B. calamita reared at different temperatures.


Wet Mass
Dry Mass
Energy
A
% Premetamorphic Value Retained
90
80
70
60
50
40
30
20
10
0
15
20
25
30
Temperature (°C)
B
% Premetamorphic Value Retained
90
80
70
60
50
40
30
20
10
0
20
25
30
Temperature (°C)

3.4. DISCUSSION.

3.4.1 The effect of rearing temperature on mass and energy changes during B. bufo and B. calamita metamorphosis.

As seen in other studies of temperature and metamorphosis (Collins, 1979; Pandian and Marian, 1985a; 1985b) an increase in rearing temperature results in a decrease in size (mass) at metamorphosis in both B. bufo and B. calamita. B. calamita generally has smaller premetamorphic tadpoles than B. bufo at equivalent temperatures, this is more prevalent at higher temperatures and is a result of faster developmental rates.

The premetamorphic mass specific energy content of both B. bufo and B. calamita is similar. Hence the total energy content of the tadpoles will decrease as mass decreases. Total energy content of tadpoles is therefore inversely proportional to temperature. This has also been shown in Rana tigrina (Marian and Pandian, 1985a).

B. calamita tadpoles are generally smaller than those of B. bufo. It follows that the total energy content of a B. calamita premetamorphic tadpole is less than that of B. bufo reared under similar conditions. It appears that energy accumulation in bufonid tadpoles is dependent on growth and developmental rates.

The have been several models that attempt to predict the required size for anuran tadpoles to metamorphose. Wilbur and Collins (1973)

described a model whereby size at metamorphosis is predictable, based on growth rates. Smith-Gill and Berven (1979) modified this model, emphasising the importance of developmental rates. Crump (1981) suggests that, as well as growth and developmental rates being determinants of mass at metamorphosis, energy accumulation is also an important parameter. It is probable that growth rates, developmental rates and energy accumulation are all important in determining the size of a tadpole at metamorphosis.

Wilbur and Collins (1973) suggest that the size of an amphibian at metamorphosis has upper and lower limits. These are set by environmental factors, the uncertainty of the larval environment, predation of larval stages and survivorship of juveniles through to hibernation. B. calamita is typically an ephemeral pond breeder (Beebee, 1983) whereas B. bufo generally breeds in more stable environments (Smith, 1951). Wilbur and Collins' model suggests that B. calamita should be smaller at metamorphosis, as is the case.

Most models that attempt to predict metamorphosis examined density effects on the size of the tadpole at metamorphosis (Crump, 1981; Smith-Gill and Berven, 1979; Wilbur and Collins,1973). In this series of experiments only one environmental factor was investigated, temperature. Wilbur (1980) suggests that under normal environmental conditions growth rates of larvae are regulated by density dependent mechanisms to levels below the physiologically maximum rate limited by the temperature regime of the pond. Although the temperature treatments used in this series of experiments do have an effect on

premetamorphic tadpole size and energy content, it must be recognised that it is only one of several variables that can affect growth and development under natural conditions.

The size (mass) and energy content of a premetamorphic tadpole is important in governing the size of the resulting toadlet. The premetamorphic masses and total energy content of B. bufo are inversely related to temperature. However, the toadlet mass (and total energy content) remains constant over a range of rearing temperatures. More efficient metamorphosis appears to compensate for small premetamorphic body size at higher rearing temperatures with a more efficient metamorphosis. Pandian and Marian (1985a) showed that metamorphic efficiency increased with temperature in R. tigrina . Although metabolic rate increases with temperature the corresponding reduction in the duration of metamorphosis results in an overall saving of energy.

Unlike B. bufo which maintains a constant toadlet mass over a range of temperatures, B. calamita has an inverse relationship between temperature and toadlet mass. There is no apparent mass loss compensation as seen in B. bufo at higher temperatures. However the actual mass specific energy content of the toadlets increases with temperature. As a result of this, there is no marked decrease in total energy content of B. calamita toadlets, and they have similar energy contents at 20-30°C.

B. bufo apparently compensates for smaller body size at the

premetamorphic stage by reducing mass loss (and hence energy loss) over metamorphosis. B. calamita appears to compensate by a more efficient energy transfer, resulting in a smaller toadlet but with an equivalent energy content to B. bufo under similar conditions.

Intraspecific studies have shown that a larger emergent toadlet can result in a fitter adult toad: postmetamorphic body size can influence body size at first reproduction, which can have an effect on male mating success and general fitness (Howard, 1979) and on female fecundity (Collins, 1975). B.bufo and B. calamita, under controlled conditions produce toadlets of different masses but comparable energy content at equivalent temperatures. Without further study it is not known whether this provides the two species with equal fitness as they progress to maturity.

Wilbur (1980) suggests a small body size at metamorphosis requires either a small size at maturity or a long period of juvenile growth with a reasonably high survival rate from metamorphosis to maturity. In bufonids there is virtually no relationship between size at metamorphosis and adult size (Werner, 1986). Shvarts and Pyastolova (1970a; 1970b) found that in Rana arvalis poor larval quality may not always cause a delayed metamorphosis.

B. calamita generally take longer to reach sexual maturity than B. bufo (Davis, 1985), although recorded ages of sexual maturity recorded for B. calamita might have been affected by social interactions at breeding ponds, older males preventing younger (although probably sexually

mature) males from breeding in small breeding pools (Davis, 1985).

There are no comparable data available for first winter survivorship of B. bufo and B. calamita. An equivalent energy content in newly metamorphosed toadlets may afford both species comparable survival ability after emergence, independent of body size. Thus, B. calamita may have physiological mechanisms that compensate for its smaller toadlet size resulting from rapid developmental rates necessitated by breeding in ephemeral pools.

Preferred body temperatures were discussed in chapter 2. B. bufo and B. calamita have PBTs of 25 and 24°C respectively (Davis, 1985). Both species' metamorphic efficiencies were highest at 25°C and did not increase to 30°C. The metamorphic efficiency values obtained were similar to those obtained by Marian and Pandian (1985) for R. tigrina. Using this and other growth and development parameters they concluded that the optimal temperature for rearing R. tigrina was 27°C. They did not investigate PBTs.

In B. bufo and B. calamita, the mean energy content of toadlets was less than that of the premetamorphic tadpole. For both species there was no statistically significant energy losses over metamorphosis at temperatures 25 and 30°C. Temperatures below this resulted in a significant loss of energy. It therefore appears that as well as being the optimum temperature for growth and development (Chapter 2), the PBT of the tadpoles is also the optimum temperature for efficient metamorphosis. It is probable that B. bufo and B. calamita tadpoles

behaviourally regulate their body temperature at 25°C when environmental conditions allow. As a result of this, growth rates, developmental rates and metamorphic efficiencies are maximised.

CHAPTER 4

4.0 The relationship between metabolic rate and growth and development in B. bufo and B. calamita tadpoles across a range of temperatures.

4.1 INTRODUCTION.

The metabolic rate of an organism is related to the sum of its physiological processes. Metabolic rate (measured as oxygen consumption) therefore has the potential to be strongly linked to an organisms growth and development. Deviation from minimal oxygen consumption may indicate the effects of many ecological and physiological factors (Feder, 1981). Prosser (1955) considered that absolute oxygen consumption might be one measure of metabolic racial adaptation.

Davis (1985) showed that oxygen consumption in B. calamita tadpoles was higher than that of equivalent B. bufo tadpoles at 10 and 20°C acclimation temperatures. He also found that B. bufo acclimated to a given temperature at a faster rate than B. calamita.

As discussed earlier (Chapter 2), B. calamita has faster developmental rates than B. bufo and reaches metamorphosis in a shorter time. Higher developmental rates as a result of warmer environmental conditions require greater energy input to sustain them. This would be supplied by an increase in oxidative metabolism and be indicated by higher

oxygen consumption rates.

There have been many studies of oxygen consumption in larval amphibians (see Feder, 1981). Most investigate the effect of developmental stage on oxygen consumption (e.g. Etkin, 1934; Fletcher and Myant, 1959; Funkhouser and Mills, 1969). There are few accounts in the literature of interspecific studies of oxygen consumption, designed to explain differences in growth and developmental rates.

In a study of Rana spp. Moore (1939) distinguished between northern and southern species and races by their rates of development. B. bufo and B. calamita have distinct developmental rates (Chapter 2), although the two species do not have latitudinally distinct distributions (Chapter 1).

The differences in developmental rates observed in chapter 2 may possibly be linked to different metabolic rates. In this chapter the metabolic rates of B. bufo and B. calamita at a range of acclimation temperatures will be investigated. These will be related to the growth and development of the species. The affect of high larval metabolic rates on subsequent metamorphosis will also be discussed.

4.2. MATERIALS AND METHODS.

4.2.1 Spawn collection and initial rearing.

For all oxygen consumption experiments collection and rearing of tadpoles was carried out as in sections 2.2.2 and 2.2.3 at temperatures of 15, 20, 25 and 30°C. Tadpoles were removed from the appropriate rearing regimes after 10 and 20 days and their oxygen consumption ($\dot{V}O_2$) measured using Warburg respirometers.

4.2.2 Oxygen consumption measurement.

Warburg constant volume respirometers (Umbriet et al, 1957) were used to determine $\dot{V}O_2$ for tadpoles of B. bufo and B. calamita at different temperatures. The apparatus consisted of seven respirometers with 20 ml flasks attached (manufactured by: B.braun. Melsungen). Six of the respirometers were used to measure tadpole respiration the seventh was used as a thermobarometer.

The temperature of the apparatus was controlled during the experiments by a waterbath consisting of a polythene tank cooled constantly by a GRANT chiller/thermocirculator and a HAAKE E3 heater/stirrer. The appropriate temperature was maintained ± 0.5°C. The apparatus was not shaken during the course of the measurements. Any disturbance artificially raises the $\dot{V}O_2$ of tadpoles in a respirometer (Feder, 1981).

Ten days after the tadpoles had been placed in the different rearing temperatures 30 tadpoles were removed from each of the rearing temperatures for VO_2 measurement (5 per respirometer flask). After a further ten days 6 of the now larger tadpoles were used at each temperature (1 per respirometer flask).

Tadpoles were removed from the rearing treatments and surface dried using the sieve method (section 2.2.4). They were then placed into beakers containing 3 ml of dechlorinated tap water. Each beaker corresponded to each respirometer flask to be used. The tadpole/tadpoles and water were then drawn up into a flexible polythene tube and pipetted into the appropriate respirometer flask. 3 ml of water was pipetted directly into the thermobarometer flask.

0.2 ml of 20% potassium hydroxide was then pipetted into the central well of the respirometer, a filter paper (Whatman No.44) wick was then placed in the potassium hydroxide.

The respirometers were then assembled and the flasks placed in the water bath at the acclimation temperature of the tadpoles (temperature of rearing). As recommended by Atlas, 1938; Funkhouser and Mills, 1969; Lewis and Frieden, 1959; Marshall and Grigg, 1980, the tadpoles were allowed to acclimatise for 20 minutes before readings commenced.

After acclimatisation, the respirometers were closed and readings commenced. Readings were taken every five minutes for thirty minutes. The tadpoles were then removed using a flexible polythene tube, surface

dried and weighed in a tared beaker of water on a top pan balance where their stages of development were also recorded. The tadpoles were then returned to the appropriate rearing treatments as part of an ongoing experiment.

The change in mm of manometer fluid (readings taken) for each respirometer was corrected against the thermobarometer reading. The six values obtained (6 x 5 minute intervals) were regressed against time. Any regressions with an r value of less than 0.95 were rejected as this suggests a faulty manometer. The actual change in mm with time was converted to mass specific oxygen consumption (ml g^{-1} hr^{-1}) by multiplying by the calibration constant (see Umbriet et al, 1957) of the particular respirometer used and the wet mass of the tadpole/tadpoles.

The rates of growth and development for the tadpoles were calculated from the measurements taken during the oxygen consumption experiments.

4.3 RESULTS.

Metabolic rate (oxygen consumption) measured at acclimation temperature increased with the acclimation temperature (Fig 4.1) in both B. bufo and B. calamita. B. bufo had a mean VO_2 of 0.148 ml g^{-1} hr^{-1} (wet mass) at 15°C rising to 0.300 ml g^{-1} hr^{-1} at 30°C. Values obtained for B. calamita at each temperature were higher than the equivalent B. bufo values. The mean VO_2 values were significantly different between the species at 15°C ($f=6.55$, $p>0.05$) and 30°C ($f=10.44$, $p>0.05$).

As VO_2 increased, there was a corresponding increase in developmental rate in B. bufo and B. calamita (Fig. 4.2). The developmental rates were obtained by recording developmental stages (Gosner, 1960) at the time of each series of oxygen consumption measurements and converting to developmental rate in stages day^{-1}. In both species there was a decrease in developmental rate above 25°C, although VO_2 increased.

At 15°C, B. calamita VO_2 was higher than that of B.bufo (0.230 ml g^{-1} hr^{-1} wet mass, compared to 0.149 ml g^{-1} hr^{-1} wet mass, however the developmental rate was lower (0.25 stages day^{-1} compared with 0.48 stages day^{-1}). At 20, 25 and 30°C both oxygen consumption and developmental rates were higher in B. calamita than B. bufo.

When oxygen consumption was looked at in relation to growth rate (Fig. 4.3) there was an inverse relationship. In B. bufo the growth rate decreased as the VO_2 increased within the temperature range 15-25°C. At 30°C growth rate was greater than at 25°C, the VO_2 was also higher.

Figure. 4.1. Mass specific oxygen consumption of B. bufo and B. calamita tadpoles acclimated to 15-30°C and measured at acclimation temperatures. Means ± 95% confidence intervals. (For sample sizes see section 4.2.2)

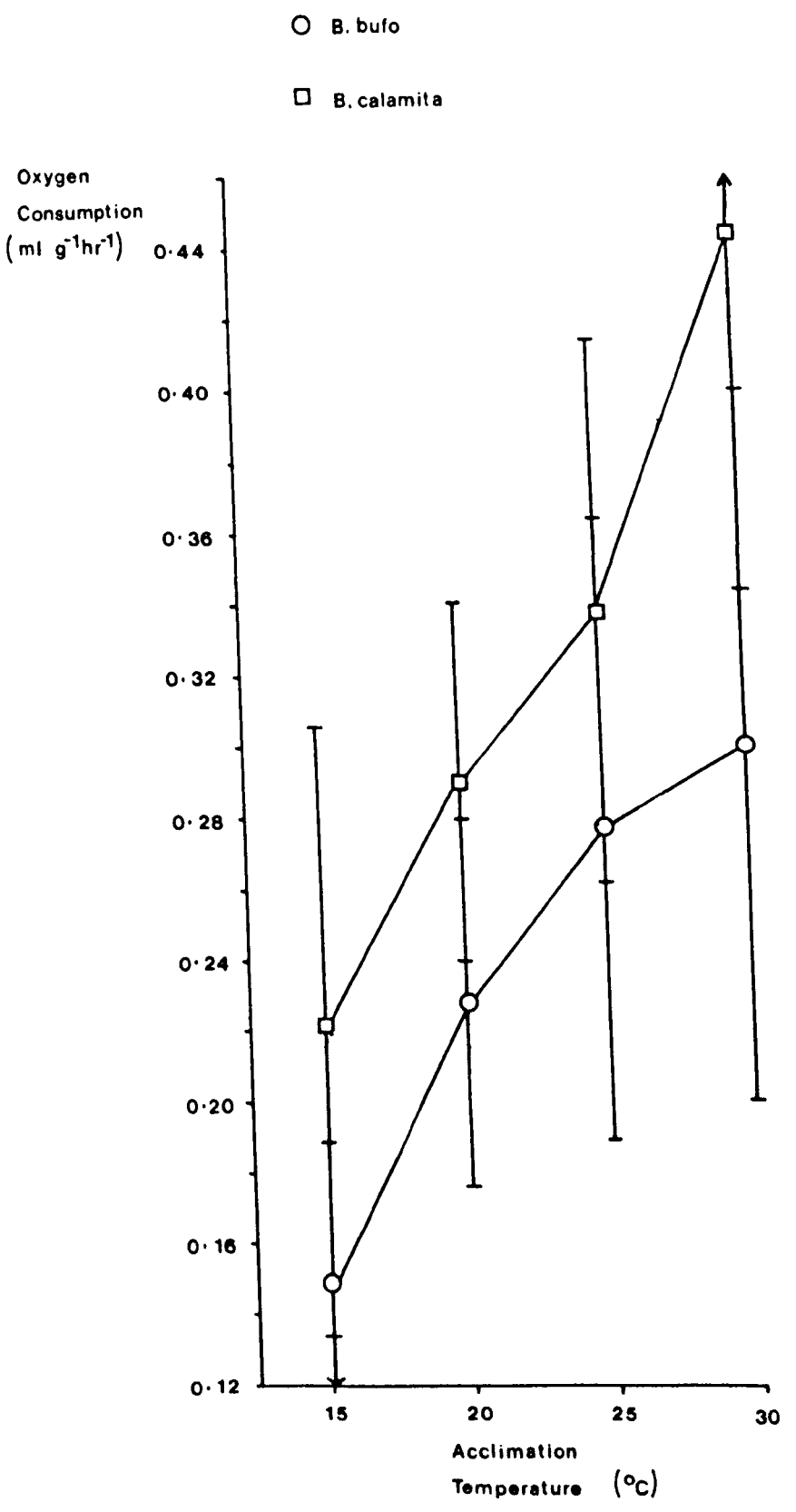

B. bufo
B. calamita
Oxygen
Consumption
(ml g⁻¹hr⁻¹)
0·44
0·40
0·36
0·32
0·28
0·24
0·20
0·16
0·12
15
20
25
30
Acclimation
Temperature (°C)

Figure. 4.2. Relationship between mass specific oxygen consumption and developmental rates of B. bufo and B. calamita tadpoles at different acclimation temperatures. Acclimation temperatures (°C) indicated on graph.

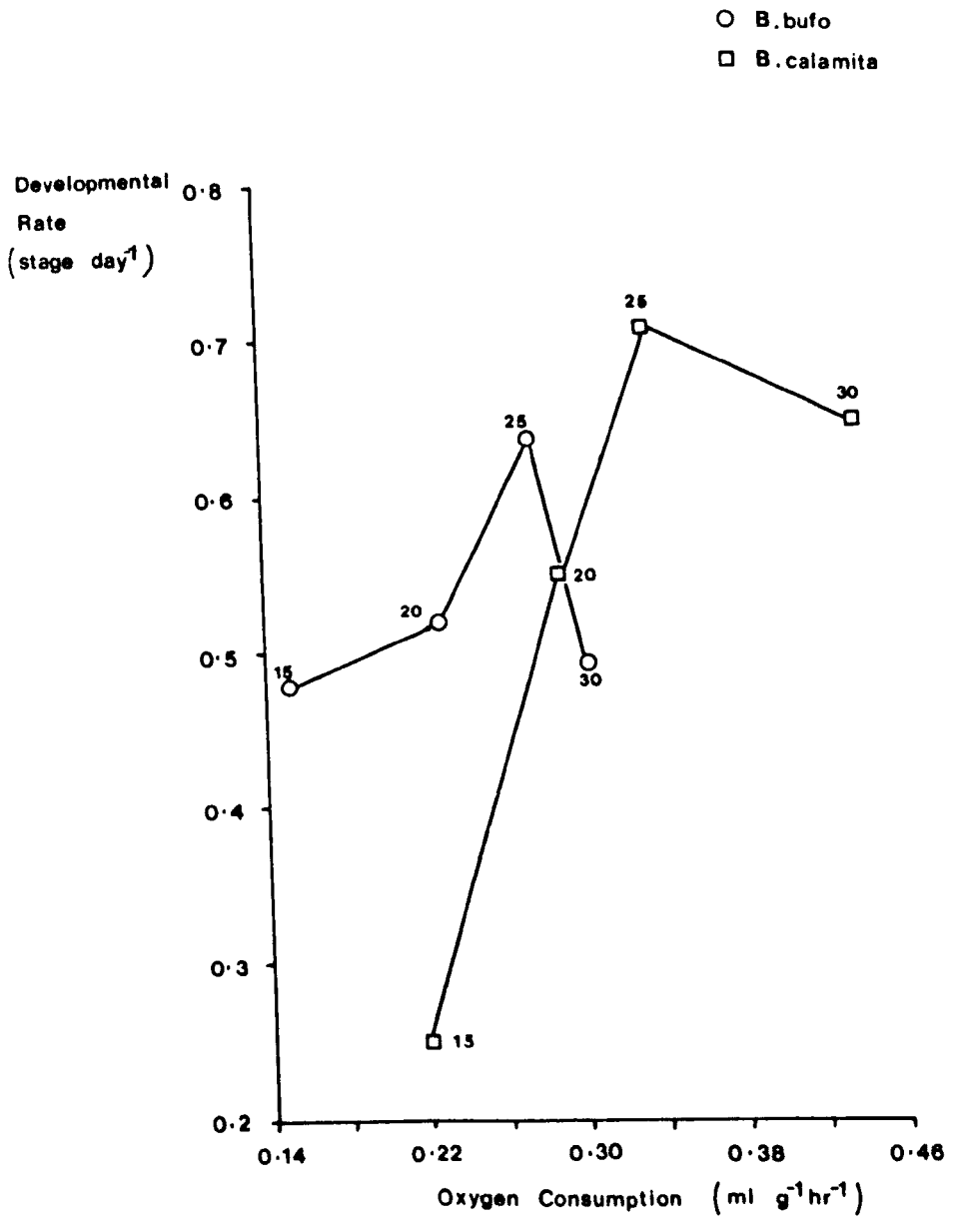
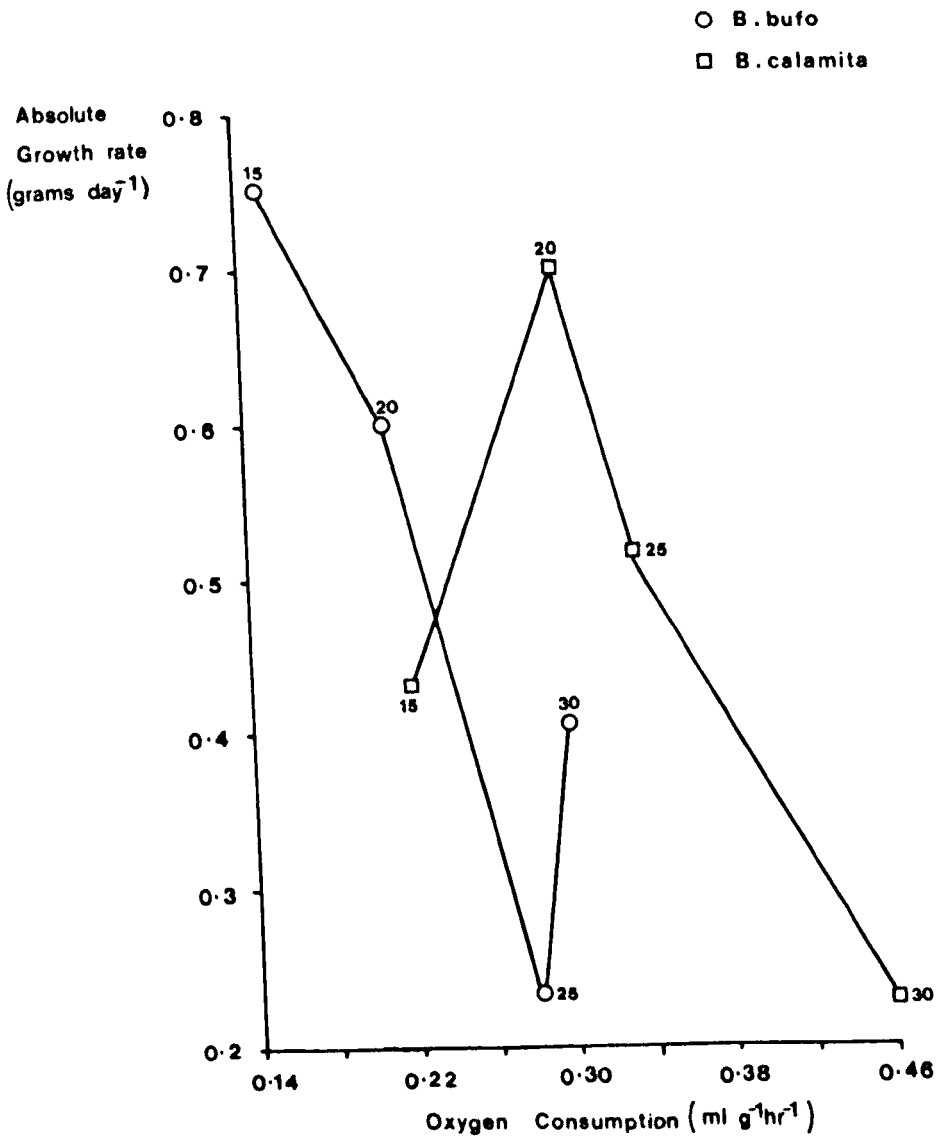

O B.bufo
□ B.calamita
Developmental Rate (stage day⁻¹)
0·8
0·7
0·6
0·5
0·4
0·3
0·2
15
20
25
30
20
25
30
15
0·14
0·22
0·30
0·38
0·46
Oxygen Consumption (ml g⁻¹hr⁻¹)

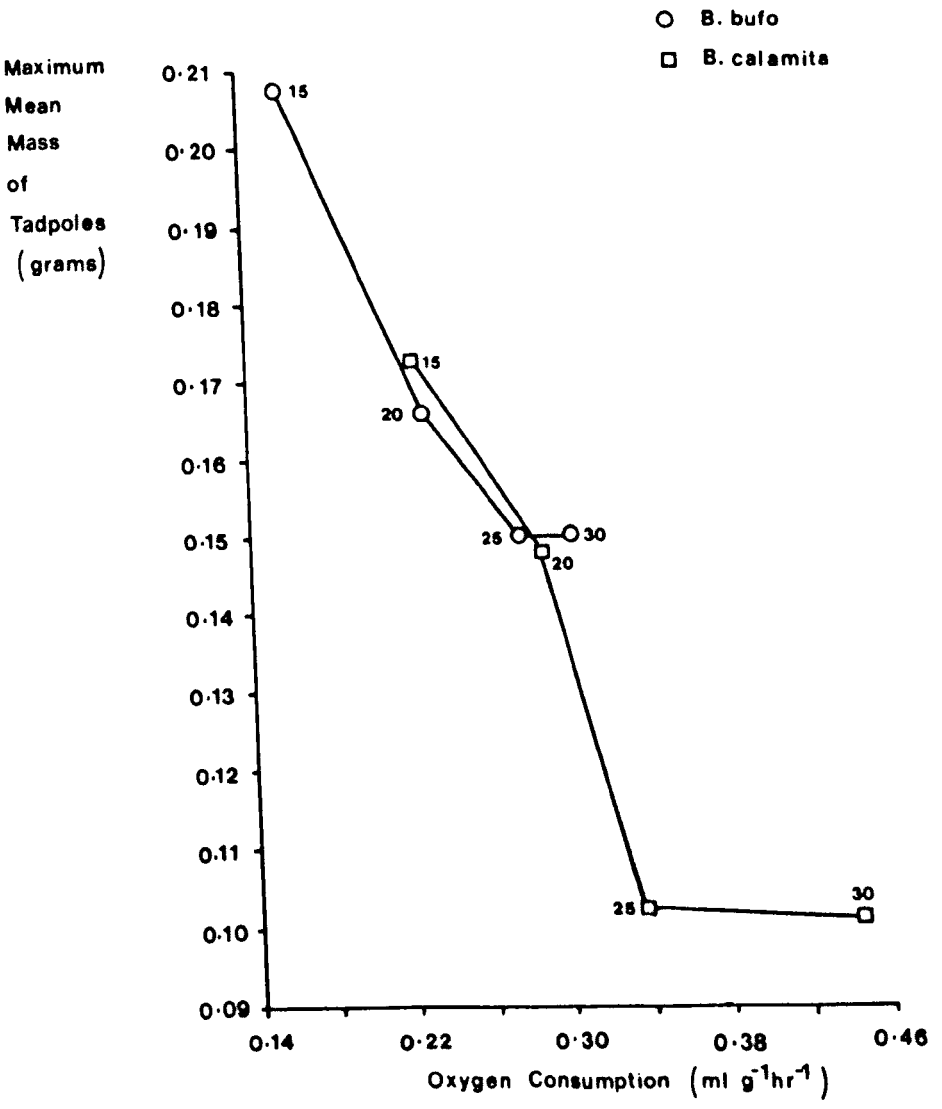
Figure. 4.3. Relationship between mass specific oxygen consumption and absolute growth rates in B. bufo and B. calamita tadpoles at different acclimation temperatures. Acclimation temperatures (°C) indicated on graph.


O B. bufo
□ B. calamita
Absolute Growth rate (grams day⁻¹)
0·8
0·7
0·6
0·5
0·4
0·3
0·2
15
20
25
30
15
20
25
30
0·14
0·22
0·30
0·38
0·46
Oxygen Consumption (ml g⁻¹hr⁻¹)

B. calamita growth rate increased as VO_2 increased from 15-20°C but above 20°C the growth rate decreased as the VO_2 continued to rise.

The mean mass of the tadpoles of both species decreased as VO_2 increased (Fig. 4.4). In both species there appeared to be a threshold of oxygen consumption, above which there was no further decrease in mass as the VO_2 continued to increase. This occurred at 25°C in both species, the VO_2 for B. bufo was 0.278 ml g^{-1} hr^{-1} wet mass and B. calamita 0.336 ml g^{-1} hr^{-1} wet mass.

Figure. 4.4. Relationship between mass specific oxygen consumption and mean mass of B. bufo and B. calamita tadpoles after 20 days acclimation. Acclimation temperatures (°C) indicated on graph.


B. bufo
B. calamita
Maximum Mean Mass of Tadpoles (grams)
0·21
0·20
0·19
0·18
0·17
0·16
0·15
0·14
0·13
0·12
0·11
0·10
0·09
15
15
20
25
30
20
25
30
0·14
0·22
0·30
0·38
0·46
Oxygen Consumption (ml g-1hr-1)

4.4 DISCUSSION

4.4.1 The relationship between metabolic rate and growth and development in B. bufo and B. calamita tadpoles across a range of temperatures.

The oxygen consumption values obtained for B. bufo and B. calamita tadpoles were similar to those obtained for other bufonid species, e.g. B. americanus (Lewis and Frieden, 1959) and B. boreas boreas (Sivula et al, 1972). The metabolic rates (VO_2) of B. calamita were higher at 15-30°C acclimation temperature than those of B. bufo. A similar result was obtained by Davis (1985) who looked at the two species acclimated to 10 and 20°C but using different oxygen consumption measurement techniques.

The results suggest that there are physiological differences between the two species during the tadpole stage of their development. At a given time and temperature B. calamita tadpoles have higher metabolic rates than B. bufo. Although the two species have differing developmental rates, stage of development is not thought to have a major effect on oxygen consumption rate, mass is a more important parameter influencing VO_2 (Feder, 1981).

Having a physiology adapted to warm conditions with higher metabolic rates should improve survival of B. calamita tadpoles in areas where they prefer to spawn (see chapter 1). B. calamita has higher oxygen consumption rates and developmental rates above 15°C than B. bufo. It

appears that the higher metabolic rates enables B. calamita to complete development in a shorter time period than B. bufo, thus giving it an advantage in ephemeral breeding ponds.

At 15°C, although VO_2 is higher in B. calamita than in B. bufo, developmental rate is lower. This suggests that another temperature related factor is influencing developmental rate. At low temperatures feeding efficiency may be reduced in B. calamita. Below 20°C B. bufo tadpoles have greater muscular performance than B. calamita (Nicolle, 1985). The reduced muscular performance at low temperatures would reduce feeding efficiency of a tadpoles. This coupled with relatively high VO_2, could result in energy being used in food gathering to the detriment of development. This effect may also be indicated by the low growth rates of B. calamita at 15°C.

Generally, absolute growth rates decrease in B. bufo and B. calamita as their metabolic rates increase. This mirrors the effect seen in developmental rates. As temperature increases so does metabolic rate. This appears to result in a shift of energy utilisation from growth to developmental processes. It is a change that B. calamita appears to achieve more efficiently than B. bufo. At temperatures of 20°C and above B. calamita has: (i) higher metabolic rates. (ii) higher growth rates. (iii) higher developmental rates.

Although B. calamita has greater absolute growth rates and faster developmental rates than B. bufo (probably as a result of higher metabolic rates), there is a cost manifested in the size of the

tadpoles. At all temperatures, B. bufo tadpoles were larger than those of B. calamita. The increased metabolism and subsequent benefits of a faster development (see chapter 2) result in smaller tadpoles. This may not be detrimental to survival if there is a mechanism that compensates for small tadpole size (see chapter 3).

There appears to be a thermal maximum for developmental rate in both species at 25°C. At temperatures above 25°C, developmental rates do not continue increasing and conversely growth rates do not decrease further in both B. bufo and B. calamita. In chapters 2 and 3 the preferred body temperatures of both species were discussed in relation to growth, development and metamorphosis. Metabolic rate continues to increase above 25°C; below this temperature it can be related to tadpole growth and development but above this there does not appear to be a relationship. Possibly, 25°C is the maximum temperature at which growth and development, as governed by metabolic rate, can proceed normally.

The relationship between tadpole mass and oxygen consumption appears to be similar for both B. bufo and B. calamita, however B. calamita occupies a higher position on the response curve. This is probably a direct effect of B. calamita's higher metabolic rates at equivalent temperatures.

CHAPTER 5

5.0. The effect of L-thyroxine on the growth and development of B. bufo and B. calamita tadpoles across a range of temperatures.

5.1 INTRODUCTION.

Many of the features of B. calamita tadpole physiology, that differ from B. bufo e.g. high metabolic rates and fast developmental rates, are known to be influenced by the hormone L-thyroxine (T_4). The effects of thyroid hormones on amphibian metamorphosis was first noted by Gudersnatsch (1914) and Allen (1929). Since then there has been considerable study of the subject, with more recent reviews by: Dodd and Dodd (1976), Etkin (1968) and Lynn and Wachowski (1951).

Huxley (1929), who worked with Rana temporaria tadpoles, noted that temperature had a marked effect on metamorphic rate which was suppressed below 5°C. He suggested that the thyroid acted as a primitive temperature regulator. Higher temperatures are known to increase the effect of the thyroid hormone L-thyroxine (Chui and Fung, 1979; Dodd and Dodd, 1976; Kollros, 1956; Lynn and Wachowski, 1951). At lower temperatures much more time is required for completion of any metamorphic event at a fixed dosage level of L-thyroxine (Lewis and Frieden, 1959). A quantitative relationship exists between L-thyroxine and its mode of action at a given temperature (Derby, 1968).

Most larval tissues become responsive to thyroid hormones at an early stage

in larval development (Etkin, 1950; Tata, 1968). Larval tissues acquire sensitivity to thyroid hormones before significant quantities are released into circulation (Lewis and Frieden, 1959). When L-thyroxine is applied exogenously, response to it has been shown to occur first when external larval gills are enclosed by the formation of the operculum (Moser, 1950). The gills are the site of maximum uptake when the hormone is administered by immersion (Ashley and Freiden, 1972).

It is well known that accelerated development and metamorphosis result from the application of thyroid hormones (see the reviews cited earlier). The actual physiological responses are not so well documented. L-thyroxine has been shown to raise metabolic rates in tadpoles (Barch, 1935; Belehradek and Huxley, 1927), reduce metabolic rate (Etkin, 1934) and also produce no change (Drastich, 1925). These widely varying observations may be due to differences in experimental technique. Funkhouser and Foster (1970) consider that L-thyroxine is necessary for the rapid bodily reorganisation that accompanies metamorphosis, the calorigenic effect of thyroid hormones in developing amphibians being secondary to the promotion of protein synthesis.

B. bufo and B. calamita tadpoles have markedly different developmental rates at similar temperatures (Chapter 2), perhaps due to dissimilarities in their thyroid physiologies. Response of tadpoles of both species to various concentrations of the thyroid hormone L-thyroxine over a range of temperatures may indicate differences in

their thyroid physiology. In this chapter the effect of L-thyroxine administration on growth, development and oxygen consumption on B. bufo and B. calamita over a range of temperatures will be investigated.

5.2. MATERIALS AND METHODS

5.2.1 Effect of L-thyroxine on growth and development of B. bufo and B. calamita tadpoles across a range of temperatures.

Spawn collection and initial rearing were carried out as in section 2.2.2. After initial rearing, the tadpoles of both species were transferred to the temperature treatment tanks. For both species and each temperature treatment, 20 tadpoles were placed in each of eight beakers containing 2 l of dechlorinated tap water at 15°C. The temperature was then raised to the appropriate rearing temperature at approximately 1°C per minute. The rearing temperatures were 15, 20, 25 and 30°C, which is the range of temperatures that allows tadpole development in both species (Chapter 2). The water was continuosly aerated.

For each of the temperature treatments, two beakers acted as controls (no L-thyroxine added), the remaining six had L-thyroxine (Aldrich Chemical Co. Ltd) added. L-thyroxine (T_4) was dissolved in a small quantity of 0.1M sodium hydroxide, and a stock solution made up with distilled water (Roberts, 1983). The stock solution of T_4 was added to the rearing water to produce concentrations of 0.0001, 0.001 and 0.01 p.p.m. T_4, two replicates for each concentration. The concentrations chosen covered the range that had an effect on amphibian metamorphosis, described by Allen (1932).

Feeding and cleaning were carried out as in section 2.2.3. During

cleaning, the four day old rearing water was discarded, the renewed water in the rearing beakers then had fresh T_4 stock solution added to produce the required concentrations.

Immersion is probably the best method for administering T_4 (Kollros, 1963). The tadpoles utilise only small amounts of the hormone and little is lost through chemical alteration of the medium. Immersion provides a relatively unvarying and continuous source of L-thyroxine (Kollros, 1963).

Recording of results was carried out as in section 2.2.4. Due to the lower number of experimental replicates, only 10 tadpoles were measured for each treatment.

5.2.3. The effect of L-thyroxine on oxygen consumption of tadpoles at different temperatures.

All oxygen consumption measurements were carried out using Warburg respirometers. The methodology was the same as that described in section 4.2.2. Oxygen consumption was measured for control tadpoles and those at 0.0001 and 0.001 p.p.m. T_4. The measurements were made at the rearing temperature (acclimation temperature) of the tadpoles, 15, 20, 25 and 30°C.

5.3. RESULTS

5.3.1. Effect of L-thyroxine on tadpole development at a range of temperatures.

At 5 and 10°C the highest concentration of T_4 (0.01 p.p.m.) did not affect B. bufo or B. calamita tadpole development (Fig. 5.1). For both temperatures, there was no significant progress in development ($p < 0.05$), and no difference between the control and the T_4 treatments ($p < 0.05$) in either species. As the high concentration of T_4 did not affect B. bufo or B. calamita development at 5 and 10°C, the effect of lower concentrations of T_4 at these temperatures was not investigated.

When reared at temperatures of 15°C and above B. bufo tadpoles developed at a faster rate when treated with T_4 (Figs. 5.2, 5.3). The 0.01 p.p.m. T_4 promoted rapid development initially. However, the tadpoles were stunted and malformed. At higher temperatures, the effects were more pronounced, tadpoles' abdomens shrank, malformed forelimbs appeared and hind limbs were distorted. These abnormalities led to high mortality. At 30°C, B. bufo tadpoles survived for 4 days, reaching stages 32-33, but grossly malformed. Tadpoles at 25°C experienced similar effects. At lower temperatures, the tadpoles survived longer periods, 12 days at 20°C, (reaching stage 37) and 24 days at 15°C, (reaching stage 35).

At 15 and 20°C (Fig. 5.2), the T_4 treatments resulted in faster developmental rates than the controls. The fastest developmental rate

Figure. 5.1. Effect of L-thyroxine (0.01 p.p.m.) on the development of (A) B. bufo and (B) B. calamita tadpoles at 5 and 10°C. Means ± 95% confidence intervals, n=10 for each point.

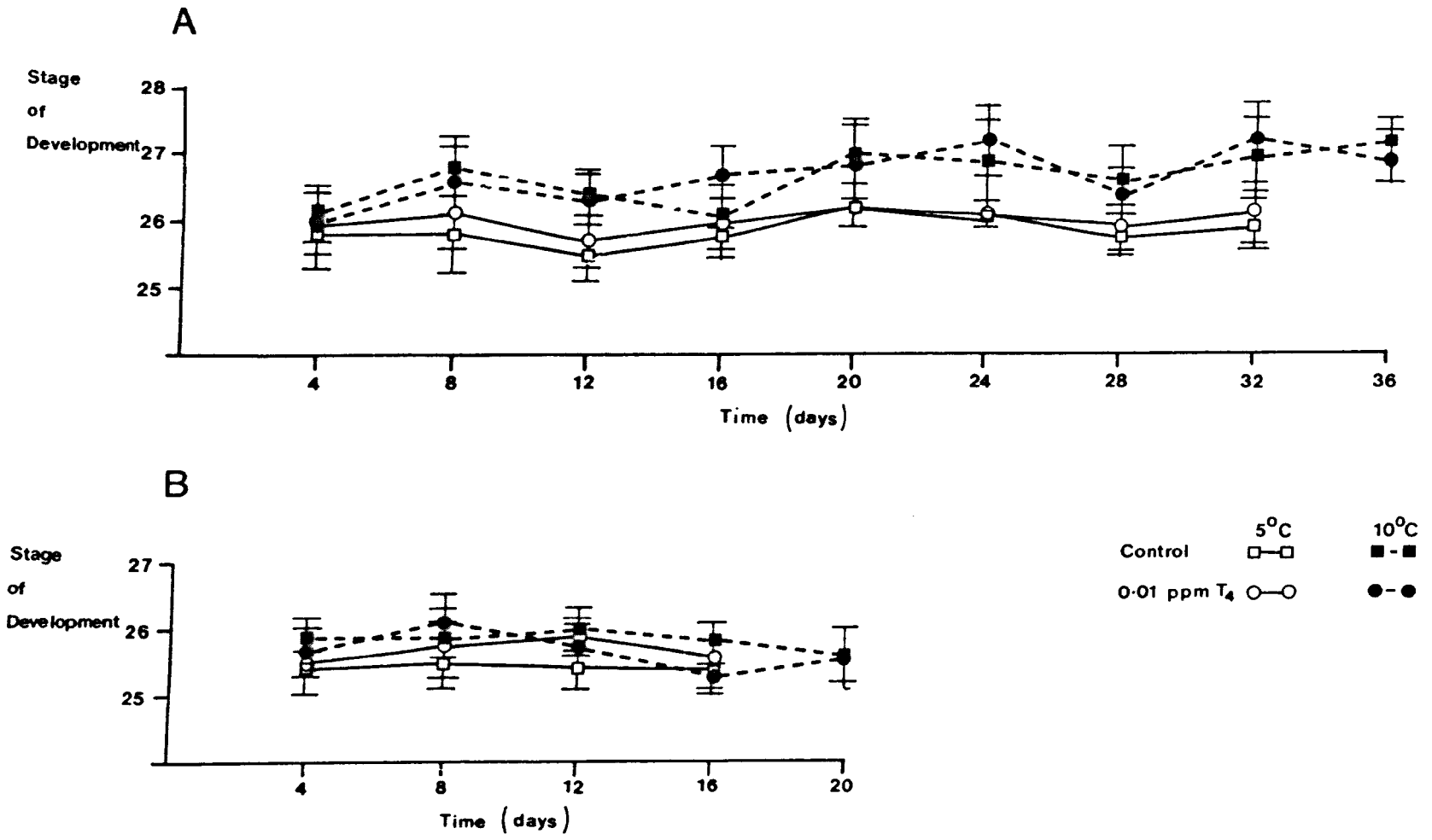

A
Stage
of
Development
28
27
26
25
4
8
12
16
20
24
28
32
36
Time (days)
B
Stage
of
Development
27
26
25
4
8
12
16
20
Time (days)
5°C
10°C
Control
0·01 ppm T4

Figure. 5.2. Development of B. bufo tadpoles at (A) 15°C and (B) 20°C when treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.

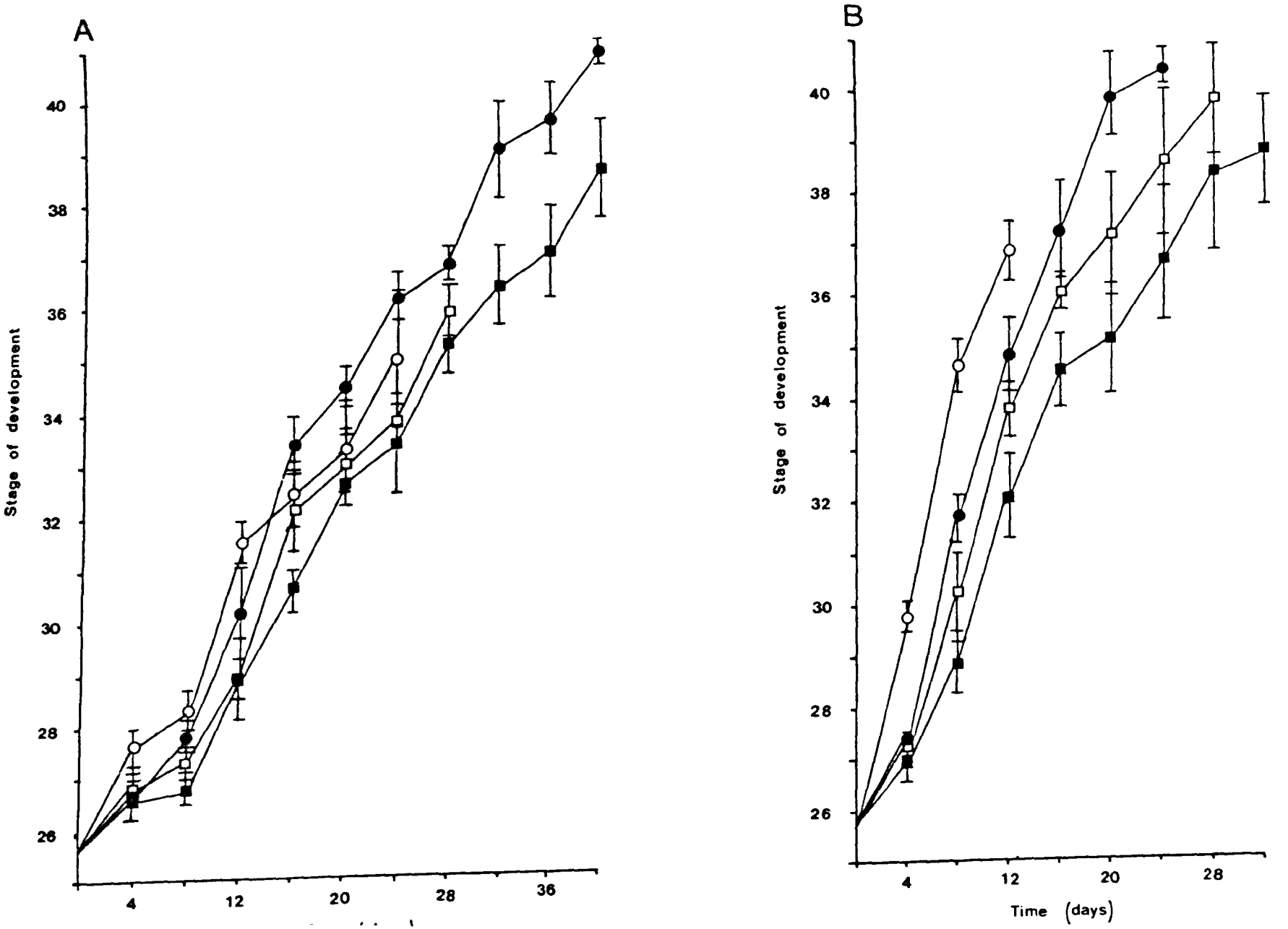
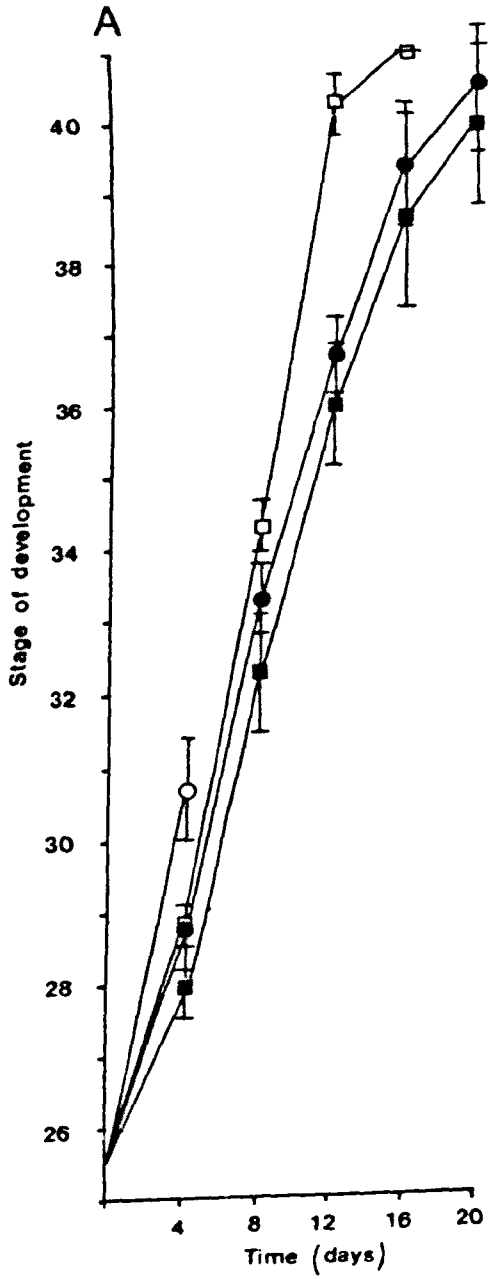
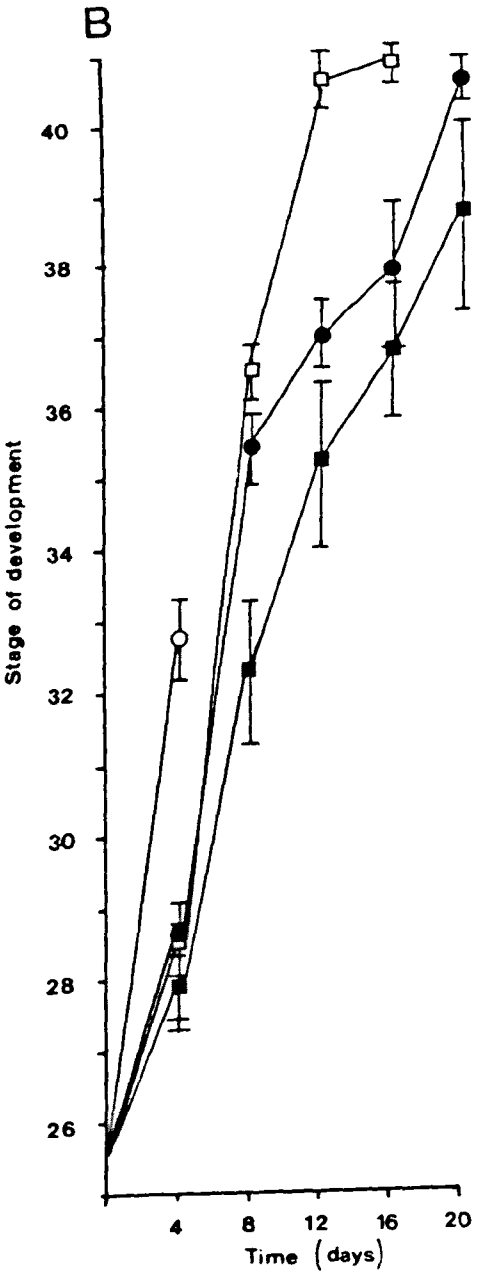

A
B
Stage of development
40
38
36
34
32
30
28
26
4
12
20
28
36
Time (days)

Figure. 5.3. Development of B. bufo tadpoles at (A) 25°C and (B) 30°C when treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.


A
Stage of development
40
38
36
34
32
30
28
26
4
8
12
16
20
Time (days)


B
Stage of development
40
38
36
34
32
30
28
26
4
8
12
16
20
Time (days)

occurred in tadpoles treated with the lowest concentration of T_4 (0.0001 p.p.m.). The development of tadpoles reared at 15°C, in the 0.001 p.p.m. T_4 treatment did not exceed stage 36. At 20°C, tadpoles reared at 0.0001 p.p.m. T_4, 0.001 p.p.m. T_4 and the controls reached metamorphosis.

With rearing temperatures of 25 and 30°C, rate of development in B. bufo increased with an increase in the T_4 concentration. At 25°C, stage 40 was attained after a period of 12 days, (0.001 p.p.m. T_4) and 20 days, (0.0001 p.p.m. T_4). The values for the tadpoles treated with 0.0001 p.p.m. T_4 were not significantly different from the controls, ($p < 0.05$). At 30°C, stage 40 was attained in 12 days (0.001 p.p.m. T_4). The tadpoles at 0.0001 p.p.m. T_4 attained stage 40 after 20 days, however the controls did not reach stage 40, mortality occurring after stages 38-39 after 16 days.

B. calamita tadpole developmental rates appeared to be proportional to the concentration of T_4 in the rearing water; the higher the concentration, the faster the development (Figs. 5.4, 5.5).

B. calamita tadpoles reared at 15°C, attained stage 40 after a period of 52 days, at 0.001 p.p.m. T_4 (Fig. 5.4). Tadpoles at 0.0001 p.p.m. T_4 and the controls did not reach this stage. At 20°C, the development of the tadpoles at 0.0001 and 0.001 p.p.m. T_4 was similar, and faster than the control, stage 40 being reached after 28 days. The results for the 25 and 30°C treatments were similar, (Fig. 5.5) tadpoles at 0.001 p.p.m. T_4 reached stage 40 after 16 days. Tadpoles at 0.0001

Figure. 5.4. Development of B. calamita tadpoles at (A) 15°C and (B) 20°C when treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.

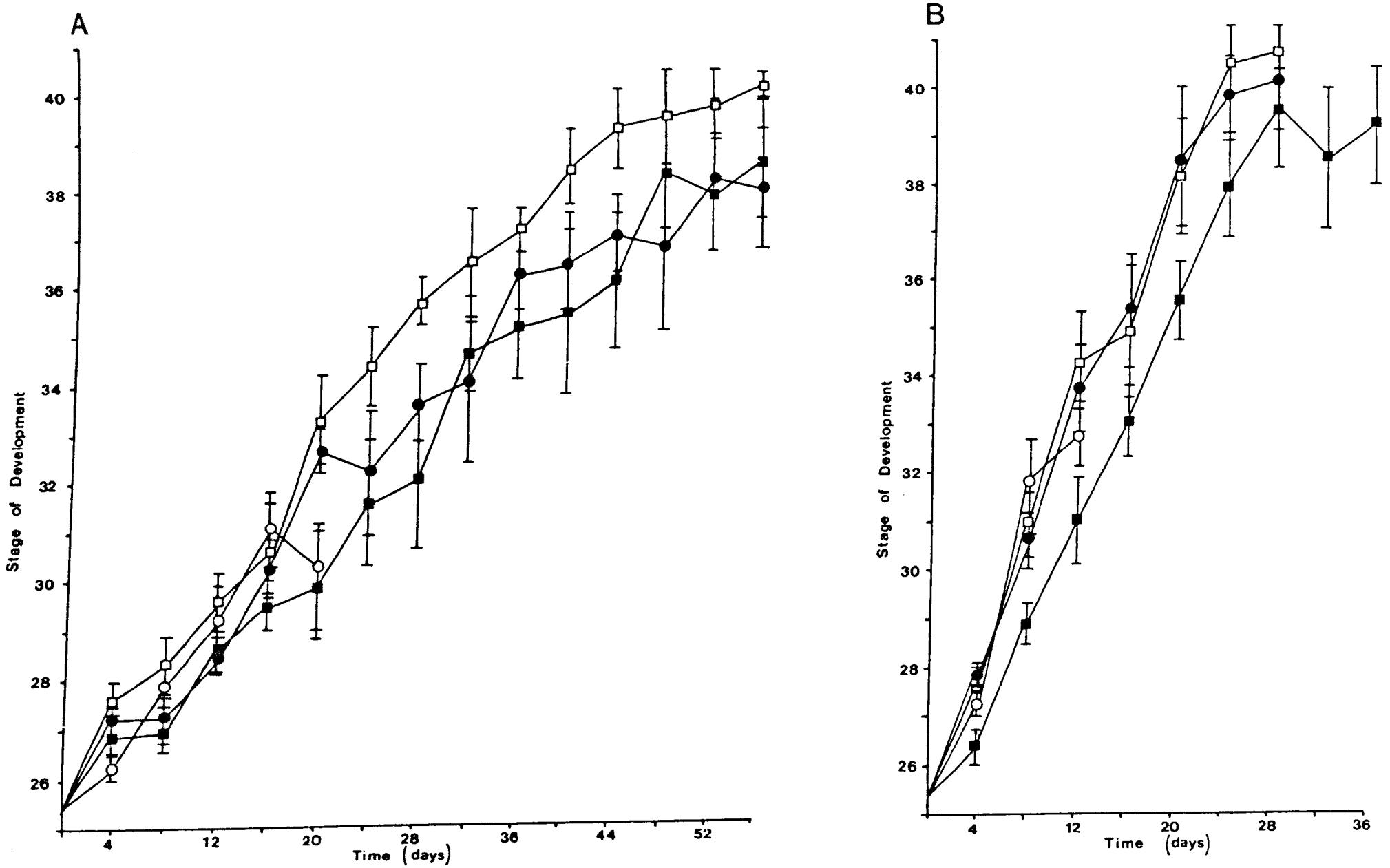
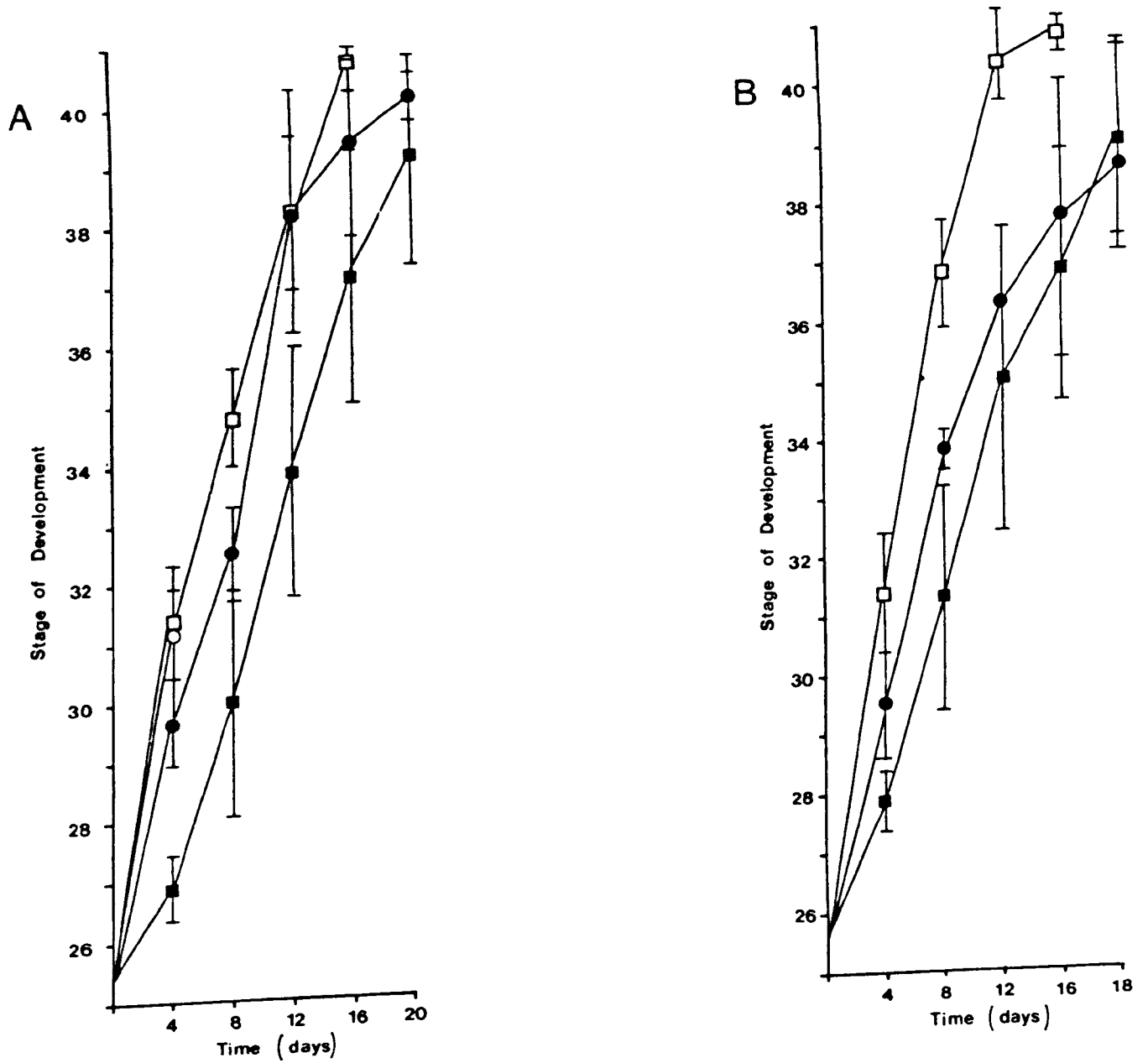

A
B
Stage of Development
Time (days)
26
28
30
32
34
36
38
40
4
12
20
28
36
44
52

Figure. 5.5. Development of B. calamita tadpoles at (A) 25°C and (B) 30°C when treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.


A
B
Stage of Development
Time (days)
26
28
30
32
34
36
38
40
4
8
12
16
18
20

p.p.m. T_4 took 20 days.

5.3.2 Effect of L-thyroxine on tadpole growth at a range of temperatures.

When reared at 5 and 10°C, 0.01 p.p.m. T_4 had no significant effect on B. bufo or B. calamita tadpole growth (increase in mass) (Fig. 5.6). The effect of lower concentrations of T_4 at these temperatures was therefore not investigated.

In general, as T_4 concentration increased, B. bufo growth rate decreased. This occurred across the temperature range 15-30°C (Figs. 5.7-5.9) although the differences were not always statistically significant ($p < 0.05$). At all temperatures, the highest concentration of T_4 (0.01 p.p.m.) resulted in very low rates of growth, and even loss of mass after an initial period of growth at lower temperatures. No tadpoles survived immersion in this concentration of T_4.

At 15°C, B. bufo growth rates were similar for control, 0.0001 and 0.001 p.p.m. T_4 treatments (Fig. 5.7). When reared at 20°C, growth rates were similar for all treatments up to 20 days, after which the controls continued to increase in mass but the T_4 treated tadpoles did not (Fig. 5.8). A levelling out, or even a drop in mass at the end of a growth curve is normally due to a loss of larger tadpoles due to metamorphosis, leaving smaller, less developed tadpoles to continue development.

Figure. 5.6. Effect of L-thyroxine (0.01 p.p.m.) on growth of (A) B. bufo and (B) B. calamita tadpoles reared at 5 and 10°C. Means ± 95% confidence intervals, n=10 for each point.

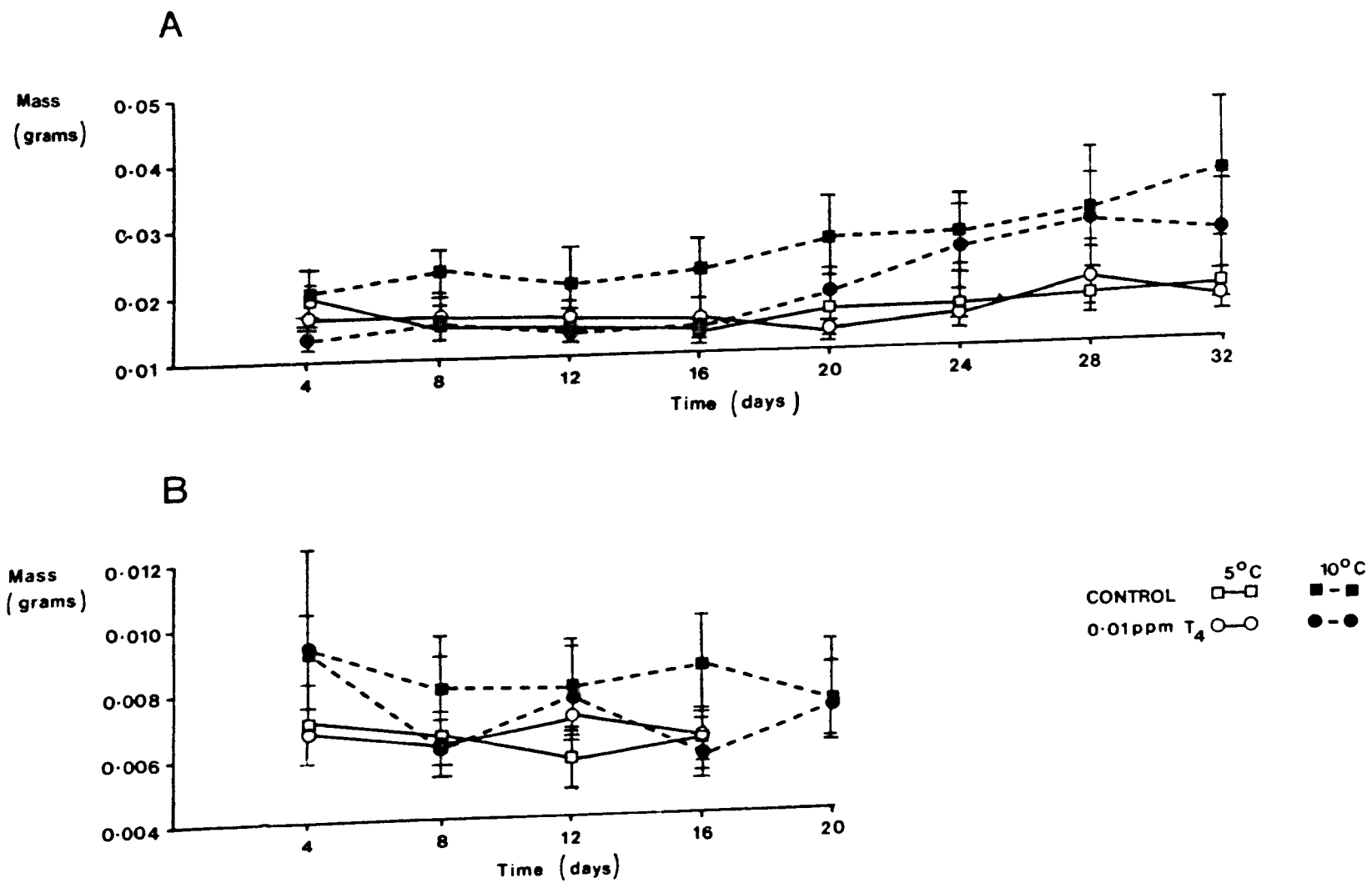

A
Mass
(grams)
0·05
0·04
0·03
0·02
0·01
4
8
12
16
20
24
28
32
Time (days)
B
Mass
(grams)
0·012
0·010
0·008
0·006
0·004
4
8
12
16
20
Time (days)
5°C
10°C
CONTROL
0·01ppm T4

Figure. 5.7. Growth of B. bufo tadpoles reared at 15°C, treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.

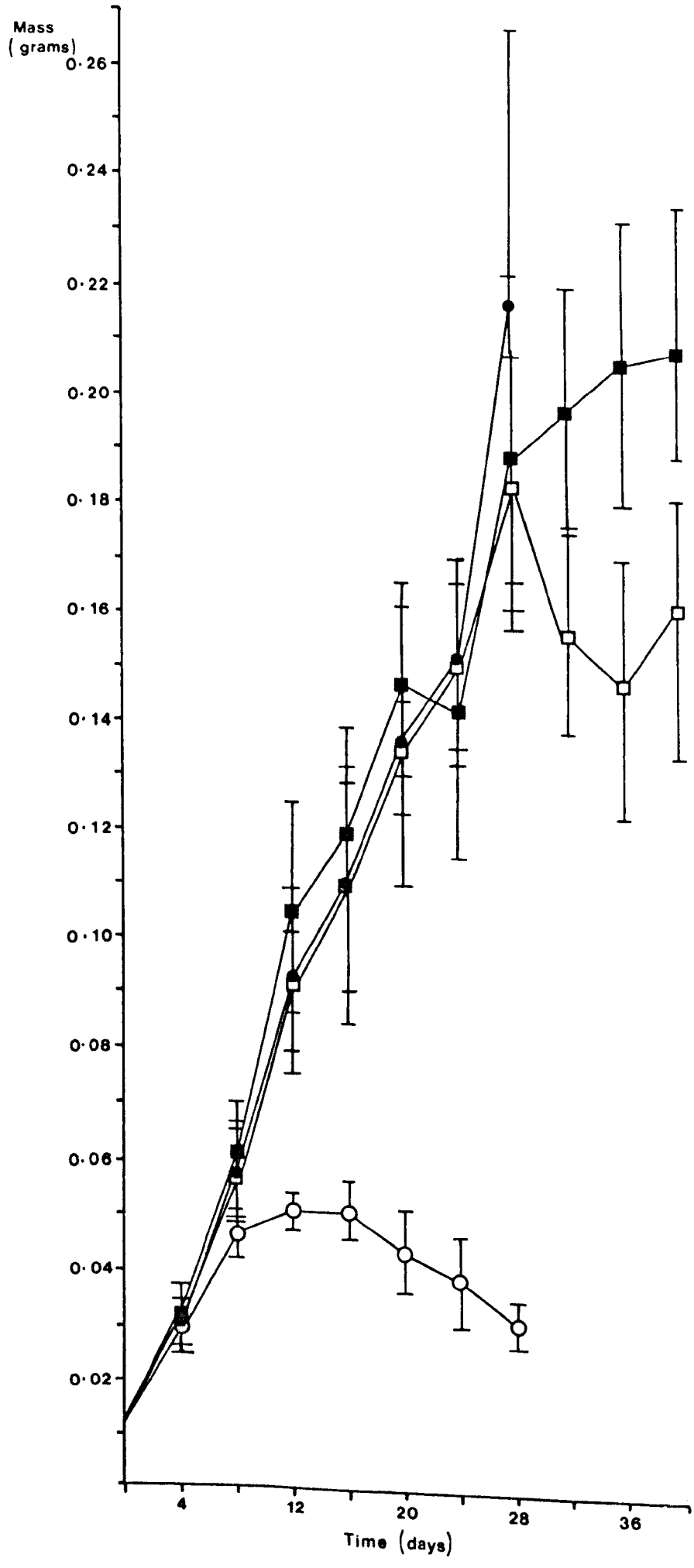
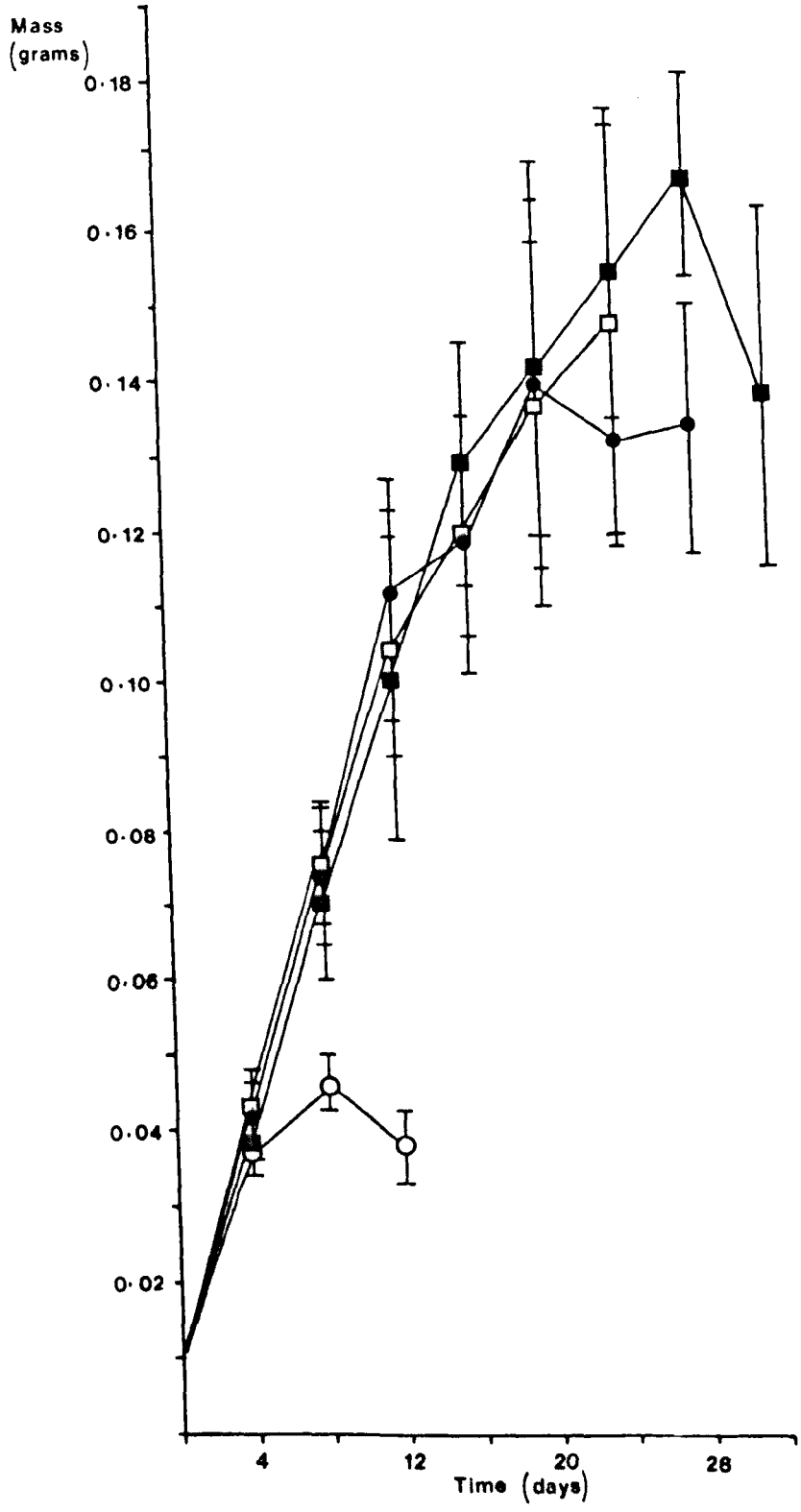

Mass
(grams)
0·26
0·24
0·22
0·20
0·18
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
4
12
20
28
36
Time (days)

Figure. 5.8. Growth of B. bufo tadpoles reared at 20°C, treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.


Mass
(grams)
0·18
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
4
12
20
28
Time (days)

Growth rates for B. bufo were similar at 25 and 30°C (Fig. 5.9), controls attaining the greatest mass after 16 days (0.15 and 0.152 g respectively). The 0.0001 p.p.m. T_4 concentration resulted in tadpoles of 0.13 and 0.133 g respectively. At 25°C, the growth rate of tadpoles in 0.001 p.p.m. T_4 was initially rapid, but began to level out after 12 days. At 30°C, growth rate of the tadpoles at 0.001 p.p.m. T_4 was similar, initially to the other treatments at this temperature. However, after 8 days the rate started decreasing.

B. calamita tadpoles growth rate decreased in general with an increase in L-thyroxine concentration, but the differences were not always statistically significant at the 95% level (Figs. 5.10 - 5.12). The T_4 concentration of 0.01 p.p.m. resulted in little or no growth at all temperatures, followed by mortality.

At 15°C, (Fig. 5.10) growth rates of the control, 0.0001 and 0.001 p.p.m. T_4 treated tadpoles was similar up to day 38. Then the control tadpoles continued to increase in mass but the T_4 treated tadpoles mass started to decrease.

At 20°C, (Fig. 5.11) growth rates were similar for control, 0.0001 and 0.001 p.p.m. T_4 treated tadpoles. The control tadpoles showed a reduction in mass over the 20-32 day period. At 25 and 30°C (Fig. 5.12), growth rates for B. calamita tadpoles at each treatment were similar. The mass increase in the 0.001 p.p.m. T_4 treatment levelled out after 12 days whilst the control and 0.0001 p.p.m. T_4 treatment

Figure. 5.9. Growth of B. bufo tadpoles reared at (A) 25°C and (B) 30°C, treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.

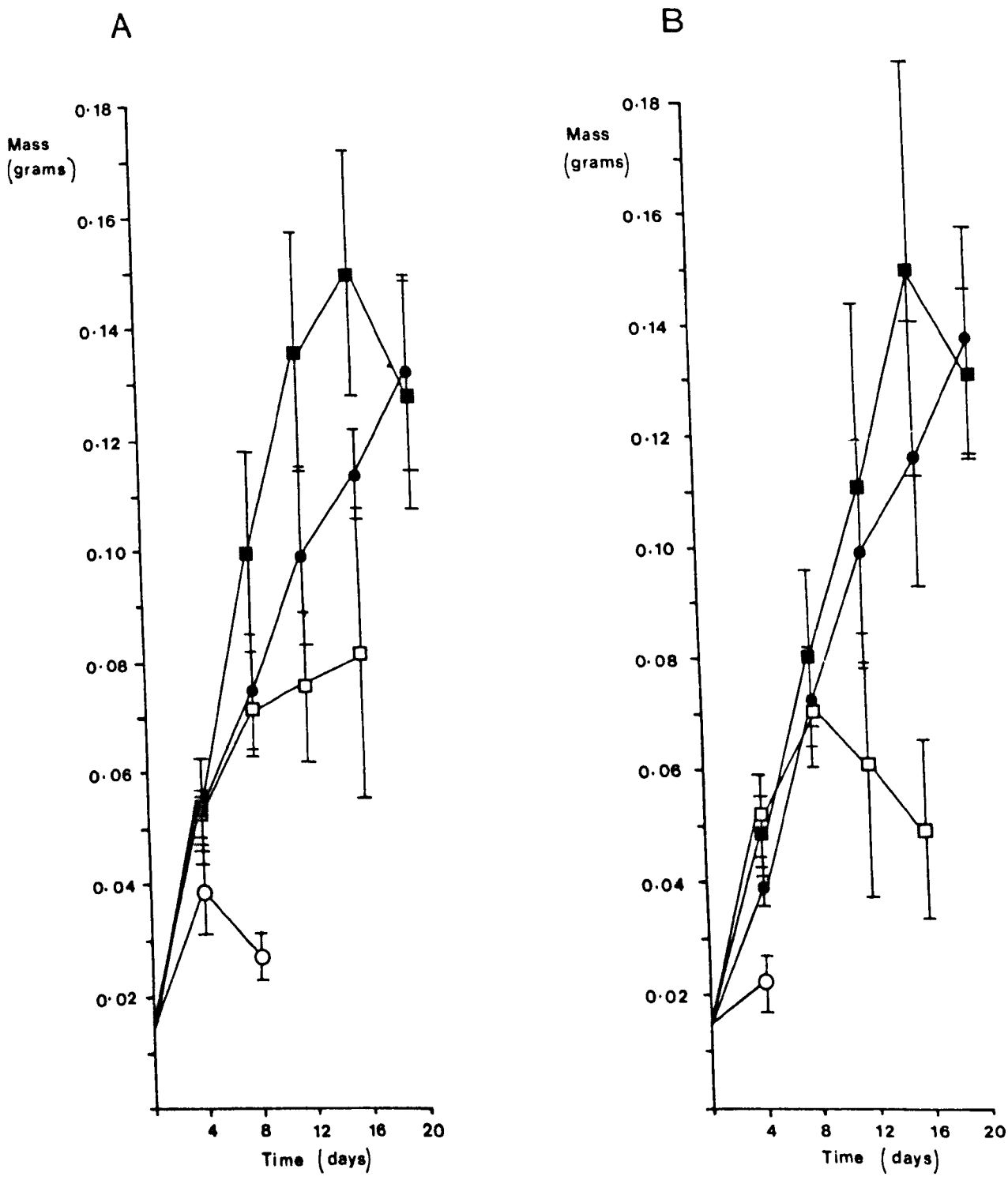

A
B
Mass
(grams)
0·18
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
4
8
12
16
20
Time (days)

Figure. 5.10. Growth of B. calamita tadpoles reared at 15°C, treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.

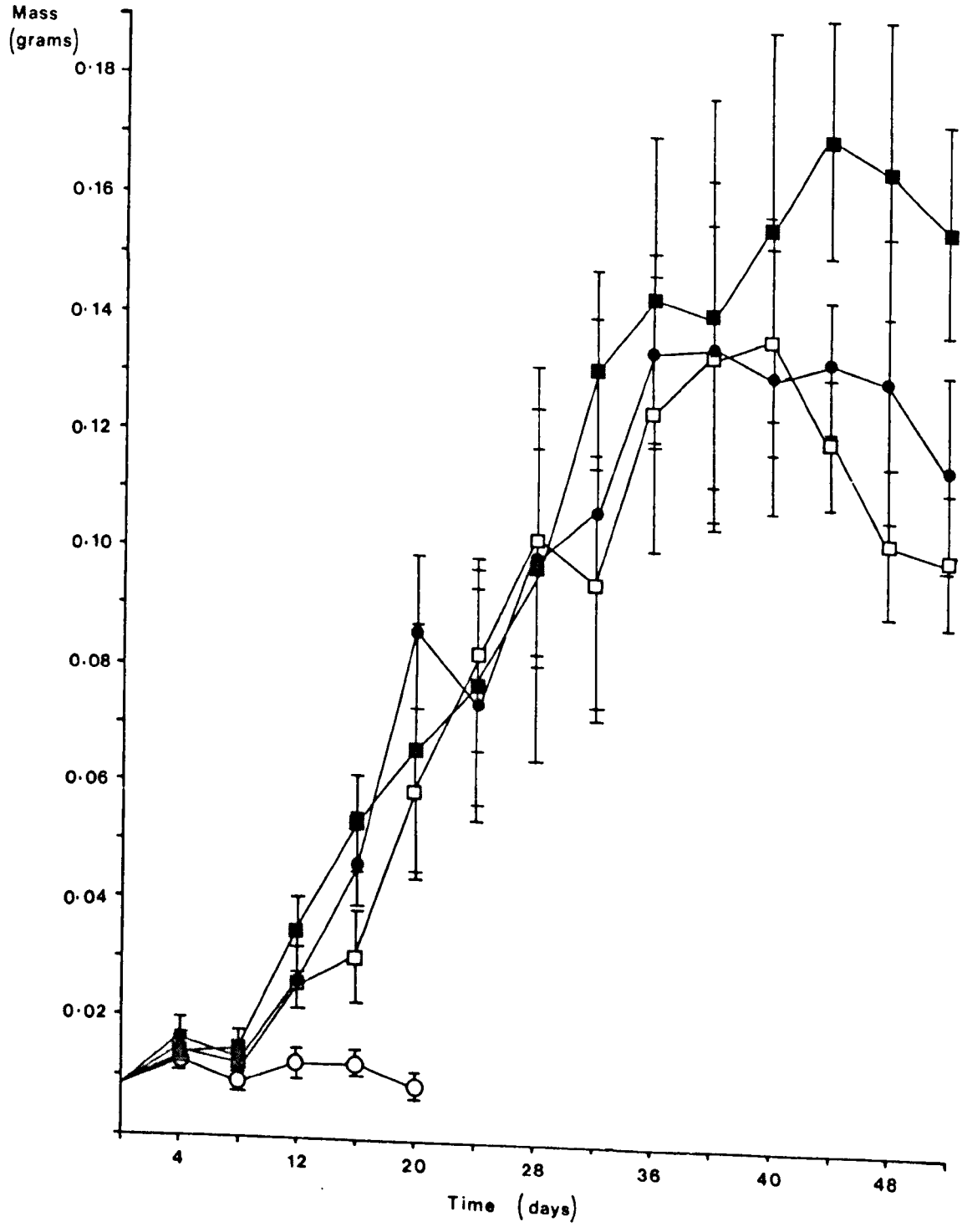

Mass
(grams)
0·18
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
4
12
20
28
36
40
48
Time (days)

Figure. 5.11. Growth of B. calamita tadpoles reared at 20°C, treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.

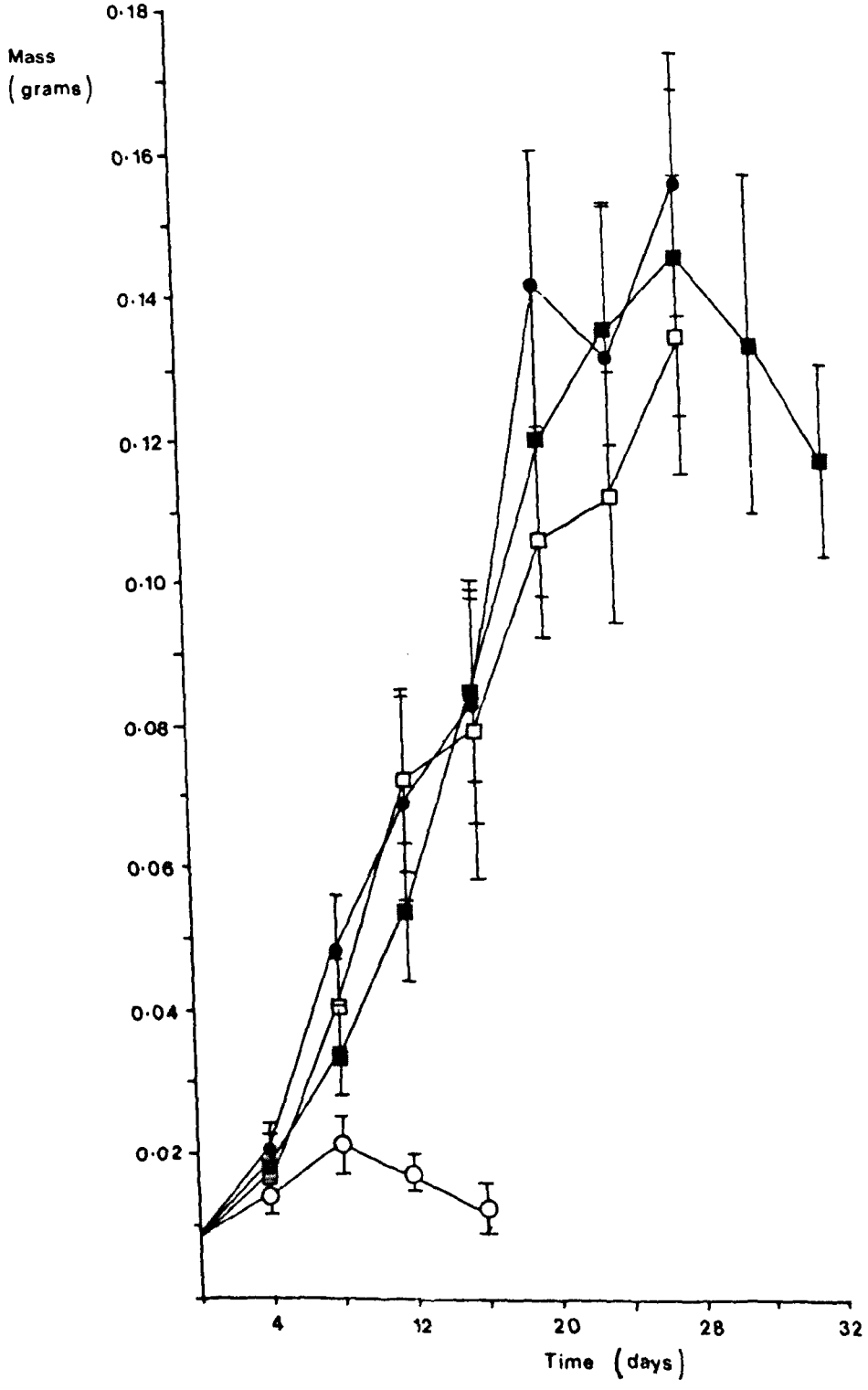
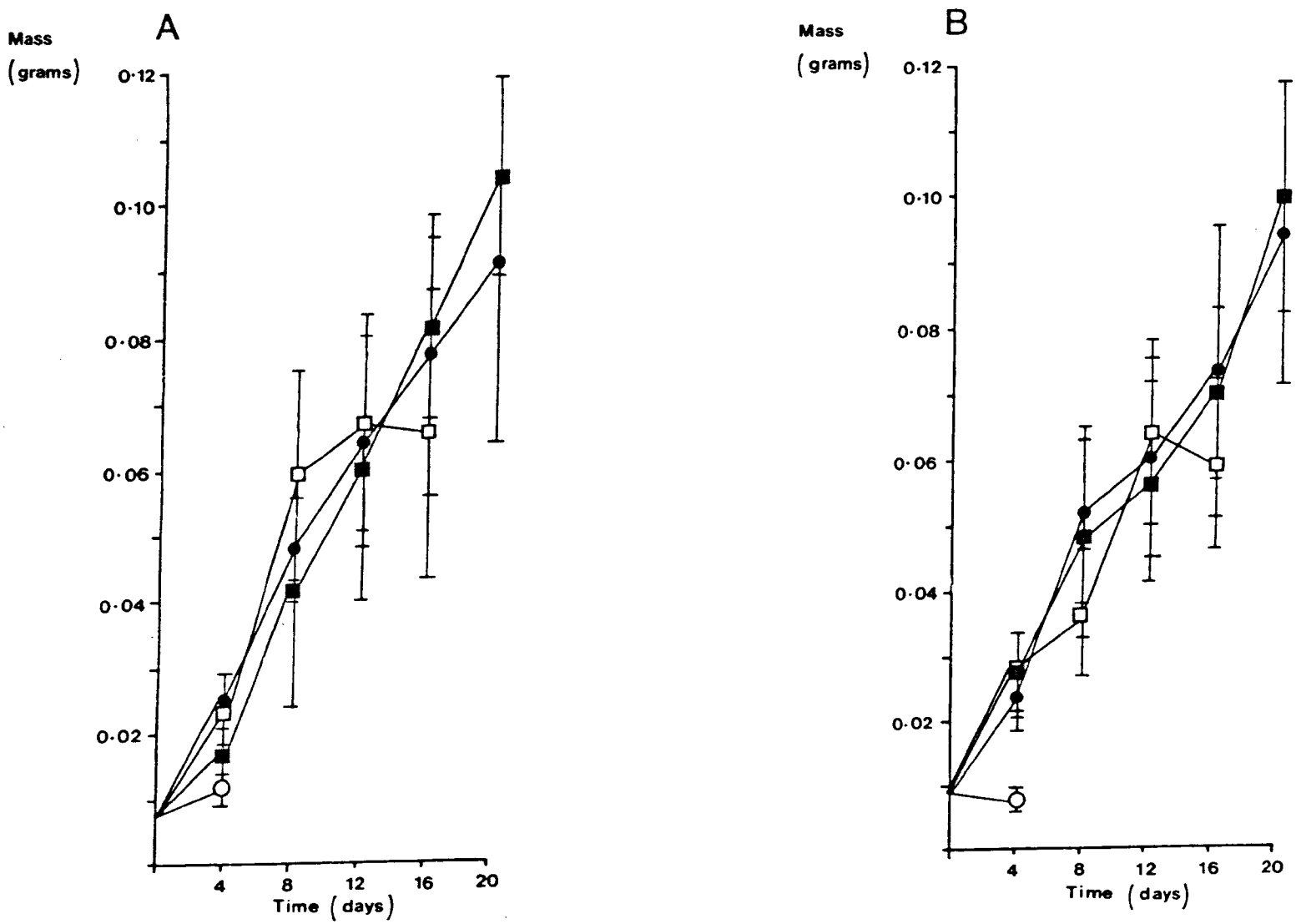

Mass
(grams)
0·18
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
4
12
20
28
32
Time (days)

Figure. 5.12. Growth of B. calamita tadpoles reared at (A) 25°C and (B) 30°C, treated with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.


A
Mass
(grams)
0·12
0·10
0·08
0·06
0·04
0·02
4
8
12
16
20
Time (days)
B
Mass
(grams)
0·12
0·10
0·08
0·06
0·04
0·02
4
8
12
16
20
Time (days)

continued increasing in mass until day 20.

In general, for both B. bufo and B. calamita, an increase in T_4 concentration led to a lower maximum mean mass (Fig. 5.13). This effect was enhanced by higher temperatures. For all the treatments, B. bufo tadpoles had higher maximum mean masses than the equivalent B. calamita tadpoles.

5.3.3 The effect of L-thyroxine on oxygen consumption of tadpoles over a range of temperatures.

B. bufo tadpole oxygen consumption rates increased with temperature for each treatment (Fig. 5.14). The 0.001 p.p.m. T_4 treatment depressed the oxygen consumption of the tadpoles, the effect being enhanced at higher temperatures. There was no significant difference between the control and the 0.0001 p.p.m. T_4 treatment ($p < 0.05$).

B. calamita tadpole oxygen consumption rates increased with temperature for each treatment (Fig. 5.15). The values obtained were higher than the equivalent values obtained for B. bufo. With the exception of the 20°C results, the oxygen consumption of the 0.001 p.p.m. T_4 treated tadpoles was lower than the controls. The 0.0001 p.p.m. T_4 treated tadpoles had lower oxygen consumption rates than the controls at 15 and 20°C but significantly higher ($p < 0.05$) at 25 and 30°C.

Figure. 5.13. Maximum mean mass of (A) B. bufo and (B) B. calamita tadpoles reared with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m T_4 (□), 0.01 p.p.m. T_4 (○) and control (■). Means ± 95% confidence intervals, n=10 for each point.

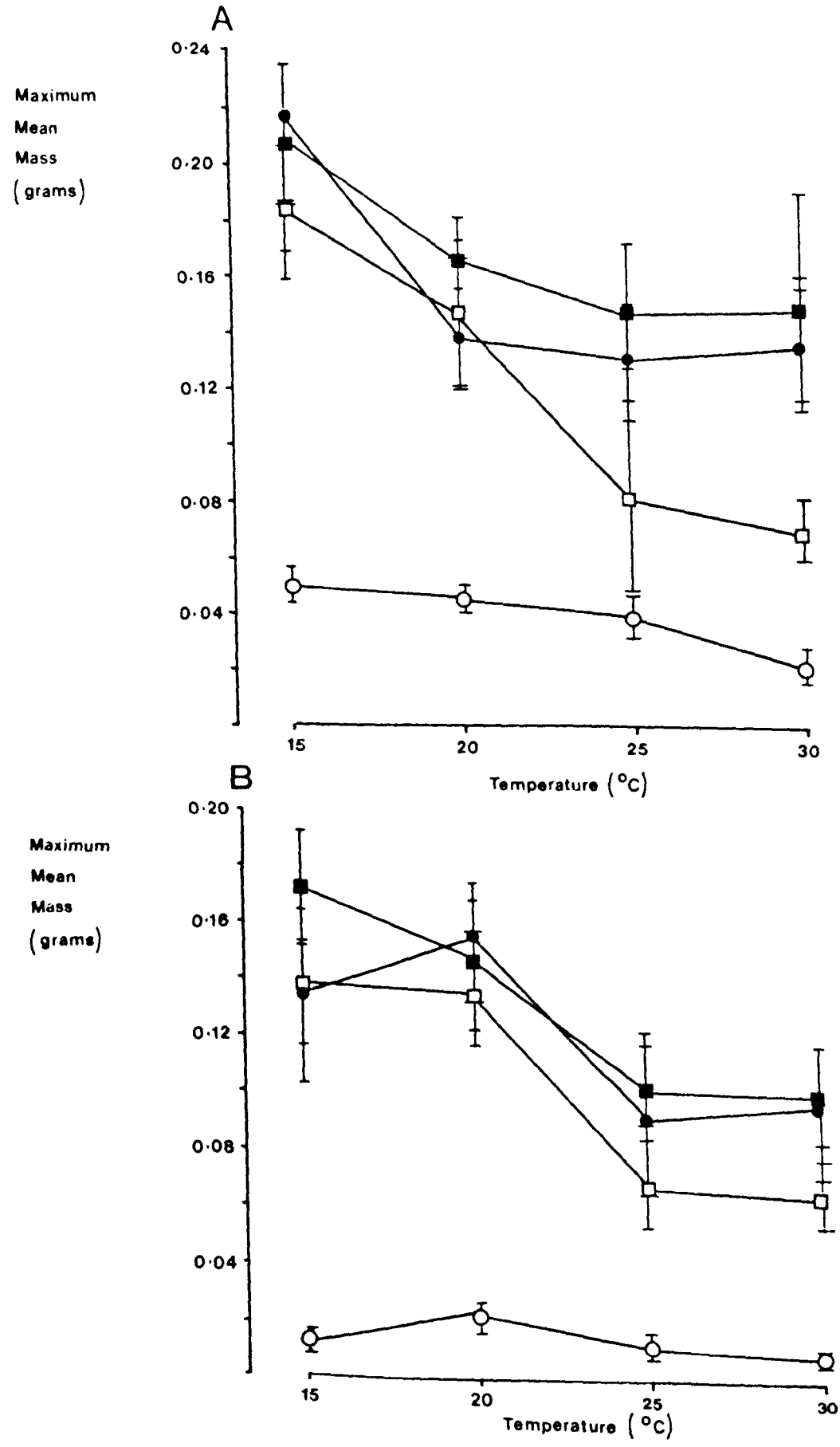

A
Maximum
Mean
Mass
(grams)
0·24
0·20
0·16
0·12
0·08
0·04
15
20
25
30
Temperature (°C)
B
Maximum
Mean
Mass
(grams)
0·20
0·16
0·12
0·08
0·04
15
20
25
30
Temperature (°C)

Figure. 5.14. Mass specific oxygen consumption of B. bufo tadpoles at a range of acclimation temperatures, reared with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□) and control (■). Means ± 95% confidence intervals. See section 4.2.2 for sample sizes.

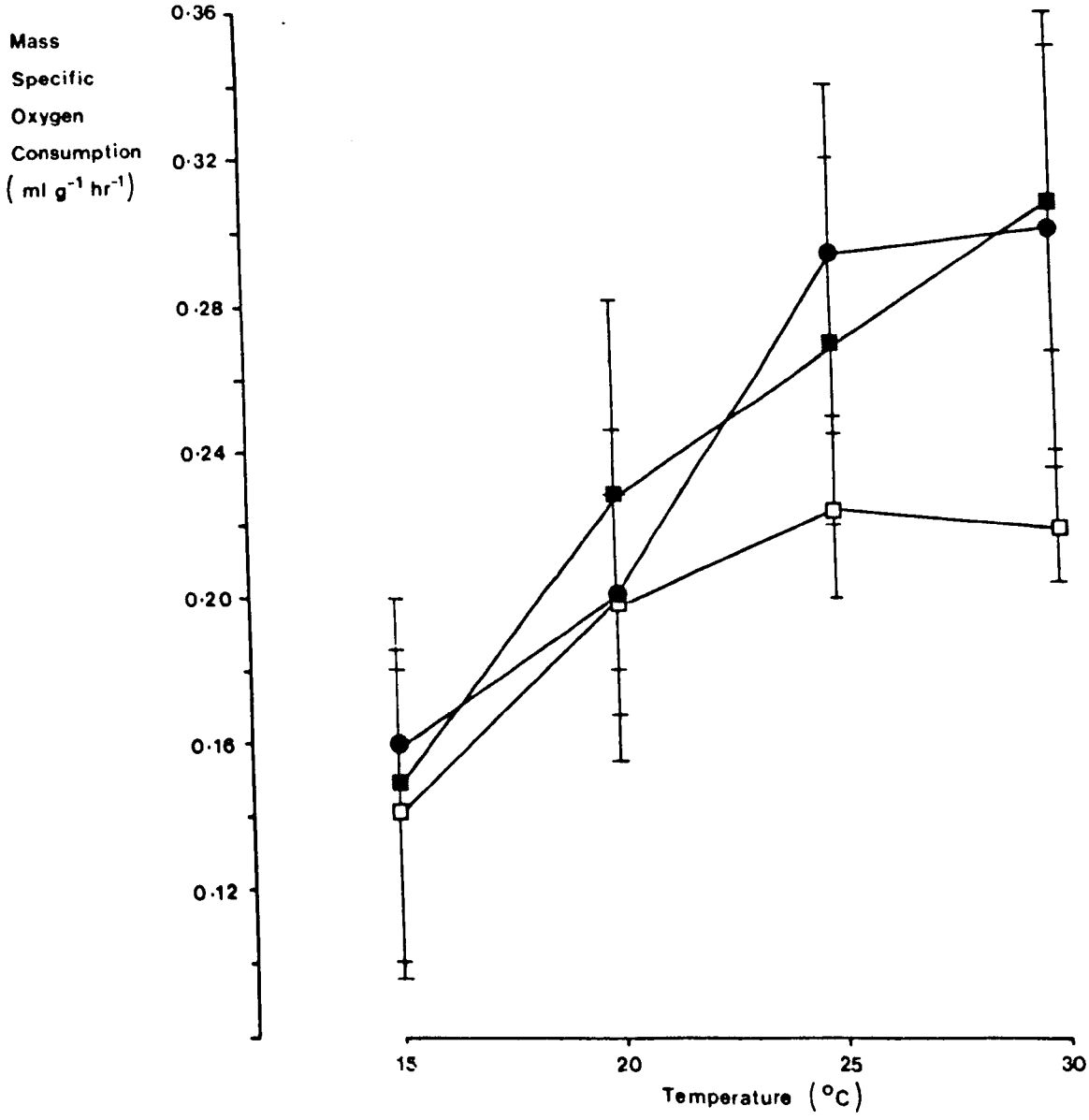
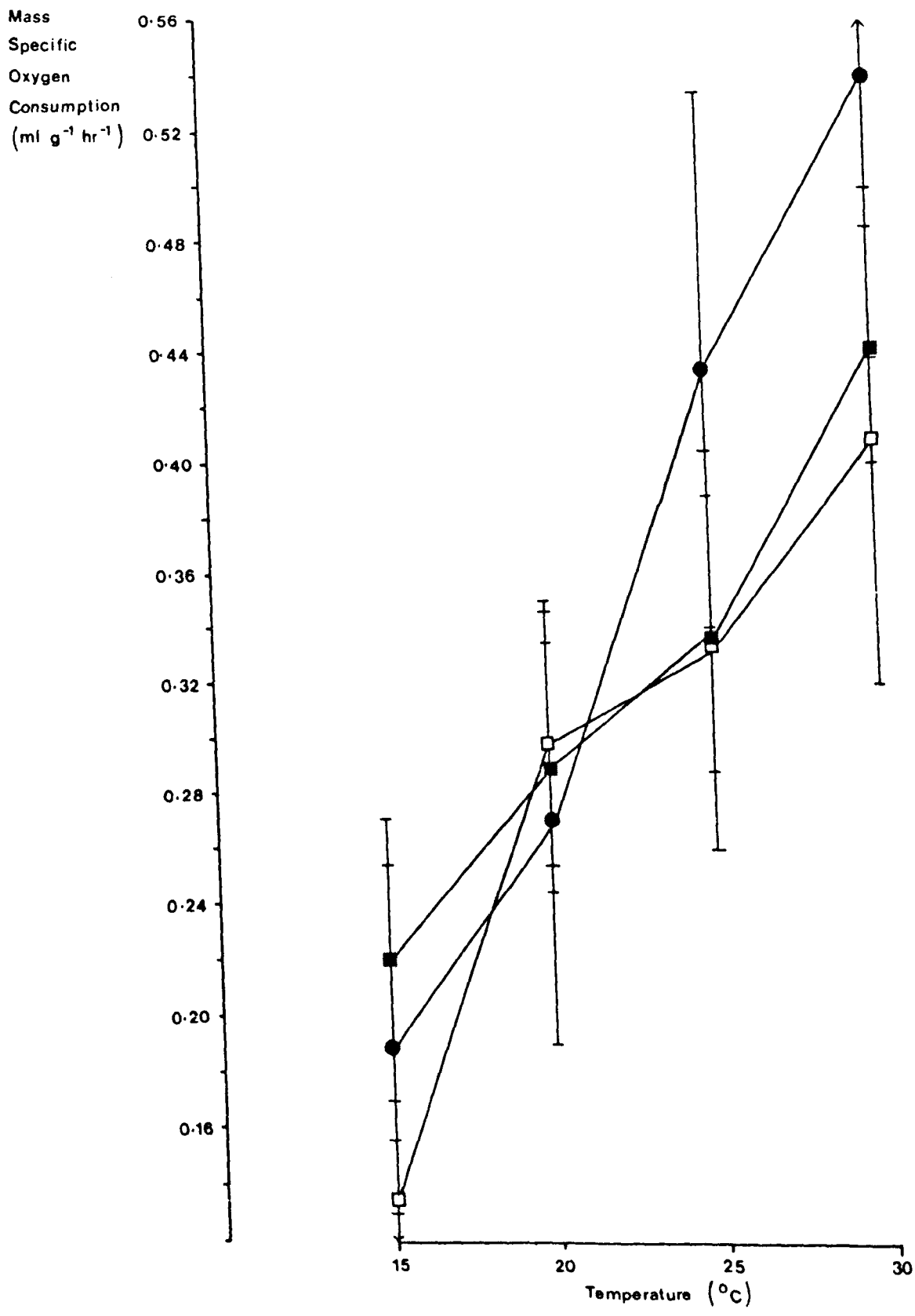

Mass
Specific
Oxygen
Consumption
(ml g-1 hr-1)
0·36
0·32
0·28
0·24
0·20
0·16
0·12
15
20
25
30
Temperature (°C)

Figure. 5.15. Mass specific oxygen consumption of B. calamita tadpoles at a range of acclimation temperatures, reared with 0.0001 p.p.m. T_4 (●), 0.001 p.p.m. T_4 (□) and control (■). Means ± 95% confidence intervals. See section 4.2.2 for sample sizes.


Mass
Specific
Oxygen
Consumption
(ml g⁻¹ hr⁻¹)
0·56
0·52
0·48
0·44
0·40
0·36
0·32
0·28
0·24
0·20
0·16
15
20
25
30
Temperature (°C)

5.4 DISCUSSION

5.4.1. Effect of L-thyroxine on tadpole development.

B. bufo and B. calamita did not respond to even the highest treatment levels of L-thyroxine at 5 and 10°C. At these temperatures neither of the species can successfully metamorphose (Chapter 2). This is possibly a result of the inability of the tadpole tissues to respond to thyroid hormones (Dodd and Dodd, 1976).

At low temperatures, hormonal signals may be received but the low temperatures prevent the execution of the message (Frieden et al, 1965). Yamamoto et al (1966) showed that a decrease in temperature reduced the L-thyroxine turnover rate and probably tissue utilisation rate of injected thyroid hormones.

If in B. bufo and B. calamita body tissues are unable to respond to L-thyroxine at low temperatures, this may explain the inability of the two species to develop and metamorphose at 5 and 10°C.

At temperatures above 10°C immersion in 0.01 p.p.m. L-thyroxine produced similar responses in B. bufo and B. calamita tadpoles; rapid development, leading to malformation and death. The detrimental effects appeared earlier (as did mortality) at the higher temperatures. Spacing of metamorphic events depend on the concentration of thyroid hormones circulating in the tadpole (Etkin, 1935). Etkin (1935) suggests there is a series of specific thresholds of L-thyroxine

concentration during development which initiate a sequence of events inherent in the tadpole tissue. As temperature increases, less time is required for the completion of any given metamorphic event at a fixed dosage of L-thyroxine.

If the concentration of L-thyroxine is so high that all the specific threshold levels are overcome, and the temperature is such that the tissues can respond then development would proceed in the majority of the tadpole tissues at one time. The step by step sequence suggested by Etkin (1935) would break down, and the tadpole, with relatively low mass would undergo the tissue differentiation of metamorphosis at a point when its own resources were insufficient to support it. This would inevitably lead to tadpole mass loss and distortion as the metamorphic sequence is disrupted resulting in high mortality.

At the 0.0001 and 0.001 p.p.m. L-thyroxine concentrations both B. bufo and B. calamita tadpoles had faster developmental rates than the controls. There was no obvious difference in the responses of the two species. Most larval tissues become reactive to thyroxine at a very early stage in larval development (Etkin, 1950; Tata, 1968). This was the case in B. bufo and B. calamita tadpoles where the effect of L-thyroxine on development could be seen at stages 26-28. Higher concentrations of L-thyroxine produced a greater effect. The metamorphic rate depends on L-thyroxine concentration and temperature (Etkin, 1935; Kollros, 1956) and a quantitative relationship exists between L-thyroxine and its mode of action (Derby, 1968).

5.4.2 Effect of L-thyroxine on tadpole growth.

There is a reciprocal relationship between the rate of tadpole development and overall body growth (Etkin and Gilbert, 1968). This is apparent in B. bufo and B. calamita. Whereas the different L-thyroxine levels significantly increased development rates over that of the controls, the growth rates of treatment and control tadpoles were similar. As in chapter 2, it is seen that the mass of the tadpole at any point is a function of the rate of development. The faster the development the lower the mass of the tadpole at a given stage.

In this series of experiments, the control tadpoles attained the highest mean masses and the 0.001 p.p.m. L-thyroxine treated tadpoles the lowest. The maximum mean mass obtained by B. calamita control tadpoles was greater than that of the 0.001 p.p.m. treated tadpoles. As temperature increased, the difference between the treatment and control remained constant. The mean masses of the control and 0.001 p.p.m. treated B. bufo tadpoles diverged as temperature increased. The maximum mass attained by the 0.001 p.p.m. treated tadpoles approached that of the 0.01 p.p.m treated tadpoles as temperature increased. This might indicate that there is a difference between the two species in their response to L-thyroxine at higher temperatures.

It is possible that the mechanism for L-thyroxine regulation involves negative feedback. When tissue receptors are saturated, thyroid stimulating hormone secretion is suppressed (Dodd and Dodd, 1976).

This suggests that there no control over exogenously applied thyroid hormones if their concentration exceeds the tissue saturation point. This may have occurred in the tadpoles treated with the highest concentration of L-thyroxine, the tadpoles' feedback mechanisms being overcome and development becoming disorderly.

Heinemann and Weber (1966) suggest that L-thyroxine exerts its metamorphic potency by controlling the release of biosynthetic messages from the genome rather than by interfering with energy metabolism. If this applies there may be inherent genetic information controlling response to L-thyroxine at higher temperatures in B. calamita. At this stage, however, this is highly speculative.

B. bufo tadpoles treated with L-thyroxine show the accelerated development and consequent reduction in mass gain that is seen in B. calamita tadpoles at increasing rearing temperatures (Chapter 2). This suggests that, in B. calamita tadpoles, L-thyroxine production or utilisation is important for its rapid development.

It should be noted that in these experiments, contrary to those in chapter 2, the rate of development of B. calamita control tadpoles was not obviously faster than those of equivalent B. bufo tadpoles. This may have been the result of increased experimental stress, or the earlier collection date of the B. calamita spawn. These differences, however, should not have affected the response of the two species to L-thyroxine.

5.4.2 Effect of L-thyroxine on oxygen consumption.

The oxygen consumption of B. bufo tadpoles treated with 0.001 p.p.m. L-thyroxine appeared to be suppressed at higher temperatures, whereas there was no significant differences between treatments and controls in B. calamita at all temperatures. The statistical variance in the results was, however, wide and no obvious trends could be discerned. As temperature increased so did oxygen consumption of controls and treatments. L-thyroxine administration has been shown to have little effect on premetamorphic amphibians (Drastich, 1925). Metabolism may be affected indirectly due to differences in body size, activity, nutrition and degree of development (Prosser and Brown, 1964).

Investigating oxygen consumption in early stages of Amblystoma tigrinum, Belehradek and Huxley (1927) found no evidence of increase in metabolic rate with L-thyroxine administration. It is probable that increase in L-thyroxine levels does not bring about a direct rise in oxygen consumption. Funkhouser and Foster (1970) consider the effect of L-thyroxine on metabolism is secondary to its effect in promoting protein synthesis. This agrees with Heinemann and Weber (1966) who suggest that L-thyroxine controls biosynthesis directly rather than via energy metabolism.

In B. bufo and B. calamita, it is possible that L-thyroxine is acting directly on protein synthesis rather than metabolic rate. Their developmental rates and masses change without significant differences in overall oxygen consumption. It appears that B. calamita is less

susceptible to high L-thyroxine concentrations at high temperatures than B. bufo. This may be a result of a modified response to L-thyroxine when subjected to higher temperatures, a possible adaptation for high developmental rates in warm, ephemeral breeding ponds.

CHAPTER 6

6.0 The effect of tadpole density on growth, development and metamorphosis in B. bufo and B. calamita.

6.1 INTRODUCTION

Changes in the density of tadpoles have been shown to have considerable effects on their growth, development and metamorphosis (e.g. Akin, 1966; Brockleman, 1969; Wilbur, 1977). The effects of differing density on tadpoles has been attributed to various factors: Competition for food (Brockleman, 1969; DeBenedictis, 1974), behavioural interactions (Rugh, 1934; Gromko et al, 1973; John and Fenster, 1975) and growth inhibitors (Richards, 1958; Rose, 1960; Rose and Rose, 1961; Akin, 1966; Shvarts and Pyastolova, 1970; Licht, 1977).

An increase in tadpole density has been shown to retard growth rate (Brockleman, 1969; John and Fenster, 1975). The decrease in growth rate is also accompanied by a reduction in developmental rates (Lynn and Edelman, 1936; Newman,1987). This results in delayed metamorphosis, with smaller sizes at metamorphosis (Collins, 1979; Semlitsch and Caldwell, 1982). An increase in density generally leads to an increase in mortality, and reduction in metamorphic success (Lynn and Edleman, 1936; Brockleman, 1969; Semlitsch and Caldwell, 1982).

Density affects under natural conditions are modified by predation, physical factors and food availability (Cecil and Just, 1979). B. bufo

generally spawn in permanent ponds (Harrison, 1985). Here the density of tadpoles at any one time will be no more than the number of individuals hatching from the spawn. The tadpole density will decrease as a result of competition and predation (Harrison, 1985). B. calamita spawn preferentially in temporary pools (Beebee, 1983). Throughout B. calamita tadpole development the pool's volume will be diminishing, thus the density of tadpoles may increase with time.

The different characteristics of the favoured spawning environments of B. bufo and B. calamita, permanent pond and ephemeral pool (Chapter 1), result in different population density changes for the two species during tadpole development. This will also affect the metamorphic success of the two species. The effect of different densities on B. bufo and B. calamita growth, development and metamorphosis will be investigated in this chapter.

6.2. MATERIALS AND METHODS

6.2.1. Effect of density on growth and development.

After initial rearing (section 2.2.2.) tadpoles were placed in shallow plastic trays (35 x 24 x 4 cm), containing 2 l of dechlorinated tap water. Tadpoles were maintained at densities of 2, 10 and 50 tadpoles l^{-1}. For both species the treatments were replicated 10 times for the 2 l^{-1} density, 6 times at the 10 l^{-1} density and 4 times for the 50 l^{-1} density.

Rearing water was renewed and trays cleaned out every four days, feeding was as described in section 2.2.3. Weighing and stage assessment was carried out every four days as in section 2.2.4.

6.2.2. Effect of density on timing of metamorphosis

On reaching stage 41 (forelimb emergence), tadpoles in the growth and development experiment (section 6.2.1) were removed. The transforming tadpoles were placed in small plastic tanks containing moist filter paper. Transforming tadpoles were removed daily and the numbers reaching stage 41 were recorded on a daily basis. When the tadpoles had fully metamorphosed (now toadlets, stage 46), they were weighed after being blotted dry with absorbent cotton. Ten toadlets were weighed for each treatment. After weighing, the toadlets were released close to the point of spawn collection.

6.3. RESULTS

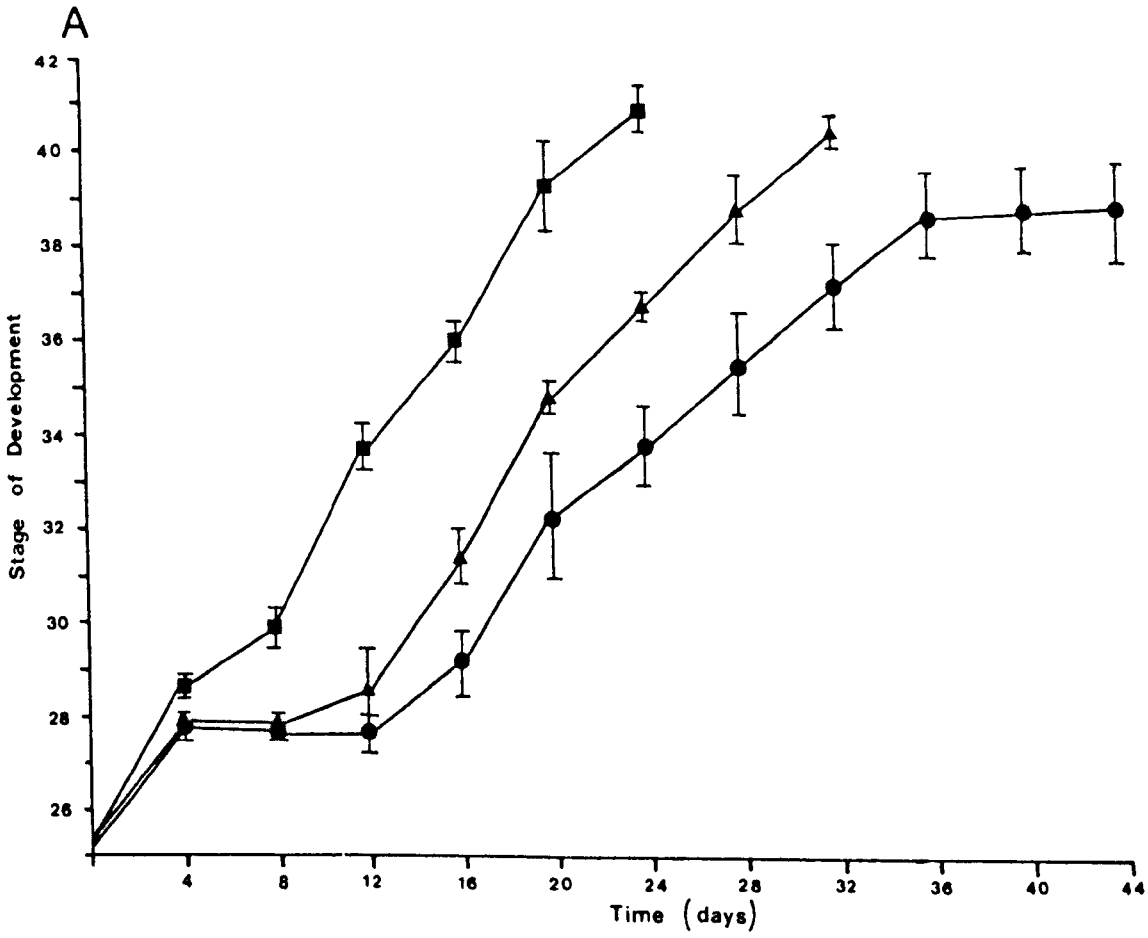
6.3.1. Effect of density on tadpole development.

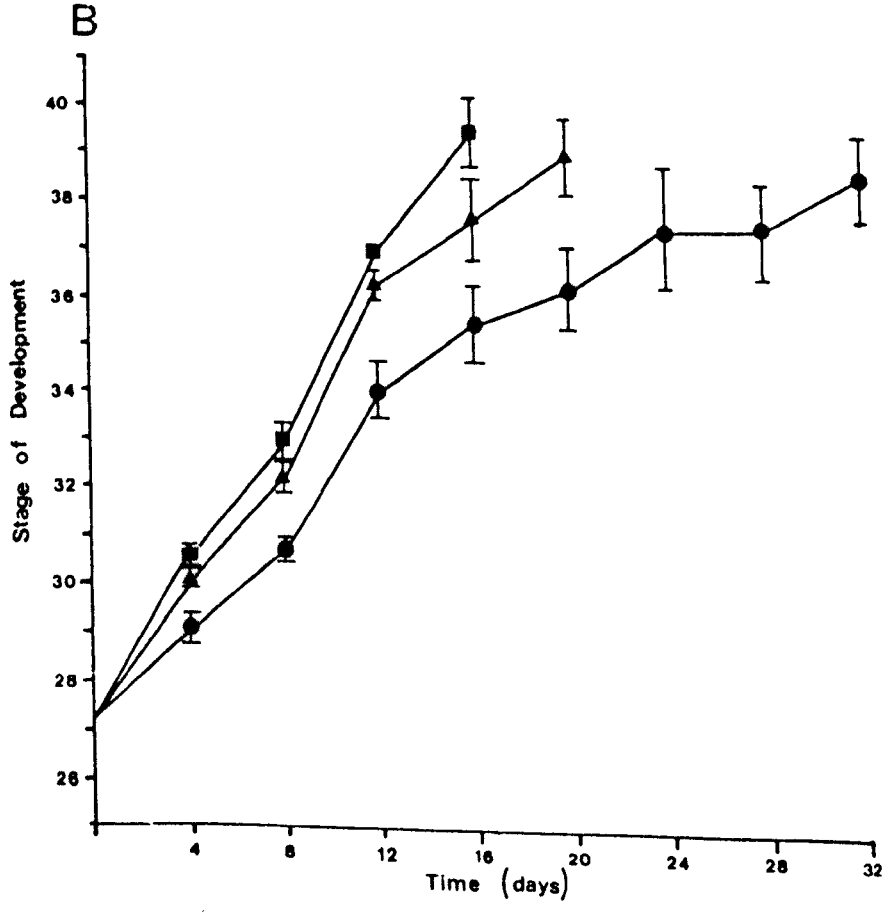
Increase in B. bufo and B. calamita rearing density resulted in a decrease in developmental rate (Fig. 6.1). There were significant differences ($p > 0.05$) in the rates of development at each rearing density.

B. bufo development at 10 l^{-1} (the middle density, MD) and at 50 l^{-1} (the high density, HD) was not significantly different ($p > 0.05$) over the first eight days. The rate of development of the MD tadpoles then increased at a greater rate than that of the HD tadpoles. At all times, the tadpoles at the 2 l^{-1} density (low density, LD) developed faster than those at MD and HD.

B. calamita tadpoles reached the specific stages of development, in all treatments, at a faster rate than B. bufo. LD and MD curves were similar, not being significantly different ($p > 0.05$) until 12 days after the start of the experiment. After this point, the LD tadpoles' developmental rate continued to be high whilst that of the MD tadpoles tailed off. Overall, B. calamita developmental rates were initially similar but at each density, diverged after stages 34-36. B. bufo developmental rates varied dramatically over stages 25-30, and continued in parallel until metamorphosis.

Figure. 6.1. Effect of density on rates of development in (A) B. bufo and (B) B. calamita tadpoles: (■) 2 tadpoles l^{-1}, (▲) 10 tadpoles l^{-1} and (●) 50 tadpoles l^{-1}. Means ± 95% confidence intervals, n=20 for each point.


A
Stage of Development
42
40
38
36
34
32
30
28
26
4
8
12
16
20
24
28
32
36
40
44
Time (days)


B
Stage of Development
40
38
36
34
32
30
28
26
4
8
12
16
20
24
28
32
Time (days)

Variation in relative times for each stage of development in B. bufo occurs mainly at stages 28-29 (Fig. 6.2). At LD development through these stages took 2-5 days, at MD and HD 11 and 13.5 days respectively. The remaining groups of stages all occupied a similar amount of time between the treatments, with the exception of stages 38-39 which increased in duration in HD tadpoles.

B. calamita development through the initial stages (28-35) was similar in duration for the three density treatments, the major differences occurring after stage 35. Stages 36-39 took 5 days at LD, 9 days at MD and 16 days at HD.

The duration of each developmental stage in relation to the total developmental time varied considerably (Fig. 6.3). B. bufo tadpoles, at LD, spent the same proportion of time in each stage grouping. At MD the greatest proportion of developmental time was spent at stages 28-29 (35%). In comparison to the LD tadpoles the proportion of time spent at stages 30-31 (20% HD, 10% MD) was considerably reduced as was the time spent at stages 34-35 (12% HD, 7% MD). The LD tadpoles again spent the greatest proportion of their development time at stages 28-29 (30%) but stages 38-39 also took a large proportion of developmental time (21%).

There was a general pattern to B. calamita development as rearing density decreased. The proportion of time spent in the early stages (28-33) was reduced whilst the percentage of time spent in stages 36-39 increased. Stages 34-35 remained similar in % duration. At MD and LD,

Figure. 6.2. Duration of developmental stages (shown in boxes) in (A) B. bufo and (B) B. calamita tadpoles reared at densities of: 2 tadpoles l^{-1}, 10 tadpoles l^{-1} and 50 tadpoles l^{-1}.

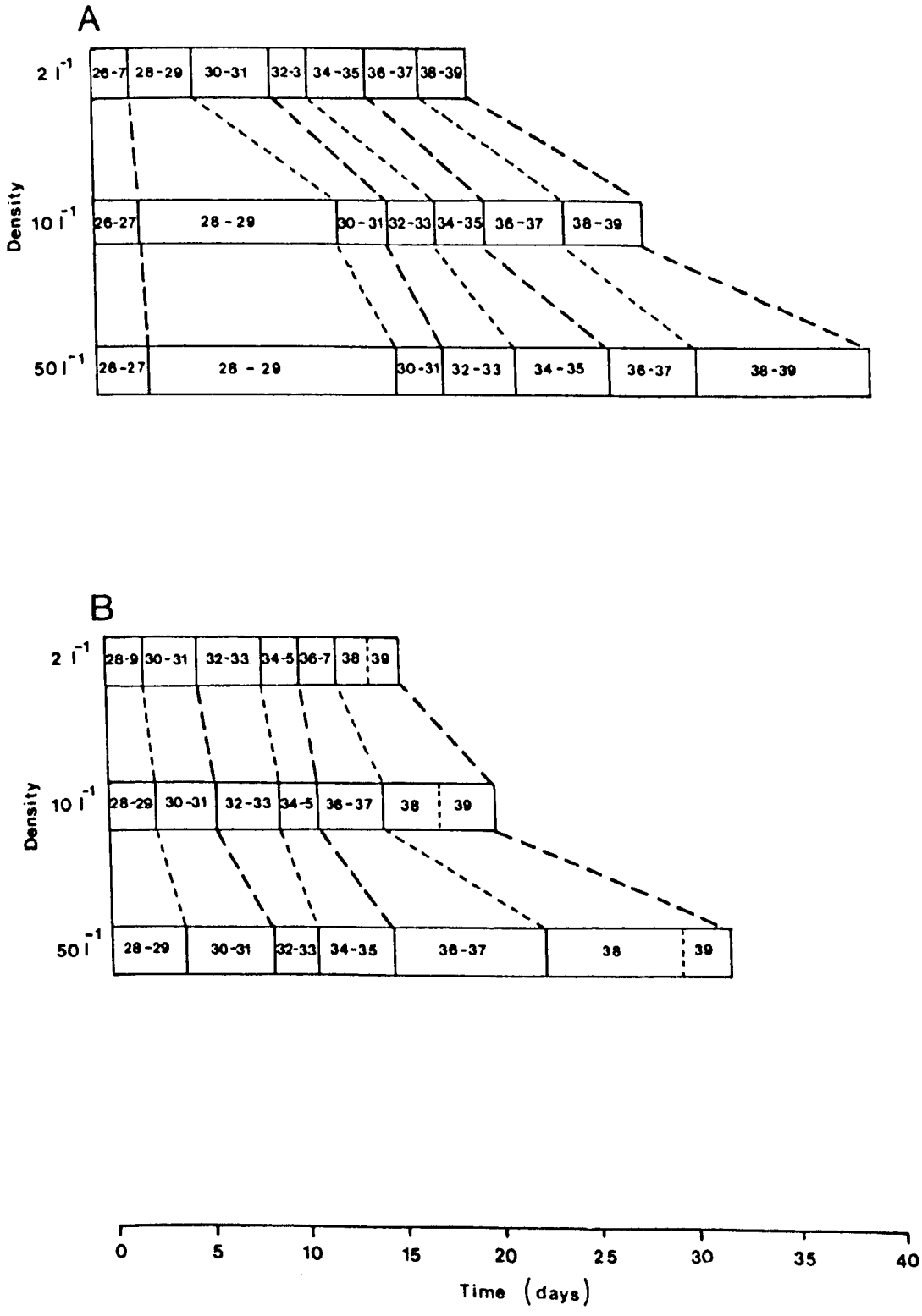
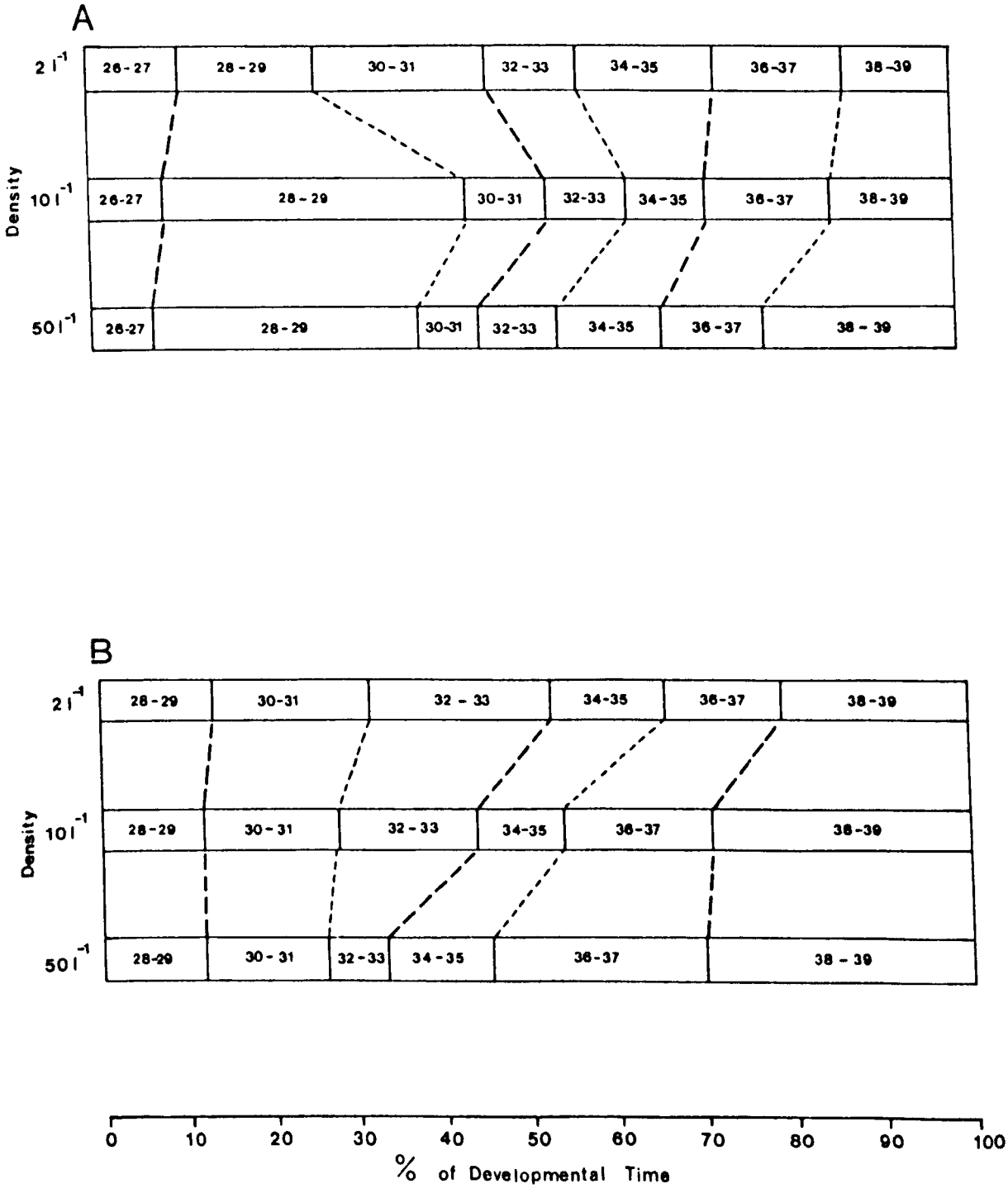

A
2 l-1
26-7
28-29
30-31
32-3
34-35
36-37
38-39
Density
10 l-1
26-27
28 - 29
30-31
32-33
34-35
36-37
38-39
50 l-1
26-27
28 - 29
30-31
32-33
34-35
36-37
38-39
B
2 l-1
28-9
30-31
32-33
34-5
36-7
38
39
Density
10 l-1
28-29
30-31
32-33
34-5
36-37
38
39
50 l-1
28-29
30-31
32-33
34-35
36-37
38
39
0
5
10
15
20
25
30
35
40
Time (days)

Figure. 6.3. Duration of developmental stages as a percentage of total developmental time in (A) B. bufo and (B) B. calamita tadpoles (stages shown in boxes) reared at densities of: 2 tadpoles l^{-1}, 10 tadpoles l^{-1} and 50 tadpoles l^{-1}.


A
2 l-1
10 l-1
50 l-1
Density
26-27
28-29
30-31
32-33
34-35
36-37
38-39
B
2 l-1
10 l-1
50 l-1
Density
28-29
30-31
32-33
34-35
36-37
38-39
0
10
20
30
40
50
60
70
80
90
100
% of Developmental Time

the greatest proportion of developmental time was spent at stages 38-39 (28% and 30% respectively). At HD, the greatest proportion of time was spent at stages 32-33 (21%).

6.3.2. Effect of density on tadpole growth.

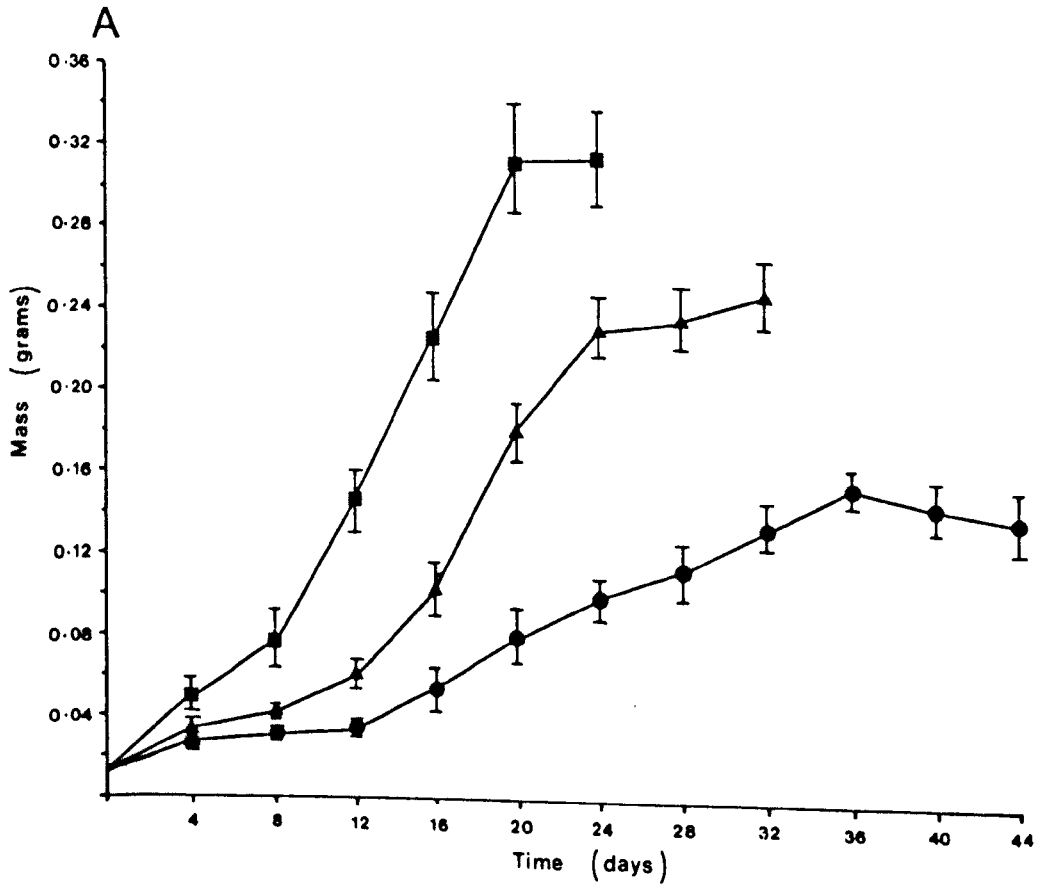
B. bufo tadpole growth was rapid at LD (Fig. 6.4), maximum mass (0.32 g) being attained after 20 days. It took 28 days for the MD tadpoles to reach their maximum mass (0.28 g). The HD tadpoles attained their maximum mass (0.15 g) after 36 days. Increasing density resulted in slower growth and reduction in maximum mass attained. All growth curves were sigmoid, tending to flatten out with increasing density.

B. calamita tadpoles reared at LD attained a maximum mass of 0.23 g after 16 days, at MD, 0.15 g after 16 days and HD reared tadpoles 0.10 g after 44 days. Unlike B. bufo, growth was linear from the start of the experimental period, followed by a tailing off as the maximum mass was approached. The tail-off was of shorter duration at LD. The MD tadpoles showed decrease in mass after the maximum mass had been attained, but this was not significant ($p > 0.05$).

6.3.3. Effect of density on tadpole mass at different developmental stages.

As tadpole density increased in B. bufo, in general the mass of each developmental stage was reduced although there were exceptions (Fig.

Figure. 6.4. Rates of growth in (A) B. bufo and (B) B. calamita tadpoles reared at densities of: (■) 2 tadpoles l^{-1}, (▲) 10 tadpoles l^{-1} and (●) 50 tadpoles l^{-1}. Means ± 95% confidence intervals, n=20 for each point.


A
0·36
0·32
0·28
0·24
0·20
0·16
0·12
0·08
0·04
Mass (grams)
4
8
12
16
20
24
28
32
36
40
44
Time (days)

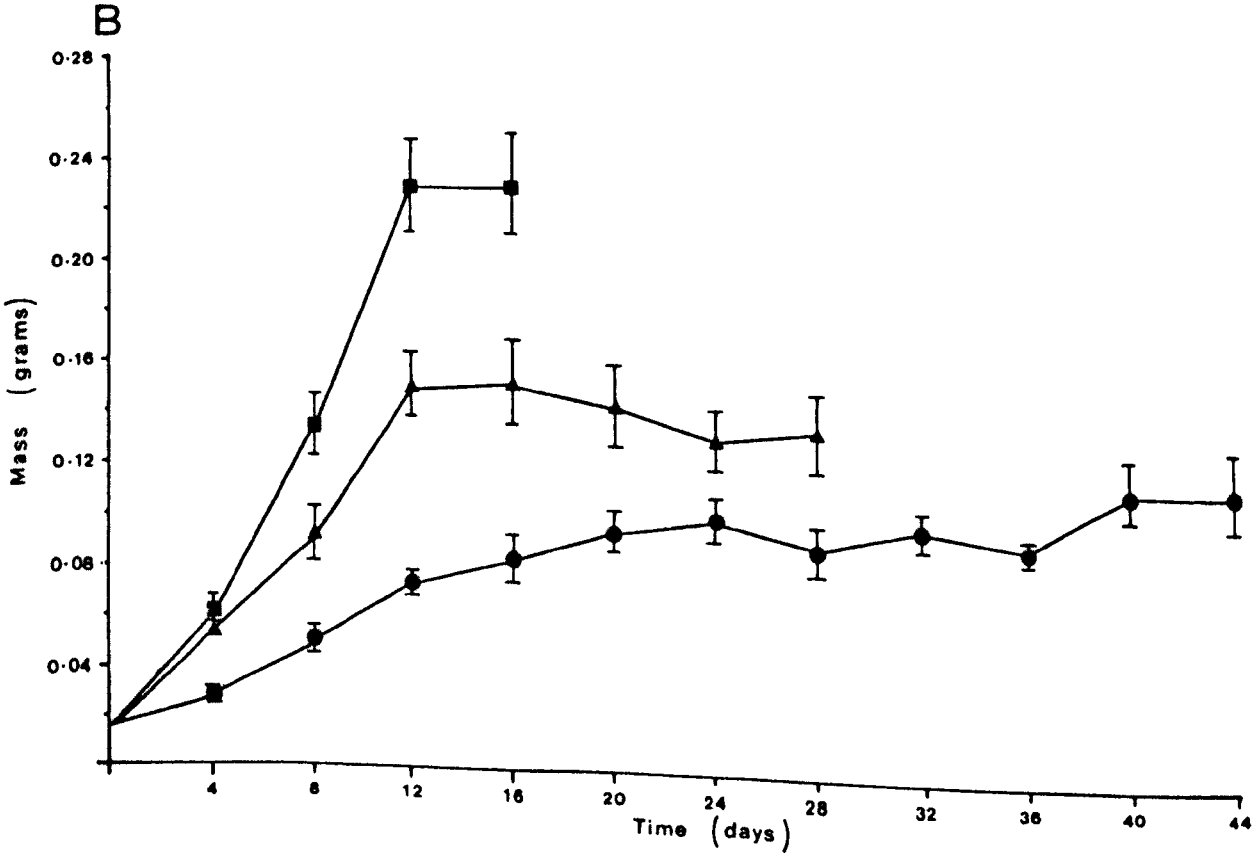
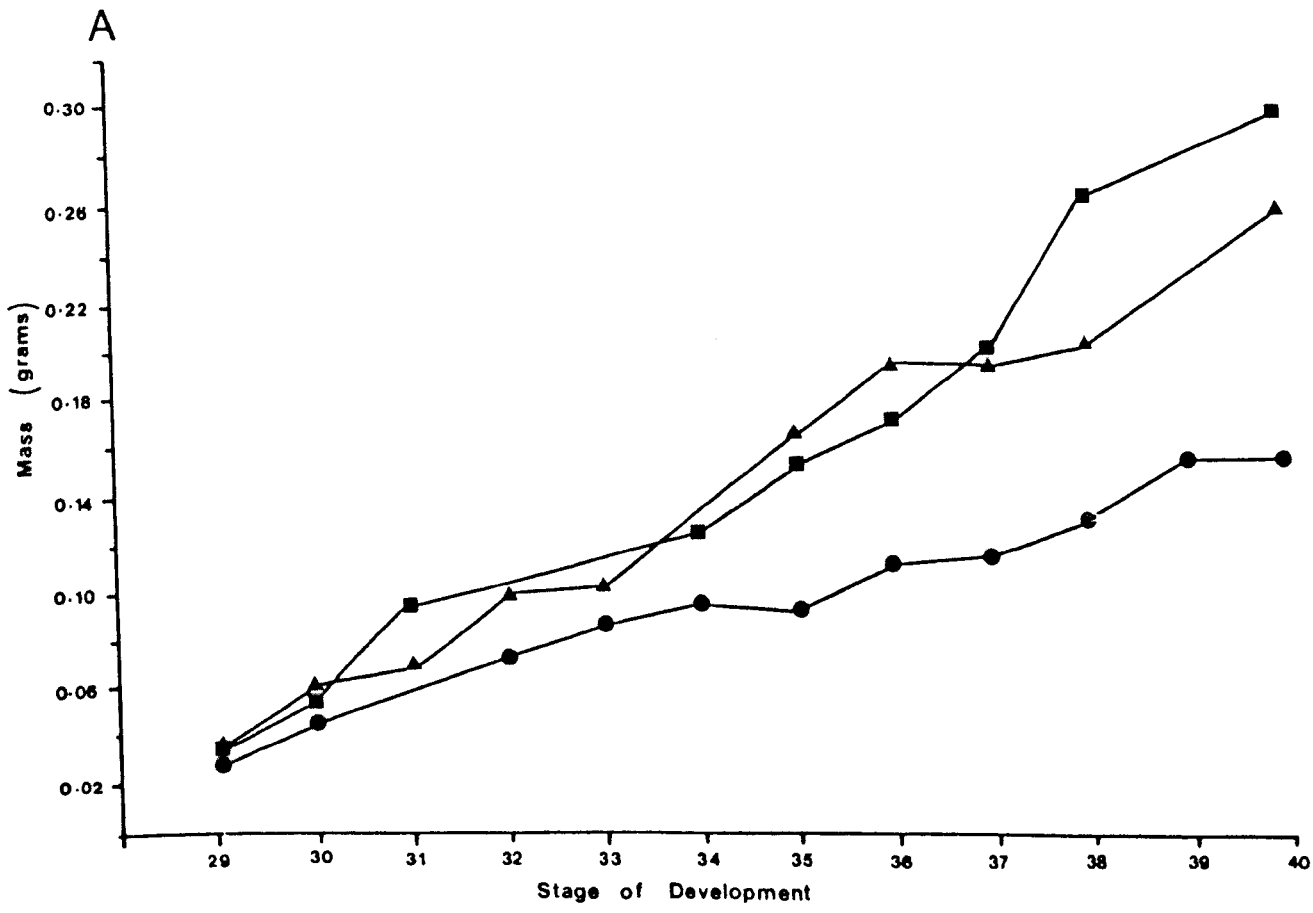
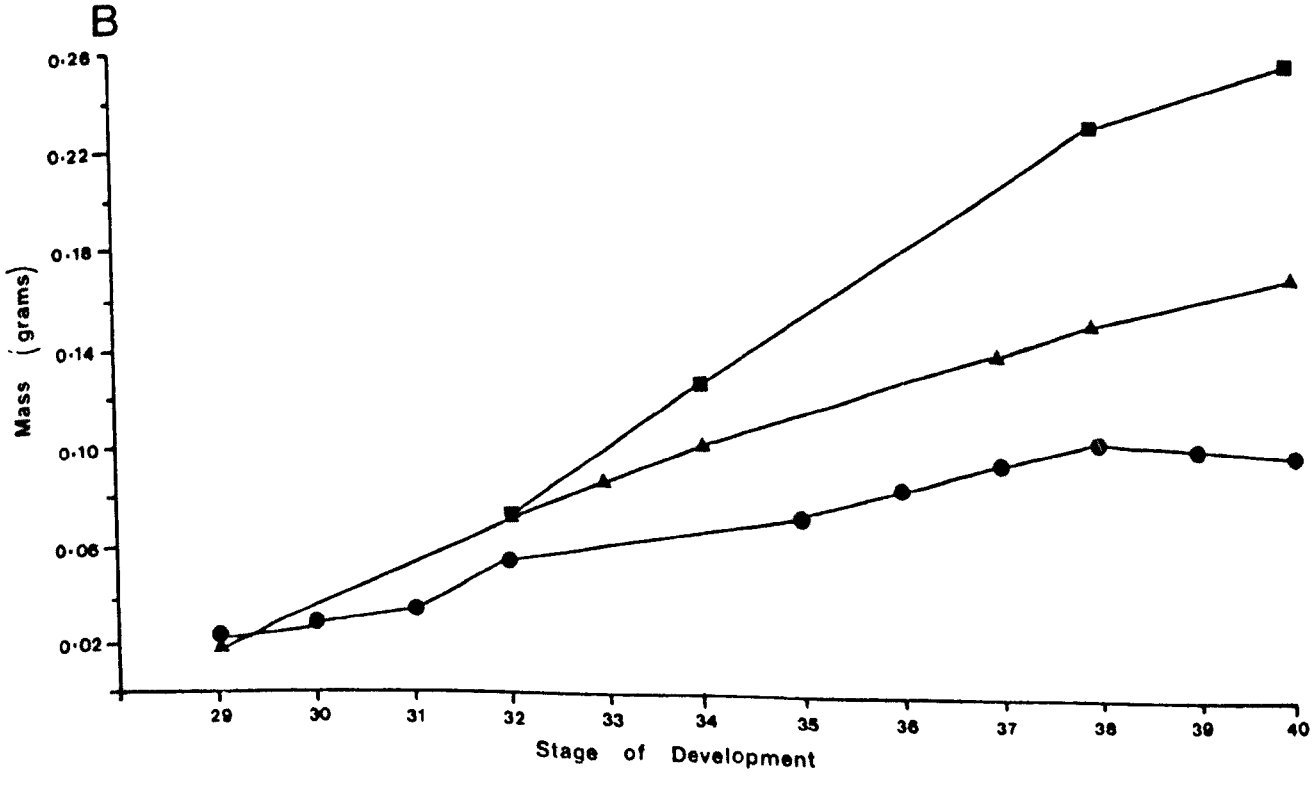

B
0·28
0·24
0·20
0·16
0·12
0·08
0·04
Mass (grams)
4
8
12
16
20
24
28
32
36
40
44
Time (days)

Figure. 6.5. Mean mass of individual developmental stages in (A) B. bufo and (B) B.calamita tadpoles reared at densities of: (■) 2 tadpoles l^{-1}, (▲) 10 tadpoles l^{-1} and (●) 50 tadpoles l^{-1}. N=20 for each point.


A
0·30
0·26
0·22
0·18
0·14
0·10
0·06
0·02
Mass (grams)
29
30
31
32
33
34
35
36
37
38
39
40
Stage of Development


B
0·26
0·22
0·18
0·14
0·10
0·06
0·02
Mass (grams)
29
30
31
32
33
34
35
36
37
38
39
40
Stage of Development

6.5). The HD tadpoles' masses at each of their developmental stages were lower than those in other treatments. The MD and LD tadpoles had similar masses up to stage 36, where the LD treatment tadpoles' stage masses increased at a greater rate than the MD tadpoles.

In B. calamita, the HD and MD tadpole masses were similar at stages 28 and 29, but after these stages the HD tadpoles always had a lower mass than the other treatments. At stage 32, the MD tadpoles had the same mass as the LD tadpoles but after this stage there was a divergence. LD tadpoles attained greater masses for the remaining stages.

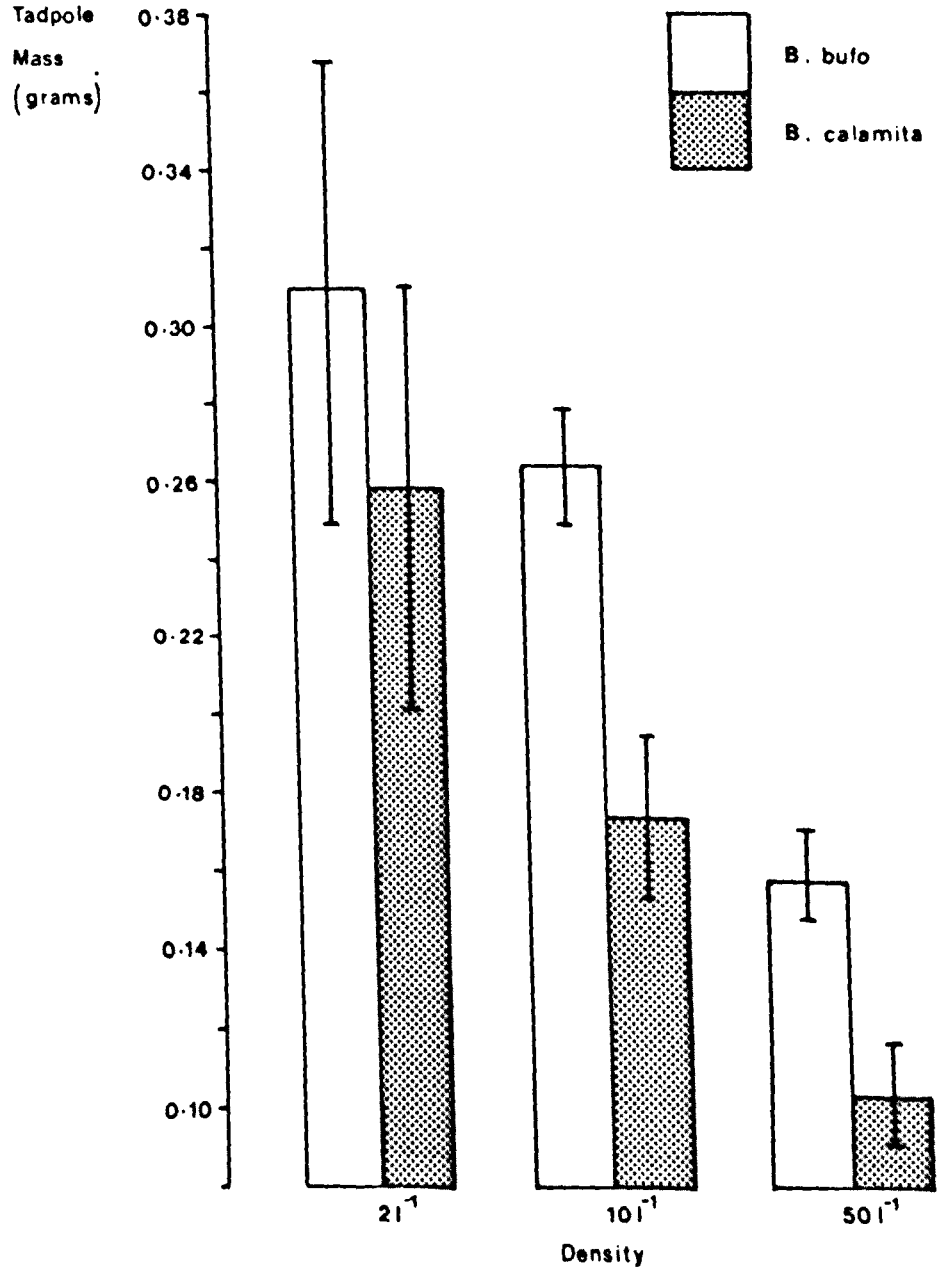
At LD, the maximum mass of B. bufo (0.31 g) was reached at stage 40. B. calamita maximum mass at LD was 0.26 g (stage 40). Both species reached a maximum mass at stage 40 (0.26 g, B. bufo; 0.17 g, B. calamita). Maximum mass of HD B. bufo was attained at stage 39 (0.16 g) whereas that of B. calamita was reached at stage 38 (0.10 g).

6.3.4. Effect of density on mass change during metamorphosis.

The mass of premetamorphic tadpoles (stage 40, Gosner, 1960) was inversely related to density (Fig. 6.6). The premetamorphic masses of B. bufo tadpoles reared at LD and MD, were not significantly different ($p > 0.05$), although the mean mass at MD (0.263 g) was lower than that at LD (0.310 g). The premetamorphic mass at HD (0.158 g) was significantly lower ($p > 0.05$) than the other densities.

Premetamorphic tadpole masses for B. calamita were significantly

Figure. 6.6. Mass of premetamorphic (stage 40) B. bufo and B. calamita tadpoles reared at different densities. Means ± 95% confidence intervals, n= 10.


Tadpole
Mass
(grams)
0·38
0·34
0·30
0·26
0·22
0·18
0·14
0·10
B. bufo
B. calamita
2l⁻¹
10l⁻¹
50l⁻¹
Density

different at all densities ($p > 0.05$). The LD treatment (0.258 g) resulted in tadpoles of greater mass than the HD tadpoles (0.105 g). The mass of MD reared tadpoles was intermediate (0.173 g).

Comparing the two species, at the lowest density B. bufo and B.calamita tadpole masses were not significantly different ($p > 0.05$). However, the mean mass of B. calamita was lower than that of B. bufo. At MD and HD the B. bufo premetamorphic tadpoles had significantly greater masses than B. calamita ($p > 0.05$).

B. bufo and B. calamita tadpoles reared at HD attained maximum mean masses that were 51.6 and 38.5% lower, respectively, than those reared at LD (Table 6.1). The tadpoles reared at MD attained maximum mean masses that were 83.9% (B. bufo) and 65.0% (B. calamita) of those attained by the LD tadpoles.

As tadpole rearing density increased, mass of resulting toadlets decreased (Fig. 6.7). In B. bufo, the greatest mean toadlet mass was attained by toadlets resulting from the LD rearing regime (0.181 g). This was significantly higher than those resulting from tadpoles reared at MD (0.134 g) and HD (0.120 g).

In B. calamita increased rearing density also resulted in significantly reduced toadlet masses ($p > 0.05$). The mean mass of toadlets resulting from LD tadpoles was 0.12 g; MD tadpoles, 0.078 g and HD tadpoles, 0.048 g.

Figure. 6.7. Mass of B. bufo and B. calamita toadlets resulting from tadpoles reared at different densities. Means ± 95% confidence intervals, n=10.

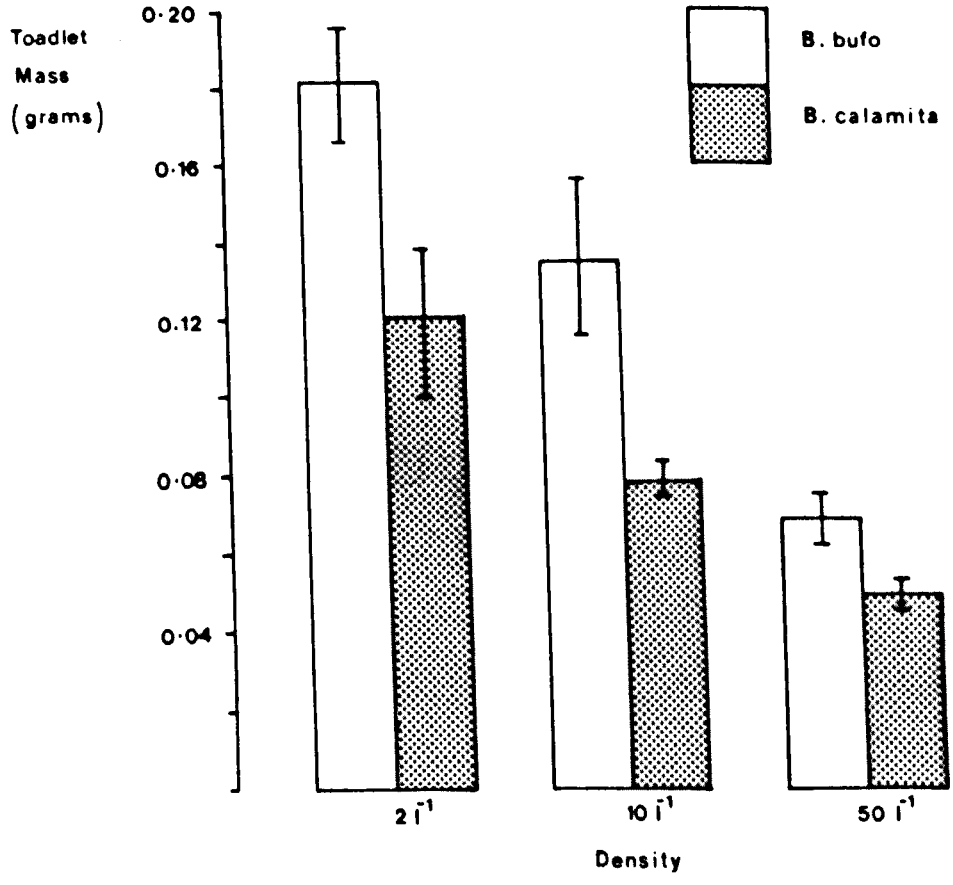

Toadlet
Mass
(grams)
0·20
0·16
0·12
0·08
0·04
B. bufo
B. calamita
2 l⁻¹
10 l⁻¹
50 l⁻¹
Density

Table 6.1. Mean masses of premetamorphic tadpoles (stage 40) reared at densities of: 2 tadpoles l^{-1}, 10 l^{-1} and 50 l^{-1}. Mass of tadpoles also expressed as a percentage of those reared at 2 l^{-1}. N = 20.

	Bufo bufo		Bufo calamita	
Tadpole Density	mass (g)	%	mass (g)	%
2 l^{-1}	0.31	100.0	0.26	100.0
10 l^{-1}	0.26	83.9	0.17	65.4
50 l^{-1}	0.16	51.6	0.10	38.5

Toadlet masses can be expressed as a percentage of the mean mass attained by toadlets resulting from the LD tadpoles (Table 6.2). In B. bufo, tadpoles reared at MD resulted in toadlets with a mean mass 74.0% the mass of those reared at LD. Those resulting from HD were 66.3% of the mean mass of the LD reared toadlets. The B. calamita toadlets resulting from MD tadpoles were 65.0% of the LD reared toadlet mass. Those reared at HD were 40% of the LD reared mean toadlet mass.

For each of the rearing densities B. calamita toadlets had lower mean masses than equivalent B. bufo toadlets ($p > 0.05$).

The amount of mass retained in the toadlet after metamorphosis (metamorphic efficiency) increased with density (Fig 6.8). B. bufo appeared more efficient, in terms of mass retention, over metamorphosis than B. calamita at each density. At HD B. bufo retained 58.1% of its prematamorphic mass, B. calamita retaining 48.1%. At MD B. bufo retained 51.1%, B. calamita, 45.1% and at LD B. bufo retained 43.3% compared to 34.2% in B. calamita.

6.3.5 Effect of density on time taken for tadpoles to reach metamorphosis.

Development to metamorphosis in B. bufo, occured at the greatest rate at LD (Fig. 6.9). Stage 41 (Gosner, 1960) was reached 25 days after the start of the experiment. In the LD treatment 39% of the successfully metamorphosing tadpoles reached stage 41 at day 25. The

Table 6.2. Mean masses of metamorphosed toadlets (stage 46) resulting from tadpoles reared at densities of: 2 tadpoles l^{-1}, 10 l^{-1} and 50 l^{-1}. Mass of toadlets also expressed as a percentage of those reared at 2 l^{-1}.

	Bufo bufo		Bufo calamita	
Tadpole Density	mass (g)	%	mass (g)	%
2 l^{-1}	0.181	100.0	0.120	100.0
10 l^{-1}	0.134	74.0	0.078	65.0
50 l^{-1}	0.120	66.3	0.048	40.0

Figure. 6.8. Metamorphic efficiency in terms of wet mass (% of premetamorphic (stage 40) tadpole mass remaining in toadlet) of B. bufo and B. calamita reared at different densities.

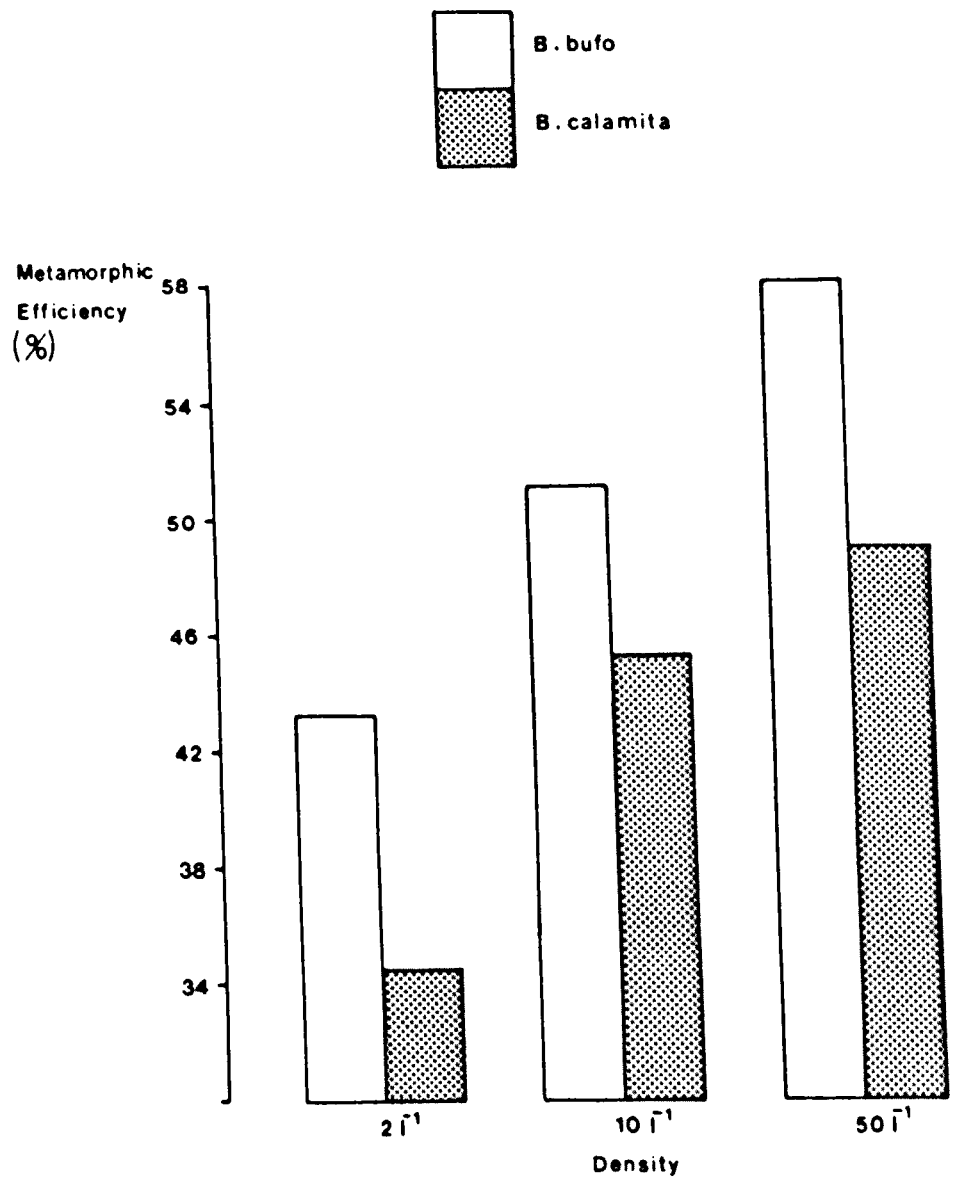
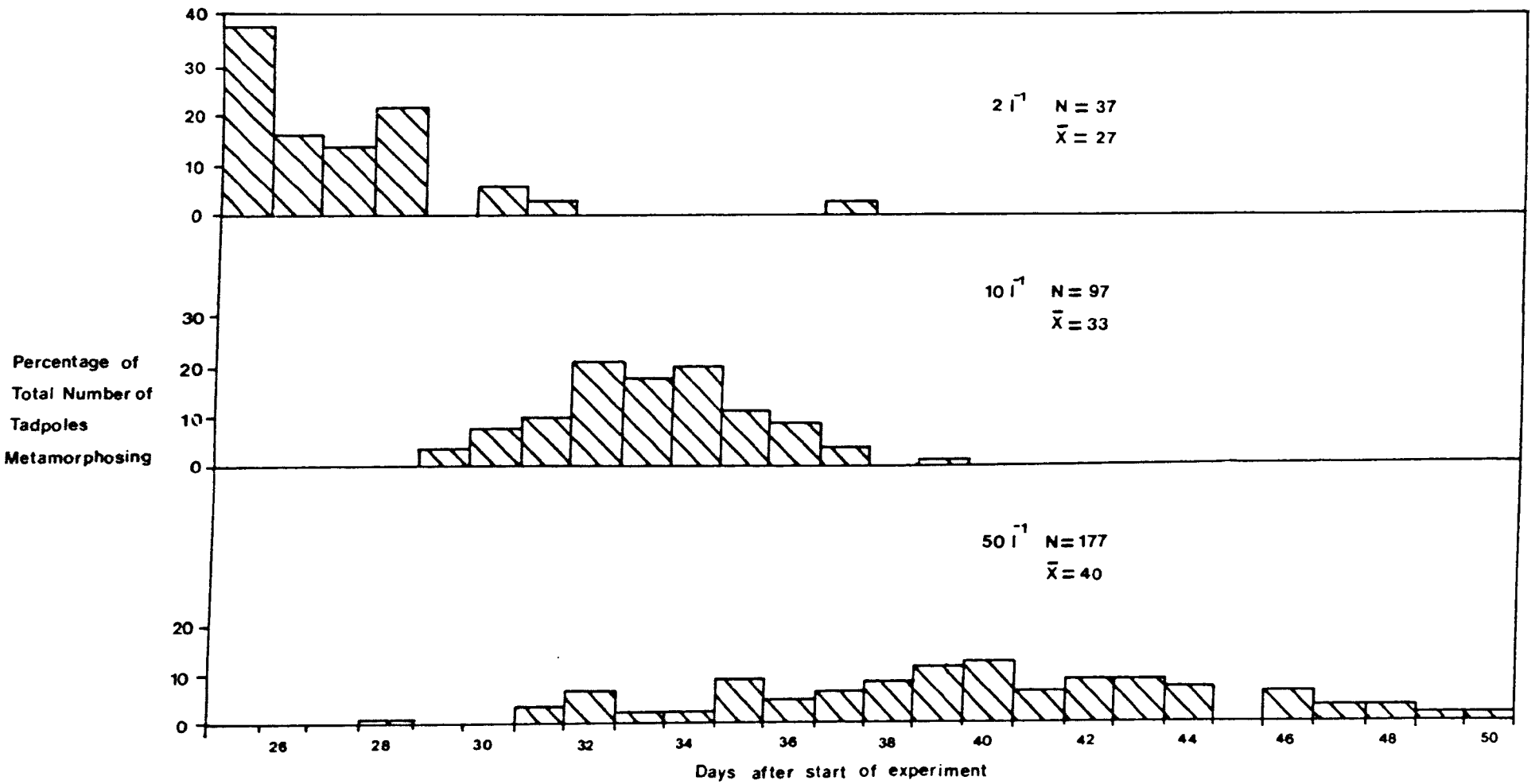

B. bufo
B. calamita
Metamorphic
Efficiency
(%)
58
54
50
46
42
38
34
2 l⁻¹
10 l⁻¹
50 l⁻¹
Density

Figure. 6.9. Time taken for B. bufo tadpoles to reach metamorphosis (stage 41) when reared at densities of 2 tadpoles l^{-1}, 10 l^{-1} and 50 l^{-1}. % metamorphosis represents the proportion of the total number of tadpoles reaching metamorphosis at each density.


40
30
20
10
0
2 l⁻¹ N = 37
X̄ = 27
30
20
10
0
10 l⁻¹ N = 97
X̄ = 33
20
10
0
50 l⁻¹ N = 177
X̄ = 40
Percentage of Total Number of Tadpoles Metamorphosing
26
28
30
32
34
36
38
40
42
44
46
48
50
Days after start of experiment

mean time period for all the tadpoles to reach stage 41 was 27 days. After the initially high proportion of metamorphosing tadpoles on day 25, the numbers reaching stage 41 decreased until day 32, when only one individual remained, reaching stage 41 at day 37. The total time period in which all the LD, B. bufo tadpoles reached metamorphosis was 13 days.

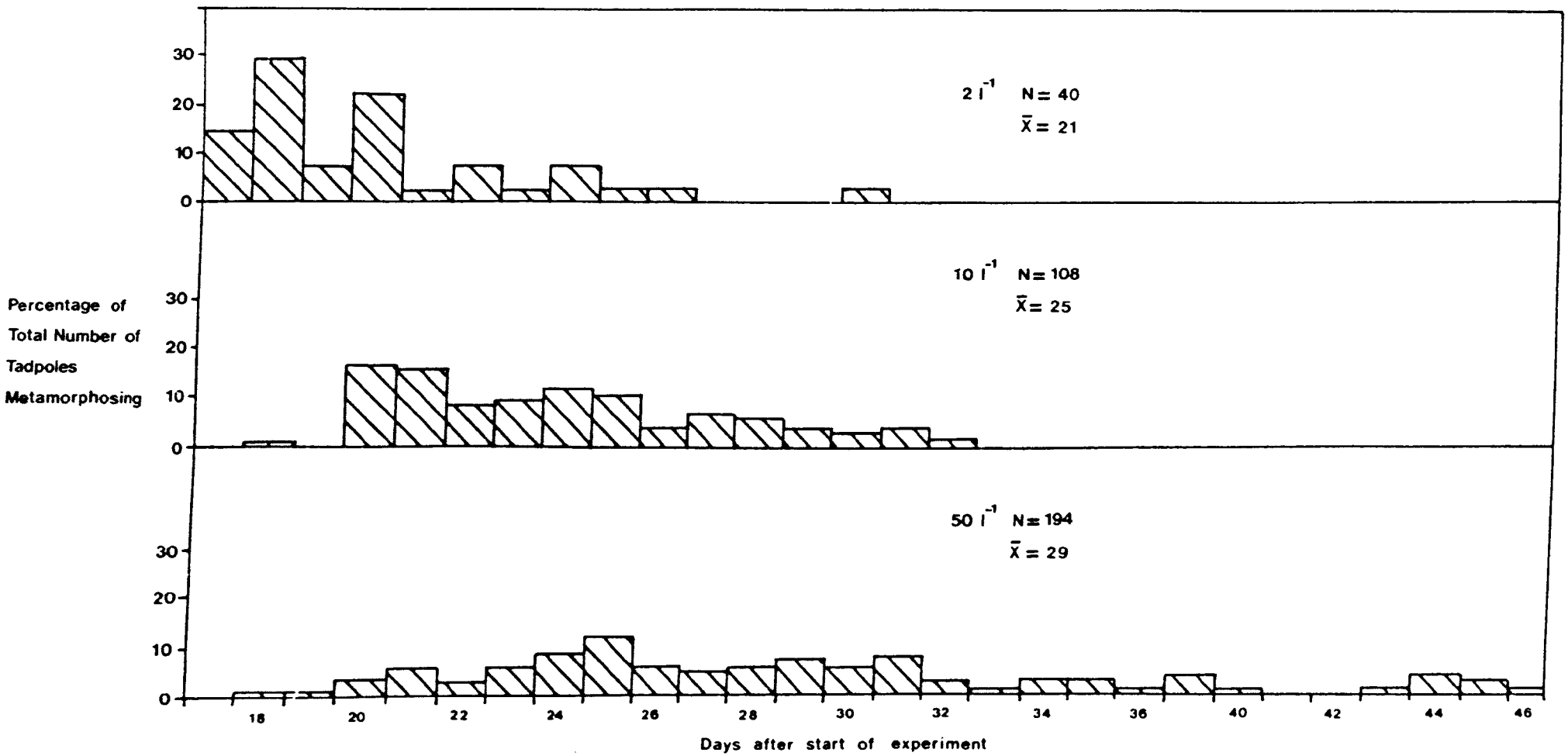
B. bufo tadpoles at MD started metamorphosing 29 days after the start of the experiment, 4 days later than those at LD. The distribution of the tadpoles reaching stage 41 was normal, the mean time being 33 days. All individuals reached metamorphosis over an 11 day period.

The HD reared tadpoles all reached metamorphosis over a 23 day period. The distribution was flattened, the mean time being 40 days. One individual reached stage 41 after 28 days. The main period for reaching metamorphosis occured after 30 days. This was 6 days later than those at LD and 2 days later than the MD tadpoles.

B. calamita tadpoles (Fig. 6.10) reached metamorphosis 17 days after the start of the experiment at LD, 20% of the tadpoles reaching stage 41 by day 18. The numbers reaching metamorphosis then decreased, the final tadpole reaching stage 41 after 30 days. The mean time taken to reach metamorphosis at LD was 21 days.

At MD the first B. calamita tadpole reached metamorphosis after 18 days, 20% of individuals had reached metamorphosis by day 21. The distribution was single tailed with initially high percentages reaching

Figure. 6.10. Time taken for B. calamita tadpoles to reach metamorphosis (stage 41) when reared at densities of 2 tadpoles l^{-1}, 10 l^{-1} and 50 l^{-1}. % metamorphosis represents the proportion of the total number of tadpoles reaching metamorphosis at each density.

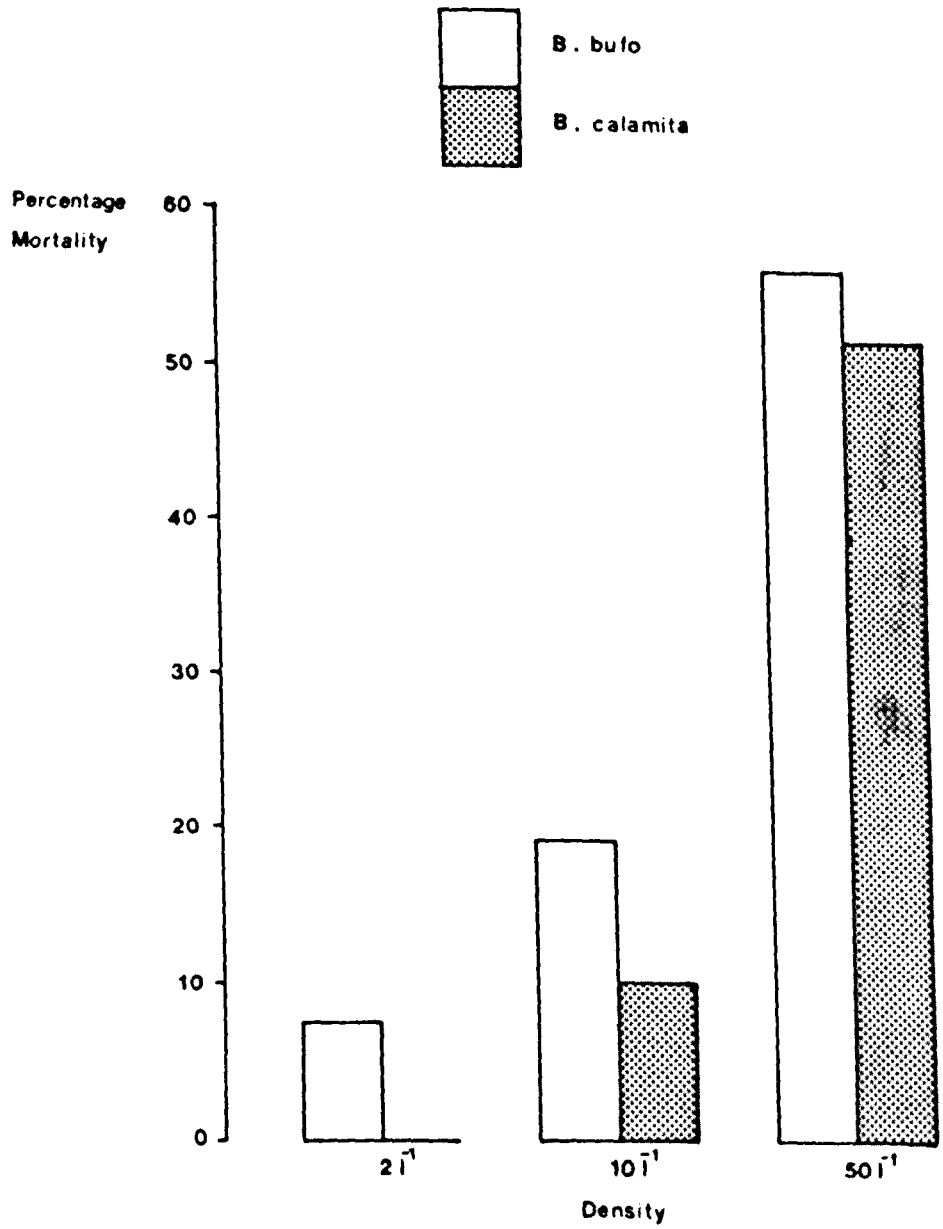

2 l-1 N= 40
X̄= 21
10 l-1 N= 108
X̄= 25
50 l-1 N= 194
X̄= 29
Percentage of Total Number of Tadpoles Metamorphosing
0
10
20
30
18
20
22
24
26
28
30
32
34
36
40
42
44
46
Days after start of experiment

metamorphosis followed by a tailing off. The final tadpole reached stage 41 after 32 days. The mean time taken by the tadpoles to reach metamorphosis was 25 days.

B. calamita tadpoles at HD reached stage 41 at day 18. Individuals reached metamorphosis daily in low numbers until 50 days after the experiment started, a 17 day period. The mean time taken by tadpoles to reach metamorphosis was 29 days.

Mortality of B. bufo and B. calamita increased at higher densities (Fig. 6.11). At LD, B. calamita suffered no mortality and B. bufo mortality was low (7.5%). As the density increased, at MD, B. bufo mortality increased to 19%. At this density B. calamita mortality was 10%. At HD B. bufo mortality was 56% and B. calamita 52%. B. calamita mortality was lower than that of B. bufo at all densities.

Figure. 6.11. Mortality of B. bufo and B. calamita tadpoles when reared at different densities.


B. bufo
B. calamita
Percentage Mortality
60
50
40
30
20
10
0
2 l⁻¹
10 l⁻¹
50 l⁻¹
Density

6.4 DISCUSSION

6.4.1 Effect of density on growth and development.

An increase in the density of both B. bufo and B. calamita tadpoles leads to a reduction in rates of growth and development. Many studies on anuran tadpoles have demonstrated this negative effect. Explanations for this effect include competition for food (Brockleman, 1969; DeBenedictis, 1974), growth inhibitors (Richards, 1958; Rose, 1960; Licht, 1967) and behavioural interactions (Gromko et al, 1973; John and Fenster, 1975). Travis (1984) demonstrated that low food levels decreased larval growth and size at metamorphosis, increasing average larval duration. Wilbur (1972) suggested that the effects of low food levels mimic that of higher densities. Travis (1984) considered the effects of low food and high density, on variation in development of tadpoles, to be separate.

In this study food was supplied ad libitum. This would suggest that behavioural interactions or growth inhibitors may have been acting. Physical factors such as environmental agitation and oxygenation of water are important in the growth and development of Alytes obstericans tadpoles (Guyetant, 1970). The effects observed in this study are probably not due to a single factor, but a combination. At high densities there would be higher incidences of physical contact between tadpoles and build up of waste materials. The trays were not aerated, oxygen supply was via diffusion across the water surface. Oxygen utilisation would be greater at higher densities and hence a reduction

in oxygen availability would occur. This may have led to reduction of growth and development.

Growth inhibition, resulting from liberated chemicals, may have been responsible for the observed effects. Small tadpoles raised in water conditioned by larger tadpoles are often stunted in relation to controls. Richards (1958) proposed that endocommensal cells were responsible for this effect. These cells were later shown to be unnecessary for the inhibition to occur (West, 1960). Rose and Rose (1961) suggested that the inhibitory compound was proteinaceous. This was confirmed by Runkova et al (1974) and Stepanova (1974). It is probable that a chemical liberated by larger tadpoles can inhibit smaller ones. In situations of high density this could result in reduced growth and development.

B. bufo and B. calamita tadpoles growth and development was reduced to lower rates at higher densities. The mass of each stage was reduced as density increased. The overall result was a smaller toadlet at higher densities. The cause of this is probably chemical inhibition, with behavioural and social mechanisms (Rugh, 1934; Lynn and Edelman, 1936; Christian, 1956) compounding the effect. Reduced oxygen levels could also contribute to reduced rates of growth and development.

The response of B. bufo and B. calamita to increased density differs. An increase in density appears to have a greater effect on B. bufo. B. calamita breed preferentially in ephemeral pools (Beebee, 1983) whereas B. bufo prefer to spawn in permanent ponds (Harrison, 1985).

B. calamita is, therefore, subject to increasing densities as breeding pools dry up. B. bufo tadpoles would be more likely to experience density reduction, as they are predated in permanent ponds. It would be disadvantageous for B. calamita to have severe intraspecific inhibition when the nature of their habitat causes an increase in density with time.

It is often proposed that the inhibition of growth at high densities is a result of larger tadpoles inhibiting smaller ones. This ensures that some tadpoles metamorphose, before ponds dry up, even when the majority perish through desiccation (Collins, 1979; Travis, 1984).

Increase in density appears to affect the developmental rates of B. bufo and B. calamita at different stages in their development. Hyla gratiosa tadpoles have their developmental rates set early in the larval period (Travis, 1984). Increased density in B. bufo tadpoles results in a marked reduction in developmental rates in the early developmental stages. These early effects result in differences in rates that are carried through to metamorphosis. Density effects in B. calamita tadpoles are more continuous. At early stages of development the effects are small, increasing as development proceeds.

Invertebrate predation is probably the most important mortality factor in B. bufo tadpoles (Mathias, 1971; Harrison, 1985). For B. calamita tadpoles, in dune slacks, it is habitat desiccation (Beebee, 1979). As the shallow, ephemeral pools used by B. calamita become vegetated, and in deeper more permanent ponds invertebrate predation becomes more

important (Banks and Beebee, 1988)). Invertebrate predators prefer smaller tadpoles (Brockelman, 1969), larger tadpoles being able to escape by virtue of size and strength. Early growth is a means of avoiding predation (Pritchard, 1965; Wilbur, 1972; Calef, 1973; Heyer et al, 1975). At high density, B. bufo tadpoles remain smaller for a longer period. This has been observed in the field (Harrison, 1985). It is probable that this is when predation has its greatest effect on population density. In a permanent pond increased density may result in longer developmental times, increased exposure to predators in later development and smaller toadlets. Smaller toadlets probably have reduced chances of survival.

In high density situations B. bufo tadpoles are maintained at small sizes, early in development. As a result of this they would be more vulnerable to invertebrate predation. Predation would reduce population density and permit increased developmental rates in the surviving tadpoles. The population would then consist of larger, faster growing tadpoles that can escape the majority of predators and metamorphose earlier in the year, enhancing post emergent toadlet survival.

Invertebrate predation is also important in B. calamita (Davis, 1985; Kadel, 1975). Fast growth in early larval stages could result in reduced mortality, larger tadpoles being less susceptible to predation (Brockleman, 1969). Desiccation is also a key mortality factor in B. calamita tadpoles. In dune slacks, desiccation may be of greater significance than predation (Banks and Beebee, 1988). A fast rate of

development through the early stages would be important for tadpole survival. This is when the pools are at their greatest volume and food availability is high. Fast early development, regardless of density, would ensure a maximum number of tadpoles available to undergo metamorphosis if the pond does not dry up completely. However, as metamorphosis is approached, at high densities, the rate of development reduction would become increasingly important in terms of population survival.

In an ephemeral pond the reduction in volume resulting in increased tadpole density often occurs when tadpoles are in the later stages of development (Davis, 1985). This could therefore be when a strategy of rapid development of a small number of tadpoles at the expense of others would be the most effective in ensuring the survival of a proportion of the population. In years when total desiccation of the breeding pond does not occur, or is late, then large numbers of tadpoles would be available to metamorphose. The density effects would not have reduced populations at early developmental stages as observed in B. bufo.

6.4.2 Effect of density on the time taken for tadpoles to reach metamorphosis.

The time taken for tadpoles to reach metamorphosis is important in terms of individual toadlet and population survival. Early metamorphosis allows a longer time period, for feeding and growth before hibernation. Collins (1979) suggests that under circumstances

in which per capita resources are high (low density or high food levels) all tadpoles will grow rapidly. There will be little variation in length of larval period or size at metamorphosis. When the per capita resources are low then tadpole growth will be low and variable. Average size at metamorphosis will be reduced with relatively greater variation around the mean.

This general effect can be seen in both B. bufo and B. calamita. However, there is a tendency in both species for variation around the mean mass at metamorphosis to be limited at higher densities. This does not fit with Collins' hypothesis, possibly because Collins did not take developmental stage into account. When looking at the effect of density on growth rates (mass gain), the stage of development must be considered. Wilbur and Collins (1973) considered growth rates were fundamental in the timing of metamorphosis but they did not take developmental rates into account. Smith-Gill and Berven (1979) considered Wilbur and Collins' hypothesis a result of a chance correlation between growth and metamorphosis. They suggest that differentiation rate determines metamorphic parameters and growth rate is dependent on differentiation, which can be influenced by food levels and density.

Wilbur and Collins (1973), may also have been observing effects of low food levels rather than density exclusively. Wilbur (1972) considered that the effects of low food levels on length of larval period mimic those of high density. Travis (1984) suggests that the effects of low food and high density are not the same.

High density has a detrimental effect on metamorphosis in B. bufo and B. calamita . It retards the onset of metamorphosis and results in a longer period of metamorphosis and higher mortality in B. bufo. In B calamita onset of metamorphosis is not retarded to the same extent as at higher densities, although the time taken for the total population to metamorphose increases. At all densities B. calamita tadpoles reached the onset of metamorphosis in a shorter time than B. bufo. In an ephemeral pond short larval period will mean greater individual survival (Travis, 1980). This would give B. calamita an advantage over B. bufo in an ephemeral environment. As well as shorter developmental periods, the distribution of metamorphosing individuals would also benefit B. calamita. Even at the highest densities the onset of metamorphosis was not delayed. At high densities of B. bufo tadpoles the metamorphosis of the total population was delayed.

When pools are rapidly diminishing, increase in B. calamita tadpole density will not effect the time taken for the onset of metamorphosis.

The inherent developmental rate of B . calamita tadpoles, regulated by environmental temperature (Chapter 1) will probably determine if the tadpoles will reach metamorphosis before the pool dries completely. At lower densities (suggesting high pond volume, thus less likelihood of desiccation), an increased proportion of the total population of B. calamita tadpoles will survive. Given the same conditions, increased density would increasingly retard B. bufo development, the population as a whole having the onset of metamorphosis delayed. The

drying up of a pond at a time near the onset of metamorphosis would possibly result in total population loss.

The effects of density on B. bufo and B. calamita tadpoles indicates that B. bufo is probably adapted to breeding in permanent ponds, density increasing developmental times. B. calamita tadpoles respond to increases in density in a way that suggests they are more suited to spawning in ephemeral ponds: fast early development with no delay in the onset of metamorphosis as a result of increased density.

CHAPTER 7

7.0 Effect of B. bufo tadpoles on the growth, development, metamorphosis and behaviour of B. calamita tadpoles.

7.1 INTRODUCTION

Intraspecific effects of density on tadpole growth and development have been investigated in many species (Alford and Wilbur, 1983; Hota and Dash, 1977; Wilbur, 1977; Berger, 1968; Lynn and Edleman, 1936) and are discussed in detail in chapter 6. Inhibition of tadpole development and growth at increased densities has been attributed to various factors: competition for food, (e.g. Brockleman, 1969) behavioural interactions, (e.g. John and Fenster, 1975) and specific growth inhibiting substances (e.g. Licht, 1967).

The various factors resulting in intraspecific density effects may also be implicated in interspecific growth and development inhibition. Richards (1958) described an endocommensal cell as a causative agent for tadpole inhibition. The cell inhibited growth in 17 species of tadpole, which represented 7 genera, 5 families and 3 suborders.

B. bufo tadpoles develop faster than B. calamita when they are in the same pools (Mathias, 1971). Mathias (1971) suggested that this was a result of B. bufo being adapted to colder environmental temperatures. However at temperatures above 15°C B. calamita tadpoles develop at a faster rate than equivalent B. bufo tadpoles (Chapter 2). This

suggests that another mechanism is operating in the pools where the two species are found together.

Heusser (1972a, 1972b) demonstrated that B. calamita tadpole growth was retarded by the presence of water conditioned by B. bufo tadpoles. Banks and Beebee (1987) suggest that in the field growth of B. calamita tadpoles is inhibited by increasing numbers of B. bufo and R. temporaria tadpoles. This could account for the observations of Mathias (1971), the inhibiting effect of B. bufo tadpoles countering the inherently faster growth and development rates of B. calamita.

B. bufo spawn approximately two weeks prior to B. calamita (Smith, 1951). A result of this is that B. bufo tadpoles are generally larger than the B. calamita tadpoles at any one time. In many anuran species larger tadpoles inhibit smaller ones intraspecifically (Gromko et al, 1973; Guyetant, 1970; Licht, 1967). If interspecific inhibition observed by Heusser (1972a, 1972b) was a result of the same mechanism as intraspecific inhibition (Chapter 6), the disparity in the two species' size could have enhanced any interspecific inhibition.

The major cause of mortality in B. calamita tadpoles is probably invertebrate predation, with 80-90% mortality (Kadel, 1975 Davis, 1985). Wilbur (1982) investigated the effect of Hyla gratiosa on H. femoralis tadpoles. H. gratiosa had a strong negative effect on the rate of metamorphosis and size at metamorphosis of H. femoralis tadpoles. In tree frog tadpoles (Hyla spp.) competition between species resulted in the inhibited species being exposed to predators

for a longer period (Morin, 1987). This reduced their chance of survivorship and increased resources available to the inhibiting species.

Smaller tadpoles are generally more vulnerable to predation (Semlitsh et al, 1988; Travis, 1985; Wilbur and Alford, 1985; Crump, 1984; Wilbur, 1983). Any inhibition of tadpole growth will result in increased exposure of the inhibited tadpole to the risk of predation. If inhibition acts interspecifically the inhibiting tadpole species may outcompete the inhibited species.

Predation is a major mortality factor in B. calamita tadpoles. The presence of B. bufo tadpoles results in increased mortality of B. calamita (Banks and Beebee, 1987). This is probably a result of interspecific inhibition.

In these series of experiments the aim is not to elucidate the specific cause of any inhibition. The effect of any inhibition will be looked at in a number of ways. Experiments using conditioned water will be used to compare B. bufo intraspecific inhibition with the effect B. bufo tadpoles have on B. calamita tadpoles. Using this method growth, development and timing of metamorphosis will be investigated.

The effect of conditioned water on B. calamita tadpoles will be compared with that of continuous exposure of B. calamita tadpoles to B. bufo tadpoles. In previous investigations of interspecific inhibition between B. bufo and B. calamita (Heusser, 1972a; 1972b)

conditioned water was used. This may not reflect the conditions experienced by tadpoles in the field. Continuous exposure to potentially inhibiting tadpoles is possibly a better method for examining the effects of interspecific inhibition.

It is possible that the inhibition of B. calamita by B. bufo may affect, directly or indirectly, tadpole behaviour. This could be indicated by changes in feeding activity or general locomotory behaviour. The inhibition of tadpoles may also result in changes in their food consumption. These possibilities will be examined.

Shvarts and Pyastolova (1970b) categorised possible inhibitory agents as: (a) Specialised inhibitors e.g. hormones, phytohormones or unicellular organisms growing in tadpole intestines. (b) Usual products of the vital functions of cells (metabolites). L-thyroxine increases metabolic rates and developmental rates (Chapter 5). Inhibited tadpoles have reduced developmental rates. The treatment of inhibited tadpoles with L-thyroxine may relieve the development retardation and indicate if thyroid suppression is a result of any inhibition mechanism.

7.2 MATERIALS AND METHODS

7.2.1 Spawn collection and initial rearing.

Spawn collection and initial rearing was carried out as in sections 2.2.2 and 2.2.3.

7.2.2 Effects of water conditioned by B. bufo tadpoles on B. bufo and B. calamita tadpoles.

After initial rearing tadpoles of both species were placed in shallow plastic trays (34 x 24 x 4 cm) containing 2 l of conditioned water. Water was conditioned by placing 1000 B. bufo tadpoles in a polythene tank containing 20 l of water, a density of 50 tadpoles l^{-1}, for a 24 hour period before the water was required. The conditioning tadpoles were removed from the water with a 1 mm nylon mesh sieve which allowed faecal material to pass through but retained the larger food particles.

Tadpoles were reared at 10 l^{-1} (6 replicates) in the conditioned water and at 10 l^{-1} (6 replicates) for controls. The trays were maintained at room temperature, 20 ± 1°C. The water in the trays was renewed every four days and the trays cleaned. Tadpoles were fed washed lettuce and ground rat pellets ad libitum. Measurements of the tadpoles were taken every four days (as in section 2.2.4).

7.2.3 Effect of continuous exposure of B. calamita tadpoles to B. bufo tadpoles.

Interspecific affects of B. bufo on B. calamita tadpoles were examined using modified plastic trays. Shallow plastic trays (35 x 24 x 4 cm) were divided centrally with a nylon mesh barrier. This was attached to the tray with silicon adhesive. The trays were filled with 2 l of water.

10 B. calamita tadpoles (stages 26-30) were placed in one half of the divided trays, the treatment tadpoles were placed in the other half. Treatments were: 25 B. bufo tadpoles; 5 B. bufo tadpoles; 25 B. calamita tadpoles and a control containing no treatment tadpoles. Each treatment had four replicates.

Tadpoles in both sides of the trays were fed washed lettuce and ground rat pellets ad libitum. Water was changed and the trays cleaned every four days. Measurements of the tadpoles were taken every four days as in section 2.2.4.

7.2.4 Effect of B. bufo tadpoles on the oxygen consumption of B. calamita tadpoles.

Divided trays (see section 7.2.3) were used for rearing B. bufo and B. calamita tadpoles. The experiment was initiated with the tadpoles of both species at stages 26-30.

A treatment containing 25 B. bufo in one side of the tray and 10 B. calamita tadpoles in the other, in 2 l of water, was set up. Controls contained 10 B. calamita tadpoles in one side of the tray in 2 l of water. Both treatments and controls had 10 replicates. The trays were cleaned and rearing water renewed every four days. Tadpoles were fed on washed lettuce and ground rat pellets ad libitum.

B. calamita tadpoles from the treatments and controls had their oxygen consumption measured 8 and 15 days after the experiment was started. The data from these two dates were pooled. Oxygen consumption was measured using Warburg constant volume manometers. Methods used are described in section 4.4.2 (Chapter 4). 5 B. calamita tadpoles were removed at random from the rearing regimes and placed in each manometer flask with 3 ml of water. Their combined mass was used to obtain a mass specific oxygen consumption. 6 replicates were used for both treated and control tadpoles on each measurement occasion (a total of 12 replicates).

7.2.5 Effect of B. bufo tadpoles on the spontaneous activity and time spent feeding in B. calamita tadpoles.

B. calamita tadpoles from the rearing regimes described in the previous section (7.2.4) had their spontaneous activity recorded 13 and 25 days after the rearing regimes were set up. The feeding activity was measured 19, 20 and 24 days after the regimes were set up.

Spontaneous activity was recorded by removing individual tadpoles and placing them in one of three plastic troughs. The troughs were 54 cm long and, semicircular in cross section, 7.5 cm in diameter. The troughs were filled with 2 l of water. The water was maintained at 20 ± 0.5°C by placing the troughs in a water bath.

The tadpoles were placed in the troughs, and left for 15 minutes to acclimatise to the experimental conditions. After the acclimatisation period the total duration of the tadpoles' spontaneous activity (in seconds) was recorded over a 300 second period. Spontaneous activity was defined as any locomotory movement in the tadpole. Three tadpoles were observed, and results recorded simultaneously.

On both recording dates 50 tadpoles from the treatment and 50 from the controls were observed. The data from the two occasions were pooled. After observation the tadpoles were returned to the appropriate rearing regime.

The feeding activity of the B. calamita tadpoles was observed in the rearing trays. In a single rearing tray a tadpole was selected at random and then observed over a 300 second period. The total time spent feeding was recorded. The tadpole was recorded as feeding on lettuce, ground rat pellets and faeces.
A total of 35 tadpoles was observed for both treatment and control tadpoles.

7.2.6 Effect of B. bufo tadpoles on food consumption in B. calamita tadpoles.

B. calamita tadpoles from the rearing regimes described in section 7.2.4 had their food consumption monitored 24 days after the initiation of the rearing regimes. The 10 treatment replicates and the 10 control trays were cleaned to remove any deposits that might have provided food to the tadpoles. The trays had their water replenished and the tadpoles returned.

A cork borer was used to cut 15 mm diameter discs from lettuce leaf lamina, 48 hr prior to the start of the food consumption experiment. The discs were soaked in water to condition them (tadpoles preferentially fed on lettuce that had been soaked in water rather than fresh lettuce pers. obs.).

Six discs were selected and placed in each tray with the B. calamita tadpoles. The discs were surface dried, then weighed on a top pan balance to the nearest 0.001 g, and assigned to a rearing tray (9 replicates for B. calamita tadpoles with B. bufo tadpoles and 9 replicated controls). Lettuce was also added to the other side of the tray, ad libitum, to feed the B. bufo tadpoles. No protein supplement was added during the course of the experiment as it would have been difficult to quantify its consumption.

After 48 hr the remains of the lettuce discs were removed, surface

dried and weighed. The wet mass of the lettuce consumed was then calculated. The tadpoles in each of the replicates were weighed at the end of the experimental period (for methods see section 2.2.4, Chapter 2) The quantity of lettuce consumed was then determined as a percentage of tadpole mass.

7.2.7 Effect of L-thyroxine on B. calamita tadpoles reared with B. bufo tadpoles.

In addition to the treatment and control trays set up as in sections 7.2.2, two similar rearing regimes were set up. These were: 10 trays containing 25 B. bufo tadpoles and 10 B. calamita tadpoles in 2 l of water containing 0.001 p.p.m. L-thyroxine (T_4) and 10 trays containing 10 B. calamita tadpoles only in 2 l of water containing 0.001 p.p.m. T_4.

The rearing water containing the T_4 was renewed every four days. All other rearing and measurement techniques were similar to those described in section 7.2.2. Oxygen consumption was measured as in section 7.2.3.

7.3 RESULTS

7.3.1 Effect of water conditioned by B. bufo tadpoles on development, growth and metamorphosis of B. calamita tadpoles.

B. bufo tadpoles showed no reduction in rate of development when reared in water conditioned by B. bufo tadpoles (Fig. 7.1). Initially, the rate of development of the treated tadpoles was greater than that of the controls, although both treated and control tadpoles reached stage 39 after 27 days.

B. calamita tadpoles treated with conditioned water showed a significant reduction in rate of development over a large part of the developmental time ($p < 0.05$). After 16 days the control tadpoles had reached stage 39, whereas the treatment tadpoles had reached stage 37.

The effect of conditioned water on rate of growth in B. bufo and B. calamita tadpoles was similar to its effect on developmental rates (Fig. 7.2). In general, treated B. bufo tadpoles attained significantly greater masses than controls ($p < 0.05$). The treated tadpoles reached a maximum mass of 0.28 g, after 28 days, the controls attaining a maximum mass of 0.24 g, after 32 days.

In comparison to B. bufo, B. calamita tadpoles treated with conditioned water had a reduced rate of growth ($p < 0.05$). After 12 days the controls had attained a mass of 0.145 g, whereas treated tadpoles, after the same time period, weighed 0.09 g. The maximum mean mass of

Figure. 7.1. Development of B. bufo and B. calamita tadpoles reared in water conditioned by B. bufo tadpoles. Means ± 95% confidence intervals, n=20 for each point.

● B. bufo control

○ B. bufo treated with conditioned water.

■ B. calamita control

□ B. calamita treated with conditioned water.

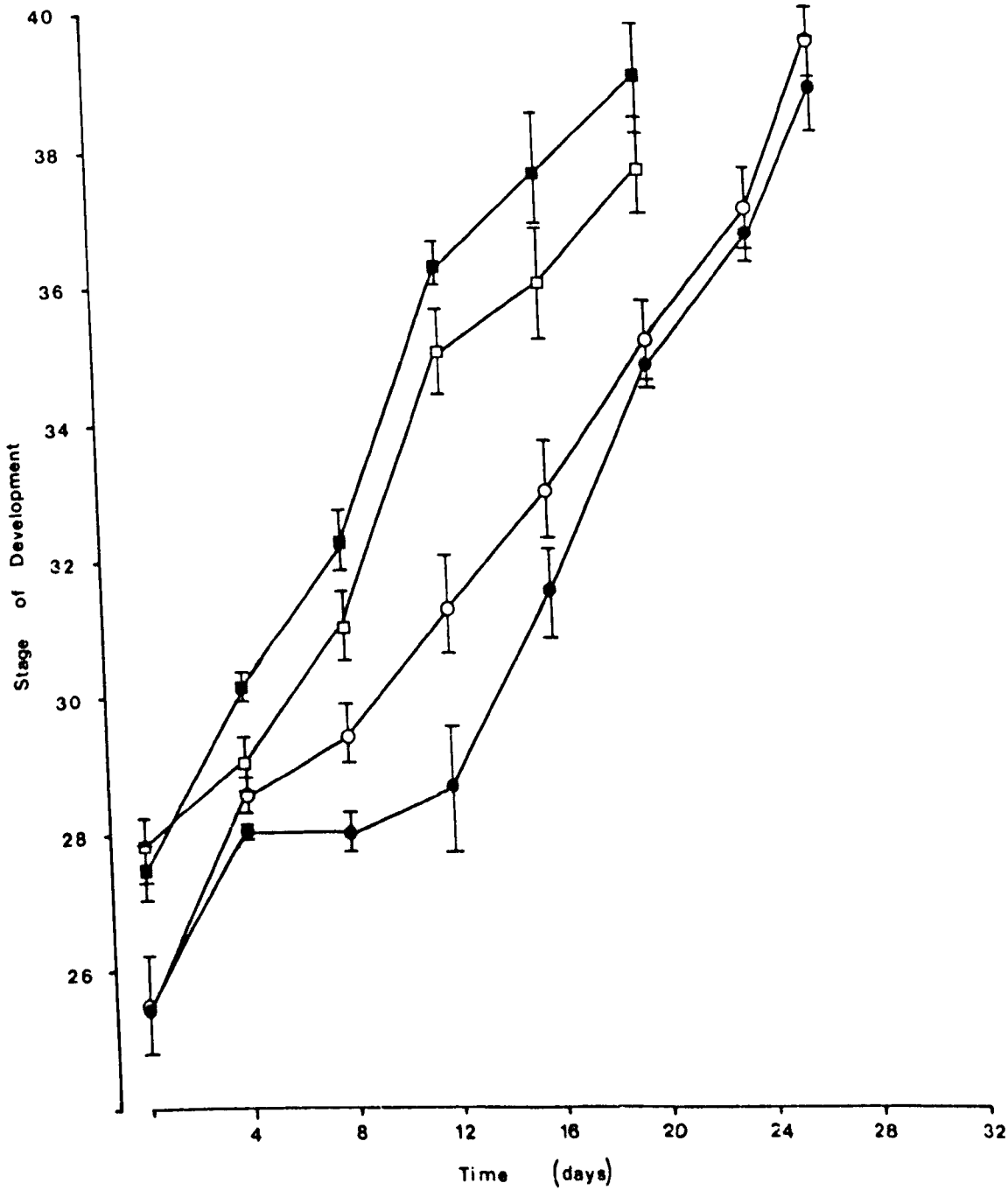

40
38
36
34
32
30
28
26
Stage of Development
4
8
12
16
20
24
28
32
Time (days)

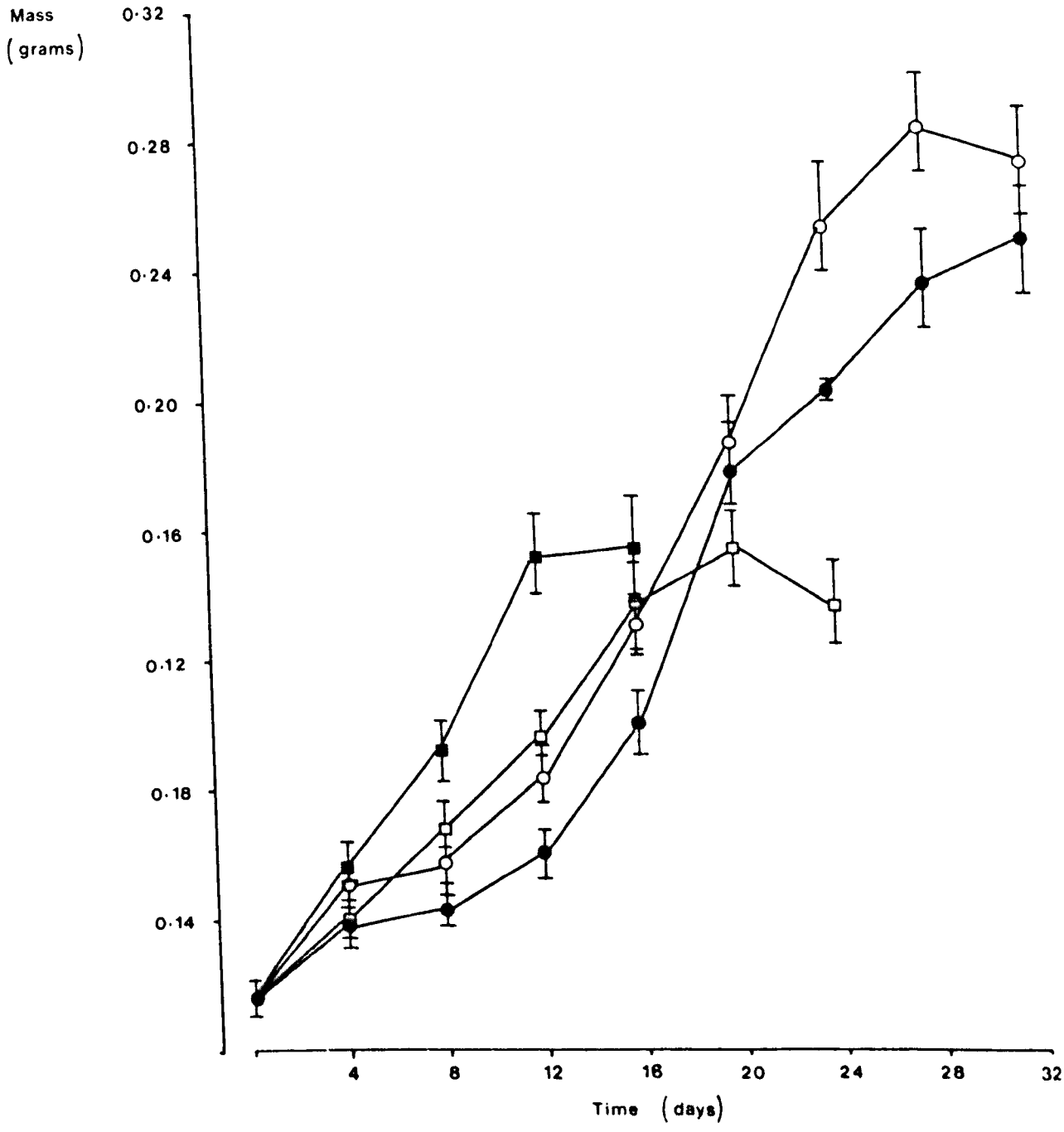
Figure. 7.2. Growth of B. bufo and B. calamita tadpoles reared in water conditioned by B.bufo tadpoles. Means ± 95% confidence intervals, n=20 for each point.

● B. bufo control

○ B.bufo treated with conditioned water.

■ B. calamita control

□ B. calamita treated with conditioned water.


Mass
(grams)
0·32
0·28
0·24
0·20
0·16
0·12
0·18
0·14
4
8
12
16
20
24
28
32
Time (days)

treated tadpoles (0.145 g) was achieved eight days after the equivalent mass was reached by control tadpoles.

Conditioned water affected growth and developmental rates in B. bufo and B. calamita . However, the mass of developmental stages for both treated tadpoles and controls, in each species, was similar (Fig 7.3). In general B. bufo tadpoles had greater masses at each stage of development than B. calamita tadpoles.

The timing of metamorphosis in B. bufo was not affected by conditioned rearing water (Fig 7.4). The metamorphosis of B. calamita tadpoles was delayed. The mean time taken by B. bufo tadpoles to reach metamorphosis in both treatment and controls was 33 days. The distribution of metamorphosing individuals with time was approximately normal. Control tadpoles started to metamorphose 28 days after the start of the experiment, and those reared in conditioned water after 27 days. The time taken, for all the tadpoles within a treatment, to reach metamorphosis was 37 days for the control tadpoles and 40 days for the treated tadpoles.

The mean time taken for B. calamita control tadpoles to reach metamorphosis was 25 days. Tadpoles reared in conditioned water took 28 days. The control tadpoles started metamorphosing 18 days after the start of the experiment, 4 days prior to the onset of metamorphosis in the treated tadpoles. Five days after the start of B. calamita metamorphosis (23 days after the start of the experiment) 45% of control tadpoles had reached metamorphosis, compared to 5% of tadpoles

Figure. 7.3. Mean mass of the developmental stages of B. bufo and B. calamita tadpoles when reared in water conditioned by B. bufo tadpoles. n=20 for each point.

● B. bufo control

○ B. bufo treated with conditioned water.

■ B. calamita control

□ B. calamita treated with conditioned water.

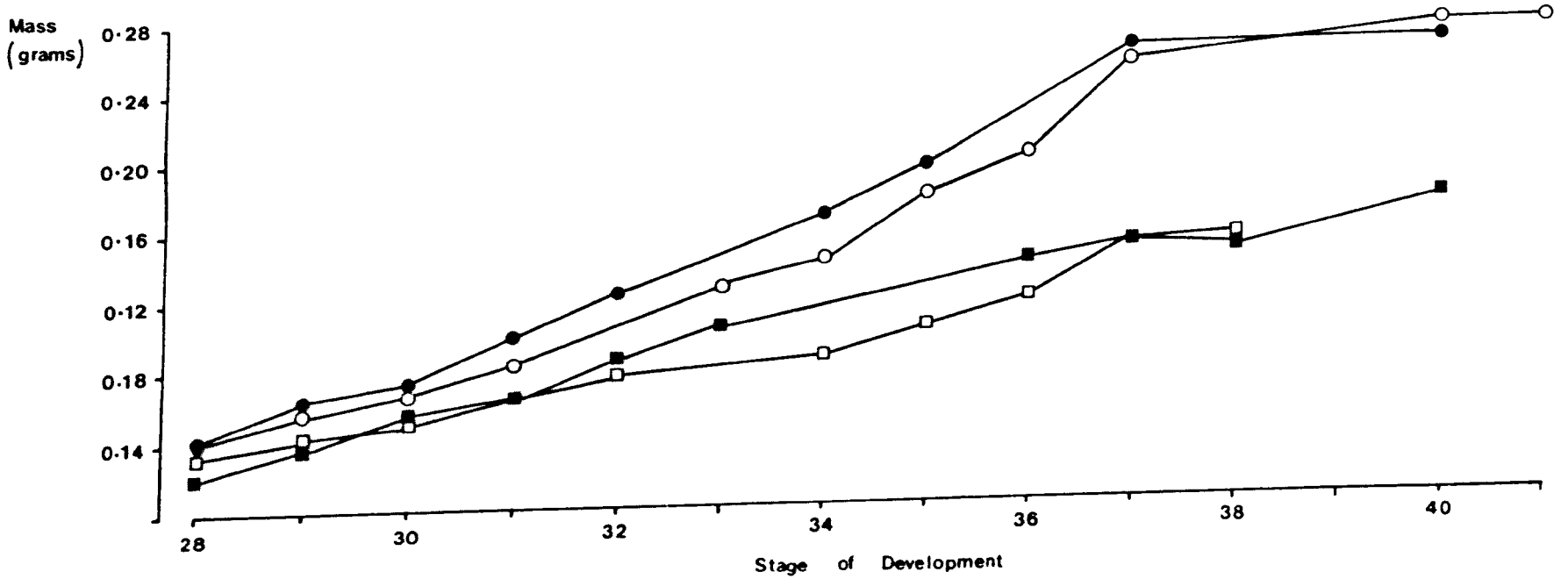
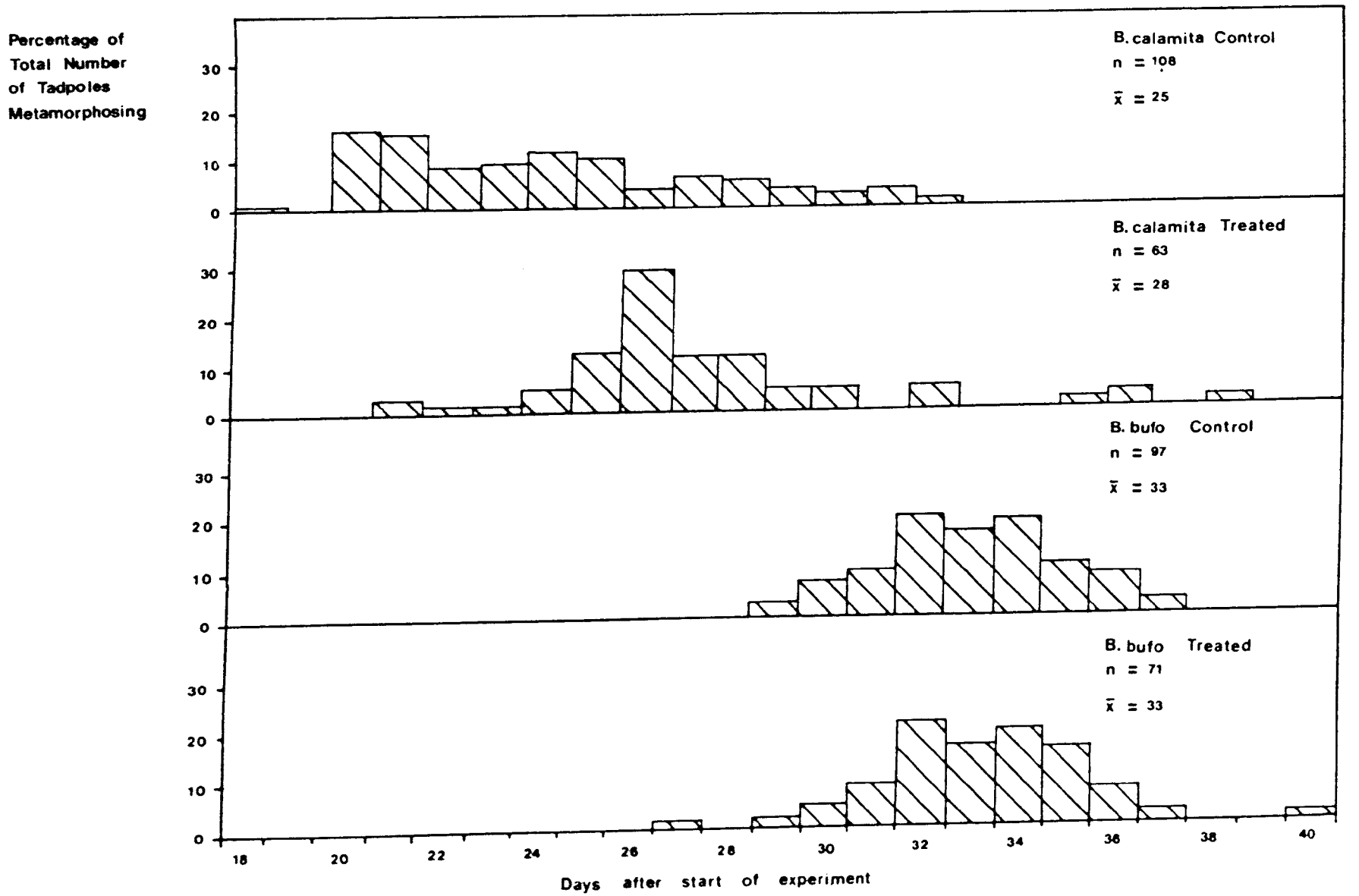

Mass
(grams)
0·28
0·24
0·20
0·16
0·12
0·18
0·14
28
30
32
34
36
38
40
Stage of Development

Figure. 7.4. Time taken for B. bufo and B. calamita tadpoles to reach metamorphosis (stage 41) when tadpoles have been reared in water conditioned by B. bufo tadpoles. % metamorphosis represents the proportion of the total number of tadpoles reaching metamorphosis in each treatment.


Percentage of Total Number of Tadpoles Metamorphosing
B. calamita Control
n = 108
x̄ = 25
B. calamita Treated
n = 63
x̄ = 28
B. bufo Control
n = 97
x̄ = 33
B. bufo Treated
n = 71
x̄ = 33
0
10
20
30
18
20
22
24
26
28
30
32
34
36
38
40
Days after start of experiment

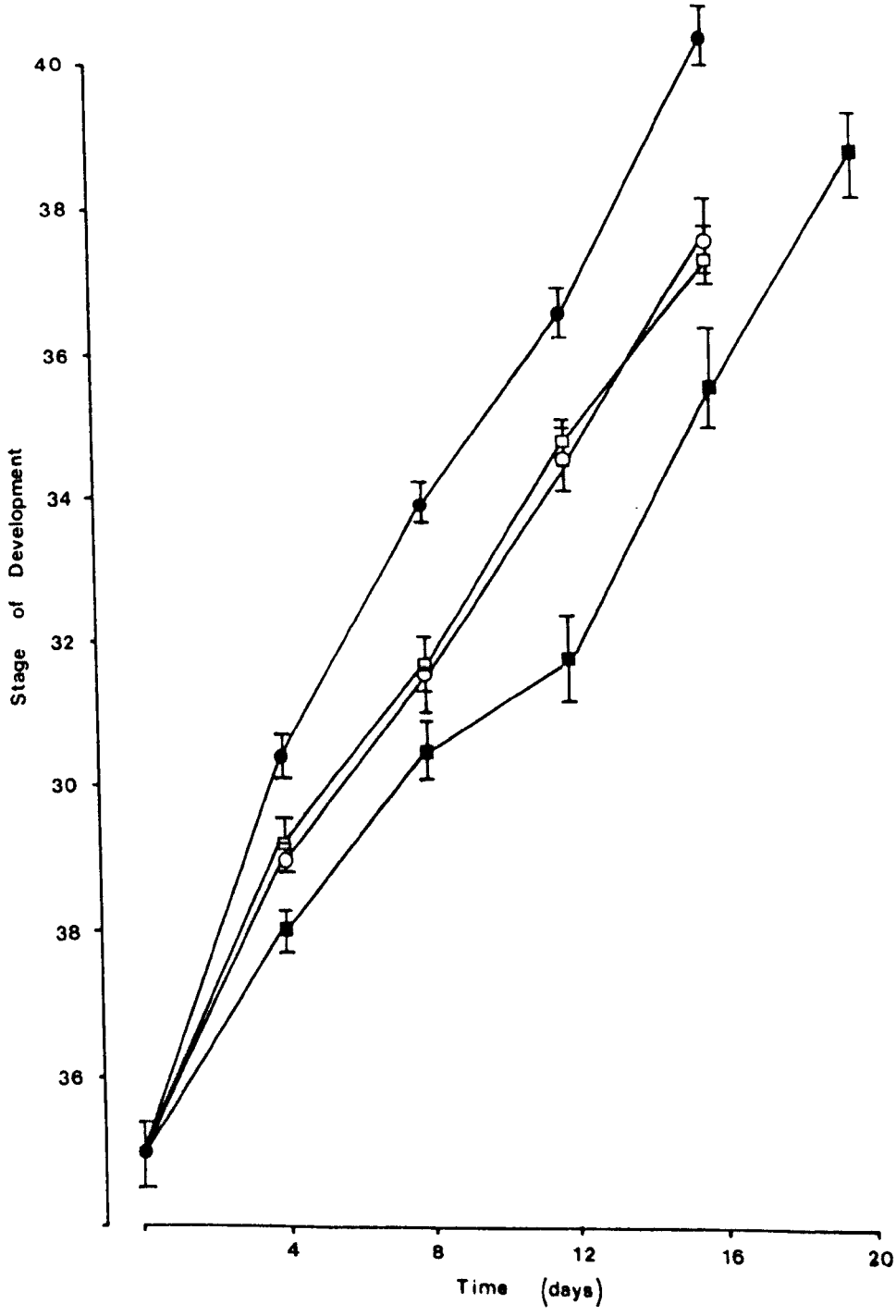
reared in conditioned water.

The distribution of metamorphosing individuals with time appeared to be one tailed in B. calamita control tadpoles. A large proportion of the tadpoles reached metamorphosis early in the time period over which all tadpoles metamorphosed. This was followed by a gradual reduction in the percentage of tadpoles reaching metamorphosis, until all the tadpoles had reached metamorphosis 32 days after the start of the experiment. The distribution of metamorphosis in the treated tadpoles was approximately normal. The greatest proportion of tadpoles reached metamorphosis 26 days after the experiment was initiated. Metamorphosis of all individuals did not occur until 38 days after the start of the experiment.

7.3.2 Effect of continuous exposure of B. calamita tadpoles to B. bufo tadpoles.

Rearing of B. calamita tadpoles with both 5 and 25 B. bufo tadpoles and 25 B. calamita tadpoles resulted in a significant ($p < 0.05$) reduction in developmental progress (Fig. 7.5). Control tadpoles reached stage 40 after 16 days. The rate of development in B. calamita tadpoles reared with 5 B. bufo tadpoles and those reared with 25 B. calamita were not significantly different ($p < 0.05$). They reached stage 37 after 16 days, 3 days after the controls attained stage 37. The B. calamita tadpoles reared with 25 B. bufo tadpoles exhibited the slowest development. Stage 38 was achieved after 20 days, 6 days later than the controls.

Figure. 7.5. Rates of development in B. calamita tadpoles reared with: 5 B. bufo tadpoles (○), 25 B. bufo tadpoles (■), 25 B. calamita tadpoles (□) and control (●). Means ± 95% confidence intervals, n=20 for each point.


40
38
36
34
32
30
38
36
Stage of Development
4
8
12
16
20
Time (days)

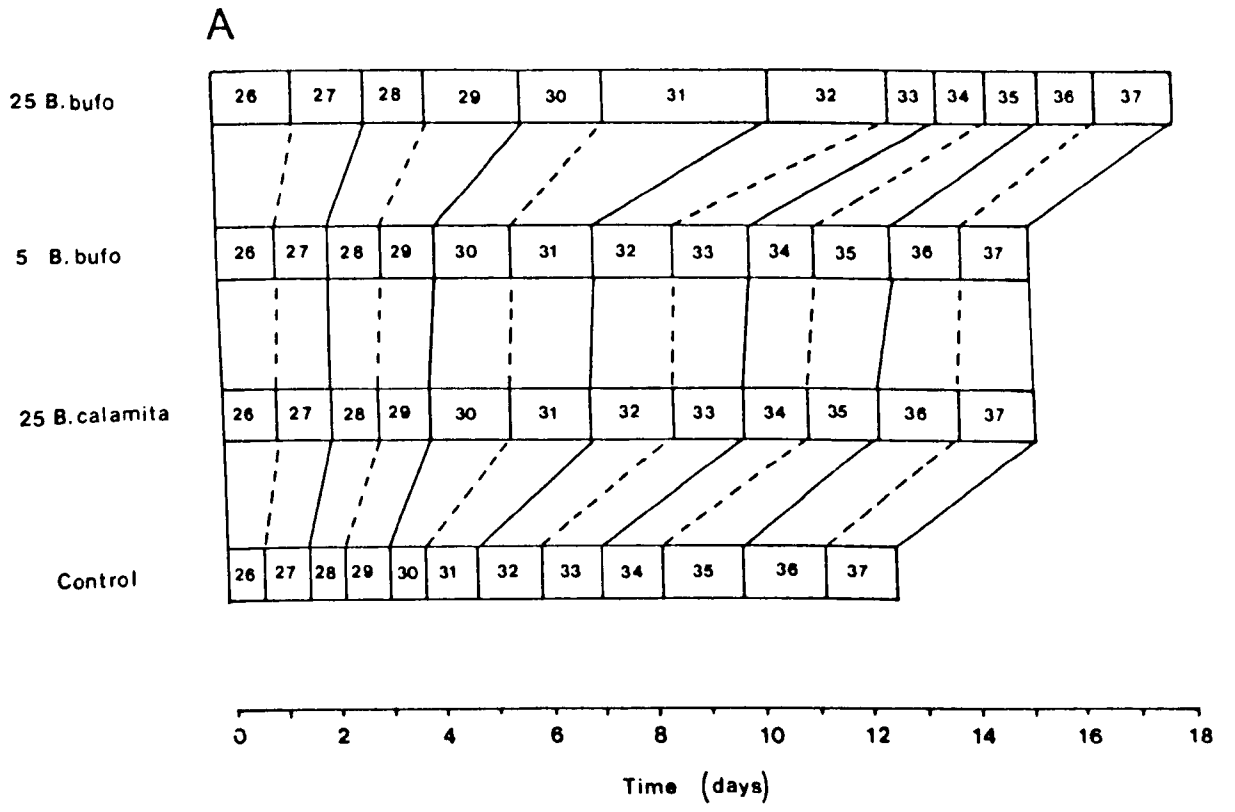
The duration of each of the developmental stages of the B. calamita tadpoles varied across the treatments (Fig. 7.6). The control tadpoles exhibited the shortest developmental times. Stages 26-37 were attained over a 13 day period. The earlier stages (26-31) were passed after 5 days. The 5 B. bufo and 25 B. calamita treatments produced similar results. The duration of stages 26-37 was 13 days, the early stages (26-31) taking 7 days for completion. The duration of stages 26-37 was greatest in the 25 B. bufo treatment, 18 days. Stages 26-31 were achieved over 11 days.

The duration of each developmental stage varied with the treatment. As the density of B. bufo tadpoles increased, a greater proportion of B. calamita developmental time was spent in the earlier stages (Fig. 7.6). Control tadpoles spent a greater percentage of their developmental time in the later stages of development. 54% of their developmental time was spent at stages 33-37. As the density of B. bufo treatment tadpoles increased, the proportion of time tadpoles were at stages 33-37 reduced to 43% (5 B. bufo) and 30% (25 B. bufo). This was reflected in a general increase in the percentage duration of each of the earlier stages (27-32). The stages that appeared to be affected by the treatments to the greatest extent were 30-32. At all times the 25 B. calamita treatment results were similar to the 5 B. bufo treatment.

The affect of continuous exposure of B. calamita tadpoles to B. bufo tadpoles on growth rates is shown in figure 7.7. For all treatments

Figure. 7.6. (A) Duration of each stage of B. calamita tadpole development when reared with 5 B. bufo, 25 B. bufo, 25 B. calamita tadpoles and a control.

(B) Proportion of time spent at each developmental stage as a percentage of the total developmental time period in B. calamita tadpoles reared with 5 B. bufo, 25 B. bufo, 25 B. calamita tadpoles and a control.


A
25 B. bufo
5 B. bufo
25 B. calamita
Control
26 27 28 29 30 31 32 33 34 35 36 37
0 2 4 6 8 10 12 14 16 18
Time (days)

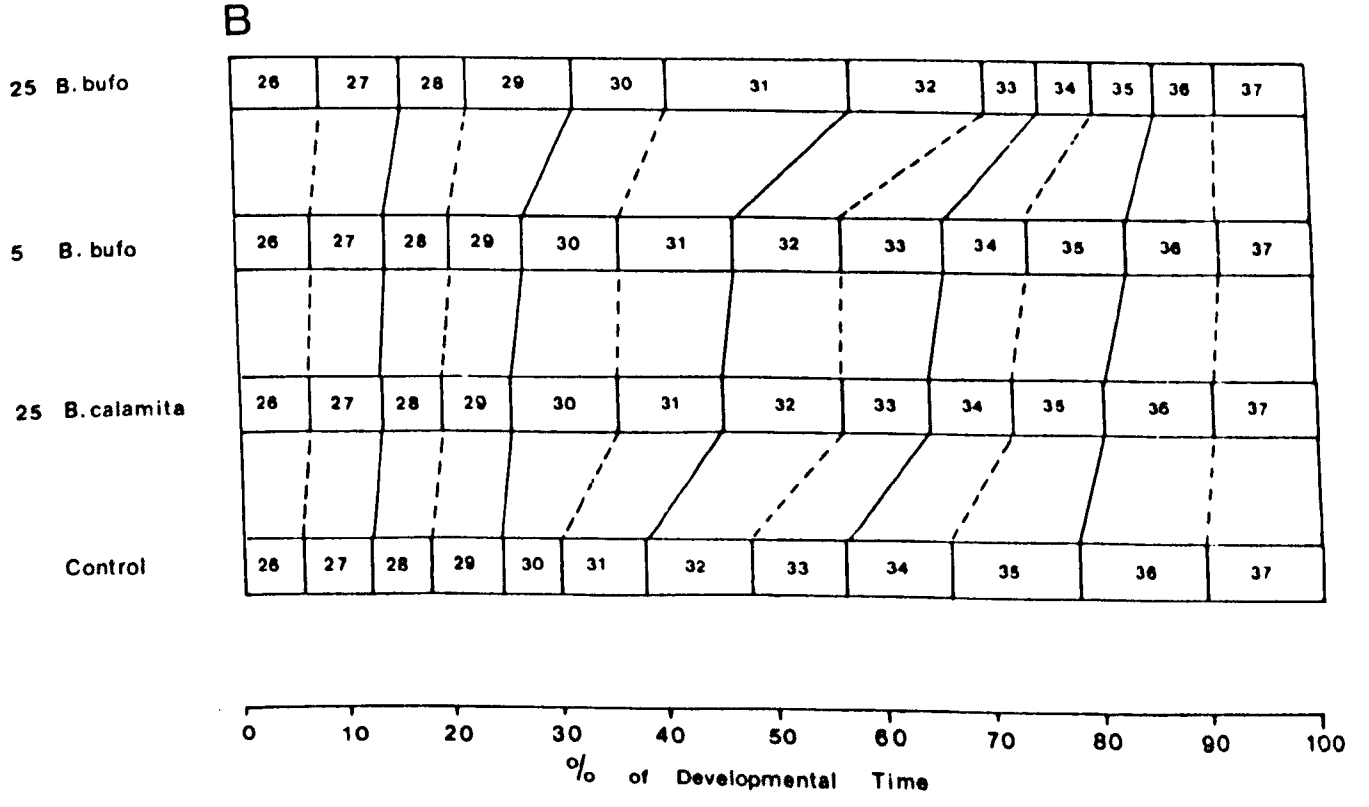
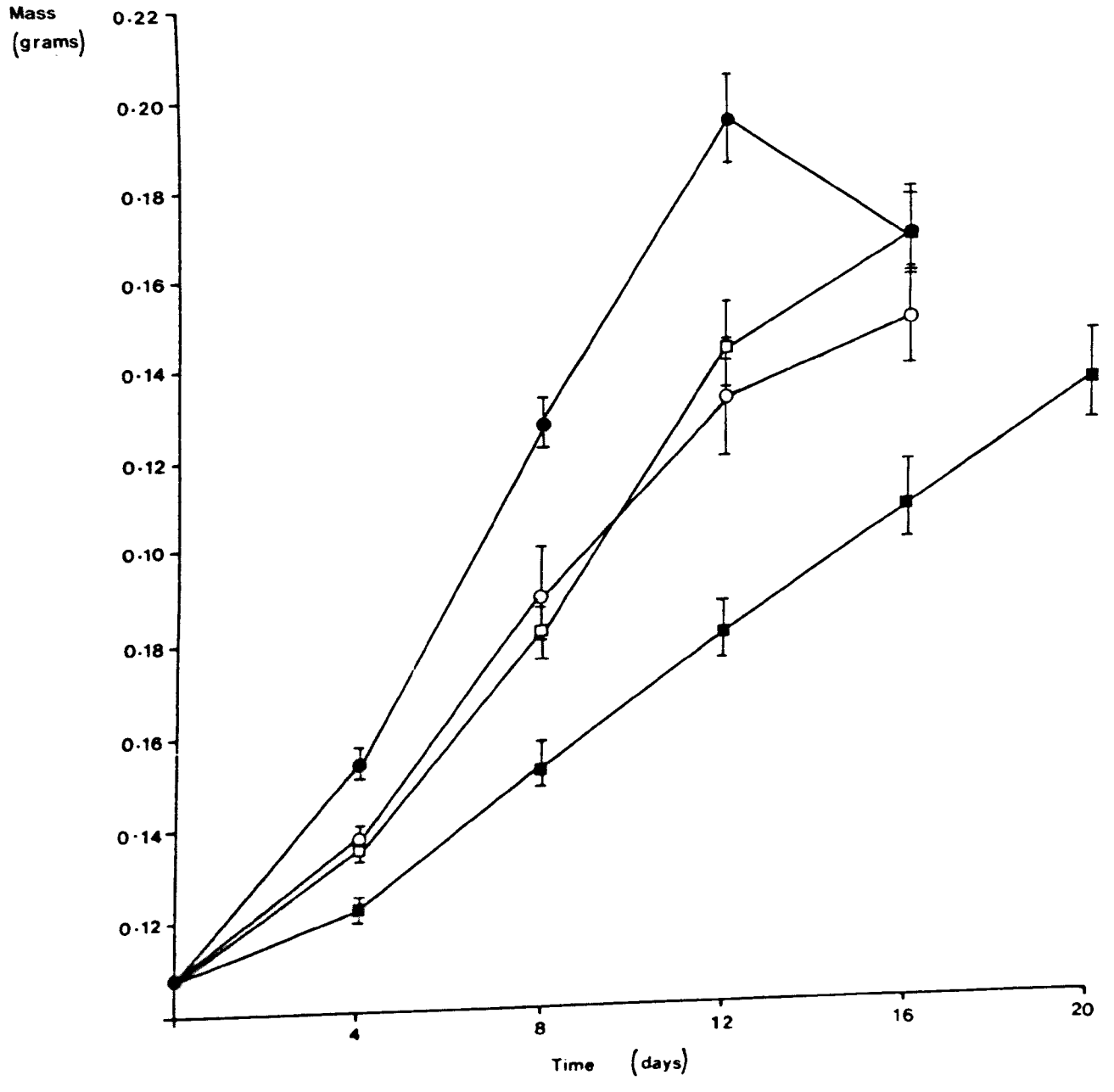

B
25 B. bufo
5 B. bufo
25 B. calamita
Control
26 27 28 29 30 31 32 33 34 35 36 37
0 10 20 30 40 50 60 70 80 90 100
% of Developmental Time

Figure. 7.7. (mass) Growth in *B. calamita* tadpoles reared with: 5 *B. bufo* tadpoles (○), 25 *B. bufo* tadpoles (■), 25 *B. calamita* tadpoles (□) and control (●). Means ± 95% confidence intervals, n=20 for each point.


Mass
(grams)
0·22
0·20
0·18
0·16
0·14
0·12
0·10
0·18
0·16
0·14
0·12
4
8
12
16
20
Time (days)

mass of B. calamita tadpoles was lower than that of equivalent control tadpoles ($p < 0.05$). Control tadpoles reached a maximum mass (0.193 g) after 12 days. The 5 B. bufo treatment tadpoles attained a maximum mass (0.148 g) after 16 days, the same period over which the 25 B. calamita treatment tadpoles reached their maximum mass (0.164 g). The maximum mass of the 25 B. bufo treated tadpoles was achieved after 20 days (0.129 g).

7.3.3 Effect of B.bufo tadpoles on the oxygen consumption, spontaneous activity, time spent feeding and food consumption in B. calamita tadpoles.

The mass specific oxygen consumption of B. calamita tadpoles reared with B. bufo tadpoles (0.174 ml O_2 g^{-1} hr^{-1}) was lower than that of control tadpoles (0.245 ml O_2 g^{-1} hr^{-1}). The difference was not significant at the 95% level (Table 7.1).

The spontaneous activity of tadpoles was similar for treatment and controls ($p < 0.05$, Table 7.2). The treated tadpoles were active for 87.5 seconds over a 300 second period (29.5% of the time). The controls were active for 85.5 seconds of the 300 second time period (28.5% of the time).

The time spent feeding by treated tadpoles was not significantly different ($p < 0.05$) from the time spent feeding by the controls (Table 7.2). The treated tadpoles fed for 38.9 seconds, 13% of the 300 second observation period. The controls fed for 14.8% of the 300 second

Table 7.1. The effect of B. bufo tadpoles on the oxygen consumption of B. calamita tadpoles.

Treatments were 10 B. calamita tadpoles reared with 25 B. bufo tadpoles. Controls were 10 B. calamita tadpoles.

Mass specific Oxygen consumption of B. calamita tadpoles (ml O_2 g^{-1} hr^{-1}).

	N	MEAN	ST DEV	SE MEAN
Control tadpoles	7	0.245	0.102	0.039
Treatment tadpoles	7	0.174	0.060	0.023

T-test of oxygen consumption: Treatment = Control

T = 1.6 DF = 12 P= 0.14 n.s.

(n.s.: no significant difference at the 95% level)

Table 7.2. The effect of B. bufo tadpoles on the spontaneous activity and time spent feeding in B. calamita tadpoles.

Treatments were 10 B. calamita tadpoles reared with 25 B. bufo tadpoles. Controls were 10 B. calamita tadpoles.

(i) Spontaneous activity, time spent active (seconds) over a 300 second period.

	N	MEAN	ST DEV	SE MEAN
Control tadpoles	100	87.5	37.1	3.71
Treatment tadpoles	100	85.5	36.4	3.64

T-test of spontaneous activity: Treatment = Control

T = 0.38 DF = 198 P = 0.71 n.s.

(ii) Time spent feeding (seconds) over a 300 second period.

	N	MEAN	ST DEV	SE MEAN
Control tadpoles	35	44.4	24.8	4.32
Treatment tadpoles	35	38.9	30.5	5.22

T-test of feeding activity: Treatment = Control

T = 0.81 DF = 68 P = 0.42 n.s.

(n.s.: no significant difference at 95% level)

observation period, 44.4 seconds.

The absolute food consumption of treated and control tadpoles (Table 7.3) was not significantly different ($p < 0.05$). Control tadpoles consumed a mean lettuce mass of 0.386 g over a 72 hr period compared with 0.636 g in the treatment tadpoles.

Food consumption of the treated and control tadpoles, when expressed in relation to the tadpoles mass, was significantly different ($p < 0.05$, Table 7.3). The control tadpoles consumed equivalent to 32.1% of their mass over a 72 hr period. The treated tadpoles consumed, over 72 hours, 45.4% of their mass.

7.3.4 Effect of L-thyroxine on growth and development of B.calamita tadpoles reared with B. bufo tadpoles.

Both control tadpoles and those reared with B. bufo tadpoles developed at a faster rate when reared in water containing 0.001 p.p.m. T_4 (Fig. 7.8). The controls treated with T_4 exhibited the fastest development, reaching stage 40 after 24 days. Over the majority of the developmental period the control + T_4 tadpoles were significantly more advanced ($p < 0.05$) than the other treatments.

B. calamita tadpoles reared with B. bufo tadpoles, and no T_4, had the lowest rates of development. These were significantly lower than all other treatments for most of the experimental period ($p < 0.05$). After 40 days the B. calamita tadpoles reared with B. bufo tadpoles reached

Table 7.3. The effect of B. bufo tadpoles on the food consumption of B. calamita tadpoles.

Treatments were 10 B. calamita tadpoles reared with 25 B. bufo tadpoles. Controls were 10 B. calamita tadpoles.

(i) Food consumption, wet weight of lettuce consumed (g) by 10 B. calamita tadpoles in 72 hours.

	N	MEAN	ST DEV	SE MEAN
Control tadpoles	9	0.386	1.87	0.62
Treatment tadpoles	8	0.363	0.07	0.03

T-test of food consumption: Treatment = control

T = 0.33 DF = 15 P = 0.75 n.s.

(ii) Food consumption, wet weight of lettuce consumed per individual B. calamita tadpole expressed as a % of its body weight (wet weight).

	N	MEAN	ST DEV	SE MEAN
Control tadpoles	9	32.1	15.5	5.18
Treatment tadpoles	8	45.4	9.18	3.25

T-test of food consumption: Treatment = Control

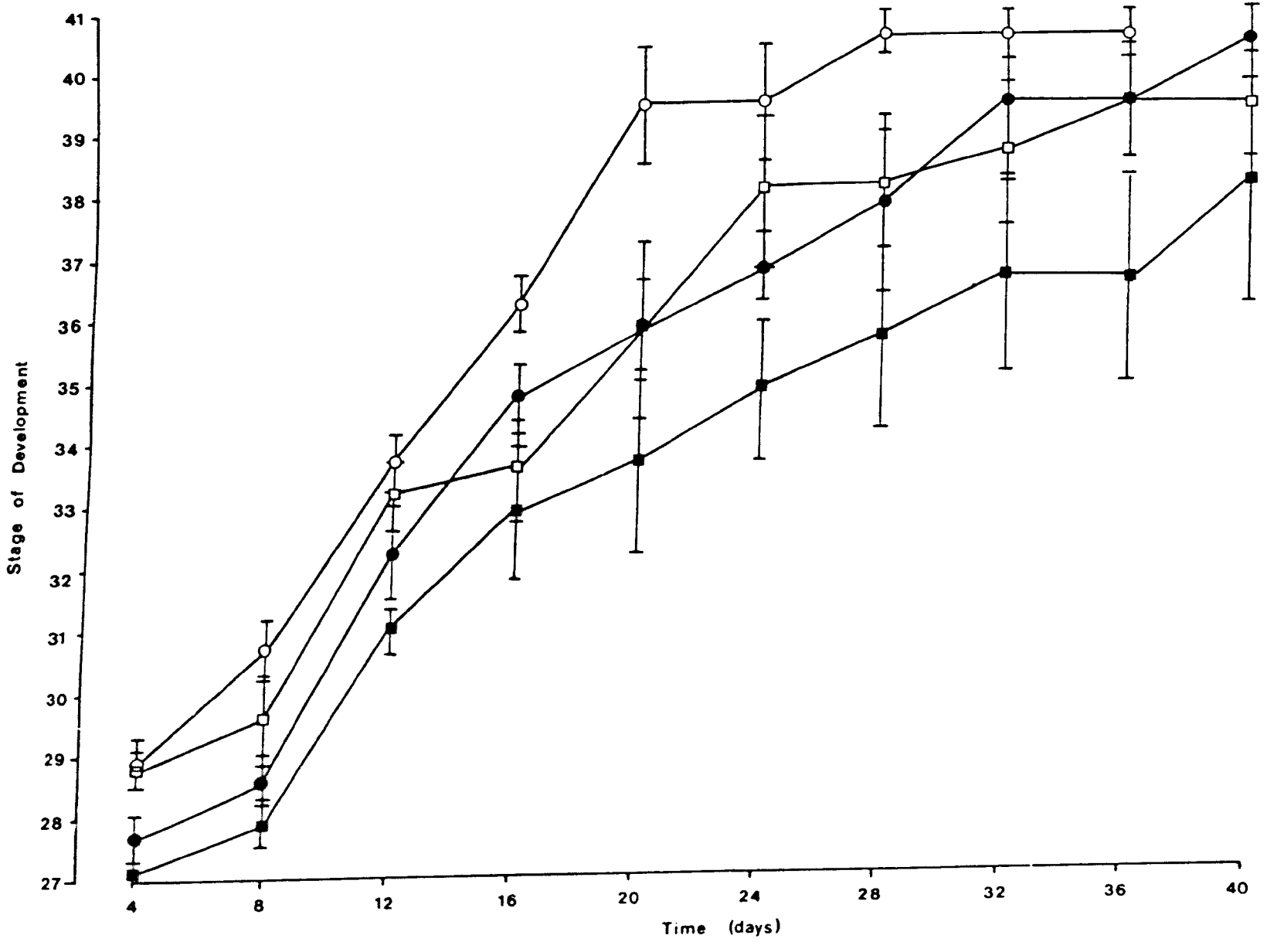
T = -2.17 DF = 15 P = 0.049 *

(n.s.: not significant at the 95% level)
(*: significant at the 95% level)

Figure. 7.8. Effect of L-thyroxine on the development of B. calamita tadpoles reared with B. bufo tadpoles (treatment), and controls with no B. bufo tadpoles. Means ± 95% confidence intervals, n=10 for each point.

● Control ○ Control + 0.001 p.p.m. T_4

■ Treatment □ Treatment + 0.001 p.p.m. T_4


Stage of Development
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
4
8
12
16
20
24
28
32
36
40
Time (days)

stage 38. The control tadpoles (no T_4, no B. bufo tadpoles), and those reared with B. bufo + 0.001 p.p.m. T_4, had similar rates of development ($p < 0.05$).

The rates of growth of B. calamita tadpoles varied considerably with the treatments (fig. 7.9). Control tadpoles and control + 0.001 p.p.m. T_4 treated tadpoles had similar rates of growth up to day 20. After stage 20 the mass of the control tadpoles continued increasing (from 0.127 g to a maximum mean mass of 0.176 g after 32 days). The mass of the control + 0.001 p.p.m. T_4 tadpoles reached a maximum (0.124 g) at 20 days, the mean mass then decreasing to 0.086 g after 40 days.

The rates of growth of B. calamita tadpoles reared with B. bufo tadpoles and those reared with B. bufo tadpoles + 0.001 p.p.m. T_4 were similar and generally lower than those of the controls. B. calamita tadpoles reared with B. bufo tadpoles + 0.001 p.p.m. T_4 attained a maximum mass of 0.117 g after 36 days, this compared with those reared without T_4, reaching 0.122 g after 40 days.

Differences in rates of development and growth for each treatment, were reflected in different masses at each stage of development (Fig. 7.10). Control and treatment tadpoles, without T_4 attained greater masses at each stage of development than those reared with T_4. The B. calamita tadpoles reared without B. bufo tadpoles (both T_4 treated and control) had higher masses at each developmental stage, than those reared with B. bufo tadpoles. In all treatments the maximum tadpole mass was attained at stage 40.

Figure. 7.9. Effect of L-thyroxine on the growth of B. calamita tadpoles reared with B. bufo tadpoles (treatment), and controls with no B. bufo tadpoles. Means ± 95% confidence intervals, n=10 for each point.

● Control ○ Control + 0.001 p.p.m. T_4

■ Treatment □ Treatment + 0.001 p.p.m. T_4

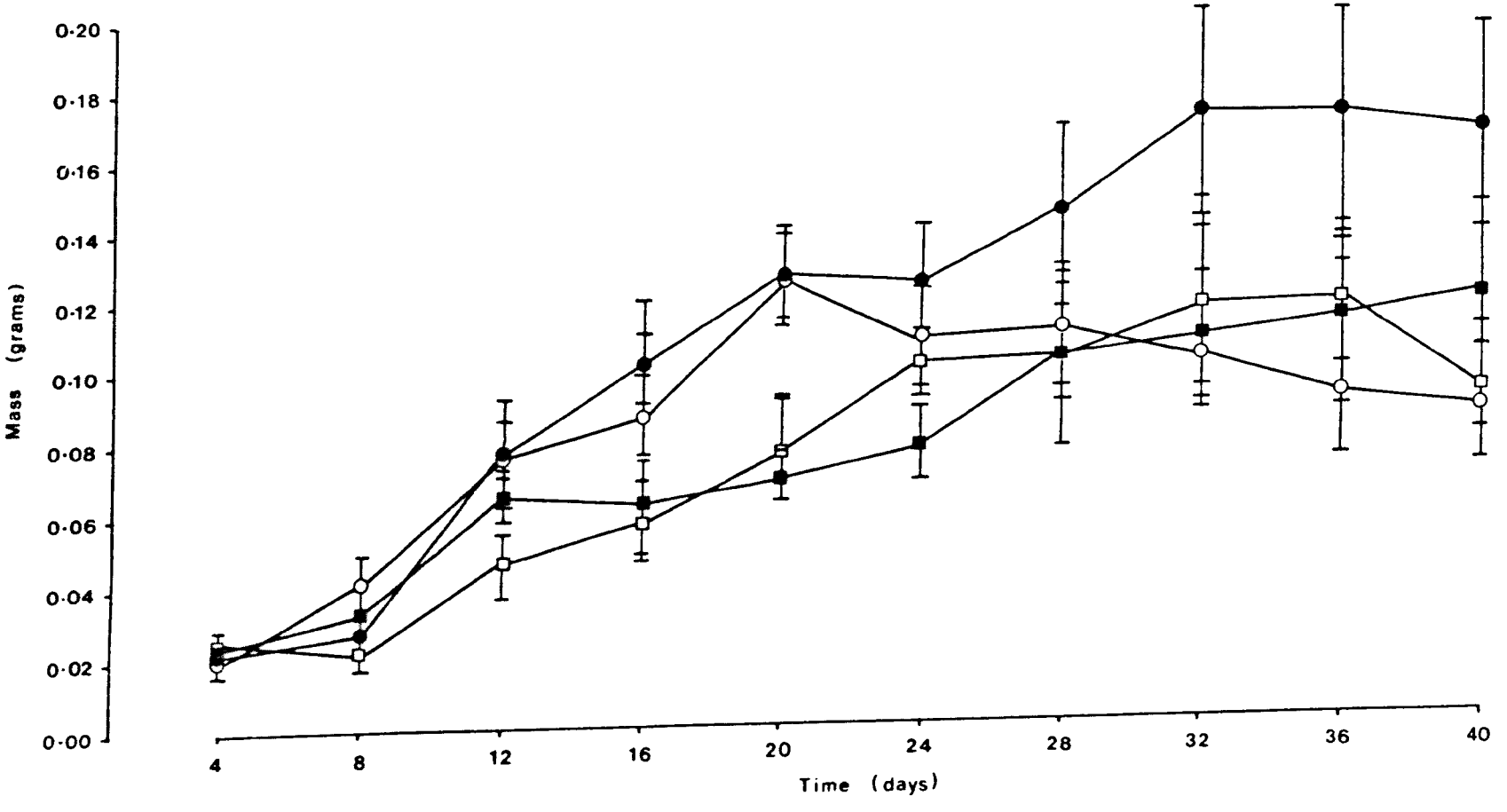
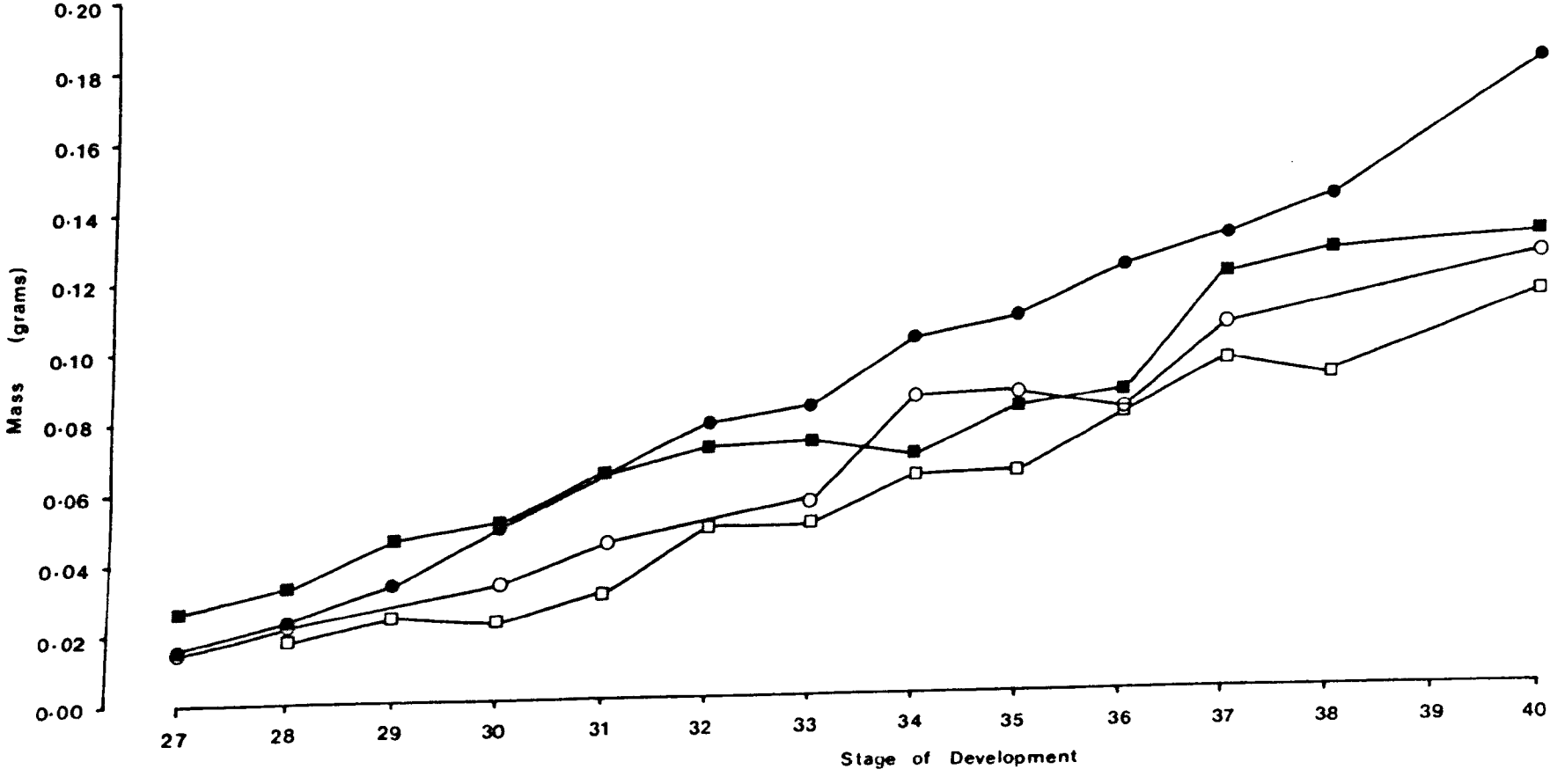

0·20
0·18
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
0·00
Mass (grams)
4
8
12
16
20
24
28
32
36
40
Time (days)

Figure. 7.10. The effect of L-thyroxine on the mass of different developmental stages of B. calamita tadpoles reared with B. bufo tadpoles (treatment) and controls with no B. bufo tadpoles. Mean values, n=10 for each point.

● Control ○ Control + 0.001 p.p.m. T_4

■ Treatment □ Treatment + 0.001 p.p.m. T_4

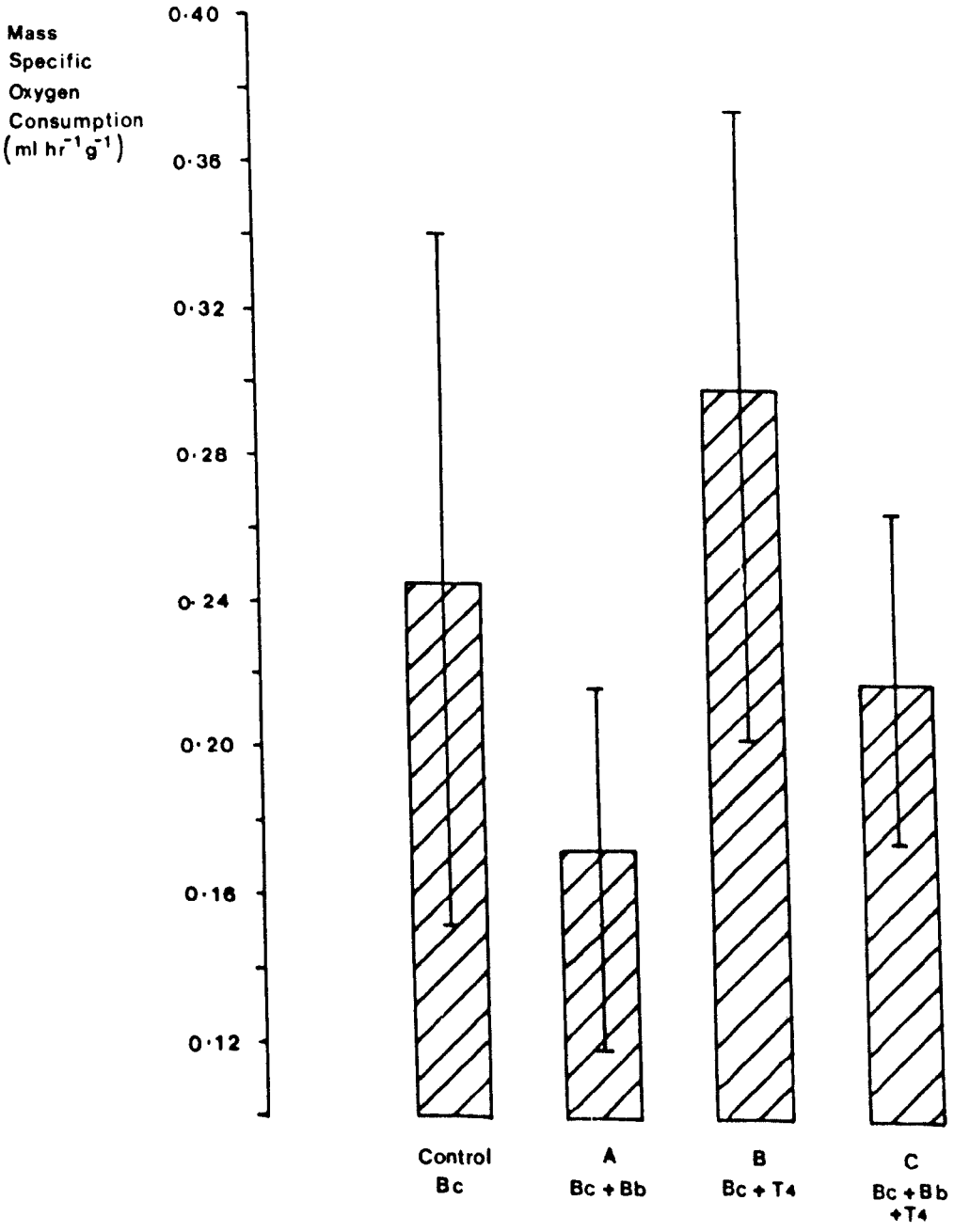

0·20
0·18
0.16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
0·00
Mass (grams)
27
28
29
30
31
32
33
34
35
36
37
38
39
40
Stage of Development

7.3.5 Effect of L-thyroxine on oxygen consumption of B. calamita tadpoles reared with B. bufo tadpoles.

The mass specific oxygen consumption of B. calamita control tadpoles and those reared with B. bufo tadpoles (0.242 and 0.170 ml O_2 g^{-1} hr^{-1} respectively), was lower than that of equivalent tadpoles reared with 0.001 p.p.m.T_4 (0.295 and 0.216 ml O_2 g^{-1} hr^{-1} respectively) (Fig. 7.11). The differences were not significant ($p < 0.05$). This may have been a result of small sample size. Addition of T_4 resulted in elevated oxygen consumption in both inhibited and non inhibited tadpoles. The reduction in B. calamita oxygen consumption resulting from B. bufo inhibition was of the same order in the T_4 treated tadpoles (inhibition resulting in 29.8% reduction) and untreated tadpoles (inhibition resulting in 26.8% reduction).

B. calamita tadpoles reared with B. bufo tadpoles in both untreated and T_4 treated water had lower mass specific oxygen consumption rates than B. calamita tadpoles reared without B. bufo tadpoles. There was a 29.8% reduction in oxygen consumption in B. bufo treated tadpoles with no T_4 in the water and a 26.8% reduction in the tadpoles reared with 0.001 p.p.m. T_4.

Figure. 7.11. Effect of L-thyroxine (T_4) on mass specific oxygen consumption of B. calamita tadpoles reared with B. bufo tadpoles. Means ± 95% confidence intervals. For sample sizes see section 4.2.2.


Mass
Specific
Oxygen
Consumption
(ml hr⁻¹g⁻¹)
0·40
0·36
0·32
0·28
0·24
0·20
0·16
0·12
Control
Bc
A
Bc + Bb
B
Bc + T4
C
Bc + Bb
+T4

7.4. DISCUSSION.

7.4.1 Effect of B. bufo tadpoles on the growth, development, metamorphosis and behaviour of B. calamita tadpoles.

The intraspecific effects of differing tadpole densities in B. bufo and B. calamita were discussed in chapter 6. When the two species come into contact, B. calamita tadpoles appear to be outcompeted by B. bufo.

Intraspecific inhibition has been observed in many species (see Chapter 6). Licht (1967) found that inhibition did not diminish when moving from intraspecific to interspecific effects of crowding. Investigations by Rose and Rose (1961) suggest that effects of tadpole inhibition are rather specific and probably reduce with phylogenic distance. Akin (1966) also concluded that inhibition effects are species-specific.

The effect of water conditioned by B. bufo tadpoles on B. calamita tadpoles was significant. The conditioned water reduced both rate of growth and rate of development in B. calamita tadpoles. This confirms the observations of Heusser (1972a; 1972b). The B. bufo tadpoles treated with conditioned water, however, showed an increase in both rates of development and growth. It is possible that the B. bufo tadpoles were not susceptible to the inhibitory substances in the conditioned water. Coprophagy, ingestion of the faecal material from the conditioning tadpoles, may have provided a food source for the B. bufo tadpoles that allowed them to develop and grow faster than

control tadpoles. Steinwascher (1978) demonstrated that Rana utricularia tadpoles grew faster when fed with faeces as well as their usual food.

The timing of metamorphosis in B. calamita was disrupted when tadpoles were treated with conditioned water. B. bufo tadpoles appeared unaffected. These results suggest that the inhibitory agent released by B. bufo tadpoles has a greater effect interspecifically than intraspecifically. The effect of the B. bufo tadpoles was also greater than the effect of an equivalent density of B. calamita tadpoles. Thus the inhibition of B. calamita tadpoles by B. bufo tadpoles is specific, rather than a result of increased tadpole density in general.

Most workers investigating both inter and intraspecific inhibition in tadpoles used conditioned water to treat experimental animals (e.g. Heusser, 1972a; 1972b). The specific substances (or organisms) that result in inhibition effects in tadpoles are still unknown. Their stability and duration of effectiveness in water may be low. The results obtained using direct treatment of B. calamita tadpoles with B. bufo tadpoles (i.e. continuous exposure to conditioning tadpoles, separated by a nylon mesh) exhibited greater inhibitory effects than the experiments utilising conditioned water. This rearing regime probably reflects the environmental conditions encountered when the two species occur together, to a greater degree than experimental methods using conditioned water. It also suggests that the inhibitory substance is not long lived away from the donor species.

It is unlikely that inhibition is a result of interspecific contacts (e.g. John and Fenster, 1975). The tadpoles that were exposed to inhibitory effects were at a fixed density. The inhibiting tadpoles were not in physical contact with them. It appears that the inhibition is a result of chemicals or organisms that can pass through the mesh divisions in the test trays.

I have discussed the different growth and developmental patterns of B. bufo and B. calamita in chapter 6. The effects seen in inhibited B. calamita tadpoles may possibly be a result of B. bufo having a tadpole development strategy that involves inhibition of early stages. B. calamita requires fast development during the early stages of tadpole life. Where the two species are found together B. calamita tadpoles may succumb to the B. bufo density regulation strategy suggested in chapter 6.

The inhibitory material from B. bufo does not appear to be specific, unlike the findings of Akin (1966) and Rose and Rose (1961). It is known that larger tadpoles inhibit smaller tadpoles of the same species (Woodward, 1987; Dash and Hota, 1980; Gromko et al, 1973; Licht, 1967). B. bufo breeds earlier in the year than B. calamita (Mathias, 1971) and therefore B. bufo tadpoles are generally larger than B. calamita tadpoles when they are found together. This could enhance any interspecific effects and could explain why B. bufo tadpoles inhibit B. calamita tadpoles to a greater extent than themselves. The results, however, indicate that 5 B. bufo tadpoles produce an inhibition effect that is equivalent to the effect of 25 B. calamita tadpoles. The

B. calamita tadpoles had a greater total mass. B. bufo tadpoles therefore appear to be stronger inhibitors than B. calamita tadpoles.

The implications for B. calamita tadpoles developing in pools already containing B. bufo tadpoles are severe. When the two species are found together the length of time taken for B. calamita tadpoles to proceed through the early stages of development will be increased. Small tadpoles are more susceptible to predation (Semlitsch et al, 1988; Cronin and Travis, 1986; Formanowicz, 1986;Travis, 1985; Crump, 1981). Larger tadpoles are more able to avoid capture, cannot be handled efficiently by small predators or may satiate predators, resulting in lower numbers being captured.

In a situation where B. bufo tadpoles are inhibiting B. calamita growth and development, the B. calamita tadpoles will remain smaller than those of B. bufo. Predators will preferentially prey upon the smaller B. calamita tadpoles. This might lead to a reduction in the predation of the larger B. bufo tadpoles. In reducing the growth rates of B. calamita tadpoles, more food resources become available to B. bufo tadpoles. Wilbur and Alford (1985) investigated the effect of B. americanus (an early breeder) on Hyla chrysoscelis which spawns later in the season. B. americanus had a significantly adverse affect on H. chrysoscelis even although the two species were not contemporous. This was interpreted as being a result of the impact of the early breeding species on the trophic structure of the pond. The impact of B. bufo tadpoles on the trophic structure of its breeding ponds is not known. It is possible that this might serve to compound any

interspecific inhibition effects.

Food was not a limiting factor in these experiments. The inhibition effects observed were a result of a substance or organism passing from the inhibitors to the test tadpoles. In a natural system this would result in more food becoming available to B. bufo tadpoles.

B. calamita tadpoles have lower levels of predator defences than B. bufo (Nicolle, 1985). In a competitive environment with B. bufo, mortality of B. calamita tadpoles as a result of predation could be severe. Beebee (1979) suggests that the decline in populations of B. calamita is a result of habitat change and subsequent appearance of B. bufo as an effective predator. It is unlikely that B. bufo is a major predator, Banks and Beebee (1987) showed that B. calamita spawn predation by B. bufo was insignificant. However, increase in B. bufo density led to reduced B. calamita growth and lower metamorphic success rates. This was probably a result of increased invertebrate predation. Morin (1987) showed that tadpoles of early breeding frogs reduced the growth and survival of late breeding frogs, which was dependent on the density of the later breeding species.

In pools without B. bufo tadpoles, predation levels are approximately 80-90% of the B. calamita tadpoles (Davis, 1985; Kadel, 1975). In ponds with both species present, if 80-90% of the total tadpole population was predated, and if B. calamita tadpoles were preferentially predated, it is likely that the mortality of B. calamita tadpoles will be severe.

As well as increasing the exposure of B. calamita tadpoles to predation, increased developmental times as a result of inhibition, lead to increased risk of tadpole desiccation in pools that are ephemeral. The duration of the tadpole stage is increased as a result of inhibition and also the pattern of metamorphosis. In an uninhibited B. calamita population a large proportion of the tadpoles metamorphose early in the total period during which metamorphosis occurs. This will enhance the population's survival should the pool dry up shortly after metamorphosis has commenced. When inhibited, the distribution of metamorphosing individuals is normal: low numbers metamorphose early in the total metamorphic period, with a peak half way through. Thus if a pool containing inhibited B. calamita tadpoles dries up near the onset of metamorphosis a much larger proportion of the population will perish.

The agent responsible for the inhibition of B. calamita tadpoles, may be specific chemicals e.g. hormones or metabolic inhibitors (Shvarts and Pyastolova, 1970 b) or internal parasites (e.g. Richards, 1962). L-thyroxine is a hormone that can promote development in tadpoles. This appears to be at the expense of growth rate (Chapter 5). It is unlikely that the inhibitory mechanism is acting directly as a suppressor or antagonist of the tadpoles' own L-thyroxine. Although the addition of L-thyroxine increased the developmental rates of inhibited B. calamita tadpoles, their growth rates were not significantly altered. The application of L-thyroxine did not therefore alleviate the inhibition totally. This suggests that thyroid suppression is not

one of the modes of inhibitory action.

Inhibited B. calamita tadpoles had a reduced mass specific oxygen consumption, although this was not statistically significant (this may have been a result of low sample size). The inhibited and control tadpoles exhibited no differences in spontaneous activity, or feeding activity, therefore the lower oxygen consumption rates observed in the inhibited tadpoles were probably differences in basal metabolic rate. A lower metabolic rate suggests that inhibition mechanism is interfering with the tadpoles' metabolism in general. Reduced metabolic rates lead to reduced growth and developmental rates (Chapter 4).

The time spent feeding in both inhibited and control B. calamita tadpoles was similar, as was the quantity of food consumed. The relative proportion of food consumed in relation to the tadpoles mass was, however, greater in the inhibited tadpoles. The inhibited tadpoles have lower metabolic rates than the controls. This should result in a lower demand for food but they are consuming proportionately greater amounts.

This implies that the inhibitory mechanism may be parasitic, within the tadpole digestive tract. Richards (1958, 1962) described an endocommensal cell, perhaps a protozoan, as the inhibitor in R. pipiens tadpoles. Other workers have also identified single cell organisms as either inhibitory parasites or carriers for inhibitory chemicals in tadpoles (Akin, 1966; Licht, 1967). Organisms obtaining nutrients from

the tadpoles' ingested food material would result in the lowering of the nutrients available to the tadpole necessitating increased consumption. Lack of sufficient assimilated food could result in the retardation of tadpole development and growth.

The inhibition of B. calamita tadpoles by B. bufo does not appear to suppress their behaviour. Their metabolic rates are depressed as well as reductions in growth and development. The inhibition effect is not removed by the addition of L-thyroxine. The reduced metabolic rates observed are probably not, therefore, a result of thyroid suppression.

It is possible that the inhibition is a result of a parasite. This would account for increased food consumption without subsequent increase in growth or development. If the inhibition was a specific chemical reducing the assimilation of food, perhaps by interfering with enzyme pathways , a similar result would be observed. The inhibition may be a result of a combination of parasite and specific chemicals.

CHAPTER 8

8.0 GENERAL DISCUSSION

8.1 Thermal physiology

The present study has shown that the thermal physiologies of B. bufo and B. calamita tadpoles are distinct. Temperature tolerance, growth and developmental rates and metabolic rates of both species differ across the temperature range 5-30°C.

The upper temperature tolerance of B. calamita spawn was greater than that of B. bufo. B. calamita spawn development occurred up to 30°C, a temperature that resulted in the mortality of B. bufo spawn. The higher temperature tolerance of spawn would allow its survival in the shallow open water bodies B. calamita prefers for breeding. In those situations water temperature may reach 30°C (Beebee, 1983).

At temperatures below 20°C B. bufo spawn develops at a faster rate than B. calamita. B. bufo tadpoles have higher developmental rates below 15°C. This may explain why B. bufo can breed earlier in the year. At temperatures above 15°C B. calamita developmental rates are greater than B. bufo. This affords them an increased chance of survival in ephemeral ponds, where they typically breed. B. bufo tends to breed in deeper, permanent ponds. Although fast development may reduce mortality due to desiccation, the greater developmental rates in B. calamita have a cost. Generally, their tadpoles are smaller than

those of B. bufo reared at equivalent temperatures, the effect being increased at higher temperatures. Tadpole mass is important in governing the size of the resulting toadlet. Energy is required for the tadpole to toadlet transformation. During metamorphosis tadpoles do not feed. Energy comes from the premetamorphic tadpole, this results is a reduction in mass over metamorphosis.

Tadpoles reared at higher temperatures have lower masses than tadpoles reared at lower temperatures. The premetamorphic mass, and energy content of B. bufo tadpoles was inversely related to the rearing temperature. Toadlet masses remained constant across the temperature range. It would appear that a more efficient metamorphosis compensates for a reduced tadpole mass at higher environmental temperatures. This compensation would probably be of benefit to B. calamita. However, unlike B. bufo, B. calamita toadlet mass decreased with temperature but there was no compensation in terms of mass. This was offset by increased metamorphic efficiency, in terms of energy transfer, which increased at higher temperatures. As a result of this, toadlets were smaller, but had an equivalent energy content to B. bufo toadlets reared under similar conditions.

The faster developmental rates in B. calamita tadpoles, increasing the chance of survival in an ephemeral environment, result in relatively small toadlets. B. calamita appears to compensate for this by having increased metamorphic efficiency in energy terms.

Rates of growth and development can be related to metabolism. In the

temperature range 15-30°C, B. calamita tadpoles had higher metabolic rates and faster development than B. bufo. Increased metabolic rates and developmental rates utilise energy that could support growth. Thus, tadpole growth in B. calamita compared with B. bufo and at higher temperatures, is reduced.

B. bufo and B. calamita tadpoles have preferred body temperatures of 25 and 24°C respectively (Davis, 1985). The tadpoles appear to select a temperature, in a thermally heterogeneous environment, that results in a fast developmental rate with minimal expense of tadpole mass. Both species' metamorphic efficiencies were greatest at 25°C, with no significant energy loss during metamorphosis. Metabolic rate increased from 15-25°C. Below 25°C metabolic rates can be related to tadpole growth and development. At 30°C there did not appear to be a relationship. 25°C is possibly the highest temperature at which growth and development, as governed by metabolic rate, can proceed normally.

The relationship between tadpole development and thyroid hormones has been well documented (see section 5.1). Differences in B. bufo and B. calamita thyroid physiology may account for distinctions in their response to differences in environmental temperatures. B. bufo and B. calamita body tissues are unable to respond to L-thyroxine at low temperatures. This might explain the inability of both species to develop to metamorphosis at 5 and 10°C. At higher temperatures, the general response of the tadpoles to L-thyroxine was increased rates of development and decreased growth. Higher concentrations of L-thyroxine produced a greater effect. When the concentration was too great

development became disorganised, resulting in tadpole mortality.

B. bufo tadpoles treated with L-thyroxine exhibited accelerated development and subsequent reduction in growth. A similar effect was observed in B. calamita tadpoles in response to increasing temperature. This suggests that in B. calamita tadpoles greater L-thyroxine production or utilisation may be an important factor in its rapid developmental rates.

The thermal physiologies of B. bufo and B. calamita can be related to their preferred breeding environments. B. calamita typically breed in shallow pools. These are warmer during the breeding season, than deeper, more permanent ponds. They breed later in the year than B. bufo, with a protracted spawning period that may last until the early summer (Beebee, 1983). The spawn and tadpoles are likely to encounter higher environmental temperatures. Thus, their ability to survive at higher temperatures is an advantage to B. calamita.

The shallow ponds preferred by B. calamita are generally ephemeral. B. calamita is at an advantage over B. bufo in this environment. Its higher metabolism and greater growth and developmental rates at temperatures above 15°C allow it to reach metamorphosis in a shorter time period than B. bufo. B. calamita would, therefore be less likely to suffer mortality through desiccation. B. bufo tadpoles can afford to have longer developmental times as they spawn earlier in the year and generally use permanent water bodies.

A consequence of the faster metabolism and increased developmental rate in B. calamita is smaller tadpoles and toadlets than B. bufo. B. calamita appears to compensate for this with increased metamorphic efficiency in energetic terms. Its smaller toadlets have comparable energy content to the larger B. bufo toadlets reared under the same conditions. This could give both species equivalent fitness in terms of toadlet survival.

8.2. Intraspecific and interspecific density effects.

Despite distinctions in the species' response to temperature, B. bufo and B. calamita may be found breeding in the same water bodies. In these situations B. bufo appears more successful (Banks and Beebee, 1988). Environmental temperature would affect base rates of tadpole development and growth. The effects of population density and competitive interactions may override these and be more important in terms of tadpole development, growth and survival.

Increase in tadpole density resulted in reduction in rates of development and growth in B. bufo and B. calamita. The species' were affected at different stages of their development.

B. bufo tadpoles responded to increased density with reduced developmental rates during the early stages. This could result in reduced population density. In the permanent ponds favoured by B. bufo (Harrison, 1985), high tadpole density would lead to longer developmental periods, increased exposure to predators and ultimately

smaller toadlets. Predation in high density populations, at an early stage, would decrease the density. This would result in a smaller population with increased rates of growth and development. Tadpoles would be larger, and metamorphose earlier. The resulting toadlets would probably be fitter, with increased chances of survival to maturity.

Unlike B. bufo, the greatest growth and development suppression in B. calamita was in the later stages of development, when reared at higher densities. This can be related to the ephemeral environments in which they prefer to spawn. An early reduction in rate of development could reduce the whole populations' chance of survival if its breeding pond dried out. Fast early development would ensure that a maximum number of tadpoles are available to undergo metamorphosis, should the breeding pool not desiccate completely. As metamorphosis is reached, at increasing density, reduction in growth and development would become more important. It would ensure the survival of some of the faster developing tadpoles, should the pool dry up completely. A fast rate of growth and development in the early stages may also reduce exposure of B.calamita tadpoles to predation.

B. bufo and B. calamita have distinct responses to increases in tadpole density. When the two species are found together B. bufo adversely affects B. calamita growth, development, metabolism and metamorphosis.

Water conditioned by B. bufo reduced growth and developmental rates in B. calamita. The conditioned water did not have a detrimental effect

on B. bufo tadpoles, growth and developmental rates were in fact increased. Timing of metamorphosis in B. calamita was also affected by conditioned water. B. bufo tadpoles appeared unaffected. This suggests that B. bufo inhibition of B. calamita is specific. The interspecific effect being greater than any intraspecific, density effects.

In their natural environment, when B. bufo and B. calamita tadpoles are sympatric, tadpoles of B. calamita are continually exposed to B. bufo tadpoles. In the laboratory, inhibition of B. calamita tadpoles was greater when tadpoles were continuously reared with B. bufo (in comparison to the use of conditioned water). This suggests that the inhibiting factor is short lived (or has reduced efficacy) away from B. bufo tadpoles.

It appears that the pattern of growth and development in inhibited B. calamita tadpoles becomes more like that of B. bufo, with development and growth suppression at early stages. B. calamita requires fast development during early tadpole stages. This may reduce predation and increase the chances of avoiding desiccation.

In the field, inhibition of B. calamita tadpoles by B. bufo results in increased B. calamita mortality (Banks and Beebee, 1988). Predators prefer smaller tadpoles (e.g. Semlitsch et al, 1988). B. calamita tadpoles that remain smaller, over a longer period of time, will be more susceptible to predation. With inhibited B. calamita tadpoles in a pond, predation of B. bufo tadpoles might be relieved. Inhibited

B. calamita tadpoles, in the laboratory, consume the same amount of food as uninhibited tadpoles. This may not occur in the field, if it does, reduced interspecific competition for food resources would only occur if inhibited tadpoles were preferentially predated.

Increased risk of desiccation in B. calamita would occur with the presence of B. bufo. The time taken for B. calamita to reach metamorphosis will increase and the pattern of metamorphosis altered. In an uninhibited population of B. calamita tadpoles a large proportion of the population reaches metamorphosis relatively quickly. The remainder reach this stage gradually.
Inhibited B. calamita tadpoles attain metamorphosis with a normal distribution. If a pool, containing inhibited B. calamita tadpoles, dries out at a time near to the onset of metamorphosis a greater proportion of the population would perish.

It would appear that B. bufo and B. calamita respond to increased density in a way that relates to their preferred breeding environments. B. bufo growth and development is reduced early in tadpole life, thus decreasing population density and allowing the remaining tadpoles to develop and grow at a greater rate. This results in toadlets of increased mass, metamorphosing at an earlier date. Predation is possibly an important factor in the regulation of population density.

B. calamita, spawning in an ephemeral environment, requires the number of tadpoles surviving to metamorphosis to be maximised. A negative response to high density, in terms of growth and development, may

ensure that a large proportion of the population reaches the later stages of development. There would be greater numbers of tadpoles available to metamorphose should the pond not dry up completely. This could compensate for years when high mortality occurs as a result of desiccation.

When B. bufo and B. calamita tadpoles are reared together, the pattern of B. calamita tadpoles development and metamorphosis becomes similar to that of B. bufo. This would probably lead to increased mortality due to predation and desiccation under field conditions. The inhibition of B. calamita tadpoles by B. bufo does not affect their feeding behaviour. Metabolic rate is depressed and growth and development rate reduced. This effect is not removed by the hormone L-thyroxine. It is possible that inhibition is caused by an endoparasite. This would account for the increased food consumption in inhibited tadpoles without subsequent increase in growth or development. The inhibitor could alternatively be a specific chemical, reducing food assimilation, perhaps by affecting enzyme pathways.

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APPENDIX I

Spawn collection dates.

Section	Species	Date of Spawn collection
2.2.1.	B. bufo	28:3:1988
	B. calamita	20:4:1988
2.2.3.	B. bufo	9:4:1986
	B. calamita	1:5:1986
3.2.1.	B. bufo	22:3:1988
	B. calamita	20:4:1988
4.2.1.	B. bufo	22:3:1988
	B. calamita	20:4:1988
5.2.1.	*B. bufo	6:4:1987
	*B. calamita	23:4:1987
	**B. bufo	22:3:1988
	**B. calamita	20:4:1988
5.2.2.	B. bufo	22:3:1988
	B. calamita	20:4:1988
6.2.1.	B. bufo	9:4:1986
	B. calamita	24:5:1986
7.2.2.	B. bufo	16:4:1986
	B. calamita	24:5:1986
7.2.3.	B. bufo	16:4:1986
	B. calamita	24:5:1986
7.2.4.	B. bufo	22:3:1988
	B. calamita	20:4:1988

* Tadpoles treated with 0.01 p.p.m. L-thyroxine.

** Tadpoles treated with 0.001 and 0.0001 p.p.m. L-thyroxine and controls.