The Complex and Dynamic Ecology of Drying Ponds: Interacting Effects of Density, Hydroperiod, and Habitat Size on the Metamorphic Traits of *Bombina orientalis* tadpoles

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This thesis is dedicated to the memory of my father,
Demosthenes Manoukis.

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ABSTRACT

Metamorphosis in larval amphibians is characterized by two important traits, length of larval period and size at metamorphosis. Their expression is widely variable and environmental factors are known to play important roles in determining them both. This study addresses the effects of three environmental factors on metamorphosis: density, pond drying (hydroperiod) and habitat size. The view that the effects of hydroperiod may be mediated by associated change in one or many environmental variables, particularly density, was specifically tested under the controlled conditions of the laboratory. Three separate experiments show that the effects of both hydroperiod and habitat size are likely to be dependent on associated change in other important environmental variables. Density showed no effect on time to or size at metamorphosis when allowed to increase with drying ($p = 0.470$ and 0.503, respectively). Drying itself also showed no effect with controlled density (experiment III, $p = 0.120$ and 0.153 on time and mass at metamorphosis), and its significance in experiment I is argued to be due to behavioral competition for food. The number of tadpoles in a tank is argued to affect competition, where tanks with more animals were consistently shown to be inferior environments. The importance of the number of individuals in each tank explains many of the results such as the strong responses to the density treatments (experiment III, $p < 0.0001$ for both mass and time) where it was confounded with higher densities. The hydroperiod treatment showed a tendency to produce larger metamorphs earlier under the drying regime with the removal of tadpoles. This result is consistent with the view that decreased numbers of tadpoles due to their removal created a superior environment, even though density did not change. In addition, the non-significance of habitat size on time or mass at metamorphosis in experiment I ($p = 0.212$ and 0.540 for analysis of means) is also likely to be due to the similarity in number of tadpoles across that treatment. Another intriguing result was the relationship between time and mass at metamorphosis in experiment I. Non-linearity, observed in tanks with slowly decreasing numbers of animals over a long (40 day) metamorphic period provides a good example of how the dynamic ecology of the tadpole's aquatic habitat may cause highly complex patterns of metamorphic expression, even within the laboratory. The action of all three variables was altered by the controlled conditions of the experiment, showing that without an ecological context their effects are almost unrecognizable. Despite this fact, the observations collected here can be interpreted in the context of environmental maintenance of plasticity, particularly adaptive plasticity.

INTRODUCTION

Science is the knowledge of consequences, and dependence of one fact upon another.
Thomas Hobbes. *Leviathan*, pt. 1, ch. 5 (1651).

Metamorphosis is the defining moment of a complex life cycle. In amphibians, the sudden shift from larvae to adult signals an irreversible change to a radically different life style. This transition is a dramatic aspect of the life cycle of species as diverse as amphibians and insects, yet the reason for its existence is still elusive. Metamorphosis represents a complex ecological situation which holds strong evolutionary implications. In order to understand the facts that account for the evolution of metamorphosis, the mechanisms that determine the process must be explored first. An ecological approach is particularly useful because it is from that vantage point that the selective forces and other factors such as the evolution of plasticity in complex life cycles will be revealed.

Though still incomplete, the body of work in this area is massive. Many studies attempt to define the pattern of metamorphic activity based on how environmental conditions play a role. This thesis seeks to expand this body of work by analyzing the effects of three environmental variables, density, pond ephemerality (hydroperiod) and habitat size, on the metamorphic character of amphibian larvae under the controlled conditions of the laboratory. Density is understood to be the measure of the number of individuals per unit volume of water. Hydroperiod refers to the permanence of an aquatic habitat in the face of evaporation. Habitat size is the amount of space available to the population, usually including features like submerged surface area and perimeter. The term "metamorphic character" of a population here will refer specifically to the observed lengths of the larval period and sizes at metamorphosis. Ultimately, this labor seeks to inform a complex and fascinating evolutionary debate over the development of metamorphic traits. Understanding the concept of an ecological niche is a first step to describing the importance of metamorphosis.

A total niche shift is often observed during metamorphosis (Wilbur, 1980), in which many aspects of an animal's mode of life are changed. Niche refers to a theoretical volume occupied by an individual, population or species in multi-dimensional space (see Futuyma, 1986). The innumerable dimensions of that space represent the infinite number of environmental gradients relevant to that species' fitness. Any individual or group of animals is able to exist and perform in a limited space of any single dimension. That area defines the species' limits along that gradient. The volume defined by the limits along each gradient is the species' niche. The niche of a pre-metamorphic frog larvae (tadpole) is

therefore very different from that occupied by the adult of the species, since it goes from being a generally omnivorous and aquatic tadpole to a generally carnivorous, tetrapodal and terrestrial adult frog. It is possible that the niche of two species of frog in the larval stage may be more similar than the niches occupied by the two species once they are adults (Wilbur, 1980). In such a case natural selection and other evolutionary processes may act in differential ways at each phase, such that competition may be important in the larval phase only, due to similar niches, leading to the regulation of the populations mainly in that part of the life cycle. The dynamic nature of the relationship between the two phases on each other as well as the effect of regulation at either or both is therefore a fertile area of evolutionary debate which may be further informed by an understanding of the ecology of metamorphosis.

Biotic and Abiotic Determinants of Amphibian Metamorphosis

The realization that metamorphosis may be determined by a number of factors, environmental and genetic, holds great importance because it is the investigation of a few environmental components that is at the heart of this work. Of the whole variety of fields relevant to metamorphosis, from molecular and developmental mechanisms to population genetic models, this section will examine environmental and ecological issues in particular. The environmental factors may be divided into two classes: biotic and abiotic.

This distinction is an ubiquitous and useful one in ecological circles. Biotic factors are generally understood to be those mediated by biological entities. This generally refers to the effect of the surrounding organisms of the same or different species on the individual. Abiotic factors, however, refer to conditions that are not affected by biological organisms but have an effect on the organism's activity. They are factors that are independent of biological events, like the amount of rainfall during a particular year. Density falls clearly into the biotic category. Pond ephemerality (hydroperiod) is argued to have aspects that straddle the distinction, despite the fact that it is abiotically determined. The third factor, habitat size, may be considered to be abiotic, but like hydroperiod it may interact with biotic aspects of the environment.

Many biotic factors have been singled out for study. These include competition (Travis, 1980 and 1984), predation (Wassersug and Sperry, 1977; Morin, 1983) and food levels (Leips and Travis, 1994; Hensley, 1993; Parichy and Kaplan, 1992a). Perhaps the quintessential biotic factor is density, often invoked as the main explanatory element in population-level models, where the number of individuals taxing a particular resource determines the rate of numerical growth of the population. Such models are generally

known as density-dependent. Abiotic factors may be narrowly understood as being density independent.

Density was an experimental factor in this study, not only because of its preeminence in the literature as a metamorphic determinant but also because it may interact with other environmental variables. One of these is hydroperiod, the second experimental factor to be discussed. The issues of interaction will be addressed below, but the main effects of density and hydroperiod alone will be discussed first.

There are many mechanisms described as being responsible for the effects of density. There is some evidence that chemical interference may be a major contributor , such as waste product accumulation (e.g., Schmuck et al, 1994) or that there may be a growth inhibitory cell in tadpole fecal matter (see Licht, 1967). It has also been argued that there may be a behavioral reasons for density effects, involving competition and interference between individuals (John and Fenster, 1974). All these are explanations for how populations of tadpoles may experience crowding and environmental stress. Whatever the mechanism, it is clear from many studies that the crowding stress in a pond should be highly related to density by volume. A change in volume, if it affects density, may have the potential to increase the density stress suffered by a population.

The resulting effects of density are fairly well understood. It has been shown that the size at metamorphosis decreases while the length of larval period increases with increasing density (Sokol, 1984; Dash and Hota, 1980; Semlitsch and Caldwell, 1982). The authors mentioned above conclude that these results on metamorphosis may be a function of the decreased growth rates associated with higher density. The Wilbur-Collins model for amphibian metamorphosis, described in detail below, holds growth rates to be a central determinant of metamorphosis. Therefore the density of a pond is expected to have a strong effect on metamorphic characteristics.

The length of time a pond exists may be affected by various factors, such as temperature, wind and rainfall. The effects of the increasing harshness of a drying habitat have been extensively studied, and results generally indicate that faster drying causes earlier metamorphosis at a smaller size (e.g., Newman, 1989), but this certainly is not a hard and fast rule (see Tejedo and Reques, 1995 for an exception). Certainly many important characteristics of a pond are changed as it dries. The question of which of those aspects contribute to producing the bulk of the effects is less often addressed, and is difficult to test experimentally. The effects of hydroperiod (broadly understood to be the effect of drying on tadpole growth and development without explicit control of other environmental variables which may be changing with drying) are often investigated in large tanks in the field (e.g., Rowe and Dunson, 1995; Newman, 1989; Dash and Hota, 1980). In many

cases the effects are produced by carefully controlled rates of drying (e.g., Semlitsch and Wilbur, 1988), but drying is still confounded with many other variables, such as density and habitat size. This may explain some of the discrepant results often obtained with respect to drying, and it is hoped that the strict separation and control of these factors may allow for a reduction in the amount of confusion over the effects of drying evident in the literature.

As a pond dries, a tadpole is affected by many changing factors. The community structure of the habitat may be changed, with increasing predator density (Pearman, 1995) or decreasing food availability (Audo et al, 1995; Parichy and Kaplan, 1992b; Leips and Travis, 1994). Physical characteristics of the pond may also change in ways known to affect tadpoles. Habitat size may decrease with a drop in volume (Pearman, 1993). Temperature regimes (Tejedo and Reques, 1995) and solute concentrations (dissolved oxygen or waste products) may also be affected. It becomes evident that the drying out of the aquatic habitat has many implications in terms of both biotic and abiotic factors. This may lead to a re-casting of the role of drying not as directly affecting tadpoles but as a change in multiple characteristics of the aquatic habitat leading to an altered metamorphic response. This study concentrates on the possibility that increased density due to drying may be responsible for many effects of hydroperiod on metamorphosis.

The interaction of density and hydroperiod is interesting on two different levels. T begin with, density is known to be a determinant of environmental quality. Higher densiti usually imply lower quality environments with higher degrees of density stress. The outcome of drying may be dependent on the strain a population is experiencing, so in that sense the effects of drying may depend on initial density. Second, the density of a pond may be dependent on the amount of drying which has occurred, due to the decrease of water volume. In other words, as the pool dries and the number of tadpoles in it remains constant, the density increases and may become a more important factor. This allows for the interpretation that the effects of hydroperiod are being mediated by an increase in density, as hypothesized by Semlitsch (1987).

The size of the habitat encompasses features like surface area with the air, submerged surface area ("hard surface") and the perimeter of the pond. These parameters may be affected by the drying out of a pond in much the same way as density, since the lowering water level may make parts of the habitat unavailable to aquatic inhabitants and change the amount of surface area, perimeter or air-water surface available to each individual. Habitat size has been a potentially important ecological variable since it has be shown to be of relevance to environmental quality. This allowed for the development of models which held the size and shape of ponds to represent heterogeneous conditions

(Pearman and Wilbur, 1990). Two ponds which may be equivalent in volume and density may affect tadpoles' growth and development in different ways due to variation in shape. This sort of effect had been observed in the past and had been interpreted largely on the basis of behavioral effects of shape. John and Fenster (1974) investigated the effect of entering partitions into a habitat and argued that the effects being observed were related to the behavioral interactions of tadpoles. This kind of micro-habitat heterogeneity is ecologically and evolutionary important, contributing, amongst other things, to the maintenance of variation in metamorphic traits. These conclusions will be re-visited below.

Pearman (1993) tested the ecological importance of habitat size by manipulating pond surface area and depth while maintaining a single density by volume. He found that *Bufo americanus* tadpoles were negatively affected by decreased interior-to-edge habitat ratios. The results for *Rana clamitans* tadpoles, however, were mixed. Pearman suggests that this may be due to differences in habitat utilization by the two species, where perimeter to volume ratio (PVR) may be more important to *Bufo* tadpoles due to their particular ecology. Qualitative observations indicated that *Bufo* larvae spend most time along the perimeter of the pond, perhaps because of the food sources that tend to aggregate there in the wild. *Rana*, on the other hand may not experience density stress in this way. It is suggested that they may be utilizing food resources in the water column, so the volume of water available to each individual is important to them. This interpretation is supported by other workers' conjectures about "niche-partitioning" in free feeding larval amphibians (Wilbur, 1980) in which inter-specific competition (competition between species) may be minimized by utilization of different microhabitats.

The density and shape of the aquatic habitat is therefore known to be of importance to the growth and development of larval amphibians. As mentioned, this study seeks to address these main effects and to probe their interaction with drying. The experiment was carried out in the laboratory, because testing how the effects of hydroperiod may be mediated by other variables such as density and habitat size can be most accurately addressed under carefully controlled parameters. In small tanks density can be controlled and responses of the tadpoles closely monitored. Using small and large tanks with equivalent volume may simulate important aspects of habitat size. There are difficulties , however, since the simplification of such a complex ecological system into a lab experiment may render the results meaningless in terms of hydroperiod effects as seen in the wild. If the lab situation proves to be reasonably faithful to field conditions, then the argument that the response to drying may depend on the situation- specific context rather than on the consistent action of a single variable, "drying" stands to gain some force.

Our understanding of the field situation can therefore be expanded greatly by an effort to control the many variables that have metamorphic effects and try to single just a few of them out at once. Still, the true characteristics of the small depressions on a rock face in Korea, the natural environment of *Bombina orientalis* (see Experimental Methods), must always be at the forefront of any effort. These pools represent a highly dynamic system. Environmental variables have effects on tadpoles, but this is also true of the animals themselves since a large part of a tadpole's immediate environment will be determined by biotic effects. The pattern of metamorphic activity may be affected by environmental factors, causing faster rates of metamorphosis in the population for example This may in turn have the effect of decreasing density, so that the system becomes one of dynamic feed-back. This is a key concept in coming to terms with the perplexing variation in responses to hydroperiod and other variables in the real world.

Ecological and Evolutionary Aspects of Amphibian Metamorphosis

The preceding section describes in some detail particular environmental factors which may have direct control over the pattern of metamorphic activity of amphibian larvae Density, hydroperiod and habitat size were all argued to be potentially important environmental variables affecting metamorphic character. This section gives attention to particular aspects of metamorphic characteristics and the reasons why they are of interest.

Two metamorphic traits were observed in response to environmental variation in density, hydroperiod and habitat size: time to metamorphosis (length of larval period) and size at metamorphosis. The time to metamorphosis is used as an indicator of the developmental rate of a tadpole, referred to as a measure of the "change of form or developmental stage with respect to time" by Alford and Harris (1988). Size at metamorphosis is taken to reflect the growth rate of the individual, the change in body size over time. These metamorphic characters display a wide degree of variability in the wild and in the lab (Wilbur and Collins, 1973) and have an extremely complex relationship to each other, mediated by the exact environmental and genetic context of the population. Researchers have shown that there is a sizable amount of evolutionary significance to measuring these variables.

The length of larval period as well as the size at metamorphosis are known to have strong effects on adult fitness. A tadpole's ability to compete intra and interspecifically (Travis, 1979 and 1984), resist predation (Wassersug and Sperry, 1977; Huey, 1980), an escape a desiccating environment (Newman, 1989; Rowe and Dunson, 1995; Tejedo and Reques, 1994) are all affected by the expression of these two metamorphic variables. A

larger tadpole may be subject to predation by only a subset of predators relative to a smaller individual, both in the water and on land. However, it is also evident that there are problems associated with larger sizes. One disadvantage may be that it takes longer to grow to a larger metamorphic size, increasing the probability of being caught in a drying pond. Spending a longer amount of time in the water also increases the individual's exposure to aquatic predators. The number of predators in the water and on land may be important in this case. The relative qualities of the aquatic and terrestrial habitats may serve to provide differential population regulation, a theme which is elaborated further at the end of this section. A summary of the effects of environmental determinants of metamorphosis and their fitness ramifications is seen below in figure 1.1.

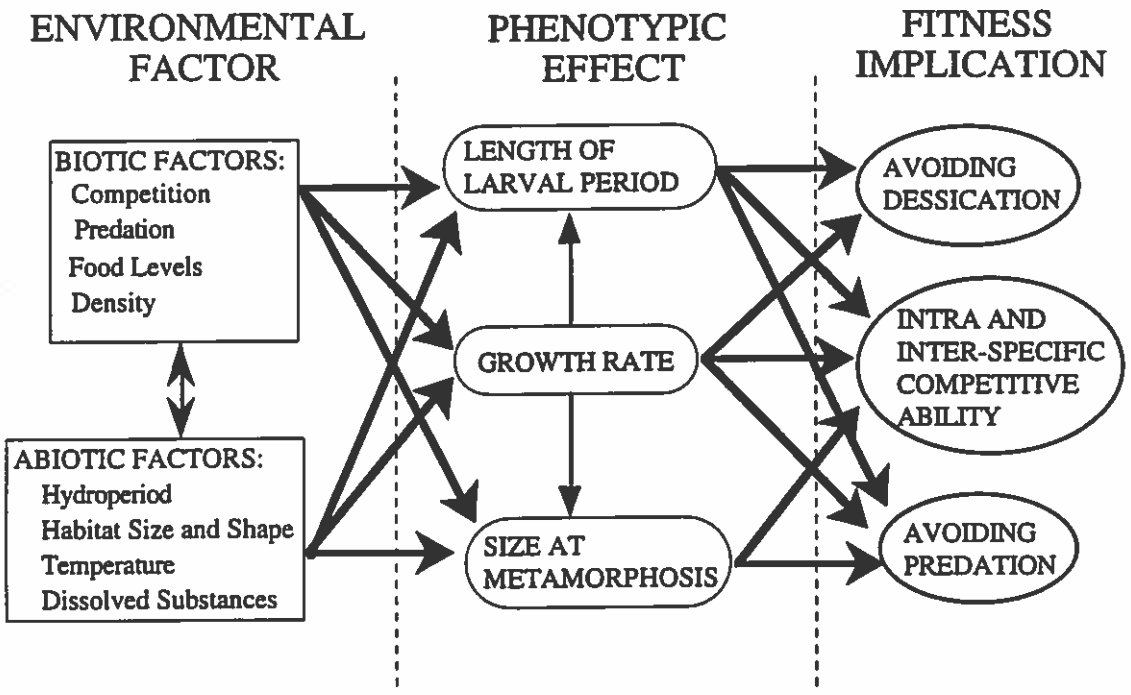

Figure 1.1: Summary of selected environmental determinants of amphibian metamorphosis and their interactions, their effects and the fitness implications of metamorphic characteristics.

To grow to the largest possible size may not be the most advantageous situation for a tadpole, especially if it takes longer to reach a large metamorphic mass. There may be a trade-off situation where tadpoles may forgo additional size for earlier times of metamorphosis. Conversely, selection may act to minimize length of larval period and maximize growth. This rationale is formalized in some optimality models (e.g., Smith and Fretwell, 1974), where calculable ideal values for fitness correlates exist. In terms of metamorphic traits, time to and size at metamorphosis would be the variables to be "balanced", limited by environmental, ecological and genetic constraints only. This might

imply that the fastest possible development to the largest size is the inevitable direction in which selective pressures act. Field observations and evolutionary theory suggest otherwise. Maintaining a wide degree of variation in metamorphic traits is the alternative to constant selection for faster development and growth. An evaluation of the processes believed to determine metamorphosis and an explanation for the observed variation is a more powerful approach to understanding the metamorphic characteristics of a population of tadpoles. The environmental maintenance of metamorphic variation is the subject of the following section.

In conclusion, metamorphic traits should be under some degree of selective pressure, however complex, which allows the evolutionary biologist to examine the relative roles of the genetic and environmental effects on size at metamorphosis and length of the larval period. Wilbur (1980) described how population dynamics may be determined by density-dependent regulation at either or both the larval and adult phases. He claims that a natural population can be placed at some location on a continuum of regulation by the two modes. Such a careful description of the population ecology of larval amphibians is argued to yield insights into the evolution of complex life cycles (life cycles with an abrupt metamorphic change). Wilbur makes the argument that the length of time spent in the aquatic phase must be selected to maximize an individual's fitness. Fully understanding the mechanisms that determine metamorphic characteristics then may have the power to explain the appearance and maintenance of complex life cycles.

Maintenance of Metamorphic Variation

Maintenance of variation in metamorphosis is believed to occur by many mechanisms. This discussion centers around maintenance of variation in development due to heterogeneous environmental conditions. This is not the only issue in maintenance of variation, and brief mention of genetic factors appears throughout, but it will be the central one since environmental factors are the focus of the study.

The central concern for this study is variation due to heterogeneous habitats. Developmental plasticity in the context of this work focuses on variation in developmental phenotype within a population of tadpoles. Developmental variation exists at many more levels, even within an individual. The many forms of developmental plasticity as well as the mechanisms responsible for their existence are reviewed by Barker (1993).

Phenotypic variation may in some cases be an adaptive condition. Models built around this premise may be said to examine adaptive plasticity. Adaptive plasticity refers to the maintenance of variation as a selective trait, so that keeping a high degree of variability

increases the overall fitness of an individual's offspring. Such systems may evolve in response to heterogeneous habitats because in an uncertain environment, variation may be favored to ensure that a higher number of offspring survive to maturity than would be possible if expression of the characteristic was uniform across all individuals. There is an increasing amount of evidence that genetic control of development and the degree of variation in metamorphic traits may indeed be selective features (Sokol, 1984; Newman, 1989 and 1992; Tejedo and Reques, 1994), hence the variability in a particular trait may be the most adaptive condition (see Kaplan and Cooper, 1984). In terms of pond drying, then, it might benefit an individual to produce offspring which vary in length of larval period given uncertainty about the permanence of a pond. If the pond dries up, the early metamorphs will survive. If it lasts longer, the "later" metamorphs will have had more time in the water to grow to a larger size at metamorphosis. In support of such a possibility, researchers have shown that the degree of genetic control over metamorphosis may be variable within a population (Newman, 1992; Lande, 1982; Berven, 1987), and is believed to vary with environmental uncertainty.

When considering factors with a genetic basis such as adaptive plasticity, it is worth remembering that the relationship between genotype (genetic make-up) and environment is rarely a direct one. Interactions between environment and sibship within a population exist in terms of metamorphic determinants (see Gaynor, 1995), hence different individuals may have different responses to the same environment. Here, yet another level of explanation for variation is presented, though its effects are not the subject of this study and so are purposely minimized (see experimental methods). The complex link between growth and development must be fully addressed when considering environmental controls on metamorphosis because that relationship will mediate any effects of these factors. The Wilbur-Collins model, below, is one interpretation of how metamorphic traits may be affected by environment, mediated by a relationship between growth and development.

The maintenance of variation by heterogeneous environmental conditions is an important source of plasticity in metamorphic traits. By demonstrating particular levels of habitat variation which are possible with respect to these experimental factors, this work provides support for the adaptation of variation as a response to ecological conditions.

The Wilbur-Collins Model

The complex relationship between development and growth that determines metamorphosis in amphibians was described in the ground-breaking Wilbur-Collins (1973) model for ecological control of metamorphosis. This mathematical model explains the link

between growth rates and ontogenetic change in a limited and particular context, and describes the response of length of larval period and size at metamorphosis to ecological factors. Their work has stood for many years as the main tool for interpretation in understanding and making predictions about metamorphosis based on ecological conditions. The measurement of time to and size at metamorphosis in this study was motivated by their conceptualization of the problem and allows for a comparison to the wide body of work already existent.

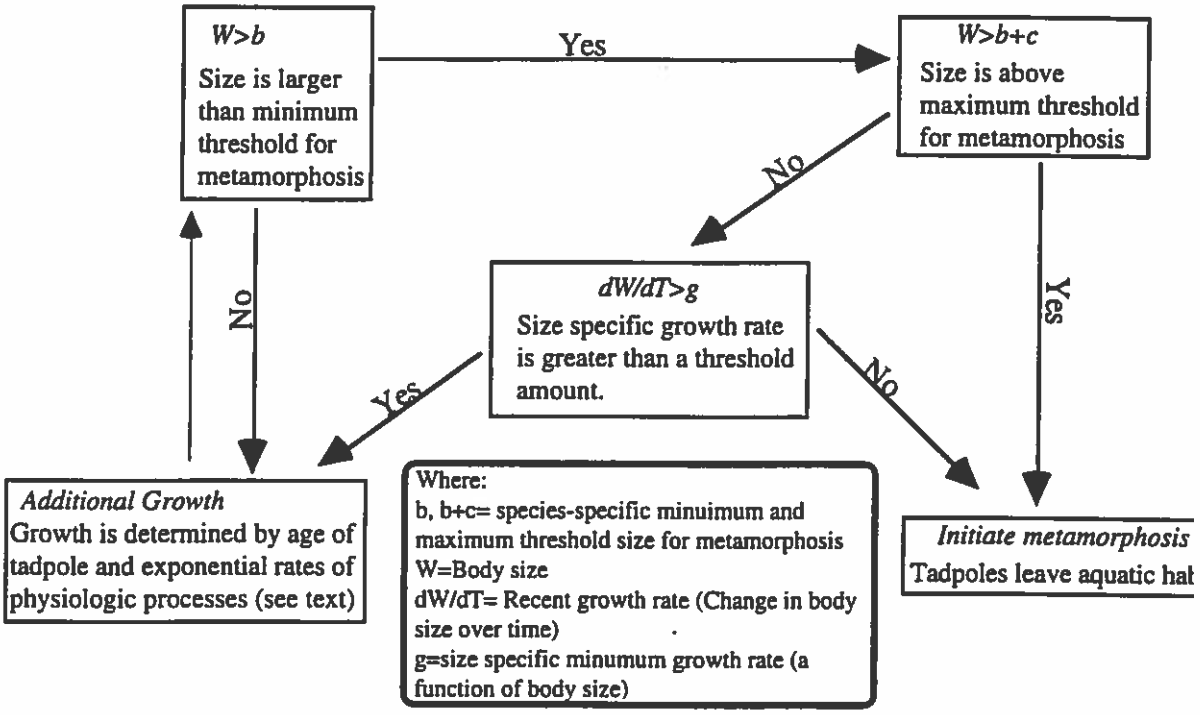

Figure 1.2: Graphical summary of the Wilbur-Collins model of ecological determinants of Amphibian metamorphosis. Several of the summary boxes above are described in greater detail in the text. (From: Wilbur and Collins, 1973)

A schematic diagram of the Wilbur-Collins model is shown in figure 1.2. A physiologically minimum threshold size is postulated (*b*) below which metamorphosis is impossible, and a maximum size (*b+c*) also theoretically exists. As seen in the figure, tadpoles will grow until minimum size is achieved. For the rest of the aquatic phase of the life cycle, the tadpole's growth will determine the exact timing of metamorphosis. The growth rate must remain above a certain amount, defined by its size, in order to continue as a tadpole. Once it drops below this size-specific rate, metamorphosis occurs. This might be taken to reflect an adaptation for larvae to respond to the quality of their aquatic habitat. If the pond is not able to support a high growth rate for the tadpole, it can respond by

on the observed metamorphic characteristics of tadpoles, supporting the theoretical model of Wilbur and Collins. They found that the timing of metamorphosis, which should be especially dependent on the rate of development, can change late in the larval period. In other words, if a tadpole's environment changes from being an initially poor one to a mucl better one metamorphosis may be put off in order to attain a larger size thus fully exploitin the aquatic habitat. Their data are argued to be evidence against another model of determination of amphibian metamorphosis (Travis, 1984) where the length of larval perio is determined very early in development then remains fixed despite any changes which might occur in the surrounding environment.

This highlights the role of growth rates as the determinant of metamorphic timing, one of the central characteristics of the Wilbur-Collins model. If this is the case, as is detailed below, then the action of any environmental factor may be understood in novel ways. A single variable such as density then may not act by affecting metamorphosis directly, it may affect tadpole growth which then in turn has some role determining metamorphic properties. The distinction is subtle, but important to an understanding of the mechanics of the timing and size at phase change.

metamorphosing and entering the terrestrial habitat in hopes of finding better conditions there.

Wilbur and Collins suggest that the maximum and minimum values also carry evolutionary significance, and that they will evolve in response to the certainty of the environment that the population experiences. A more uncertain environment will produce a greater range of variation in time to and size at metamorphosis, c . Recall the discussion of developmental plasticity as a selective trait in the previous section. The opportunity exists for canalizing selection in an environmentally homogeneous habitat. Canalization is defined as selection against more developmentally plastic genotypes and so the population tends to have less variation in metamorphic traits when the environment is predictable and stable (Kaplan and Cooper, 1984)

Ecologically, the implication of these relationships is that factors that affect the growth rate of a tadpole are likely to influence the time to and size at metamorphosis, given that the evolutionary role of the larval aquatic phase is to maximize growth and size (Wilbur, 1980). This conclusion about the role of the larval stage seems to have been especially borne out by experimental proof that both length of larval period and size at metamorphosis have significant impacts on the adult fitness of an individual (see Semlitsch et al, 1988).

Control of growth rates is more complex than only environmental factors. Wilbur and Collins suggest in their model that the physiological characteristics of the growth process occupy a central position (see box 1). As always, however, over-arching genetic issues exist even in terms of physiology, and have received much attention in the debate over control of metamorphic timing (see Semlitsch and Ryer, 1992; Travis et al, 1987 and Lande, 1982).

Alford and Harris (1988) showed that previous life history has a strong effect

Box 1: Specifying growth rate. (From: Wilbur and Collins, 1973)

Growth rate is predicted in the model as the balance of two exponential rates, an exponential growth rate [1] and an exponential dampening of this rate [2] due to developmental and physiological factors, leading to the observed sigmoid growth pattern observed in many larval amphibians:

$$dW/dt = \gamma W_t \qquad [1]$$

$$d\gamma/dt = -\alpha\gamma \qquad [2]$$

Both of these rates may be resolved into the Gompertz function [3]:

$$W_t = W_0 \exp\left[(A/\alpha)\left(1-e^{-\alpha\gamma}\right)\right] \qquad [3]$$

Where: W_t is body mass at time t , W_0 is body size at time zero, A is the value of γ , which is the exponential growth rate at t=0, α is the exponential dampening of γ and e is the base of natural logarithms.
Finally, the dependence of the growth rate on time is described by [4], giving us the following growth pattern:

$$\gamma = Ae^{-\alpha t} \qquad [4]$$

Predicting growth rates becomes even more complex than this "physiological" model, above, when environmental factors are taken to be affecting growth also.

Summary

The combined effects and often the interaction of biotic and abiotic factors are main contributors to any observed pattern of metamorphosis of a population of larvae. Factors like density (Dash and Hota, 1980; Sokol, 1984; Semlisch and Caldwell, 1982), pond size (Pearman, 1994 and 1995), food levels (Leips and Travis, 1994; Hensley, 1993; Parichy and Kaplan, 1992), sibship effects (Jazienski, 1988; Hokit and Blaustein, 1994), previous life history (Alford and Harris, 1988), and hydroperiod (Tejedo and Reques, 1994; Semlitsch, 1987) are all known to have effects on the length of the larval period, size at metamorphosis and the growth and the fitness (broadly understood as the survival) of larval anurans. Their mode of action is not only direct, but may be mediated by their effect on growth rates (Wilbur and Collins, 1973). In addition to these, genetic and physiological characteristics play important roles, but are not the focus of this study.

The evolutionary significance and interpretation of metamorphosis can be attained by measuring the impact of environmental controls on the metamorphic character of a species or population. The direct fitness effects of length of larval period and size at metamorphosis allow for the existence of selective pressures and the evolution of plasticity in those traits.

This study addresses the effect of density, hydroperiod and habitat size on metamorphic character and the mode of action of hydroperiod specifically by controlling density. It is hypothesized that the effects of drying are highly related to a change in density within the pond, therefore any factor affecting density will be relevant to metamorphic character. The effects of density may not be limited to those caused by its increase with drying, but may be involved in a dynamic loop since an increase in metamorphosis due to drying may in turn decrease the aquatic density, which will then affect the metamorphic timing of the remaining tadpoles. Habitat size is also relevant in this dynamic scenario, since the effects of density may be directly related to the way in which tadpoles are experiencing density in the pond. If the animals are utilizing the surface area of a pond most intensely and that is not affected by hydroperiod, then one might expect a different outcome of desiccation if its effects are density dependent. This work examines the dynamic and complex nature of metamorphosis in amphibian complex life-cycles.

EXPERIMENTAL METHODS

Bombina orientalis and the Reed College Colony

Bombina orientalis Boulenger (Anura: Discoglossidae) is a widely distributed species, found throughout temperate East Asia. The frogs used in this study were collected at the Samhwa-sa site in S. Korea, by Dr. Robert H. Kaplan. Since their arrival at Reed College the frogs have been kept under constant environmental conditions described below and have been fed crickets (regularly supplemented with vitamin powder).The field site consists of a cold mountain stream running over a granite rock face, in the mountains above Samhwa Temple in Kangwon province. In this locality the frogs breed in water-filled depressions in the rock (Parichy and Kaplan, 1992), but they face considerable environmental uncertainty. There is known to be much variation in the temperature regimes, ecological composition, density and volume across different ponds (Kaplan, 1989; Gaynor, 1995). Uncertainty comes in many forms, from hazards of predation by insect larvae or other anuran species' tadpoles (Kaplan, 1992) to the permanence of a particular pool. The river has been known to flood over and wash away tadpoles on occasion, but extended rainless periods may also cause total pond dissection even after the wet breeding season in late April (Kaplan, personal communication).

The adults themselves do not spend a great portion of the year in the pools, but the males aggregate there and call to attract females out of the surrounding deciduous woods. After breeding the frogs return to the woods, to be followed weeks later by any froglets that survive to the terrestrial phase of their life cycles.

B. orientalis adults from the permanent colony at Reed College were bred on the 12th of March 1996 (for experiment I) and on the 13th of September 1996 (for experiments II and III) by injection with Human Chorionic Gonadotropin (between 0.10-0.15 cc, 250-375 I.U. per individual). Almost all the same adults were used in both breedings, to minimize genetic effects. Each breeding pair was placed in a large (1 L) covered fingerbowl containing approximately 400 mL aged tap water, which was left in a cabinet overnight. The following morning the eggs were collected and placed in the common environment of a large holding tank (approx. 4 x 3 x 0.2 m) filled to an approximate 1.5 cm depth with aged tap water and filtered tap water (1:4). They were all placed into the same environment to avoid possible variation in the environmental quality of their small fingerbowls and to completely mix up the sib groups so responses to different environmental conditions could

be avoided. The experiment was set up in a temperature controlled (set to 24°C) room wit a regular photoperiod of 12L:12D.

Three days after laying most eggs were undergoing neurulation, by the ninth day most had hatched and, for experiment I, on the twelfth day they were placed in their experimental environments, already able to eat boiled spinach. For experiments II and III they were entered into the experimental tanks on the tenth day, because they were already the feeding stage at that point. At the start of experiment I, a random sample (n=12) showed that the tadpoles were between stages 23-25 (Gosner, 1960) and had an SVL between 60 and 78 mm. A similar sample of animals used in experiments II and III were a similar stages when they were randomly assigned to treatment tanks.

Experiment I

The first experiment started on the 12th of March, 1996, with the breeding of 19 pairs of *B. orientalis* and ended on the 17th of May, 1996, when almost all tadpoles had metamorphosed (see figure 2.0a). For most of the aquatic phase of their life-cycle the animals were kept in glass aquariums under one of eight combinations of the experimental variables detailed below.

As a safeguard, particularly against possible spatial variation in temperature, a blocking variable was included besides the experimental factors. Two blocks were set up, dividing the upper shelves of the experimental area into one block and the bottom ones int the other (see figure 2.0 b). This was done because temperature gradients often exist vertically, even under controlled laboratory conditions. In addition to this precaution, the temperature of the air at the four corners of the experimental area was monitored with alcohol thermometers in small conical flasks filled with water and stopped up.

Throughout the experiment the tadpoles were to be fed boiled spinach *ad libitum*. This proved rather difficult to keep up with at times, but an attempt was made to at least keep the amount of food for each animal roughly equivalent. The water in the tanks was never changed or supplemented so that solute concentrations in the tanks (which may mediate the effects of density) were not affected beyond the experimental design.

Figure 2.0 a): *Bombina orientalis* tadpoles immediately after metamorphosis during experiments II and III.

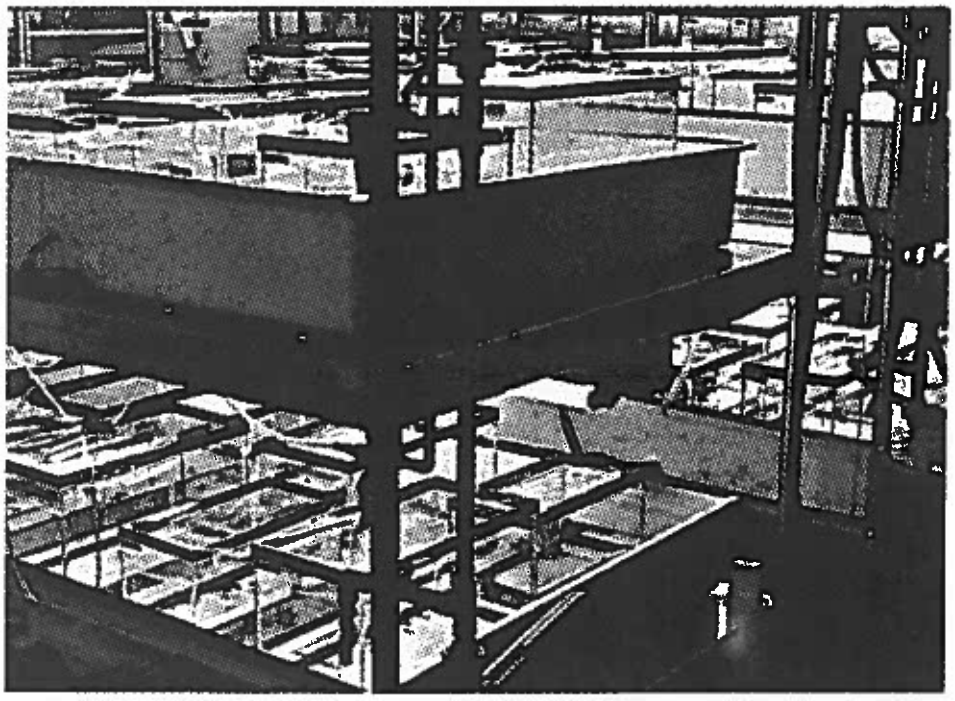

Figure 2.0 b): Physical set up of experiment I in Reed College amphibian lab. Blocks are set vertically (Block A is above, B below). For experiments II and III only the top pair of shelves were used (Block A: Background; Block B: Foreground).

Physical and Statistical Design

The statistical design of experiment I is shown in figure 2.1. Three fixed level variables with two levels each (density, hydroperiod and habitat size) were the experimental variables for this experiment. Under the density variable half the tadpoles

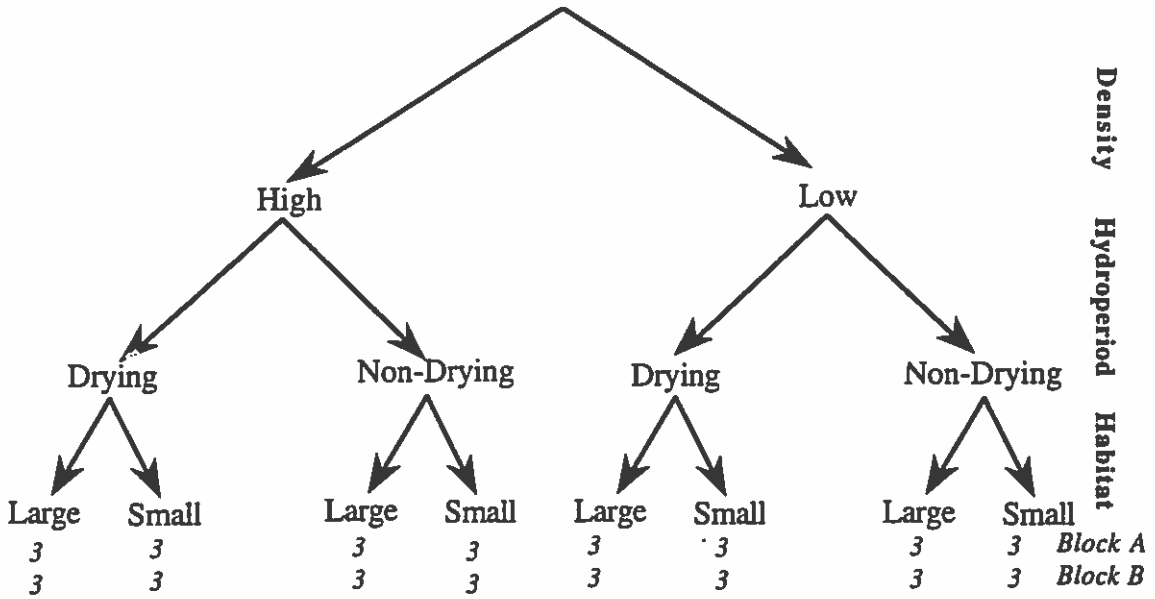

Figure 2.1: Graphical rendition of the design of experiment I. Variables and levels for each of the variables are shown on the right hand side, sample sizes (number of tanks) are indicated on the arrows and at the terminals for the treatment combinations. Water volume in drying tanks went from 8.0 to 2.0 L.

were subjected to high density (5 tadpoles/ L) and the other half to low density (2.5 tadpoles/ L) conditions. This involved initially placing 40 tadpoles in the high density tanks and 20 in the low density ones.

In terms of hydroperiod, drying (over 5 weeks) or non-drying groups were set up. The drying tanks had their absolute water volume reduced from 8 L to 2 L over a period of five weeks by removing 400 mL of water on Tuesdays and 800 mL on Fridays. These values were chosen to allow the removal of a whole number of tadpoles from both high and low density tanks and so drying could be carried out over 5 weeks. In order to maintain density by volume, 2 tadpoles per 400 mL were removed for the high density tanks and 1 tadpole per 400 mL for the low density tanks. The number of animals in the high density drying tanks went from 40 to 10 by the end of the drying, while for the low density drying tanks it went from 20 to 5 individuals. Tadpole removal was carried out by dragging a small net through those tanks and removing the first animals netted, ensuring that they were randomly chosen and no bias entered into the treatments. Loss of water to the environment

by evaporation was avoided by covering the tanks with plastic wrap and only removing this cover for brief manipulations such as feeding.

Habitat size and shape were varied to test if *Bombina* tadpoles are experiencing density by volume or if some aspect of niche subdivision plays importantly. At issue here is whether the volume of water available to each tadpole (density) is the most important indicator of crowding or if some other aspect of the aquatic environment (such as surface area under water or perimeter length) is more important. In order to address the issue, two sizes of tank were used, large (40 L capacity: W 24cm x L 49cm x H 27cm) and small (20 L capacity: W 19cm x L 39cm x H 25cm). If some other aspect of the environment besides water volume was most important to the larvae one would expect to detect a difference between these two treatments.

Within each spatial block the experimental tanks were systematically set out to maximize the distance between tanks with the same treatment. This sort of array was chosen over randomizing locations because of the small total number of replicates in each block (3) which increases the probability that two or more tanks of the same treatment will end up next to each other, thus confounding spatial and experimental effects.

The effects of the experimental treatments were recorded by measuring the SVL (Snout to Vent Length) and tail length of a random sample of five tadpoles from each tank once a week. The first five netted tadpoles were placed ventral side up on a petri dish and photographed with an identifying label and a small ruler in the frame. The resulting slide was measured using a microscope interfaced with an Apple Macintosh computer as described in Phillips and Kaplan (1987), which gave measurements to the nearest 0.01mm.

The onset of metamorphosis was defined by the emergence of at least one forelimb (Gosner stage 31; Gosner, 1960). After the first metamorphosed tadpole was found the tanks were monitored three times a day (6 h between each day check, and 12 h overnight) and any tadpoles found to be beginning metamorphosis were removed and photographed as described above (see figure 2.0b) for recent metamorphs). In addition to the length measurements at metamorphosis, each tadpole was carefully dried on a piece of damp filter paper to remove excess water then placed on a tared weighing boat on a digital balance (Mettler model PC440). The time of removal (time to metamorphosis) and mass of each tadpole were thus recorded in addition to the length data

Experiments II and III

Experiment II was designed to test how *Bombina orientalis* tadpoles under the controlled laboratory conditions described in experiment I, would respond to hydroperiod

if density was not controlled as the water volume decreased. An interaction of density with hydroperiod effect was hypothesized from the results of experiment I, so experiment III was set up to further probe the effects of density and hydroperiod on larval growth and development.

Many of the other aspects of the experiments were kept the same as experiment I: once more, tadpoles were fed boiled spinach *ad libitum*: and the temperature, photoperiod and cling wrap cover on each tank were the same.

Temperature differences across tanks were carefully monitored by using a digital thermometer to measure the water temperature in each of the 28 tanks three times over the course of the experiment. The exact density in each of the tanks was more closely followed by recording the number of tadpoles in each one any time tadpoles were removed due to metamorphosis or water removal in the drying treatment.

Physical and Statistical Design

Experiment II had tadpoles at a fixed initial density, 3.75 tadpoles/ L (n=30 tadpoles per tank) and two hydroperiod treatments (drying or non-drying), as shown in figure 2.2. In order to simulate conditions tadpoles experience in the field under a desiccation situation, density was allowed to increase as water volume decreased. This was carried out by simply not removing tadpoles as water was extracted. Experiment III had tadpoles randomly assigned to one of six cells resulting from the combination of two factors: density (three levels: 6.25, 3.75 or 1.25 tadpoles/ L) and hydroperiod (two levels: Drying or Non-Drying). The design of experiment III is seen in figure 2.3, overleaf. Note that in this experiment the density in the drying tanks was controlled by the removal of tadpoles in order to follow exactly the design of experiment I.

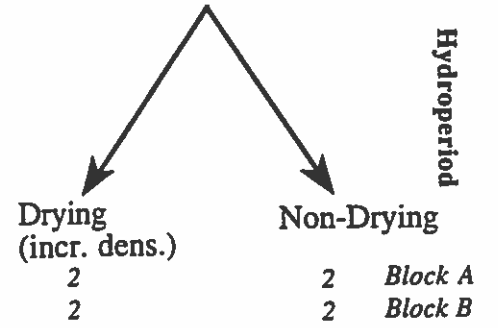

Figure 2.2: Design of experiment II. Th initial number of tadpoles in all tanks is and none are removed. Sample sizes at bottom, as fig. 2.1

Both experiments were run together, so all tanks were split among two spatial blocks in a systematic array together (see figure 2.0b). The drying tanks in both experiments had their water volume lowered from 8 L to 2.4 L over a period of 4 weeks by removing 800mL twice a week, a slightly shorter hydroperiod than the first experiment's. This change was introduced in order to attempt to complete the desiccation of the drying tanks before the first metamorphs appeared. It also allowed for extraction of a whole number of tadpoles

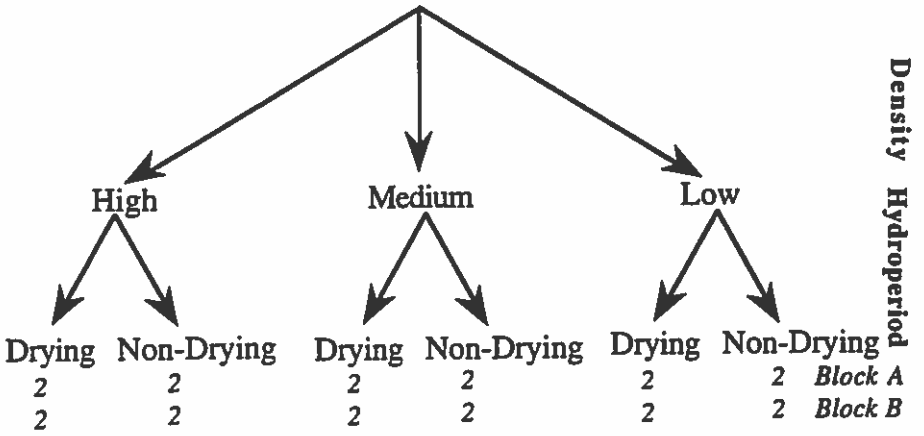

Figure 2.3: Experimental design for experiment III, testing the effects of density and hydroperiod. Fixed treatments shown at left, sample sizes at bottom.

with each water removal. Another difference to experiment I was that since habitat size was no longer an experimental variable only large (40L) tanks were used for both experiments II and III. The response variables for these two experiments were only time to and mass at metamorphosis, both recorded in the same manner as for experiment I.

It is worth noting that one of the cells in experiment III is the same as in experiment II. The medium density, non-drying cell in experiment III is equivalent to the fixed density non-drying tanks in experiment II. These were, in fact, the same tanks, but the data obtained from them was used in different analyses since they were part of both designs.

Statistical Analysis

The weekly length measurements in experiment I were analyzed using the mean snout-vent lengths (SVL) and tail lengths (TL) for each tank. The data were graphically displayed as a growth trajectory split by the most significant factors. Each week's results were also analyzed using ANOVAs for the main experimental treatments and their interactions. During week 3 measurements were only taken from tanks with 5 or more tadpoles remaining, leading to a reduction in the number of mean values during that week. This criterion was later changed, so the sample size for week 4 was 48 tank mean values again. This meant that the sample size for the size of tadpoles in some tanks was less than 5 late in the metamorphic period, but the effects of this reduction was relatively minor.

For the metamorphic data of experiment I, the body length, mass at and time to metamorphosis response variables were meant to reflect carefully controlled experimental treatments. This expectation was only partially met when tadpoles began metamorphosing

earlier than expected and the total time of metamorphic activity was longer than originally anticipated. Density decreased in the non-drying tanks as tadpoles metamorphosed while in the drying tanks it was possible to keep the actual density near the planned level by removing water but not tadpoles. This situation was extended over at least a month due to the length of the metamorphic period.

Several strategies were employed to cope with the change in density over the metamorphic period. One attempt to keep the measured variables as responses to the originally planned conditions of experiment I was to analyze a constrained dataset (the data from only the first 25% of metamorphs). In this way the metamorphic responses would be to the treatments before densities had changed and remained changed for long periods of time due to the removal of metamorphosed animals. It is important to note that the first quarter of the total observations recorded were used, not the observations collected during the first quarter of the total duration of metamorphic activity. This meant that no constraints were placed on the possible values of time to metamorphosis. The means of the first 25% of the tadpoles from each tank was quantitatively analyzed with ANOVAs.

Analyzing the means for each tank controls pseudo-replication problems, and by entering a single value for each tank it is possible to detect and control for possible confounding factors across tanks. This approach therefore benefits from avoiding a phenomenon known as pseudo-replication, where two tanks which are replicates and therefore should be equivalent in fact are not. If one of the tanks has conditions which have a systematic effect on the inhabitants and produces smaller tadpoles, for example, all of its individual products should not be directly compared to those of the other tank. By taking the means, this effect is minimized and controlled for.

There are also potential problems with examining the means only. The results show that the total variation is compressed by taking the means for each tank to such an extent as to cause distortion of the actual relationship between time to and mass at metamorphosis. Using all the values within tanks allows for the examination of the entire scope of variation (see figure 2.4, overleaf).

Fitting regression models to the metamorphic responses of each individual to the first experiment's treatments addressed the entire variation in both time and mass variables, despite potential problems of pseudo-replication. There are several advantages to using regression in this case. The exact density of tadpoles in each tank over time was inserted as a term in the model. Pseudo-replication was partially controlled though nesting and a qualitatively non-linear relationship between time to and mass at metamorphosis was also examined through the insertion and testing of various quadratic terms in the model. More details of these aspects of the regression follow.

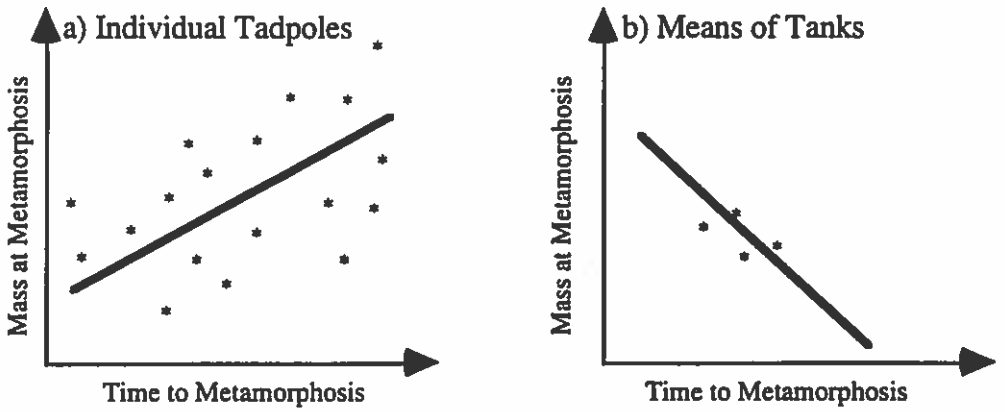

Figure 2.4: Schematic representation of the change of a positive relationship between time and mass at metamorphosis for the individual tadpoles (a)) to a negative relationship between the means for the tanks (b))

Since changes in density were considered to be of potentially great importance in the response of metamorphic variables, some attempt had to be made to exactly quantify the number of tadpoles in each tank over time. An estimate for the exact density was arrived at by calculating the absolute number of tadpoles at each time, since volume was known. Subtracting the number of metamorphs which had emerged at a given time from the total number of metamorphs recorded to have left from that tank gave the number of tadpoles estimated to be in the tank at that time. This could be divided by the known water volume to give the estimated density as shown below ([1]).

$$\text{Estimated Density at time t} = \frac{[(\text{Total Metamorphs}) - (\text{Number of Metamorphs to time t})]}{\text{Volume at time t}} \qquad [1]$$

It is important to highlight, however, that this estimated value does not include any animals that did not survive to metamorphosis- since there was no record of their leaving the tank.

The effects of tank on the relationship of time to and size at metamorphosis was minimized in the analysis of the metamorphic characteristics of individual tadpoles by nesting the "tank" variable within the "hydroperiod" categorical variable. The "tank" variable simply consisted of using each tank's unique identification number to distinguish between tanks. Nesting allowed for testing the magnitude of variation which is due to tadpoles within tanks, in addition to tadpoles within hydroperiod (Sokal and Rohlf, 1969). While not permitting a detection of correlations within tanks (i.e. longer times are associated with higher masses) it does control for variation across tanks. A degree of correlation within tanks is expected, since the metamorphosis and removal of animals

diminished the density in the tank, and density is believed to be playing a central role. In response also to pseudo-replication issues, a graphical examination of the metamorphic times and masses split by tank was utilized to give some reassurance that correlations of metamorphic characteristics within tanks are fairly consistent in all tanks.

In order to address an apparently non-linear relationship between time to and mass at metamorphosis (which is especially apparent in the non-drying/high density treatment combination) a quadratic term was considered for the model. One would expect there to be a low p-value associated with a quadratic "time" ($(time)^2$) term if the relationship between those two variables was indeed non-linear for that treatment combination. This expectation may be confounded by the entry of the continuous density variable, however. It may have been that density would "explain" the non-linearity between time and mass, thus rendering the quadratic time variable unimportant.

A note about the "time" and "density" variables used in the models is also needed. It was statistically expedient to use a centered measure for each of these for ease of comparison of the coefficients. In this way, the values of the coefficients can be compared directly without having to account for the change in overall mean values across treatments. This meant subtracting the individual value for time or density from the mean value for the whole experiment then using that result as the value for time to metamorphosis or density at any particular time. This value was squared to obtain the quadratic terms.

Adding a continuous variable for number of tadpoles was contemplated in light of subsequent findings. This would not be necessary, however, if the number of tadpoles and the density by volume in a tank are very highly correlated. This correlation analysis was performed and is reported in the results.

For experiments II and III the mean values of time to and mass at metamorphosis were used because they were more reliable and representative than those for experiment I. Also, metamorphosis occurred over a much shorter period of time, making the period of reduced control over density relatively shorter. It was therefore also sufficient to use the categorical version of density in an analysis of variance of the mean time to and mass at metamorphosis for experiment III. In analyzing the mean time to metamorphosis for experiment II, however, it was noticed that the two levels for the variable had very different amount of variation, a condition known as heteroscedasticity (Sokal and Rohlf, 1969) and a violation of the assumption of equal variance of the analysis of variance. The raw data for time to metamorphosis were considered for transformation. The variance of the mean time to metamorphosis was not large enough to cause significant change in the qualitative result of the ANOVA (A. Jones. pers. comm.), however, so the untransformed data were used.

The data obtained from all three experimental designs were analyzed using three statistical packages: Abacus' concepts Statview 4.5 and SuperAnova generated most of the graphical analyses and the ANOVA tests and S-Plus (1988, 1995 MathSoft, Inc. and AT&T) was used for the regression models and the F-test.

RESULTS

The results of the three experiments described in the experimental methods are organized below into three main sections, one for each experimental design. Analysis of the results of experiment I is further divided into pre-metamorphic growth, analysis of mean metamorphic response and an examination of the time and mass at metamorphosis for each individual tadpole. Experiment II is presented first as an analysis of the experimental treatments followed by an examination of changes in density over the experiment.

Experiment I

Experiment I measured the effects of density, hydroperiod, habitat size and their interaction on larval growth and metamorphic traits. The responses to these treatments were measured in two ways. The first was the size of tadpoles before metamorphosis, and the second was the metamorphic character (size and time) of the tadpoles. Note that for this experiment the drying regime tanks were density controlled though the removal of tadpoles with decreasing water levels. The presentation of the resulting analyses was carried out as described above.

The Effects of Experimental Variables on Larval Growth Prior to Metamorphosis

The effects of the experimental variables on the mean snout- vent length (SVL) and tail length (TL) of each tank before metamorphosis were examined using analysis of variance (ANOVA). The block variable was excluded from the analyses because it was not significant in any of its terms at any time. Temperature, the other non-experimental variable, was analyzed for all three experiments and found to not be important either (see Appendix B). ANOVAs were performed on the mean SVL and TL for each week separately, and the entire set is included in appendix D. Week 1 represents the beginning of the experiment (its measurements were collected about six days after placing of the tadpoles in the tanks). Week 1 has no significant terms, and by week 5 many metamorphs had already been observed. Density had the strongest effect, yielding significant differences in weeks 2, 3 and 4 for both SVL and TL (table 1). The agreement between the two response variables is quite good except for the significant effect of habitat size and shape on tail length in weeks 2 and 3, an effect which was not observed on SVL.

Figure 3.1 shows main effects of the experimental variables over the five weeks during which larval size was recorded. Figure 3.2 is a plot of all the second order terms in

the ANOVAs over all five weeks. The three-way interaction plot is displayed in appendix D.

Figure 3.3 shows the mean snout-vent length for the 12 tanks against time elapsed from the beginning of the experiment, with 95% confidence intervals. The SVL and TL measurements yielded qualitatively equivalent relationships (TL not shown). Examination shows a tendency for larger size of animals in the drying treatment as compared to the non drying hydroperiod being obvious only in the high density regimen. Further, in the high density condition (3.3 a) the difference between hydroperiod treatments tends to decrease over time (note the convergence of the size of drying or non-drying tadpoles by week 5, reflected in ANOVA for SVL for week 5).

Figure 3.3 also reveals that animals under the low density treatment grew to a large size more quickly, while their high density counterparts grew more slowly to approximately the same level (at around 14.5- 15.0 mm SVL).

Table 1: ANOVAs for effects of experimental treatments and interactions on SVL and TL of larvae in experiment I. For the entire 5 weeks, refer to appendix D.

		Response: SVL				***Response: TL***			
WEEK 2- 324hrs									
Source	**df**	**S.S.**	**M.S.**	**F**	**P**	**S.S.**	**M.S.**	**F**	**P**
Density (D)	1	15.132	15.132	22.174	0.0001	19.258	19.258	11.459	0.0016
Hydroperiod (HY)	1	1.266	1.266	1.854	0.1809	2.893	2.893	1.721	0.1970
Habitat Size (HSS)	1	1.314	1.314	1.926	0.1729	6.668	6.668	3.968	0.0532
D* HY	1	0.320	0.320	0.468	0.4978	0.066	0.066	0.039	0.8444
D * HSS	1	1.002	1.002	1.468	0.2328	0.159	0.159	0.095	0.7601
HY * HSS	1	0.312	0.312	0.457	0.5032	0.036	0.036	0.021	0.8849
D* HY * HSS	1	0.023	0.023	0.034	0.8555	0.200	0.200	0.119	0.7319
Residual	40	27.298	0.682			67.225	1.681		
TOTAL	*48*	*46.667*		R^2	= 0.415	*96.505*		R^2	= 0.303
WEEK 3- 492hrs									
Source	**df**	**S.S.**	**M.S.**	**F**	**P**	**S.S.**	**M.S.**	**F**	**P**
Density (D)	1	3.500	3.500	11.860	0.0021	5.258	5.258	6.717	0.0160
Hydroperiod (HY)	1	0.466	0.466	1.579	0.2210	0.824	0.824	1.052	0.3152
Habitat Size (HSS)	1	0.086	0.086	0.292	0.5939	3.421	3.421	4.370	0.0474
D* HY	1	0.166	0.166	0.563	0.4602	0.079	.079	0.102	0.7528
D * HSS	1	0.056	0.056	0.190	0.6670	0.306	.306	0.391	0.5379
HY * HSS	1	1.4E-5	1.4E-5	4.6E-5	0.9946	1.1E-4	1.1E-4	1.4E-4	0.9907
D* HY * HSS	1	0.122	0.122	0.414	0.5263	0.262	0.262	0.335	0.5681
Residual	24	7.083	0.295			18.788	0.783		
TOTAL	*31*	*11.479*		R^2	= 0.388	*28.938*		R^2	= 0.363
WEEK 4- 660hrs									
Source	**df**	**S.S.**	**M.S.**	**F**	**P**	**S.S.**	**M.S.**	**F**	**P**
Density (D)	1	6.145	6.145	6.234	0.0167	2.788	2.788	0.887	0.3520
Hydroperiod (HY)	1	1.629	1.629	1.652	0.2060	0.108	0.108	0.034	0.8542
Habitat Size (HSS)	1	0.407	0.407	0.413	0.5242	6.160	6.160	1.959	0.1693
D* HY	1	1.702	1.702	1.727	0.1963	0.031	0.031	0.010	0.9208
D * HSS	1	0.316	0.316	0.321	0.5743	0.006	0.006	0.002	0.9651
HY * HSS	1	0.021	0.021	0.021	0.8860	0.523	0.523	0.167	0.6854
D* HY * HSS	1	0.097	0.097	0.098	0.7557	0.756	0.756	0.241	0.6264
Residual	40	39.431	0.986			125.74	3.144		
TOTAL	*48*	*49.748*		R^2	= 0.207	*136.12*		R^2	= 0.363

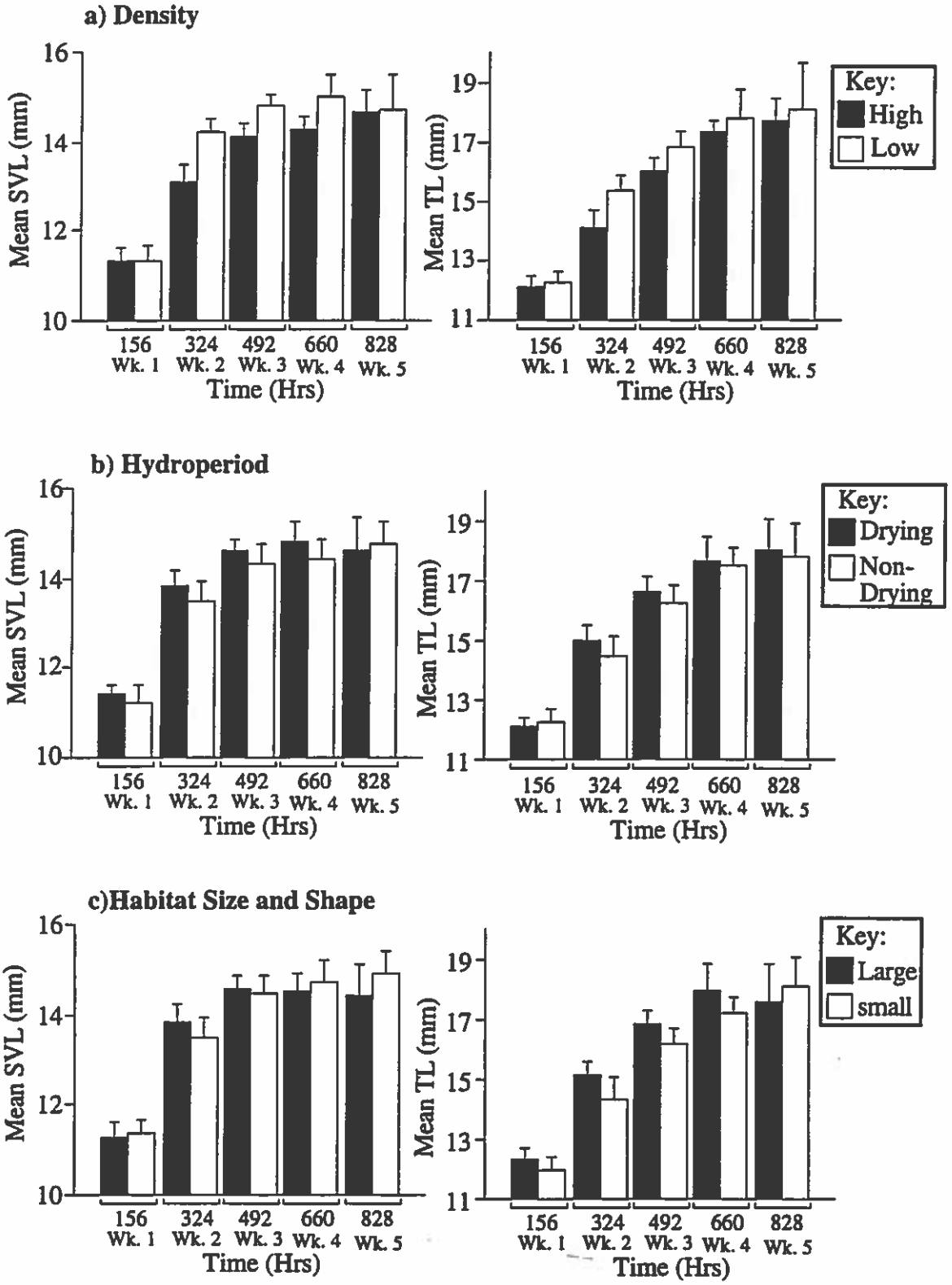

Figure 3.1: Plots of main effects of experimental variables on snout-vent (SVL) and tail (TL) lengths during experiment I. a) density, b) hydroperiod and c) habitat size and shape.

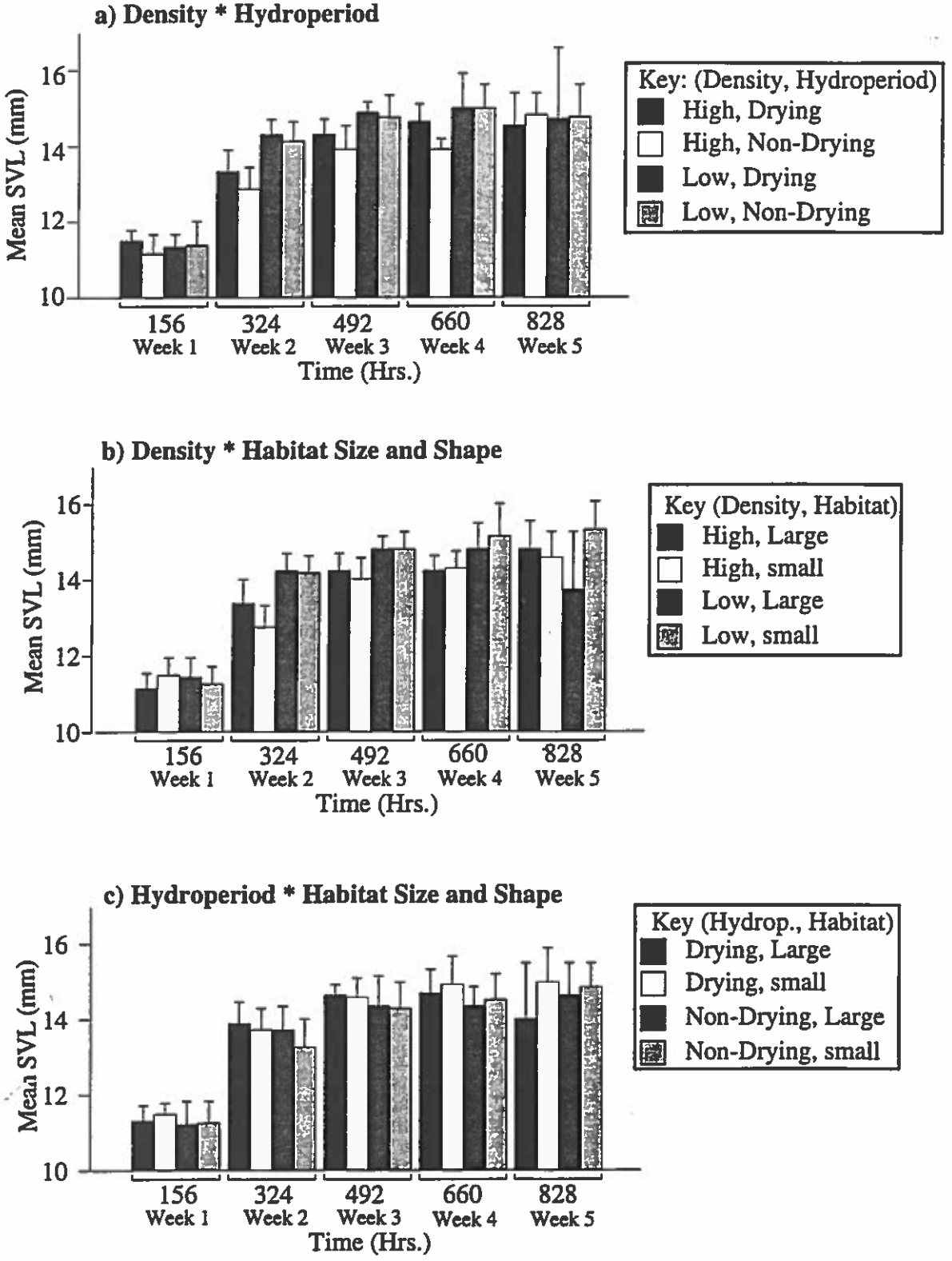

Figure 3.2a: Second order interactions of the data for mean snout-vent length of larvae over time in experiment I. a) Density*Hydroperiod, b) Density*Habitat Size and Shape and c) Hydroperiod*Habitat Size and Shape.

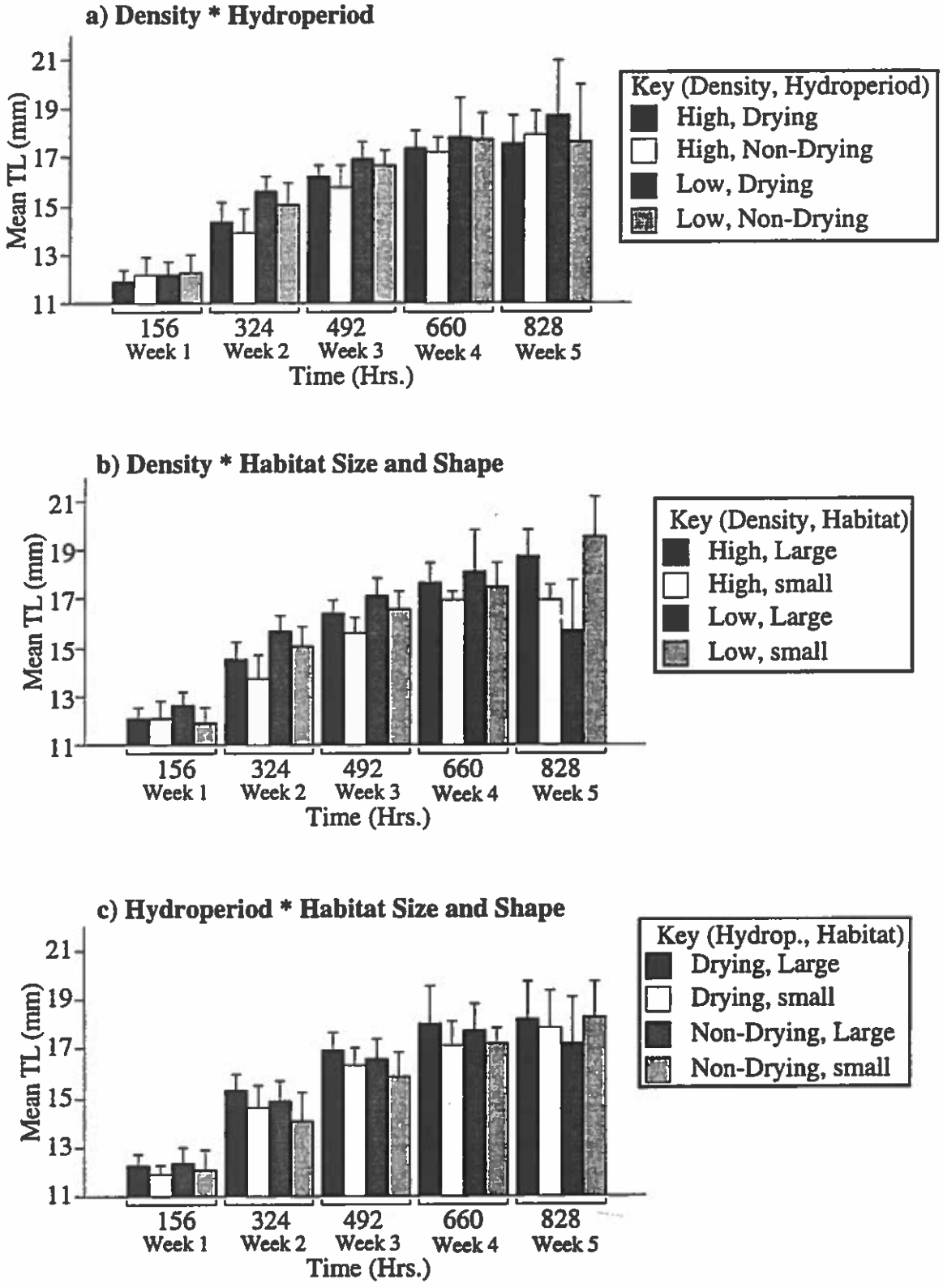

Figure 3.2b: Second order interactions of the data for mean tail length of larvae over time in experiment I. a) Density*Hydroperiod, b) Density*Habitat Size and Shape and c) Hydroperiod*Habitat Size and Shape.

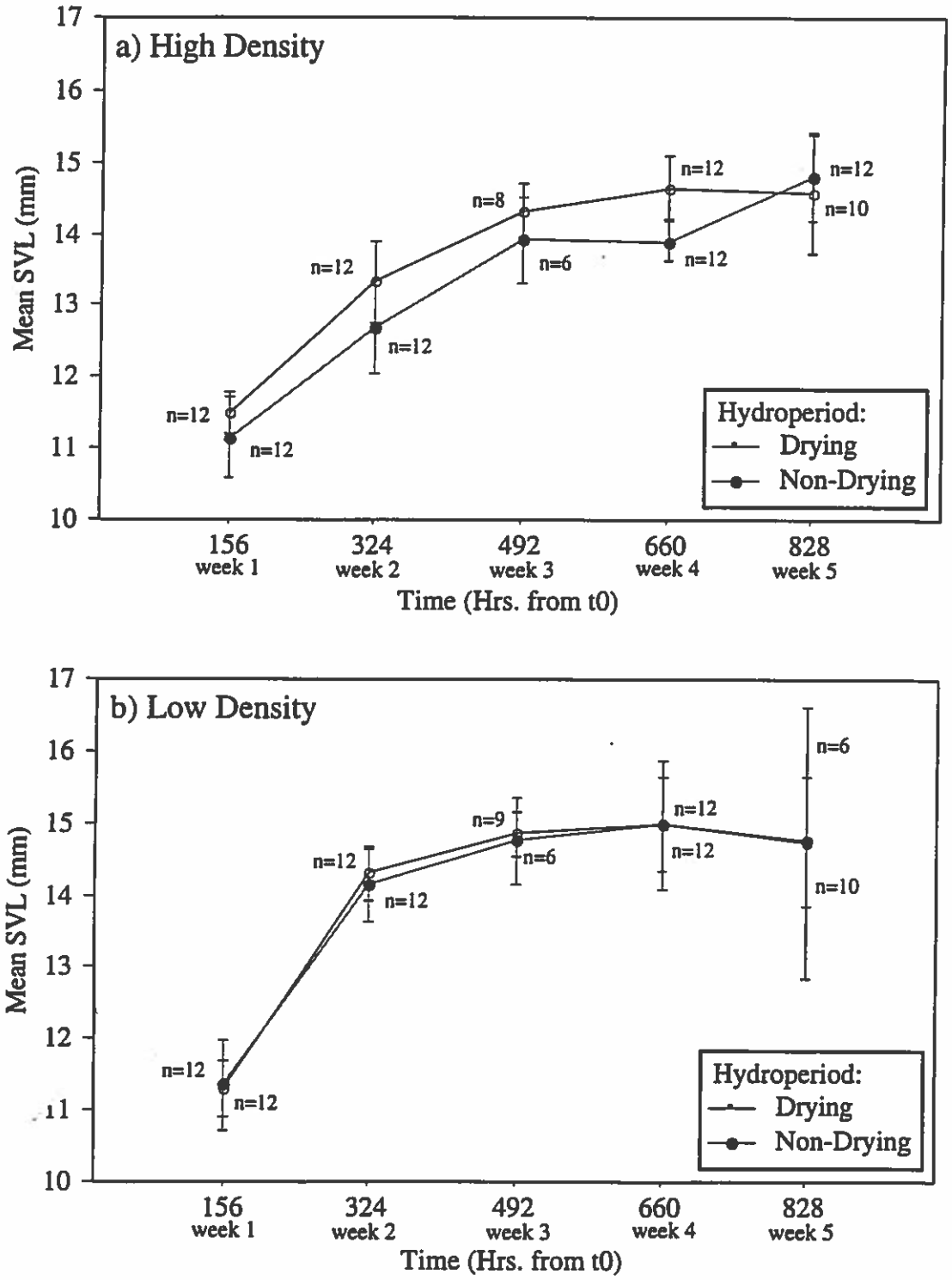

Figure 3.3: Mean SVL of tadpoles over time. Error bars indicate 95% confidence intervals. a) High density treatment tanks, split by hydroperiod. b) low density tanks, split by hydroperiod.

Mean Metamorphic Response to Experimental Variables

Mass at metamorphosis was chosen as a general measure of size because it held a direct and predictable relationship to all the measurements of size at metamorphosis. This approach is supported by figure 3.4, where the log transformed length measurements (SVL, tail and total lengths) are plotted against the log of mass at metamorphosis. They are closely related ($r = 0.787$ for log-Mass and log-SVL; $r = 0.647$ for log-Mass and log-TL; $r = 0.758$ for log-Mass and log-Total Length). Because of the closeness of the relationships between mass at metamorphosis and the other length variables at metamorphosis, mass is used as the sole measurement for "size" in all subsequent analyses.

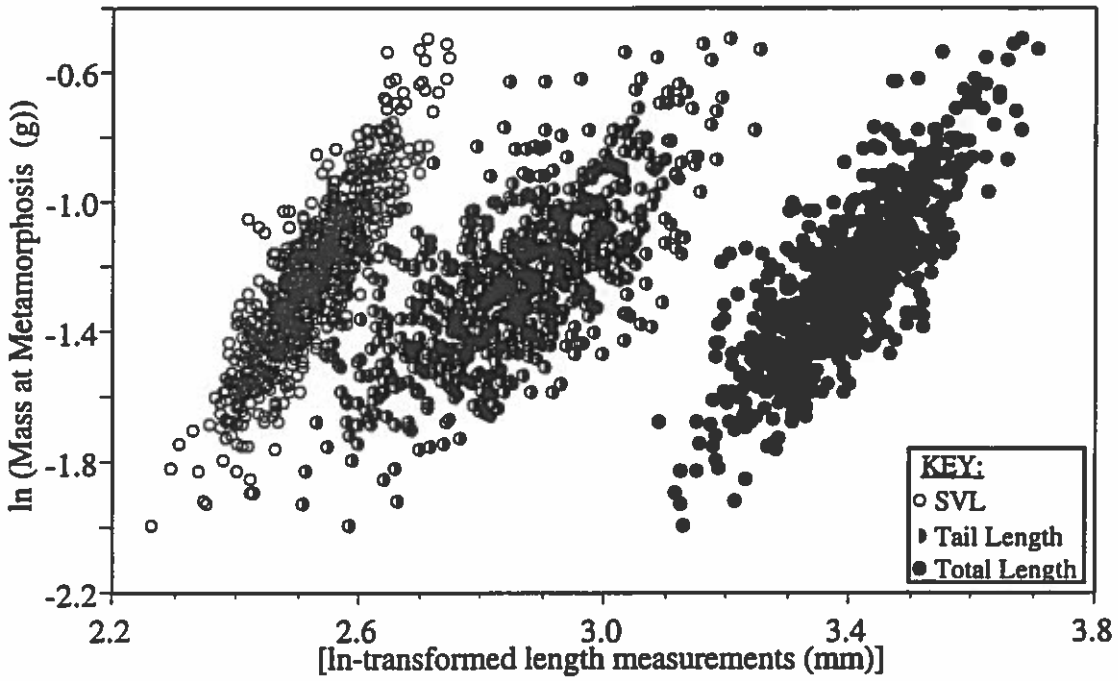

Figure 3.4: Log-transformed length measurements (SVL, Tail and Total lengths) at metamorphosis plotted against log of mass at metamorphosis. Due to the recording techneque used whenever more than one tadpole metamorphosed from a particular tank in a single time interval the length measurements and the mass measurements were not paired. In those cases the mean lengths and mean mass for all the tadpoles metamorphosing from that tank at that interval were used.

The first 25% of the metamorphic data from each tank were analyzed to control for the metamorphosis and removal of tadpoles, which altered density. These mean values were quantitatively explored with ANOVAs. Table 2 and figure 3.5 show the results of a full interaction ANOVA for the mean mass at metamorphosis of the first 25% of the data from each tank. All the experimental factors were included, but the block factor was not since it was not statistically significant as a main effect or in any interaction.

Table 2: ANOVA for main effects and interactions of experimental variables on mean mass at metamorphosis for the first 25% of tadpoles from each tank.

Factor	d.f	S.S	M.S	F-Value	P-Value
Density	1	0.034	0.034	38.905	<0.0001
Hydroperiod	1	0.004	0.004	4.109	0.0494
Habitat Size	1	3.3×10^{-4}	3.3×10^{-4}	0.383	0.5396
Density*Hydroperiod	1	0.001	0.001	0.606	0.4408
Density*Habitat Size	1	0.001	0.001	1.192	0.2815
Hydroperiod*Habitat Size	1	1.5×10^{-4}	1.5×10^{-4}	0.168	0.6837
Density*Hydroperiod*Habitat Size	1	0.001	0.001	0.663	0.4203
Residual	40	0.035	0.001		
TOTAL:	*48*	*0.0765*			Model R^2 =0.535

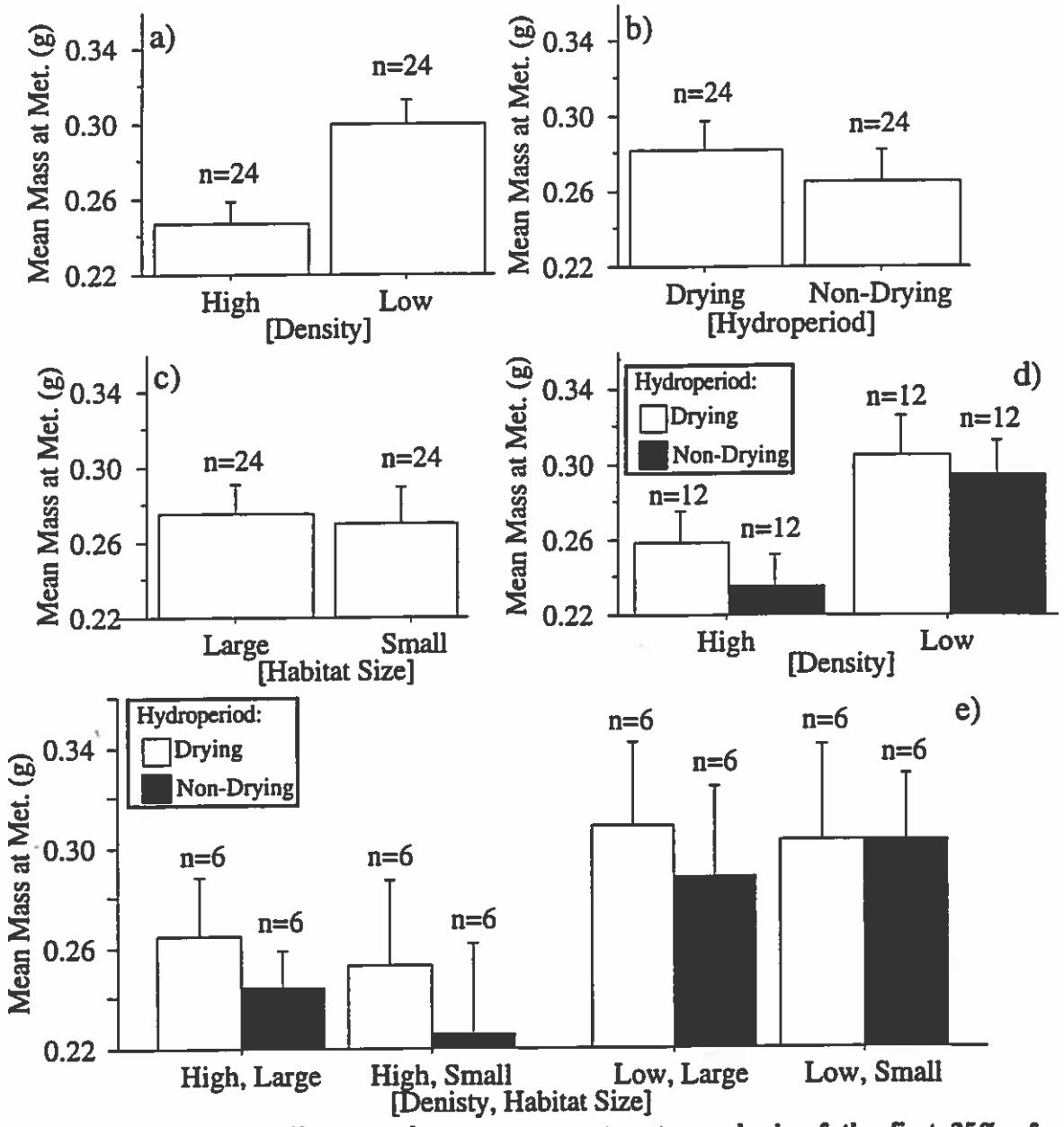

Figure 3.5: Graphs of effects on the mean mass at metamorphosis of the first 25% of animals from each tank. a) Density main effect, b) Hydroperiod main effect, c) Habitat Size main effect, d) Density/Hydroperiod interaction and e) three-way interaction. All error bars indicate 95% confidence intervals.

The density and hydroperiod main effects are statistically significant at the α=0.05 level. This is seen in the graphs for the density and hydroperiod main effects (figure 3.5a and b), where the difference in the mean sizes at metamorphosis shows that the high density tadpoles metamorphosed at a smaller size than the low density ones. In addition, the non-drying hydroperiod tadpoles were smaller at metamorphosis than the ones subjected to the drying treatment. The habitat size main effect is not statistically significant (p=0.5396), as shown in figure 3.5c. None of the interaction terms including habitat size are statistically significant either. Note the non-significant trend for the hydroperiod effect to be most important in the high density tanks (see figure 3.5d). Overall, the model presented in figure 3.5 has explains over 50% of the variation observed in the mass at metamorphosis (see R^2 value, table 2).

Table 3 and figure 3.6 is the full interaction ANOVA and plots for the effects of the experimental treatments on time to metamorphosis in hours (see overleaf). Once again, the block variable was excluded due to its non-significance. The density main effect remains statistically significant (p<0.0001), but the hydroperiod main effect is not (p=0.1227). The high density tadpoles took longer to metamorphose (see figure 3.6 a) as compared to their low density counterparts. The drying hydroperiod animals appear to have metamorphosed earlier than the non-drying ones (see figure 3.6 b), albeit not statistically significantly so at the 0.05 level. Once again, habitat size failed to produce a detectable effect either as a main term or in any of its interactions, so no plots other than its main effect were produced. The interaction between density and hydroperiod is much less convincing (see figure 3.6 d). Overall, this model accounted for 42.4% of the variation observed in the data, slightly lower than the model for mass at metamorphosis.

In summary, both the drying and low density effects produced larger metamorphs at an earlier time than the non-drying and high density treatments. The next section further probes the effects of the experimental treatments though the analysis of the metamorphic traits of individual tadpoles instead of the means for each tank.

Table 3: ANOVA for main effects and interactions of experimental variables on mean mass at metamorphosis for the first 25% of tadpoles from each tank.

Factor	d.f	S.S	M.S	F-Value	P-Value
Density	1	32654.70	32654.70	23.779	<0.0001
Hydroperiod	1	3414.569	3414.569	2.486	0.1227
Habitat Size	1	2210.525	2210.525	1.610	0.2119
Density*Hydroperiod	1	261.698	261.698	0.191	0.6648
Density*Habitat Size	1	1179.137	1179.137	0.859	0.3597
Hydroperiod*Habitat Size	1	287.839	287.839	0.210	0.6496
Density*Hydroperiod*Habitat Size	1	434.259	434.259	0.316	0.5770
Residual	40	54931.26	1373.281		
TOTAL:	*48*	*95373.99*			Model R^2 =0.424

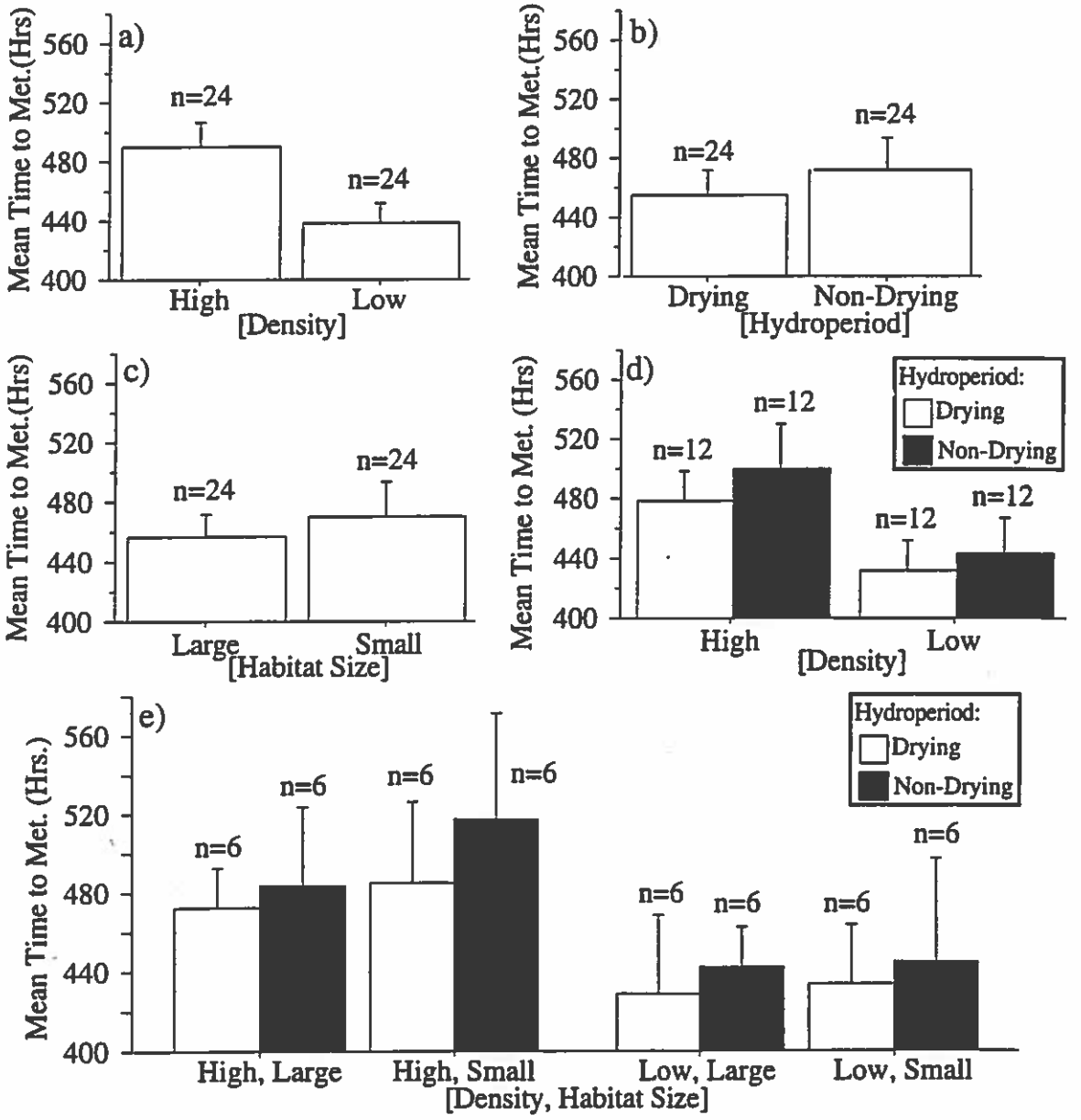

Figure 3.6: ANOVA table and graphs of effects on the mean time to metamorphosis of the first 25% of animals from each tank. a) Density main effect, b) Hydroperiod main effect, c) Habitat Size main effect, d) Density/Hydroperiod interaction and e) three-way interaction. All error bars indicate 95% confidence intervals.

Metamorphic Characteristics of Individual Tadpoles

The data collected though the period of metamorphic activity in experiment I can be understood on several levels of the hierarchical structure of the experimental design, as indicated in the statistical analysis section of the experimental methods. The full extent of the variation in time to and mass at metamorphosis is reduced in the mean values, so analysis of the metamorphic characteristics of individual tadpoles may yield further details on the effects of experimental variables. Figure 3.7 shows a comparison of the time to and size at metamorphosis for each tadpole in experiment I (3.7a) and the mean values for these variables for each of the tanks (3.7b). The reduction in variation from taking the means is so severe that the relationship between time to and size at metamorphosis is negative in some treatments where it is strongly positive for the individual observations within tanks. In other words, taking means of both variables leads to the positive relationship between time to and size at metamorphosis for individual tadpoles being subverted to a negative relationship. On this basis values of time and mass at metamorphosis for individual tadpoles is worthy of further attention.

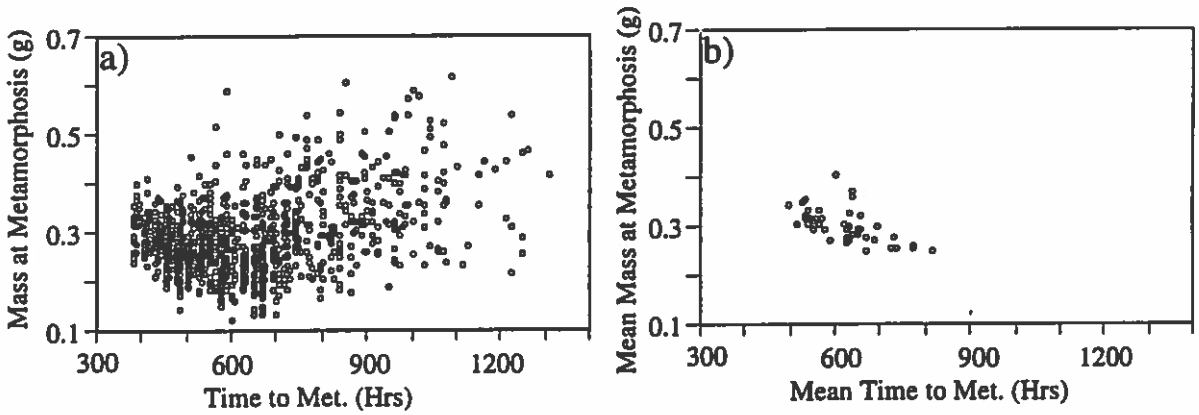

Figure 3.7: Plots of length of larval period (time to metamorphosis in hours) against mass at metamorphosis (in grams) for a) individual tadpoles and b) the mean values for each tank.

Figure 3.8 (overleaf) shows the mass at and time to metamorphosis for each individual tadpole, split by various treatment combinations. The number of tadpoles in each treatment combination is not constant, this being an inherent feature of the experimental design. That fact has the effect of decreasing the resolution of the results in some of the treatments. This is especially obvious in the low-density cases and in the drying tanks (figure 3.8a and b) as compared to the high density ones (figure 3.8c and d). All treatment combinations show positive relationships between length of larval period and size at metamorphosis.

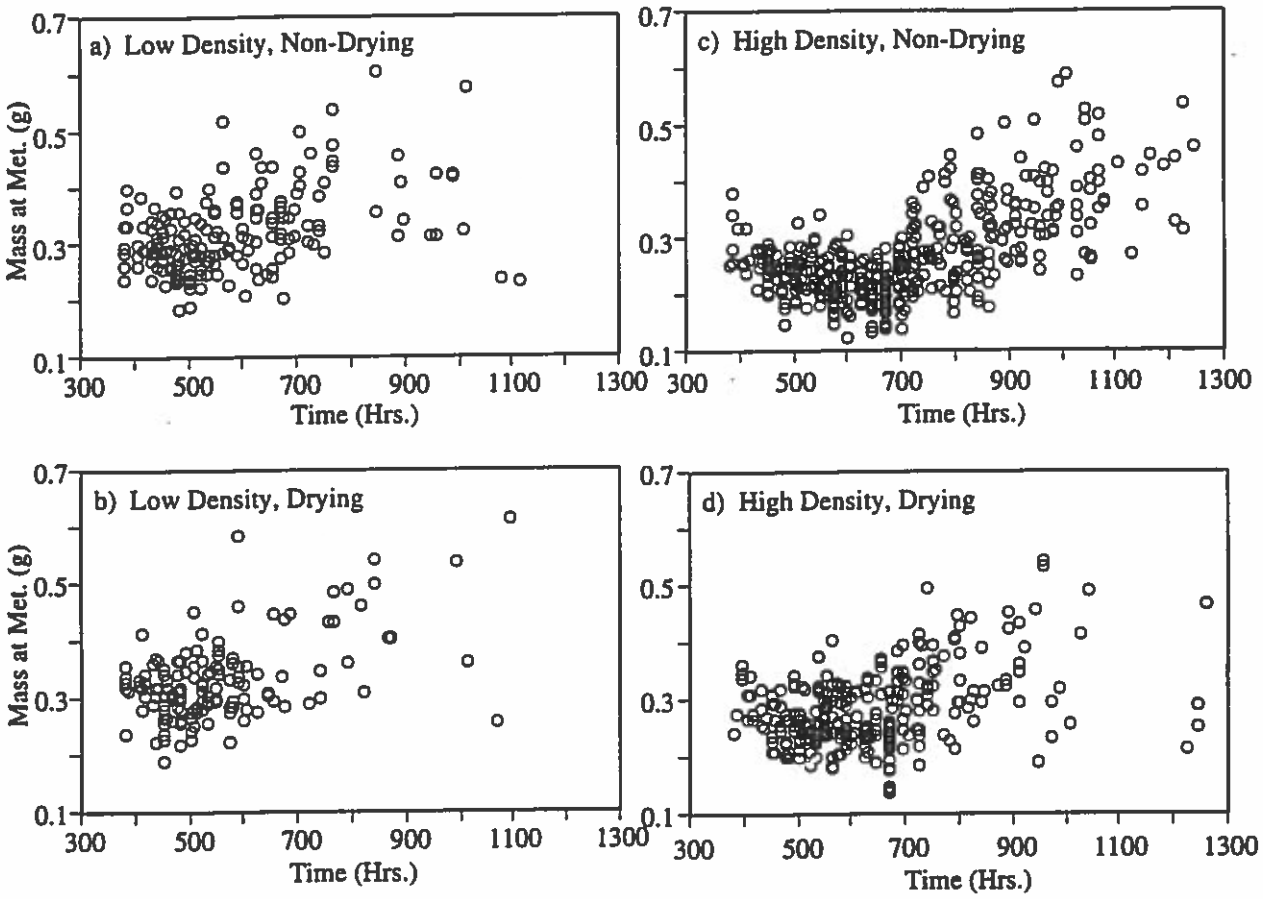

Figure 3.8: Plots of length of larval period against mass at metamorphosis for different treatment combinations of density and hydroperiod. Combinations as labeled.

A direct comparison of habitat size levels once again did not show any large difference between the two, so this factor was excluded (analysis not shown). A very striking feature, however, is the apparent non-linearity between length of larval period and mass at metamorphosis in the high density treatment, especially in the high density non-drying tanks (figure 3.8c). The non-linearity is not as crisp for the high density/drying tanks (figure 3.8d). For equivalent plots of this data for each individual tank, please refer to appendix A. Typical results for single tanks in the high density, nondrying treatment combination are presented below, in figure 3.9. Note that there appears to be a consistent relationship between the two patterns (time versus mass and time versus density) for these examples. From such plots, use of the continuous measure of density rather than just the categorical version seemed expedient, as argued in the experimental methods section.

Splitting plots of time to and mass at metamorphosis for each treatment combinations by tank number allows for a qualitative appraisal of weather or not the distribution of points within the overall spread is biased by tank, as argued in the

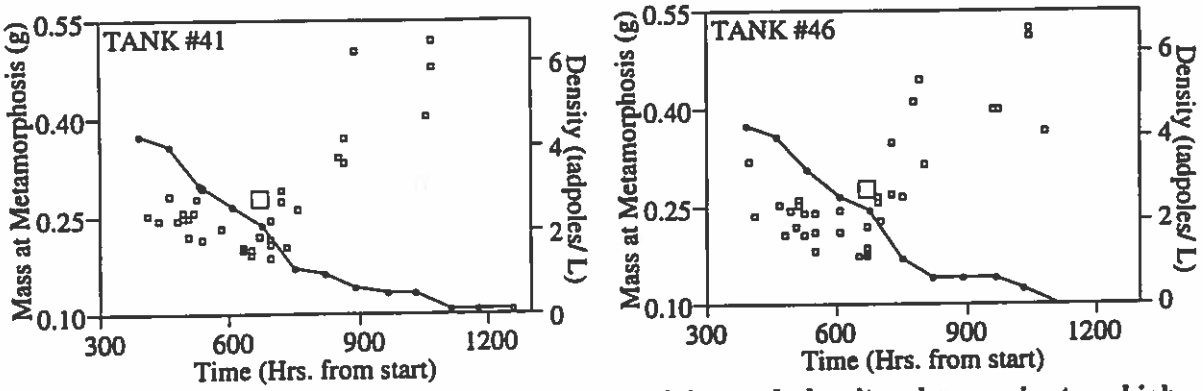

Figure 3.9: Typical patterns of metamorphic activity and density changes in two high density, non-drying tanks from experiment I. Full results for every tank in the experiment are presented in Appendix A. Points connected by a line represent the estimated density; small scatter points show values of time and mass for individual tadpoles and the large point represents the mean time and mass for the tank.

experimental methods section. There is no evidence that conditions in any particular tank were so different as to cause all the metamorphs from that replicate to occupy only one portion of the distribution (plots are presented in Appendix C). Instead, the points from any one tank are generally spread out over the whole range of the distribution.

Regression models were fit to the data on mass at metamorphosis for individual tadpoles. These included two continuous variables (time to metamorphosis and estimated density) and two categorical ones (hydroperiod and tank). For minimizing pseudo-replication, as argued in the experimental methods, the tank variable was nested in hydroperiod. Quadratic terms were also included to test for non-linearity between continuous variables. The formulae are presented below, in Figure 3.10.

Figure 3.10: Formulae for regression models fit to entire metamorphic data of experiment I.

Model 1:

$$Mass = Time*Hydroperiod(Tank) + Time*Density + Time^2*Hydroperiod(Tank) + Time^2*Density + Density*Hydroperiod(Tank) + Density^2*Hydroperiod(Tank) + Density^2*Time + Density^2*Time^2$$

Model 2:

$$Mass = Density*Hydroperiod(Tank) + Density^2*Hydroperiod(Tank)$$

The complete output of the two models is presented in appendix E.

The full regression model that was examined (Model 1) included the various important terms described in the statistical analysis section of the experimental methods. The entire model may be summarized as follows: mass at metamorphosis is dependent on density and/or density squared, time and/or time squared and "tank nested in hydroperiod".

Each of these variables also interact, though no interaction higher than the second order (two way) were allowed.

Each coefficient will not be described in great detail, though some general observations will be made about the fit of the full model. First, it appears that time, density, and hydroperiod play a significant role in determining the mass at metamorphosis of any particular tadpole (see appendix E). Their exact interrelationships are extremely complex and cannot be fully addressed here because of the strong correlations of some of the variables. Model 1 was constructed in order to address specifically if the effect of time on metamorphosis was dependent on the density changes that may have occurred. A correlation of density and time show them to be highly correlated (Table 4), so it is possible that the changes in density over time were responsible for the effect on metamorphosis.

Table 4: Correlation of Time and Density.

	Time~Density
Correlation Value	-0.532
Fisher's r to z (p-value)	<0.0001

In order to ascertain if their effects are important independently, the full model (model 1) was compared to a nested model (model 2) which did not have any of the "time" terms included. The resulting F-test is reported below, in table 5.

Table 5: F-test for the difference between the fit of models 1 and 2.

Model	Test	Resid.Df	RSS	Df	Sum of Sq	F Value	Pr(F)
1		1003	2.940010				
2	- all time variables	1015	3.315191	12	0.3751809	10.66624	0

The very low p-value for the F-test indicates that time is indeed having some effect on mass at metamorphosis, and that all the effects are not simply density dependent.

In general, it also appears from both models 1 and 2 that the nested effect of tank was unimportant in determining the mass at metamorphosis, except for in certain treatment combinations (see appendix E). Overall, however, it is probably safe to say that the effects of tank were at most very small and restricted to certain cases, such as the drying tanks where smaller sample sizes may have had an effect biasing the significance of that term.

Given this general situation, it is difficult to accurately assess the relative importance of the quadratic terms in the model. It is at least possible to say that there is some evidence of non linearity in the relationships between variables, since so many of the quadratic terms and some interactions came up significant (see Appendix E). Perhaps the most important aspect to highlight is that density was an important variable in determining

an individual's mass at metamorphosis, but that it may have affected the relationship between time and mass in unknown ways.

As described in the experimental methods, the correlation between density and the number of tadpoles in the tank became of importance later in experiments II and III. Instead of substituting the "density" variable with "number of tadpoles", they were compared with a correlation test. The correlation of number of tadpoles to density is perfect for the non-drying tanks (since the volume does not change and so density is a linear transformation on the number of tadpoles). Table 6 (below) shows a correlation test for these two variables in the drying tanks only, revealing the strong and consistent relationship between the two. There is no biological reason to believe that they might be acting in different ways, so in this case the "density" variable may be taken to be equivalent to "number of tadpoles".

Table 6: Correlation value and p-value for the relationship between estimated density and the number of tadpoles in any particular drying tank over time. They are almost perfectly related even for the drying tanks.

	Estimated Density~Number of Tadpoles
Correlation Value	0.959
Fisher's r to z (p-value)	<0.0001

Experiment II

Experiment II was designed to test the effects of drying with increasing density by comparing drying to non-drying tanks which had no tadpoles removed throughout the experiment. This situation closer approximates the action of drying as experienced in the field, where dramatic increases in density accompany the evaporation of water in a pond. The analysis of the resulting data is presented in two parts. First, the effects of drying on time and mass at metamorphosis are examined. Second, the increase in density that ensued as a result of drying was quantified.

Analysis of Hydroperiod Without Density Control

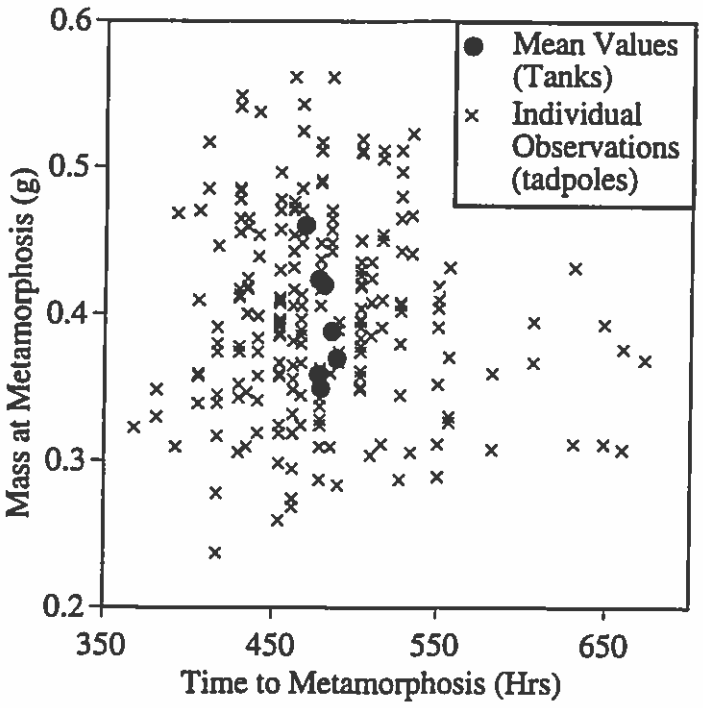

Figure 3.11: Comparison of mean tank values for time to and mass at metamorphosis with those from individual tadpoles.

Once more, an analysis of the mean values of time to and size at metamorphosis was carefully considered for its advantage ensuring the independence of each observation in the statistical test. Figure 3.11 shows a scattergram of the length of larval period against the mass at metamorphosis for each individual tadpole and the means of the 8 tanks in experiment II.

The spread of the mean mass at metamorphosis by tank is quite even and representative of the spread of the entire data (individual tadpoles). The mean values for time to metamorphosis appear to be more compressed by comparison, and are not as good a representation of the spread of the individual observations. The reason for this observation is the greatly reduced length of metamorphic period in experiment II. This fact indicates that use of the times to metamorphosis for each individual tadpole must remain a possibility if the compression of the means proves to be confounding.

Overall, however, the mean values are reasonably representative the entire data allowed for the use of the mean values only in this instance. While some trends may have been obscured using the mean time to metamorphosis, this was not found to be the case.

Qualitative conclusions of the effects of the variables on time and mass on individual observations compared to the means showed no difference, so the more conservative estimate of the means was used. This allowed the analysis of the results of experiment II to circumvent problems of pseudo-replication. The means for time to metamorphosis were slightly heteroscedastic but not to an extent that would distort the qualitative results of the ANOVA (A. Jones, Personal communication).

Table 7: One-way ANOVAs for metamorphic characteristics in experiment II: effect of hydroperiod (drying with increasing density or non-drying) on a) Mean Mass at metamorphosis and b) Mean Time to metamorphosis.

a) Response: Mean Mass at Metamorphosis(g)

Factor	d.f.	S.S	M.S	F-Value	P-Value
Hydroperiod	1	0.0008	0.0008	0.5085	0.503
Residual	6	0.0097	0.0016		
TOTAL:	*8*	*0.0105*			Model R^2 - 0.078

b) Response: Time to Metamorphosis (Hrs)

Factor	d.f.	S.S	M.S	F-Value	P-Value
Hydroperiod	1	22.781	22.781	0.594	0.4702
Residual	6	230.158	38.360		
TOTAL:	*8*	*252.939*			Model R^2 - 0.090

ANOVAs for the mean size at and time to metamorphosis are presented in Table 7. Figure 3.12 shows plots for the effects of the hydroperiod experimental variable. The "block" variable was excluded from the analyses since it did not have a significant effect and was a non-experimental variable. Temperature was also found to not have a significant effect on mass or time (see Appendix B). The analyses of experimental variables show no effect of drying with increasing density (no removal of tadpoles) versus nondrying (fixed number of tadpoles) on either time to ($p = 0.4702$) or mass at ($p = 0.503$) metamorphosis. In addition to this they explain very little of the observed variation (see R^2 values, Table 7).

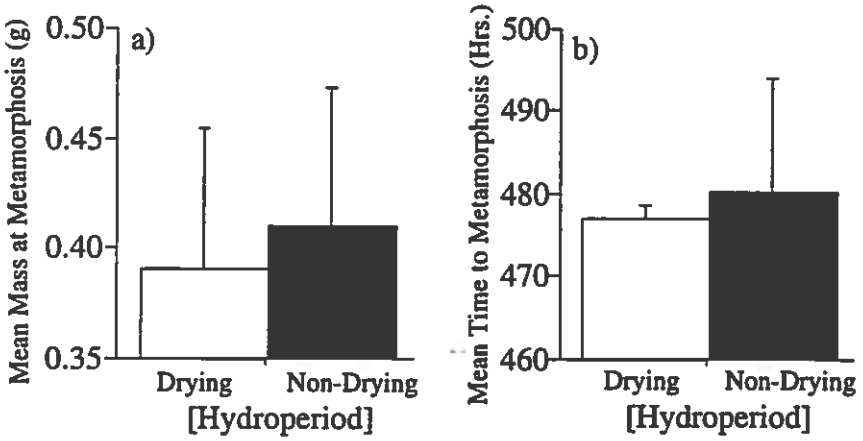

Figure 3.12: Main effects of drying on a)mass at and b)time to metamorphosis in experiment II. Note that the drying treatment in this case entailed an increase in density. Error bars indicate 95% confidence intervals

A Closer Look at Density

It was originally expected that the great increase in density in drying tanks over the course of experiment II would have a large effect on the comparison of metamorphic characteristics of tadpoles in the two treatments. The difference in density at the time of metamorphosis is shown by plotting the mean density of animals in the tanks in each treatment over the metamorphic period. This is shown in figure 3.13a. The number of tadpoles in the tanks at those times was also of interest, so the mean absolute number of individuals in tanks of each of the two treatments was also plotted (figure 3.13b)

This large difference in the mean density is contrasted with the comparability of the mean number of tadpoles in the drying and nondrying tanks over the period of metamorphosis (figure 3.13b). Both these conclusions were statistically tested using one-way ANOVAs. The difference in average density was found to be statistically significant ($p<0.0001$) but the difference in the average number of tadpoles was not ($p=0.2929$).

In order to calculate the average density of tanks in each of the two treatments, the density of the tank at the time of metamorphosis of each tadpole was employed. This was necessary because a density value was usually recorded only upon the metamorphosis of an animal. The equivalence of sample sizes (112 and 106 values for density) for each of the hydroperiod treatments indicates that there is no biasing of one treatment over the other by increased sampling. The number of individuals in each tank was used to calculate the density, so the number of times density was sampled was equivalent to the sampling times for numbers of tadpoles.

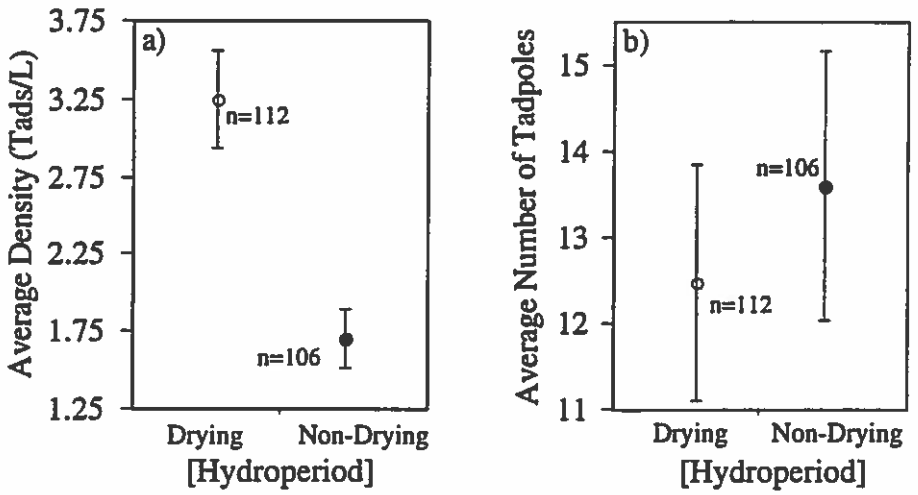

Figure 3.13: Plots of the mean (a) density and (b) number of tadpoles of tanks in each of the experimental treatments for experiment II. "n" indicates the number of sampling times of density and number used to produce the mean value and the 95% confidence intervals (indicated by error bars).

Experiment III

Experiment III was set up to test the effects of density on metamorphic responses. Experiment I showed complex relationships between time and mass at metamorphosis over as the metamorphic period progressed. It is possible that these patterns were caused by changing densities as tadpoles were removed from the experiment, and this is the issue experiment III explicitly addresses. The experimental design included three density levels (high, medium and low) and two hydroperiod levels (non-drying and drying, again with density control).

Analysis Density and Hydroperiod Effects

Analyses of variance of the mean values for time to and mass at metamorphosis were carried out to investigate the effects of the experimental variables on metamorphosis. This method was appropriate on the basis of figure 3.14, where the spread of the 24 values for mean time and mass at metamorphosis is representative of the overall distribution of individual observations. The relationship between time and mass is negative for the mean values, but in this case it is not clear that the relationship between the individual tadpole observations is positive (compare to experiment I). The results of the ANOVAs are shown in Tables 8 and 9, below.

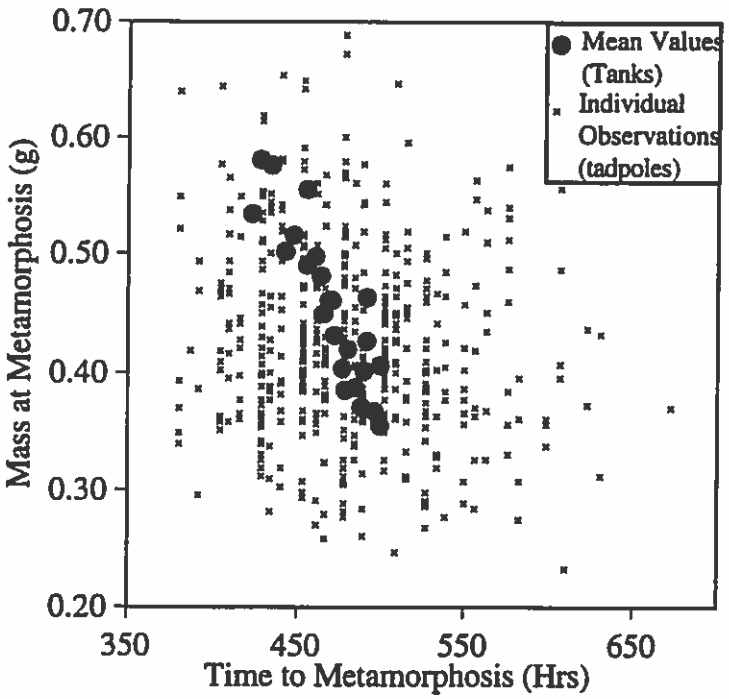

Figure 3.14: Comparison of values of time to and mass at metamorphosis for means and for individual tadpoles in experiment III.

The ANOVAs show that there is a strong main effect of density on both mass at (Table 8) and time to (Table 9) metamorphosis, where higher densities lead to smaller sizes at metamorphosis and longer lengths of larval periods. These effects are statistically significant and are graphically summarized in figures 3.15a for mass at metamorphosis and figure 3.16a for time of metamorphosis.

The effect of hydroperiod was not statistically significant at the 0.05 level, but a trend was apparent where the drying tanks had shorter larval periods and larger masses at

Table 8: ANOVA for effects of density and hydroperiod on mass at metamorphosis in experiment III.

Factor	d.f.	S.S	M.S	F-Value	P-Value
Density	2	0.0728	0.0364	26.4463	<0.0001
Hydroperiod	1	0.0031	0.0031	2.2238	0.1532
Density*Hydroperiod	2	0.0027	0.0013	0.9798	0.3945
Residual	18	0.0248	0.0014		
TOTAL:	*24*	*0.1034*			Model R^2 - 0.760

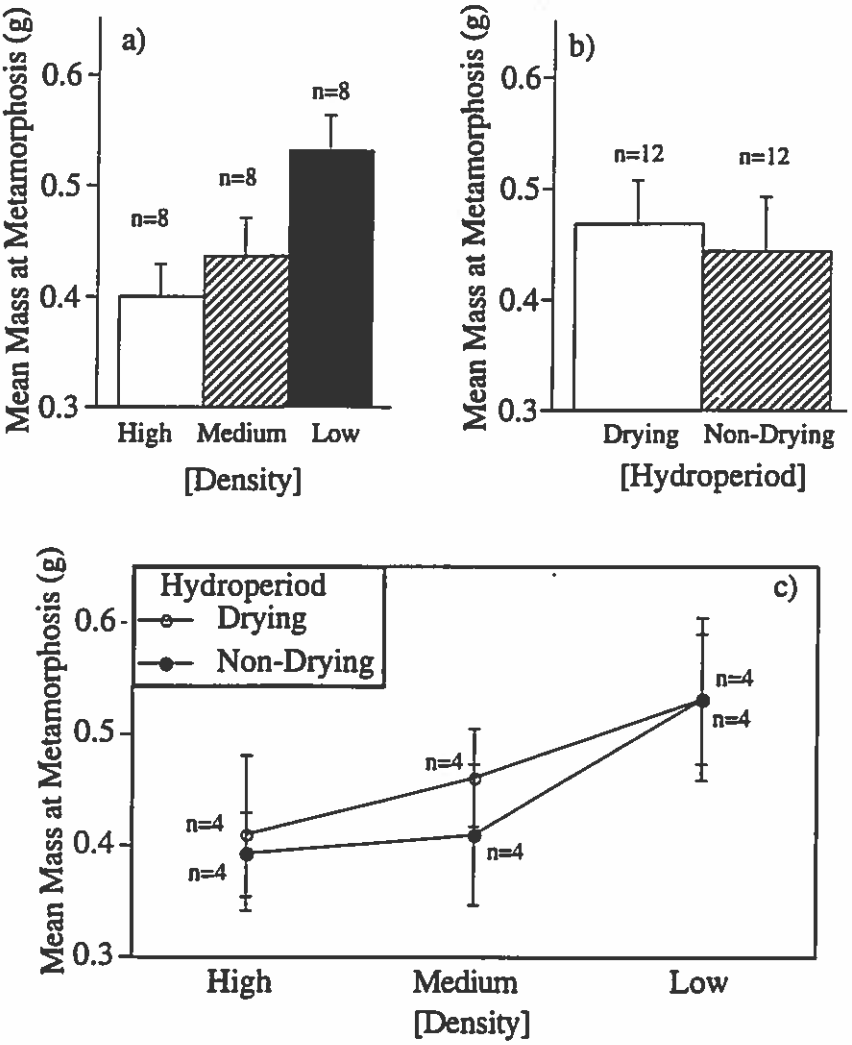

Figure 3.15: Analysis of Variance of the mean values per tank for experiment III: the main effects (a) density and b) hydroperiod) and interaction (c) of hydroperiod and density on mass at metamorphosis. All error bars indicate 95% confidence intervals

metamorphosis (see figures 3.15b and 3.16b and Tables 8 and 9). The interaction of density and hydroperiod was non-significant for both variables (figures 3.15c and 3.16c). Both models account for much of the variation in the data (see R^2 values, Tables 8 and 9).

Once more, block and temperature were excluded from the analysis due to their non-significance. These results qualitatively agree with the ones in experiment I. Interpretations of the relationships between variables and their effects on metamorphic traits are offered in the discussion section.

Table 9: ANOVA for effects of density and hydroperiod on time to metamorphosis in experiment III.

Factor	d.f.	S.S	M.S	F-Value	P-Value
Density	2	8885.41	4442.71	36.434	<0.0001
Hydroperiod	1	324.87	324.87	2.664	0.1200
Density*Hydroperiod	2	147.72	73.86	0.606	0.5564
Residual	18	2194.89	121.94		
TOTAL:	*24*	*11552.89*			Model R^2 - 0.810

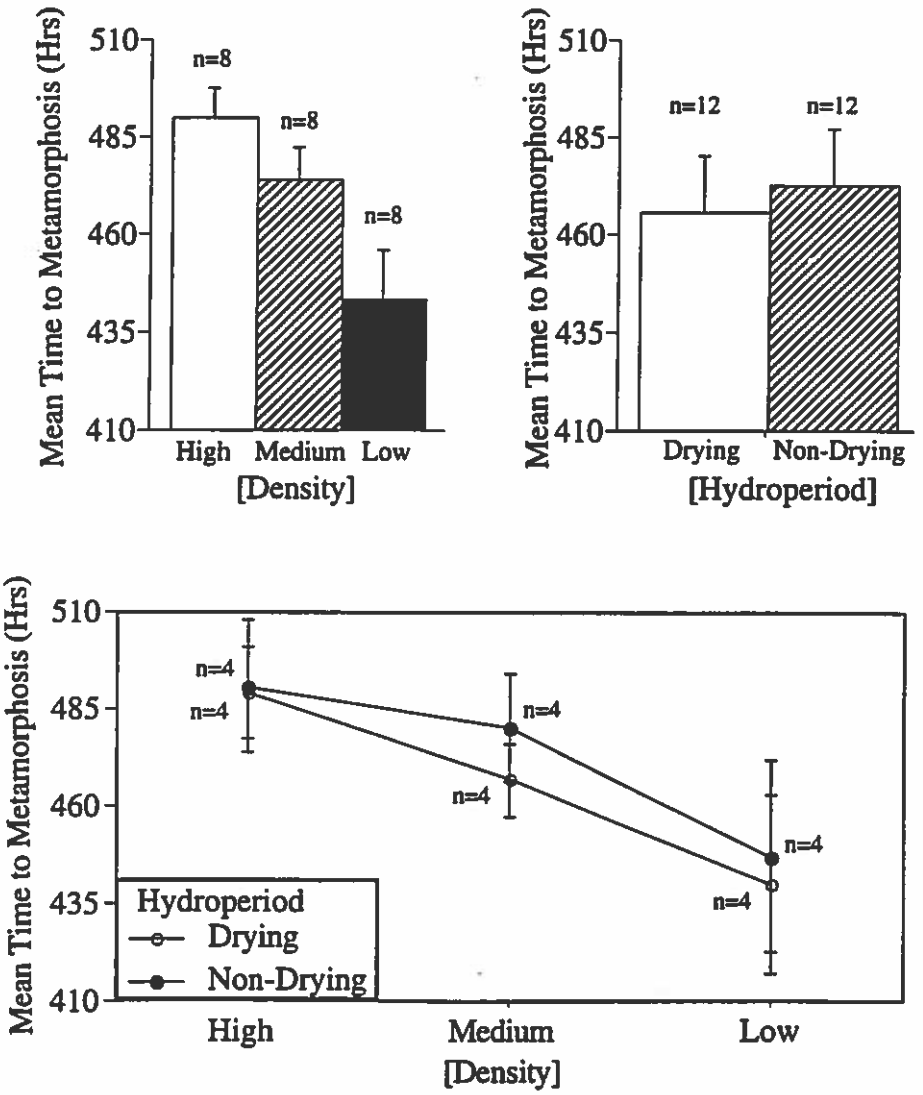

Figure 3.16: Analysis of Variance of the mean values per tank for experiment III: the main effects (a) density and b) hydroperiod) and interaction (c) of hydroperiod and density on time to metamorphosis. All error bars indicate 95% confidence intervals

DISCUSSION

Science has "explained" nothing; the more we know the more fantastic the world becomes and the profounder the surrounding darkness.
Aldous Huxley. *Along the Road*, pt. 2, "Views of Holland" (1925).

The objective of this study was to investigate an important fact of the existence of tadpoles: ponds sometimes dry out. The fine points of what the exact effects of drying are in terms growth and development are of interest in understanding evolutionary events. The mechanics of what aspect of drying produces the response to the fact is of ecological importance. An understanding of both evolutionary and ecological issues culminates in an improved comprehension of the existence of complex life cycles.

The aquatic habitat of larval amphibians is a dynamic and complex one. There are many factors and interactions over time which form a mosaic of particular conditions, conditions which then affect the expression of many traits of the population. Ultimately, explanation of all possible implications may be humanly impossible, but the rewards have already been great even for the small distance we have traversed.

The study of metamorphosis has been a rich field, allowing for the creation of models as simple and symmetrically beautiful as that of Wilbur and Collins (1973) out of what at times may seem like a morass of confusion. Their model has spurned many experiments and tests, and undoubtedly has allowed for increased appreciation of complex life cycles. This experiment has revealed a few more issues surrounding environmental variation and metamorphic patterns which may make our understanding of metamorphic activity and complex life cycles in general a little clearer.

Experimental Results

Growth Characteristics In Experiment I

The results and interpretation of the growth of tadpoles prior to metamorphosis in experiment I is integrally tied to their metamorphic characteristics. It only does us harm to separate the two if we forget that growth and development are linked in complex ways and that growth may be the mechanism for timing metamorphosis, as argued by Wilbur and Collins (1973). With this reaffirmation, this section will proceed to describe and interpret the growth data so it can later be linked to the metamorphic characteristics of the tadpoles.

Snout to vent and tail lengths are known to have implications for the fitness of amphibian larvae, since larval morphology affects the swimming ability of a tadpole. In *Bombina orientalis*, sprint speed is positively related to tail length and negatively related to

body width (Barker, 1993). Sprint speed in tadpoles has fitness implications in ability to resist predation (see Kaplan, 1992) and is related to reproductive success, predation success and social dominance (see Howell, 1994). There are also implications of size during metamorphosis and afterwards, in the terrestrial phase of the life cycle, but this will be addressed in the discussion of the results of the metamorphic responses.

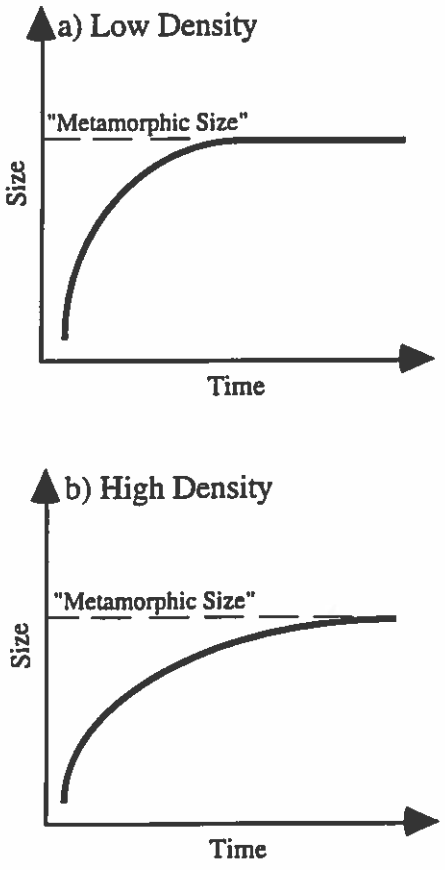

Figure 4.1: Growth of tadpoles under different density treatments in experiment I. a) Low density, b) High density

A link between morphology and performance implies that evolutionary pressures may shape the morphological expression of a population. Clearly, for *B. orientalis* there are fitness implications associated with larger sizes, where larger tail lengths tend to cause higher fitness. Natural selection occurs if the morphological traits in question are heritable. Since tail length and SVL were correlated in this study, this observation of larval morphology and size may in this case be taken to show that larger larval sizes result in higher fitness. By extension, higher growth rates may be argued to be beneficial. This is a reasonable conclusion not only on the basis of the preceding argument that larger larval sizes have a positive impact on the individual's fitness, but also because higher growth rates translate to a larger metamorphic size. How growth rate relates to length of larval period is a more complex problem, and these results are interpreted in the next section.

Density had a strong, consistent effect on both tail and snout- vent lengths in the growth data. After the first week (when tadpoles were entered into treatments) density limited the growth that tadpoles were experiencing. As expected (Dash and Hota, 1984; Semlitsch and Caldwell, 1982; Sokol, 1984), higher densities yielded lower sizes at many of the weeks, and therefore probably lower growth rates. The density differences in experiment I had an effect on the growth trajectory of the tadpoles. The animals in the high density treatments seemed to grow more steadily to a level plane, while the low density ones grew quickly at first then leveled off (see figure 4.1). This may be an indication that animals in the low density tanks were reaching a "metamorphic" size earlier than their high density counterparts. Interpretations of the effects

of density on growth and development are expanded in the following section with the discussion of the metamorphic responses.

The hydroperiod variable showed no statistically significant difference in each weeks data for SVL or TL. Still, it may be said that the effects of hydroperiod were slightly more pronounced in combination with high density (see figure 3.2a and b). While that interaction term was not significant for any of the weeks, the qualitative observation that tadpoles seemed to be larger in the drying tanks than the nondrying tanks of the high density treatment is remarkable. This is an unexpected result, because the tadpoles in the drying habitat would be expected to be under higher stress. This should lead to lower growth rates and earlier metamorphosis at a smaller size (e.g. Newman, 1989). Once again, the metamorphic responses to the treatments must be discussed before further examination. If the tadpoles in the drying treatment do turn out to have experienced higher sizes and growth rates together with the metamorphic responses which accompany these traits (shorter times and larger sizes) then one conclusion may be that the effects of hydroperiod appear to be dependent on density increases. This argument would be based on the non- agreement of the responses to drying observed in other experiments with those observed here, where density was controlled for.

The habitat size variable did not show as strong an effect as might be expected if there had been differential use of parts of the habitat in this case (the "niche-partitioning" argument, see introduction). Habitat size did come up as having a statistically significant effect on tail length in week 3, but this was a relatively minor effect. It is possible to attempt to explain this result in terms of locomotor needs in spaces of different depths, but this would probably be an over- interpretation of the data. One would expect a much stronger result if habitat size was indeed a variable of much importance, and in general it does little service to attempt to make inferences from this result which subsequent data do not bear out.

Metamorphic Characteristics in Experiments I, II and III

The results of the three experiments with respect to metamorphosis may be summarized in terms of the main hypotheses being tested. Perhaps the most burning question this thesis seeked to address was the possibility that the effects of hydroperiod were mediated by changes in other variables, particularly density (but possibly also habitat size). It was hypothesized that by controlling density as drying was artificially simulated the effects of hydroperiod and crowding would be effectively separated. If the hydroperiod effect was dependent on an increase of density, there would be no difference between

hydroperiod treatments. Other researchers (e.g. Semlitsch, 1987) have suggested that the action of hydroperiod may be mediated by density, so controlling it was a way to address the high degree of variability in the outcome of drying in studies in the field. Tejedo and Reques (1995), for example, found a negative relationship between length of larval period and size at metamorphosis for individual tadpoles. This is exactly the opposite result obtained in many other studies (e.g. Newman, 1989; Collins, 1979) which show that the later metamorphosis occurs, the larger the resulting froglet (figure 4.2). Tejedo and Reques attribute this difference to temperature regimes over the seasons because the length of the metamorphic period was great. Thus, the tadpoles that metamorphosed at the end of the period were exposed to colder temperatures which may have caused their reduced size. Other studies which have found non-positive relationships between time and size at metamorphosis also attribute the result to other environmental variables, such as decreasing food, sibship effects (variation among families in response to hydroperiod) or increasing concentrations of growth inhibitors (see Tejedo and Reques, 1995).

The effect of hydroperiod was not a significant force in any of the analyses of metamorphic responses, coming up as statistically significant only in the first experiment. This initially might lend credence to the possibility that many of the effects of hydroperiod in the field may have been related to density increases. The direction of the relationship between drying and non-drying tanks was not completely as expected, however. In agreement with the preliminary indication from the growth data, tadpoles in drying tanks were metamorphosing earlier and at a larger size. This was an indication that perhaps things were not occurring as expected.

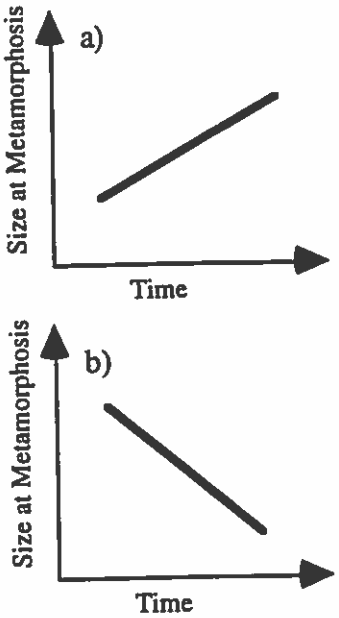

Figure 4.2: Conflicting results i field. a) positive relationship be time and size at metamorphosis Newman, 1989) and b) negative relationship between the two (e. Tejedo and Reques, 1995)

In both experiments I and III the density-controlled drying regimes produced metamorphs earlier that their non-drying counterparts. This conformed to expectations from other studies (Newman, 1989; Rowe and Dunson, 1995) where according to the predictions of the Wilbur and Collins model the rapidly degrading conditions of drying pools would decrease growth rates and so induce metamorphosis earlier.

If the above explanation fit this study's results, a trade-off would be expected, where the tadpoles in the drying tanks would emerge earlier due to lower growth rates but

then also be smaller at metamorphosis. The density-controlled drying tanks produced larger metamorphs, however, in complete opposition to expectations of the Wilbur-Collins model and other the other empirical studies which showed drying habitats to be more environmentally stressed (e.g. Newman, 1989). This indicates that the tadpoles in the "drying" tanks experienced a higher growth rate, reaching a larger mass at an earlier time. The drying habitats appeared to be overall superior environments. Further agreement was observed in the data from experiment III, which tested hydroperiod and more finely probed density by including three density levels instead of only two.

Is this proof that controlling density radically altered the effects of drying? Before answering the question, the results of the density treatments must be visited to bring the whole picture into focus. Higher densities were expected to produce smaller metamorphs at a later time, a common result with many interpretations. These effects are believed to be mediated by factors ranging from chemical inhibition (Sokol, 1984) to food level depletion (Dash and Hota, 1980) to various forms of competition (Tejedo and Reques, 1994). Whatever the exact mechanism in each case, the effect of "crowding" is important and is observed here in both experiments I and III. The Wilbur and Collins model accounts for this sort of response in terms of decreased growth rates in higher density environments right from the beginning, leading to smaller masses and longer times to metamorphosis in higher density tanks.

The mode of action of density and hydroperiod treatments was revealed by the results of experiment II. In these tanks, tadpoles in the drying treatments were expected to suffer a considerable increase in density stress as water volume decreased and their numbers stayed fixed. While their density did increase dramatically through the experiment as planned, it did not have any effect on their metamorphic characteristics. This single result showed that density was not in fact the variable responsible for affecting time to and mass at metamorphosis. The interpretation of all the results now had to be revisited because in experiment II metamorphic characteristics were not being affected by actual density. Experiment II was also the only one where the number of tadpoles in the drying tanks was not correlated with the density of the tank.

It appears that the number of animals in a tank, not their density, was in fact the limiting factor for the tested variables. All the experimental results can be explained in terms of this critical observation, as follows. The density variable in experiments I and III behaved as expected because higher densities always entailed a higher number of animals in the tank. In experiment II, however, the number of tadpoles in each of the treatments was equivalent but the densities varied by a great amount. No significant differences in

metamorphic characteristics were observed. This implies that the number of tadpoles, instead of the density, was in fact the factor having the most effect on metamorphosis.

The hydroperiod results corroborate this view and can now be more easily understood. The density-controlled drying tanks had tadpoles removed during drying to keep density constant. This meant a lower absolute number of tadpoles in the drying tanks over time than in the non-drying tanks, which had no animals removed. Thus, the drying tanks produced larger froglets at an earlier time because of the lower number of tadpoles due to their removal during drying. In effect, the drying habitat was a "superior" environment. If this view hold true the effects of the third variable of experiment I, habitat size, can be expected to be strongly affected also.

Indeed, there was no detectable effect of varying shape and volume parameters on the growth and development of the tadpoles. This was an unexpected result because of previous work which had shown that niche partitioning may have a large role to play in how tadpoles of different species experienced density stress (Pearman, 1993), as already mentioned in the previous section.

The absence of a difference may be due to *Bombina* tadpoles actually experiencing crowding though density, either by chemical or other interferences, as argued in the introduction. At the same time, these results may have been observed because the ecological factors that make habitat size important are not present under controlled laboratory conditions. Pearman (1995) extended the importance of habitat size to community ecology. He showed that habitat size is relevant not only to the amount of space available to each tadpole but also because it may affect the ecological composition of the pond. The ability of a predatory beetle to colonize a pool and feed on tadpoles was affected by the size and shape of the pool, so in that indirect sense there was a strong effect of shape and size on the growth and survival of larval amphibians. Morin (1983) found that for several anuran species the mass at metamorphosis was increased by higher predation pressures, suggesting that intraspecific competition was higher with higher densities of predators. In other words, the predators were mediating the level of competition between tadpoles of the same species. These levels of ecological complexity were absent from the glass aquariums set up in the controlled conditions of the lab, perhaps accounting for the absence of effect of the habitat size variable.

This explanation appears to be the correct one, in support of the hypothesized importance of the number of tadpoles per tank. The results from experiment I show that the larvae in the tanks appear to not have been experiencing density-stress in any habitat related way because there was no effect of large or small tanks on their metamorphic characteristics. The number of tadpoles in the large and small tanks was equivalent

throughout, accounting for the absence of an effect. If the number of tadpoles per unit surface area or some other shape or size difference was crucial, one would expect to see different responses to tank size. Since the volume of water per individual did not have a strong effect, as seems to be the case from the results of the other variables, then it would be hard to rationalize a crowding effect of some other aspect of the habitat.

The strong effect of number of tadpoles per tank and the irrelevance of the density of tadpoles per unit volume or other space measurement is likely to be related to competition for food. Food levels were not variable across treatments, all animals were fed *ad libitum*. This means that there was always some amount of boiled spinach in the tank. This implies that the amount of food was unlimited to each tadpole, and so was absolutely not a factor. It is possible, however, that this was not the case since there must have been some amount of competition for access to the clumps of boiled spinach, and the aggregation of many tadpoles around each clump was commonly observed. This may have made behavioral interference and competition more intense in tanks with more animals in them than in the ones with a lower absolute number of individuals in them.

It is unlikely that chemical interference (e.g. Schmuck et al 1994) was responsible for the effect of number of tadpoles on growth and development. If this had been the case, volume would have been an important factor and density per unit volume would have acted as expected. This leaves very little to account for the strong effect of the number of tadpoles in a tank except for issues relating to food levels and competition. There may have been differences in the amount of boiled spinach available to each tadpole depending on their numbers in the tank. There were a few times when there was no spinach at all in some of the tanks, especially in experiment I. These were very minor in terms of the entire length of the experiment, but the fact that they occurred may be some indication that the amount of food per individual may have been varied. Audo et al (1995) found strong effects of food deprivation on metamorphic properties, which shows that food levels may have been the critical factor in this case. The issue of food availability is also important in the discussion of length of metamorphic activity, below, and more research in this area is clearly needed.

There may have been behavioral interference for the food that was available, irrespective of its supply, worsening the situation for animals in tanks with many tadpoles in them. The absolute number of competitors may be significant due to such interference over access to clumps of boiled spinach despite the fact that clumps were always present. There are still many questions to be answered with respect to effects of behavior on the growth of tadpoles. John and Fenster (1973) argued for a psychological effect of habitat subdivisions which resulted in differential growth rates. The possible competition of tadpoles for food seems to be at least as plausible a scenario, and merits further attention.

In conclusion, the experimental design and execution led to confounding treatments. The effect of density was not as important as the number of tadpoles in the habitat, possibly because of behavioral competition for food. By extension, the other two experimental variables also were not as expected, but valuable information is yet to be distilled from them.

Duration of Metamorphic Activity in Experiments I and III

Experiment III was designed to investigate the possibility that changing densities had a strong effect on the metamorphic pattern of experiment I. Despite good agreement in the relationships of experimental variables with each other and their effects on time to and size at metamorphosis, there was major divergence in the amount of time metamorphs appeared in the tanks, which will be referred to as the metamorphic period for this discussion. The difference was impressive, with the animals from experiment I metamorphosing over a period of approximately 40 days, while for experiments II and III only about one quarter of that amount of time was observed between the emergence of the first and last metamorphs. The shortness of the period for experiment III had the effect of rendering any patterns of metamorphic activity similar to that of experiment I non-existent.

The relationship of length of larval period to mass at metamorphosis for some of the treatment combinations in experiment I remains fascinating even though experiment III did not yield further clues to its explanation. Specifically, the non-linearity between the time to and size at metamorphosis observed most strongly in the high density non-drying tanks of experiment I are at issue. The relationship between the two variables changes from longer times meaning smaller sizes (at first) to a positive relationship where increasing times lead to larger sizes. This was initially interpreted in terms of the possible effect of changing densities in the non-drying tanks as metamorphosed animals were removed (see figure 4.3). As density decreased and crowding stress was reduced, the relationship between time and mass at metamorphosis was altered from a negative to a positive one. It was hypothesized that this effect was not observed as strongly in the drying tanks because the control of density was

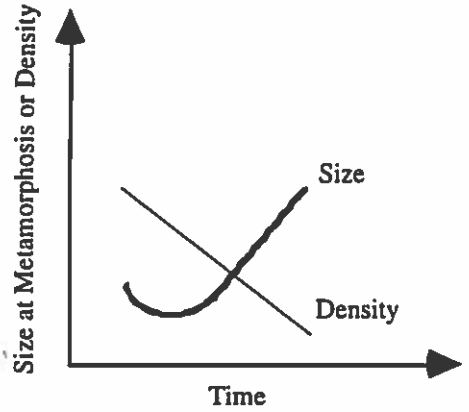

Figure 4.3: Schematic of hypothesized relationship between time and size at metamorphosis mediated by density changes

possible by removing water but ceasing to remove tadpoles once metamorphic activity became extensive. This would lead to the numbers in the drying tanks remaining higher than in the non-drying tanks for longer. The fact that no non-linearity was observed in the low density tanks was initially believed to be due to the fact that these tanks already started at lower numbers, hence only the positive portion of the relationship was evident in the results. The non-linearity may be viewed, in summary, as the effect of the slow release of the remaining tadpoles from conditions of high crowding. This may have been responsible for the change in the relationship between these two variables, a situation of "ecological release" for the remaining tadpoles.

Ecological release generally refers to changing ecological conditions with the effect of releasing a population from formerly constraining pressures. In this case, the decrease in density is believed to have allowed for increased times to and sizes at metamorphosis, a change which would not have occurred if density had remained at its original level. Such situations are sometimes observed in the field (see Mueller, 1994 for an example), usually in association with the introduction of exotic species to different habitats. It is possible that a careful investigation of the responses of tadpoles in temporary ponds to lowering densities might provide an opportunity to observe a more natural occurrence. Changes in numbers of tadpoles in the tanks was the main interpretational tool for understanding the patterns of metamorphosis seen in experiment I, but there are other relevant issues.

The effects and causes of late metamorphosis may not be a straight-forward issue of changing densities. The evolutionary history of the species plays importantly in its response to remaining as a tadpole for an extended period. For this reason, there may be population-specific genetic and evolutionary reasons for the observed response. Such effects are not the central concern of this study and were minimized by mixing sibs and using the same individuals for breeding both times (see experimental methods). The fact remains that the effect of time was found to be important independent of density changes in the regression models presented in the results. The reason for this observation may lie in non-environmental factors. Wilbur and Collins (1973) and others give some attention to physiological processes that may determine the pattern of growth (and metamorphosis) over time, and these are given some attention in box 1 of the results. It is likely that the age of a tadpole will have an important role to play in its metamorphic traits in conjunction with the environment surrounding the individual. Hensley (1993) describes the loss of phenotypic plasticity over time in tadpoles. This implies that an individual tadpole's ability to respond to its environment may be affected by its age, and he then connects this observation to the Wilbur-Collins model, pointing out that responses to changing growth rates may be dependent on the developmental stage of the tadpole. Such issues are

peripheral to the main focus of this study, but illustrate that many non-environmental evolutionary factors remain unresolved with respect to metamorphic characteristics.

The incredibly reduced length of the metamorphic period in experiment III did not allow the detection of any pattern between time to and mass at metamorphosis. It was expected that the high density tanks would display significant non-linearity and with a turning point in the curve later than that observed for the medium density tanks, since it would take longer for the densities to reach the level where length of larval period and size at metamorphosis became positively related (see figure 4.3). The low density tanks in experiment III were expected to show a purely linear relationship, since the starting density was already below the level at which positive correlations were observed. In most cases, however, the pulse-like nature of the metamorphic activity compressed the variation of the length of larval period to such an extent as to make any effect of one variable on the other undetectable (figure 4.4, overleaf). Note, however, that the effect of density on mass and on time to metamorphosis in experiment III was as expected, with higher densities producing smaller metamorphs at a later time.

The reduction of the length of metamorphic period in experiment III may have reduced the amount of time that changes in density (or more likely, number of tadpoles) would have been able to affect the mass at metamorphosis. Indeed, the time during which tadpoles metamorphosed out of tanks in experiment I was about four times that of experiment III, as shown in figure 4.4, overleaf. While this might explain why the non-linearity was not observed, it does not give an explanation for why the period of metamorphic activity was so abridged.

There are not many likely explanations for the difference in duration of metamorphic activity between the two experiments. The argument about the difficulty of keeping all tadpoles fed *ad libitum* and the potential variation in the amount of boiled spinach available per capita in tanks with more animals gains some strength. This is because experiment III had only 28 tanks in the design, while experiment I included 48 tanks. It is likely that the amount of food per tadpole was higher for the animals in experiment III than for those in the first experiment, and they suffered fewer hours with exhausted food supplies. Such a difference in feeding was minor, but may have been responsible for the large difference in metamorphic duration, indicating that there may be an important interaction between metamorphic period and food level. Once more, chemical effects are not likely because of the absence of a density effect in experiment III. The food level per individual problem, compounded by possible behavioral interferences in tanks with more animals must have played the most important role.

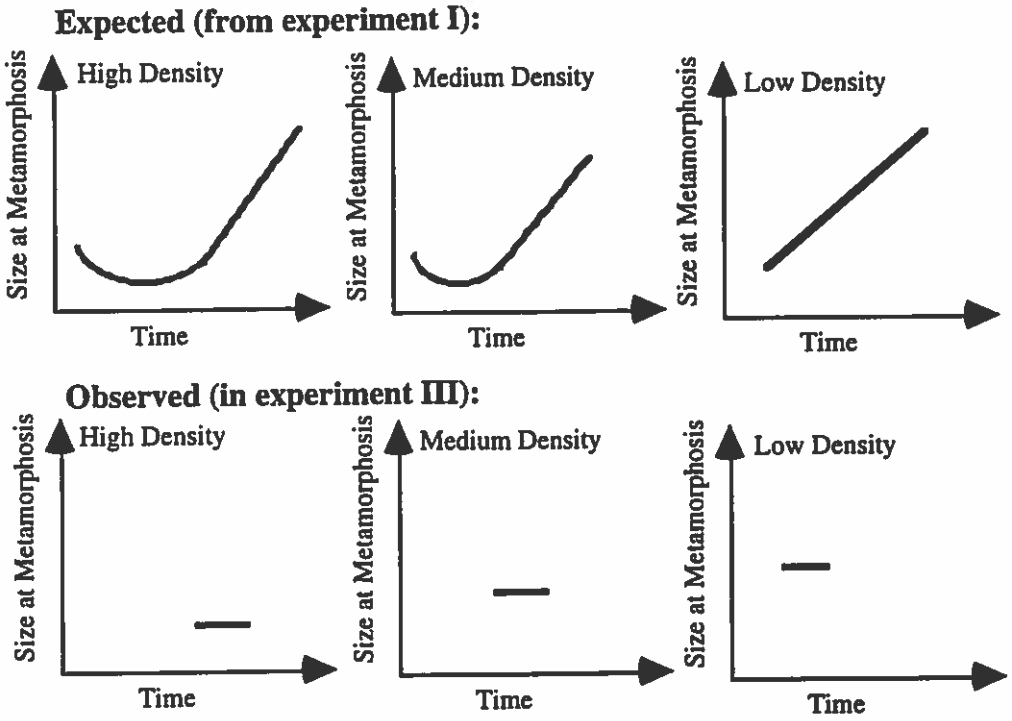

Figure 4.4: Expected and observed relationships between time and size at metamorphosis in experiment III. The expectations are based on release of the relationship between time and size from constraints of higher density

If a small difference in the ability to keep the animals fed all the times together with competition was capable of producing such a marked effect, food levels should be more carefully regulated in the future on a per capita basis. Certainly the potential interaction between food levels and metamorphic activity should be further investigated in this sort of laboratory set up because it may give answers about the intraspecific competition tadpoles may experience for food. Also, such an experiment might determine if the non-linearity observed between time and mass at metamorphosis is really a product of crowding and if it is mediated by food levels.

Evolution and Ecological Dynamics

What remains is to interpret the experimental results of this thesis in terms of ecological situations in the field and to apply this understanding to reach an insight into evolutionary issues. Both the length of larval period and the size at metamorphosis of amphibians have been shown to be consequential to the success of the individual, as argued in the introduction. This experiment was set up to minimize the effect of non-experimental

variation on the response of these two variables, by carrying the protocol out under controlled laboratory conditions.

The experiments revealed that under optimal conditions tadpoles were maximizing the size at metamorphosis and minimizing the length of the larval period. In this case, the only experimental variable which had a consistent and significantly strong effect was density, therefore the above conclusion is derived from the metamorphic response of tadpoles under "lower density" conditions. It became apparent that density per unit volume was not the actual determinant of stress in a tank, but rather the number of animals in each tank. The reason for this is probably competition and behavioral interference for food, as described in the preceding section. This does not invalidate the conclusion that under lower stress conditions tadpoles responded with shorter larval periods and larger sizes at metamorphosis.

Whatever the reason for the observed importance of the number of tadpoles in each tank, interpreting higher numbers of animals as inferior environments puts the results in good agreement with the Wilbur-Collins model. Superior environments would lead to higher growth rates (as observed in the growth data for tanks with less tadpoles in them). These higher growth rates would yield larger sizes at metamorphosis, as observed. Also, if the growth rate difference between a high and low density tanks was large enough, the low density tanks would produce metamorphs earlier since they would reach the metamorphic threshold earlier and possibly also approach the maximum size for metamorphosis. Thus, the results of this experiment are consistent with, and interpretable though, the Wilbur-Collins model.

Determining the effects of hydroperiod independent of density changes was one of the principle objectives of this work. Specifically, it was possible that the effects of hydroperiod were to a large degree mediated by ensuing increases in density, as hypothesized by Semlistch (1987) and others. In this series of experiments, the effects of hydroperiod were trivial given control of density. The effects of hydroperiod were also non-existent under increasing density conditions (experiment II). In some sense, the effects of density may be said to have never been truly tested because of the extreme simplicity of lab conditions, where crowding was not at all related to the number of individuals per unit volume. On the same token, however, hydroperiod was not a significant environmental factor given total control of all other variables. The actual drying of the environment seems to have no effect on the growth or development of larvae of this species. The effects of hydroperiod in the field are very likely mediated by changes in other variables which are associated with decreases in volume. This fact also holds true for the test of the habitat size experimental variable.

Habitat size did not have a strong effect on the growth or development of the larvae. Pearman argued that the basis for his observation of the importance of habitat size lay in the utilization of different parts of the niche in variable ways inter- specifically (Pearman, 1993) and in differences in the ecological community supported by different ponds, especially with regard to predator densities (Pearman, 1995). In this case, the variable he observed was not tested. The controlled conditions of the laboratory made ecological considerations such as these non existent, to the point where it appears that competition for boiled spinach was responsible for most of the observed results.

The simplification of complex ecological conditions to single factors appears to have defeated its own purpose to some extent. This study demonstrates that changes in volume by drying and changes in habitat size do not have effects on growth and development of the larvae of *Bombina orientalis* in isolation of all other environmental changes which accompany them. Whether or not the effects of drying are mediated by increases in density particularly cannot be ascertained from these experiments, though increasing density remains a likely candidate along with several other variables which cause increased environmental stress in the field. The limitations of testing ecological variables in the absence of all other factors are thus revealed.

As often is the case, the role of each part alone is not equivalent to their roles in the total. If a hypothetical investigator was to test a number of environmental variables in to the complete exclusion of all others, he or she might conclude that hydroperiod is not important, and thus should not be observed. In order to gain full understanding, however, the interaction and interdependence of certain variables must be accounted for. In this case, it is clear that hydroperiod must act by affecting some other environmental variable through the decrease in water volume. Increasing density stress, changing temperature or the ecological community are potential factors that merit further attention. In this light, the drying of the pond is very important, despite its triviality on its own. Hydroperiod and habitat size are important because of the dependence of other variables on them.

Unfortunately, this renders the issues a good deal more complex. What should be realized is that the nature of the aquatic habitat of larval amphibians must remain at the forefront of the tadpole ecologist's research. In addition to the issues of interaction described above, the dimension of the dynamic nature of the habitat must be considered. It is clear that many environmental factors have effects on the metamorphosis of tadpoles to froglets, but the very fact that metamorphosis in anurans entails a radical niche shift necessarily implies that the environment will be changed by the metamorphic response of the animals to the environment. An illustrative scenario, relevant to this study's topic, is helpful. As a pond dries due to low rainfall, the growth rate of tadpoles within the pond is

decreased because of increased density stress. This produces a response: tadpoles metamorphose earlier. This is not the end of the effect of drying, however. Due to the early metamorphosis of those tadpoles responding to stress, the remaining tadpoles begin to experience lower strain, a situation of ecological release. These remaining larvae are then free to grow for a longer time, at a higher rate, to a larger size.

The issue of environmental change over time directly affects the interpretation of the results of the length of metamorphic period of experiment I. As argued in a preceding section, the extended amount of time during which metamorphosis was observed in experiment I appears to have been responsible for significant changes in the conditions in those tanks. Even under the sterile conditions of glass aquariums, it appears that the decrease in the number of tadpoles had the effect of changing the metamorphic size of the remaining tadpoles in the high density treatment.

Much of the wide degree of variation in metamorphic characteristics of populations in the field can be understood as being due to differences between ponds and the dynamic nature of habitats. The extreme variability across ponds and within ponds over time itself serve as a refutation of the principles of optimality. Given the extreme amount of variation in conditions an ideal metamorphic character for an individual becomes impossible, in addition to the issues of evolutionary constrains mentioned above. Also, the reduction in variation of metamorphic traits becomes undesirable in terms of individual fitness. As eloquently argued elsewhere (see Kaplan and Cooper, 1984), the best response to high environmental variability may be variability itself.

Arguments for the importance of developmental plasticity are thus reinforced by the results of this research which demonstrates that environmental conditions will affect metamorphic character in complex and interacting ways. Variation in habitat conditions both across ponds and within a single pond over time due to its dynamic nature have been shown to have the potential to produce extreme and consequential heterogeneity. In this mosaic of environmental conditions plasticity and variation is the expected outcome, and it is clear here that there is a sound ecological reason for the evolution of such plasticity. The mode of action of many environmental variables is clearly not well understood as these results show, none of this changes the fact that strong responses to them are apparent and have significant evolutionary implications.

Conclusion

The variation and interaction of environmental and ecological conditions across ponds and over time must be considered when attempting to understand the evolutionary processes responsible for the existence of metamorphosis. This study showed that the effects of hydroperiod which have been extensively documented in the field are very likely to be largely mediated by associated change in other variables with drying. This is a good indication that the high degree of variation in results of ecological studies on metamorphosis in the field must be due to the many permutations of relevant environmental conditions. A view of how dynamic change in a system may have unexpected effects was afforded by the extended duration of metamorphic activity in experiment I and the likely change in the relationship between time and mass at metamorphosis due to decreasing numbers of animals in the tanks.

These results serve to highlight that maintenance of variation in metamorphic traits may be effected by variation in environmental conditions. Plasticity in these traits is thus revealed to be potentially an adaptive state for populations of larvae in the field. Other evolutionary issues regarding the debate over the fixation of developmental rates by endocrine or physiological processes are briefly addressed, and serve as a reminder that there are other important non-environmental causes and effects of plasticity.

Under the controlled conditions of this experiment none of the ecologically important variables tested here acted exactly as expected. While all the above conclusions are supported by the results of a highly contrived lab situation, important issues surrounding this and other such tests should receive attention. First, it can not be said that the designs executed here represent a faithful reconstruction of the conditions of the field. Second, it gives reason for pause when imagining that conclusions derived from tests of isolated variables in the lab will always faithfully represent the effects of those variables in a more complex situation. As long as the limitations of lab studies are well known and remembered, then their results may be used to tentatively interpret observations of the more complex field situation. This exercise should eventually lead to a better understanding of the interactions and variations which are most relevant to the situation of tadpoles in real ponds. If such principles are well discerned, the evolutionary processes that shape the existence of metamorphosis will be further illuminated. It is my hope that this study showed how the many complexities of complex life cycles might be addressed and that it may contribute to an ever expanding literature on the subject. Full comprehension of this important area may have implications on our understanding of plasticity and the evolutionary processes that shape it.

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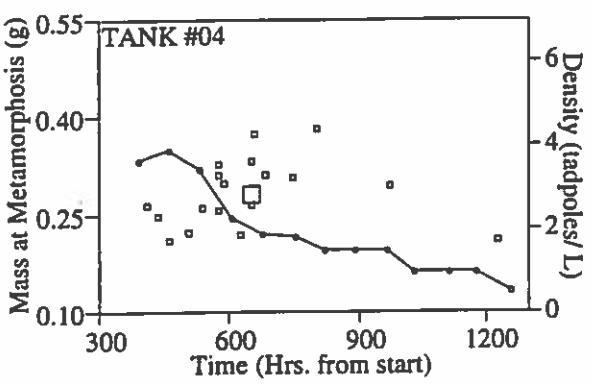
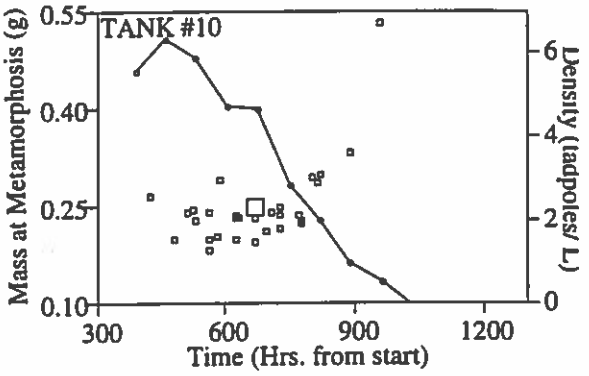
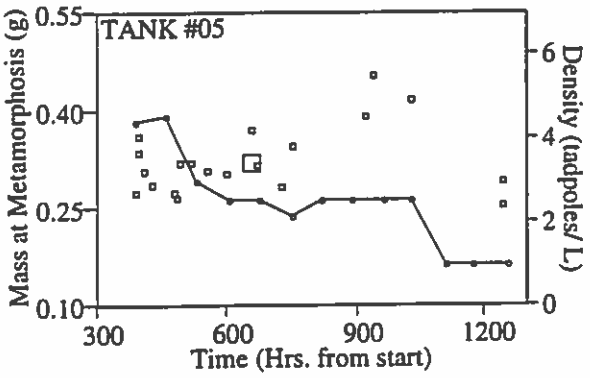
APPENDIX A

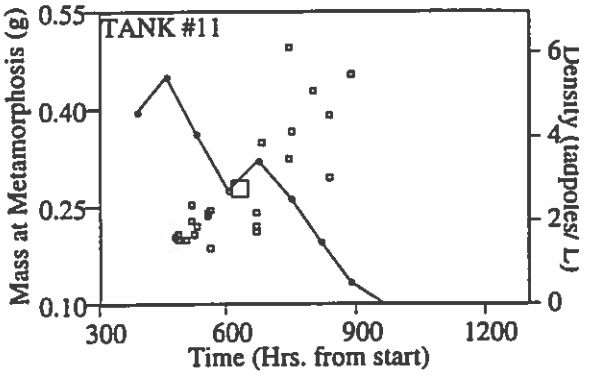
The sacttergrams of time versus mass at metamorphosis for each tank in each of the eight experimental treatment combinations in experiment I are presented below. Each also has the estimated density plotted over time, for comparison of the pattern between the two relationships. Treatments are arranged from the the expected most to least harsh combinations. The decrease in density associated with change in metamorphic traits is especially notable in the high density, non-drying tanks.

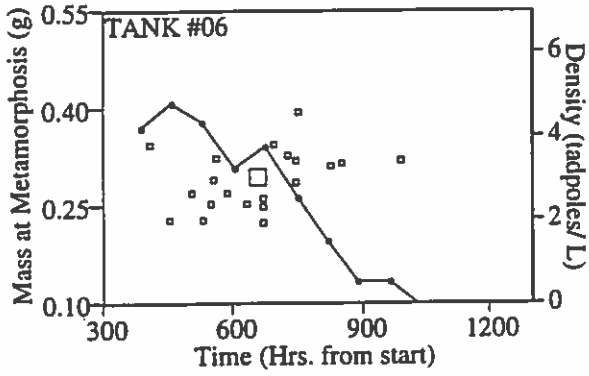
High Density, Drying, Small Habitat

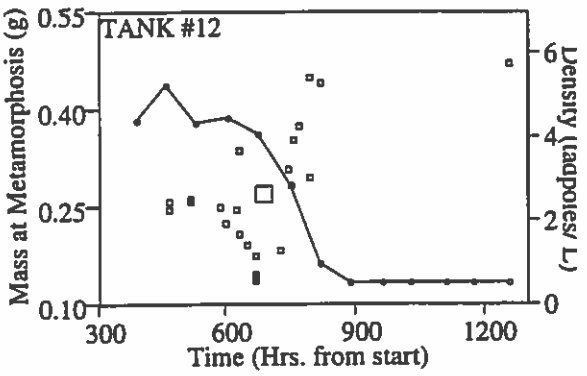










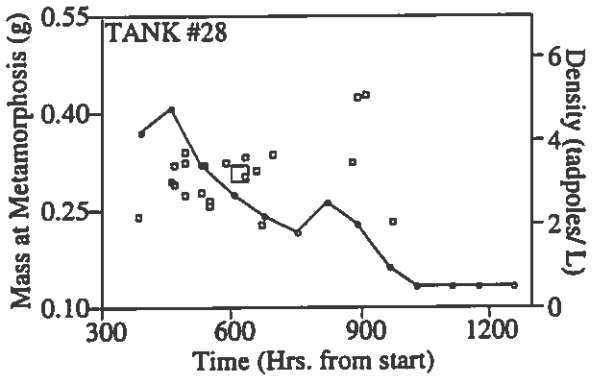


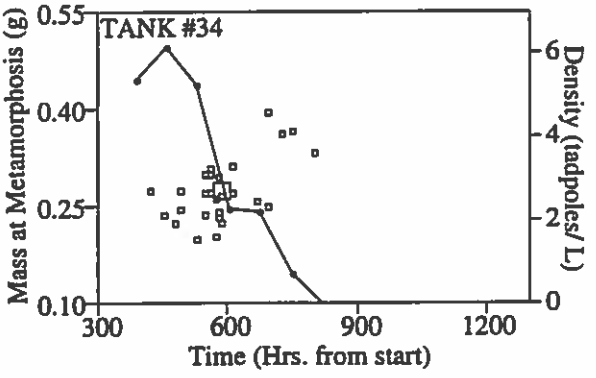
BLOCK A

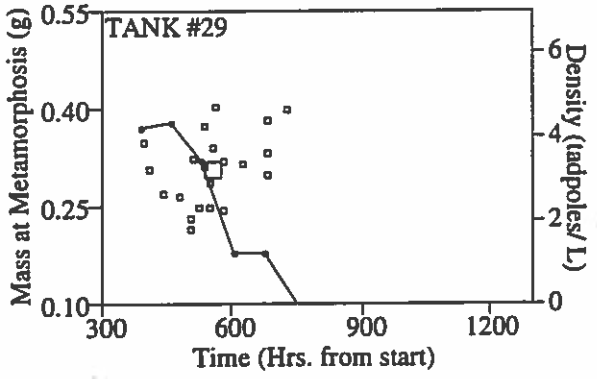
BLOCK B

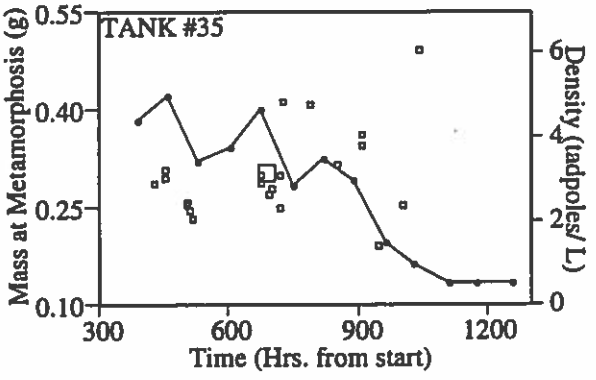
KEY:
- Individual Tadpoles
- Mean Value for Tank
- Estimated Density

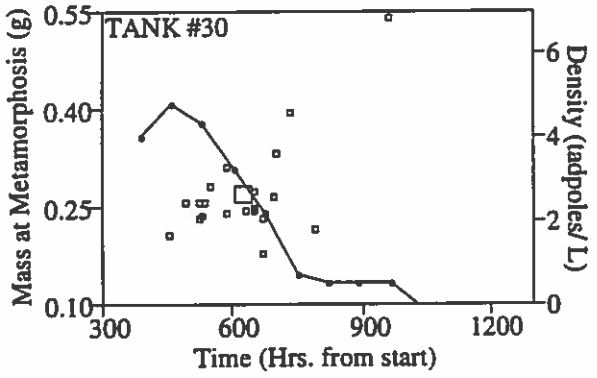
High Density, Drying, Large Habitat

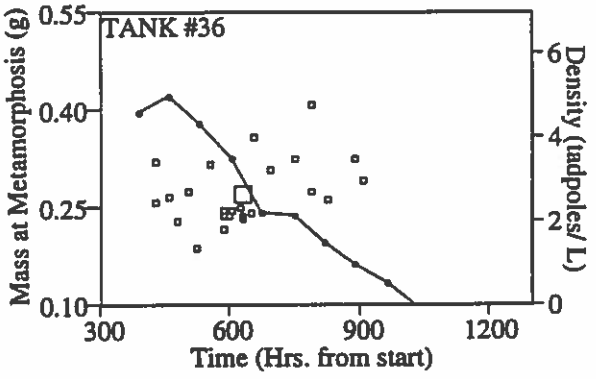










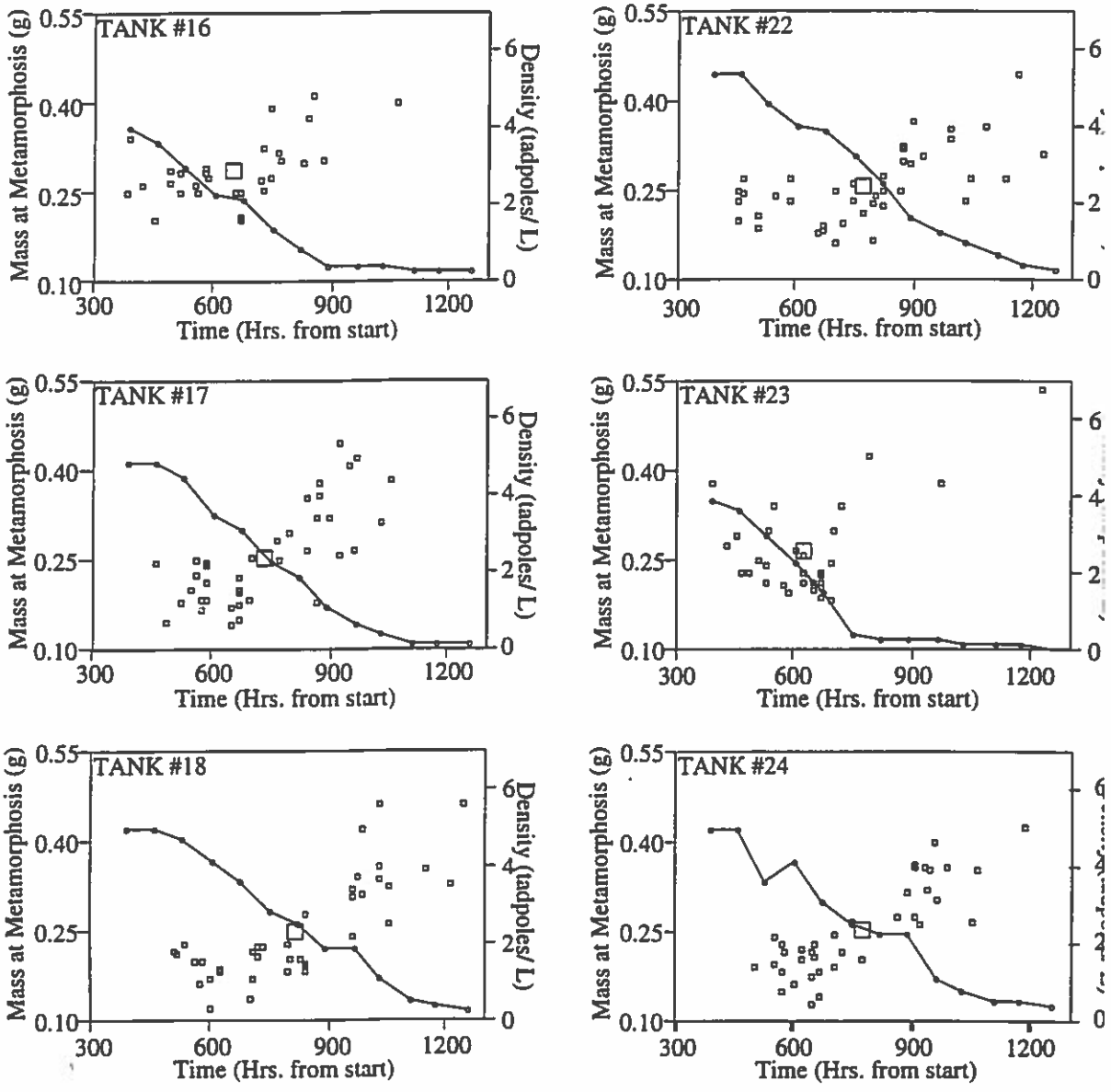


BLOCK A

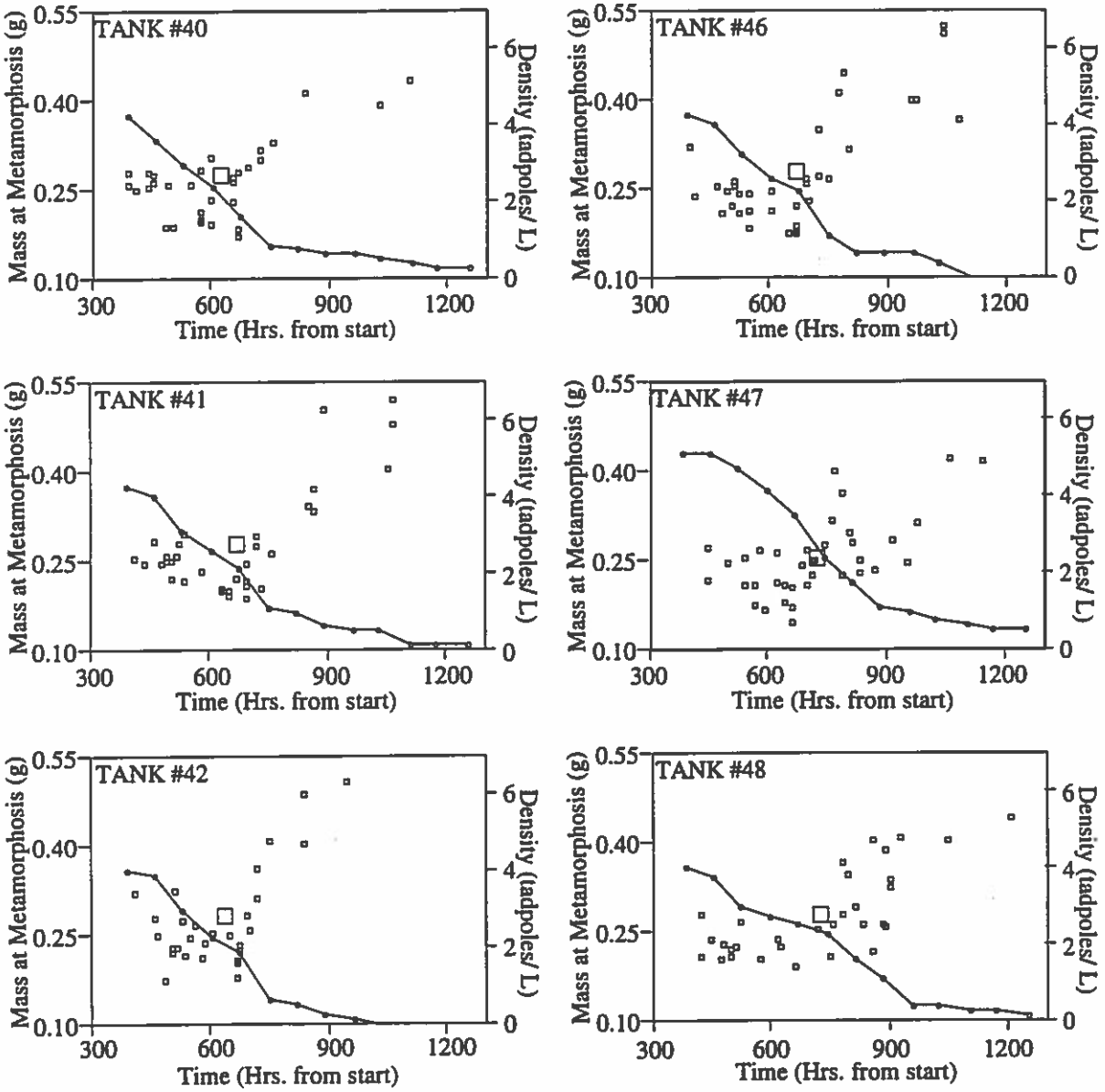
BLOCK B

KEY:
- Individual Tadpoles
- Mean Value for Tank
- Estimated Density

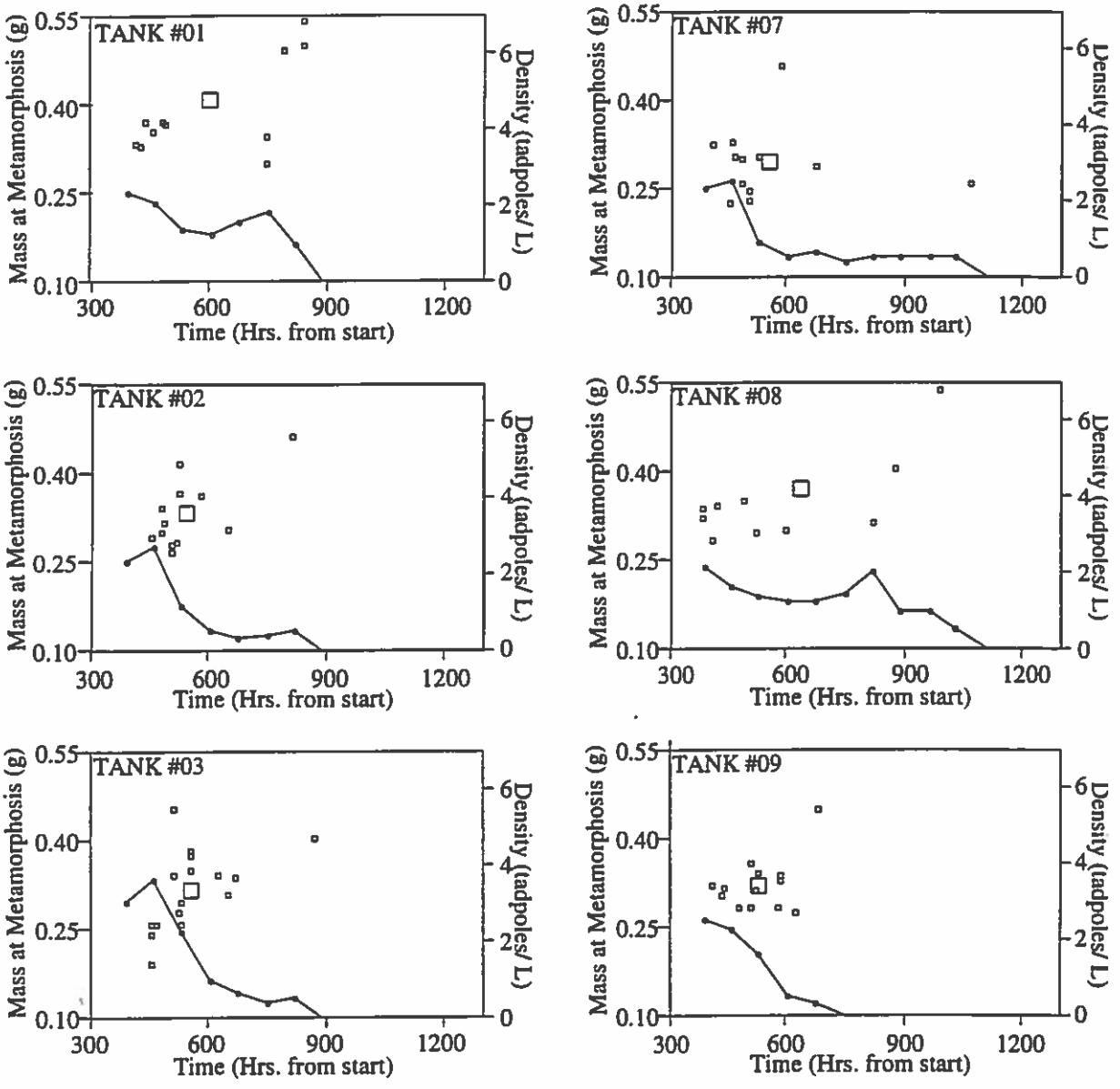
High Density, Non-Drying, Small Habitat



High Density, Non-Drying, Large Habitat



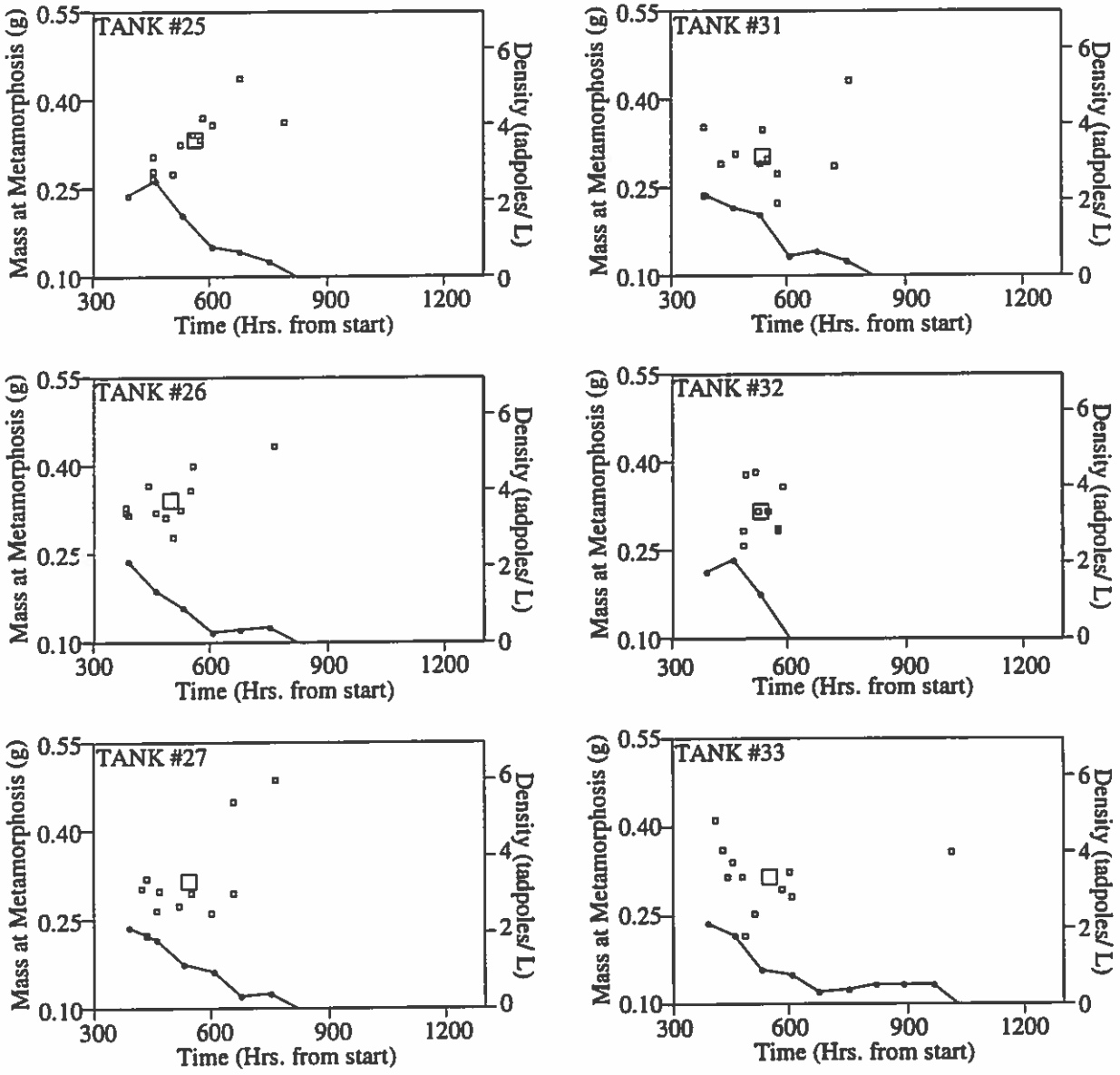
Low Density, Drying, Small Habitat



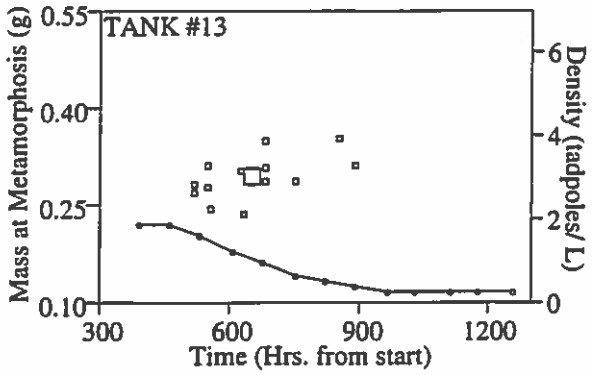
BLOCK A BLOCK B

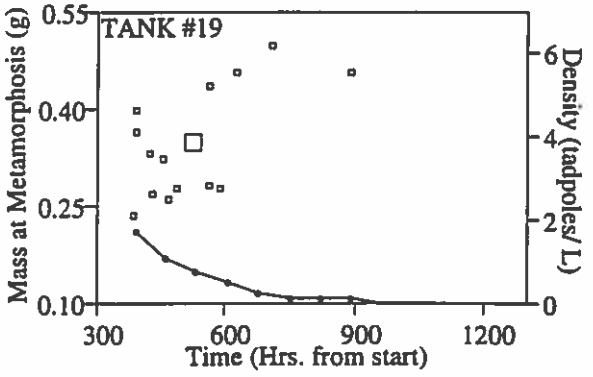
KEY:
- Individual Tadpoles
- Mean Value for Tank
- Estimated Density

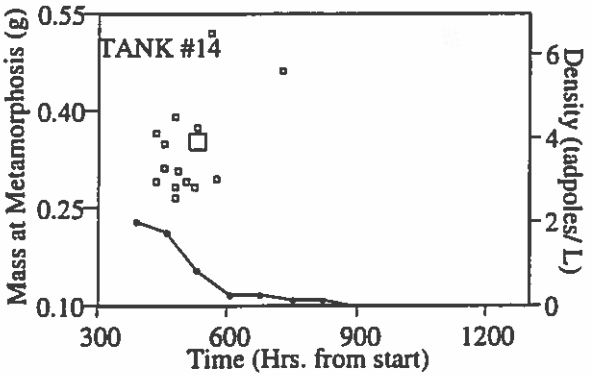
Low Density, Drying, Large Habitat

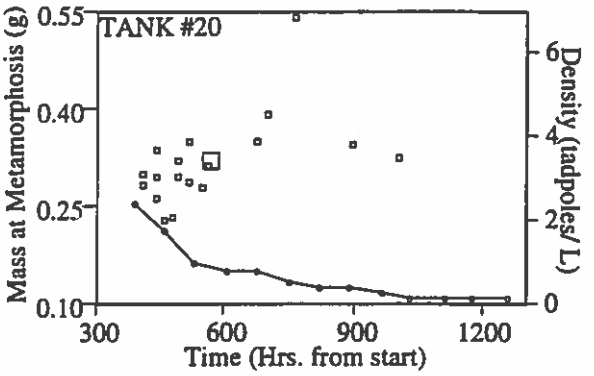


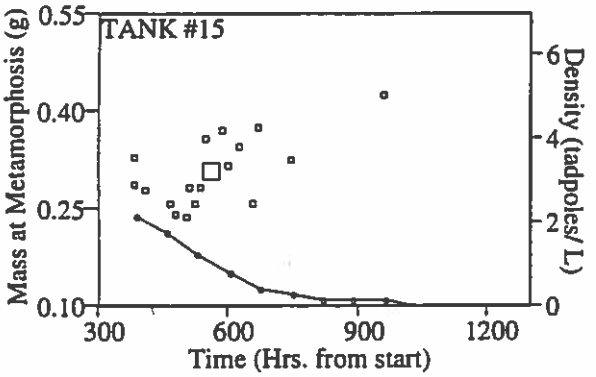
Low Density, Non-Drying, Small Habitat

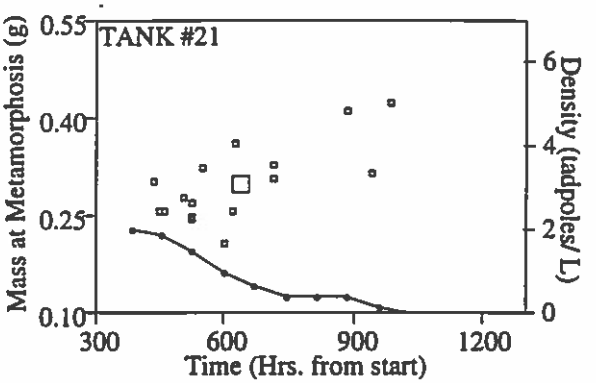










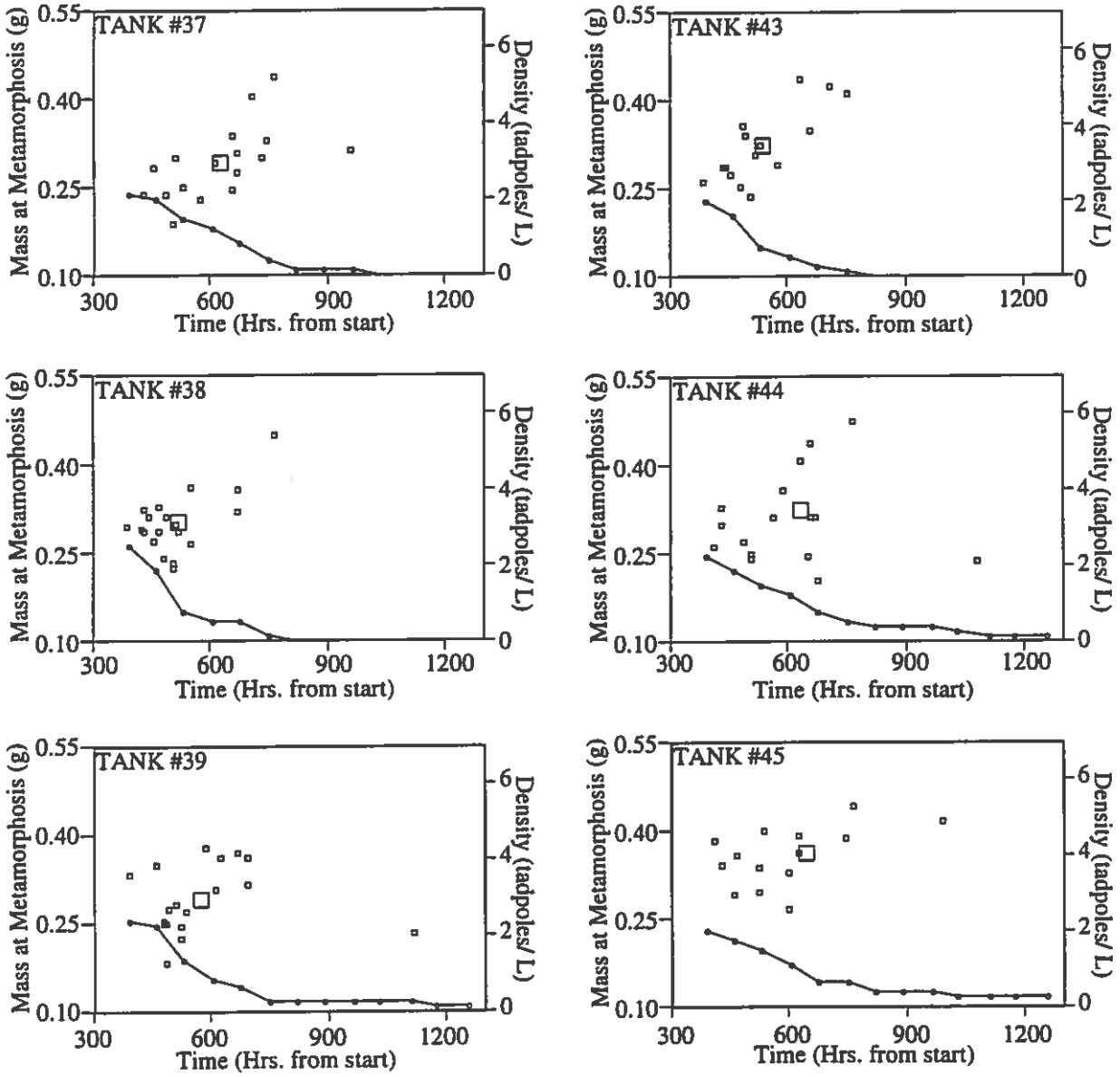


BLOCK A

BLOCK B

KEY:
- Individual Tadpoles
- Mean Value for Tank
- Estimated Density

Low Density, Non-Drying, Large Habitat



BLOCK A

BLOCK B

KEY:
- Individual Tadpoles
- Mean Value for Tank
- Estimated Density

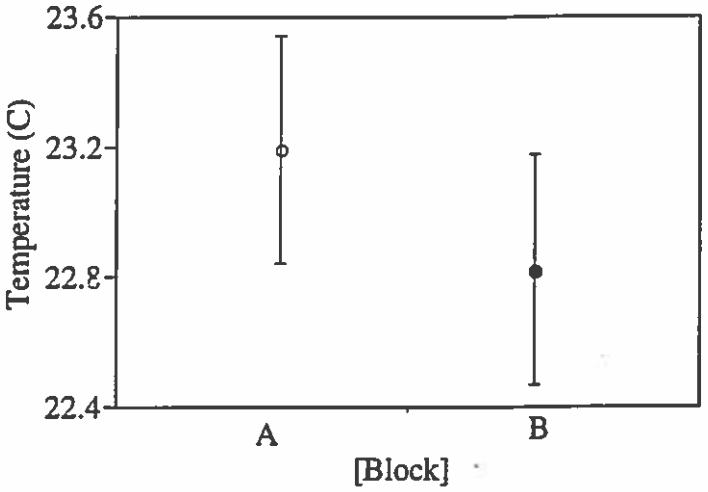
APPENDIX B

Temperature variation across the space of the lab was monitored in all three experiments. For experiment I atmospheric temperature was recorded at regular intervals in two points of block A and two in block B. These data were analysed with respect to block using an ANOVA. For experiments II and III the water temperature was measured in each tank three times during the duration of the experiment. This allowed for the direct comparison of the effects of temperature on mass and time at metamorphosis though linear regression. There were no significant trends in effects of temperature differences in any of the experiments.

EXPERIMENT I

ANOVA for the variation in temperature across blocks.

Factor:	d.f.	S.S	M.S	F-Value	P-Value
Block	1	1.650	1.650	2.366	0.1308
Residual	46	32.078	0.697		
TOTAL	*47*	*33.728*			



Plot of main effect of block on temperature

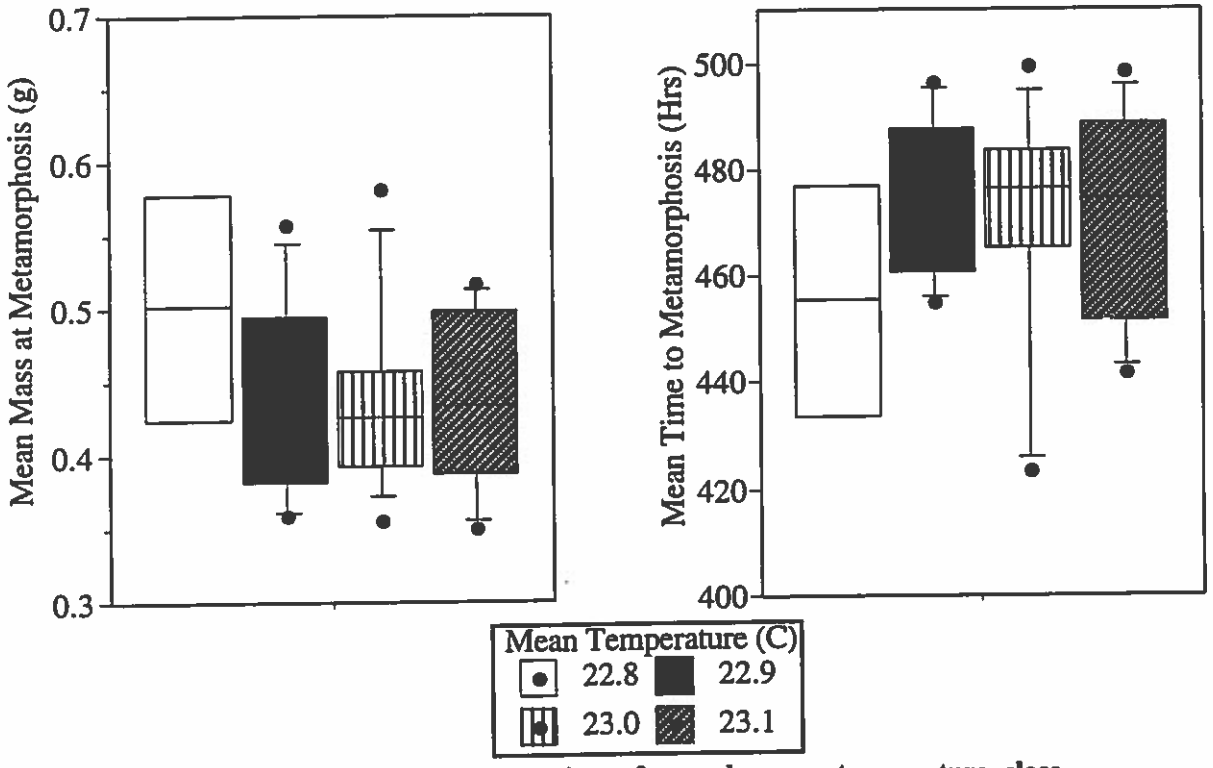
EXPERIMENTS II AND III

Temperature~Mass at Metamorphosis

Factor	Coeffiecient	St. Error	Std. Coeff.	t-Value	P-Value
Temperature	-0.140	0.142	-0.190	-0.986	0.3330
Intercept	3.662	3.260	3.662	1.123	0.2716

Temperature~Time to Metamorphosis

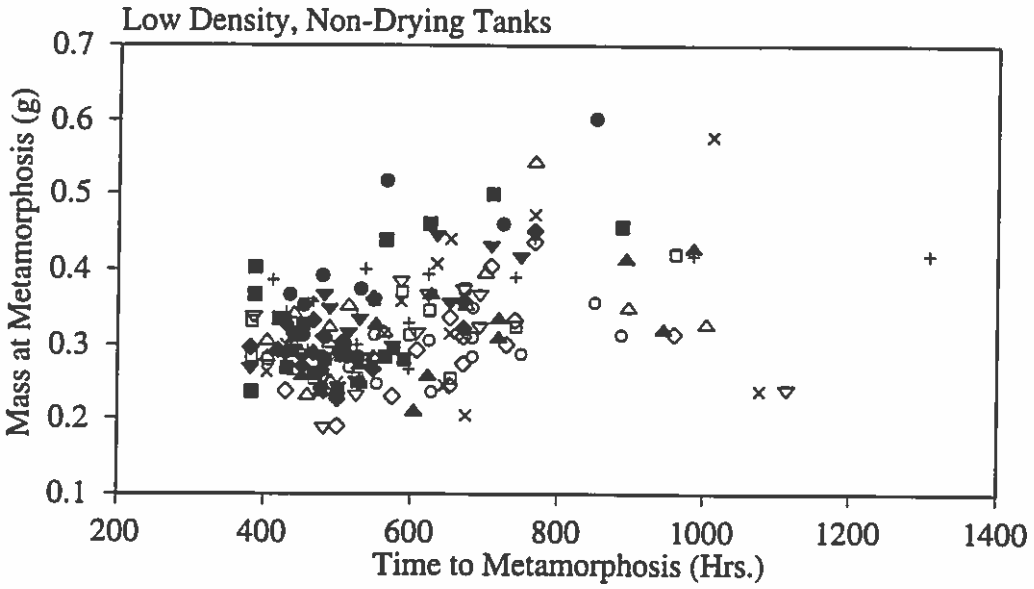
Factor	Coeffiecient	St. Error	Std. Coeff.	t-Value	P-Value
Temperature	17.728	44.545	0.078	0.398	0.6939
Intercept	62.624	1024.057	62.624	0.061	0.9517

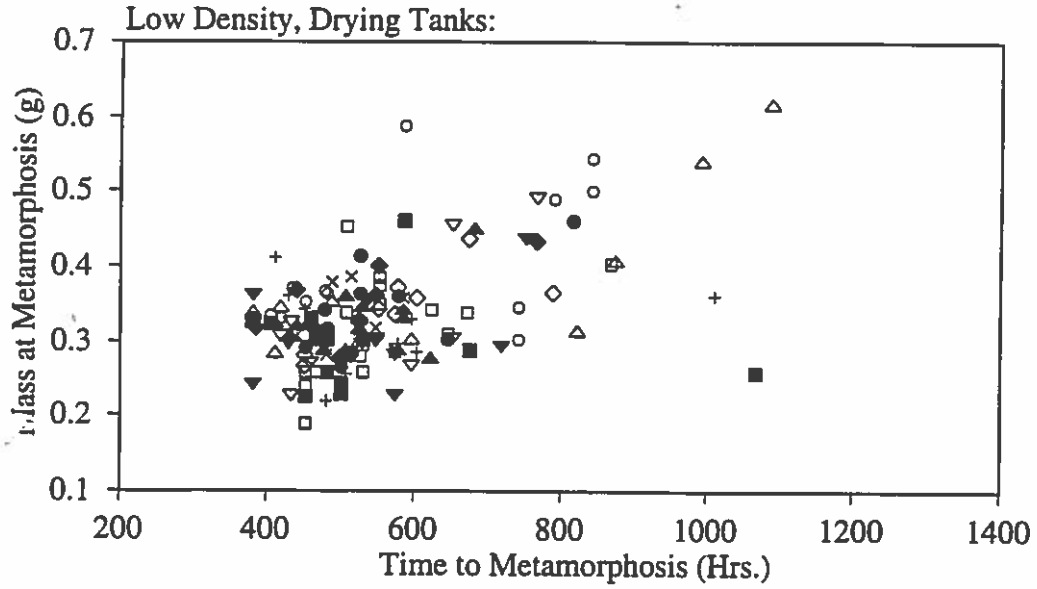


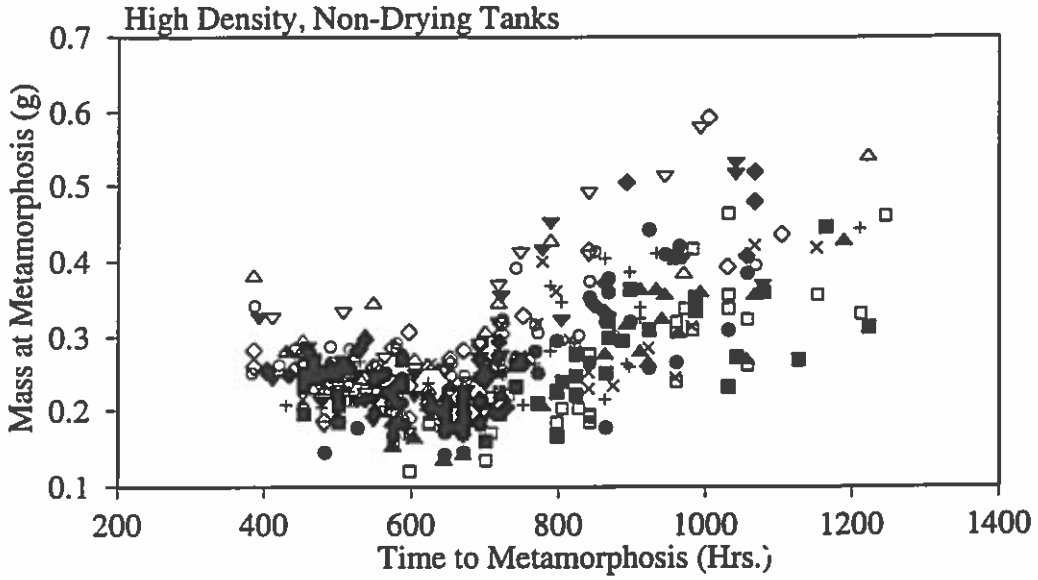
Spread of mean masses and mean times for each mean temperature class

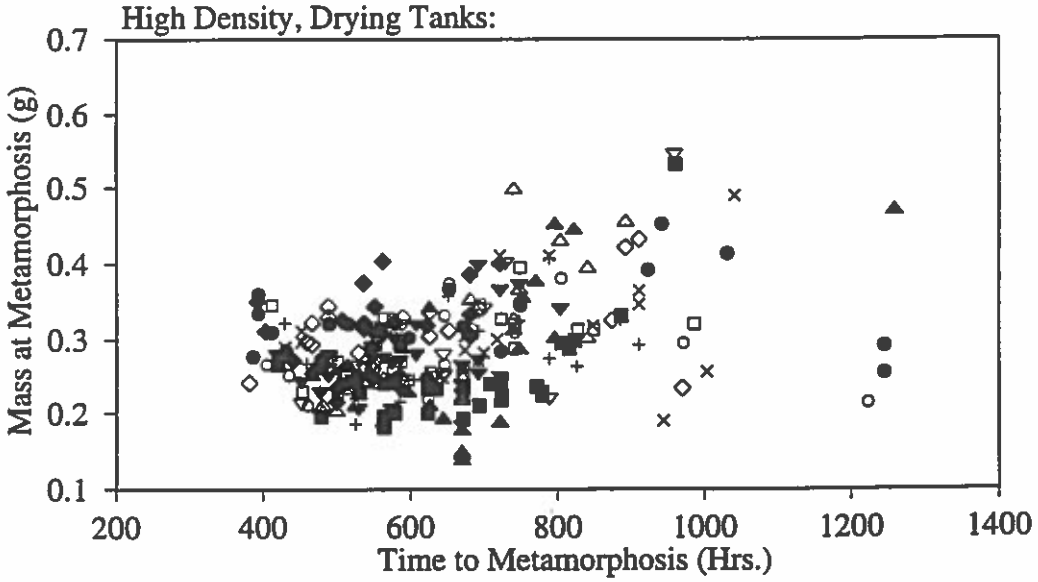
APPENDIX C

Scattergrams of time against mass at metamorphosis are presented below. The points are split by tank, for a qualitative comparison of the spread of individual points within the distribution of all the data for that treatment combination. The symbols in the graphs are not keyed for precise identification of each tank, since they are meant for comparing the spread of each tank only.


Low Density, Non-Drying Tanks
Mass at Metamorphosis (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
200
400
600
800
1000
1200
1400
Time to Metamorphosis (Hrs.)


Low Density, Drying Tanks:
Mass at Metamorphosis (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
200
400
600
800
1000
1200
1400
Time to Metamorphosis (Hrs.)


High Density, Non-Drying Tanks
Mass at Metamorphosis (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
200
400
600
800
1000
1200
1400
Time to Metamorphosis (Hrs.)


High Density, Drying Tanks:
Mass at Metamorphosis (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
200
400
600
800
1000
1200
1400
Time to Metamorphosis (Hrs.)

APPENDIX D

Ten ANOVA tables for the effects and interactions of the main experimental variables on snout-vent length (SVL) and tail length (TL) of the animals in experiment I prior to metamorphosis are shown below. The data from week 6 was not included due to vary small sample sizes that week, making confidence intervals very large. The three-way interaction plots are also shown. For plots of the main effects, please refer to the results section.

WEEK 1

Response=Mean SVL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	0.002	0.002	0.004	0.9531
Hydroperiod	1	0.326	0.326	0.551	0.4622
Habitat Size	1	0.087	0.087	0.147	0.7031
Density * Hydroperiod	1	0.550	0.550	0.930	0.3406
Density * Habitat Size	1	0.868	0.868	1.468	0.2328
Hydroperiod * Habitat Size	1	0.023	0.023	0.039	0.8447
Density * Hydroperiod * Habitat Size	1	0.139	0.139	0.236	0.6299
Residual	40	23.654	0.591		
TOTAL	*48*	*25.649*		R-Squared	= 0.078

Response=Mean TL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	0.319	0.319	0.345	0.5603
Hydroperiod	1	0.290	0.290	0.314	0.5784
Habitat Size	1	1.321	1.321	1.429	0.2390
Density * Hydroperiod	1	0.156	0.156	0.168	0.6838
Density * Habitat Size	1	1.521	1.521	1.644	0.2071
Hydroperiod * Habitat Size	1	0.063	0.063	0.068	0.7949
Density * Hydroperiod * Habitat Size	1	0.830	0.830	0.898	0.3490
Residual	40	36.986	0.925		
TOTAL	*48*	*41.486*		R-Squared	= 0.108

WEEK 2

Response=Mean SVL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	15.132	15.132	22.174	0.0001
Hydroperiod	1	1.266	1.266	1.854	0.1809
Habitat Size	1	1.314	1.314	1.926	0.1729
Density * Hydroperiod	1	0.320	0.320	0.468	0.4978
Density * Habitat Size	1	1.002	1.002	1.468	0.2328
Hydroperiod * Habitat Size	1	0.312	0.312	0.457	0.5032
Density * Hydroperiod * Habitat Size	1	0.023	0.023	0.034	0.8555
Residual	40	27.298	0.682		
TOTAL	*48*	*46.667*		R-Squared	= 0.415

Response=Mean TL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	19.258	19.258	11.459	0.0016
Hydroperiod	1	2.893	2.893	1.721	0.1970
Habitat Size	1	6.668	6.668	3.968	0.0532
Density * Hydroperiod	1	0.066	0.066	0.039	0.8444
Density * Habitat Size	1	0.159	0.159	0.095	0.7601
Hydroperiod * Habitat Size	1	0.036	0.036	0.021	0.8849
Density * Hydroperiod * Habitat Size	1	0.200	0.200	0.119	0.7319
Residual	40	67.225	1.681		
TOTAL	*48*	*96.505*		R-Squared	= 0.303

WEEK 3

Response=Mean SVL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	3.500	3.500	11.860	0.0021
Hydroperiod	1	0.466	0.466	1.579	0.2210
Habitat Size	1	0.086	0.086	0.292	0.5939
Density * Hydroperiod	1	0.166	0.166	0.563	0.4602
Density * Habitat Size	1	0.056	0.056	0.190	0.6670
Hydroperiod * Habitat Size	1	1.371E-5	1.371E-5	4.645E-5	0.9946
Density * Hydroperiod * Habitat Size	1	0.122	0.122	0.414	0.5263
Residual	24	7.083	0.295		
TOTAL	*31*	*11.479*		R-Squared	= 0.388

Response=Mean TL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	5.258	5.258	6.717	0.0160
Hydroperiod	1	0.824	0.824	1.052	0.3152
Habitat Size	1	3.421	3.421	4.370	0.0474
Density * Hydroperiod	1	0.079	.079	0.102	0.7528
Density * Habitat Size	1	0.306	.306	0.391	0.5379
Hydroperiod * Habitat Size	1	1.085E-4	1.085E-4	1.386E-4	0.9907
Density * Hydroperiod * Habitat Size	1	0.262	0.262	0.335	0.5681
Residual	24	18.788	0.783		
TOTAL	*31*	*28.938*		R-Squared = 0.363	

WEEK 4

Response=Mean SVL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	6.145	6.145	6.234	0.0167
Hydroperiod	1	1.629	1.629	1.652	0.2060
Habitat Size	1	0.407	0.407	0.413	0.5242
Density * Hydroperiod	1	1.702	1.702	1.727	0.1963
Density * Habitat Size	1	0.316	0.316	0.321	0.5743
Hydroperiod * Habitat Size	1	0.021	0.021	0.021	0.8860
Density * Hydroperiod * Habitat Size	1	0.097	0.097	0.098	0.7557
Residual	40	39.431	0.986		
TOTAL	*48*	*49.748*		R-Squared = 0.207	

Response=Mean TL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	2.788	2.788	0.887	0.3520
Hydroperiod	1	0.108	0.108	0.034	0.8542
Habitat Size	1	6.160	6.160	1.959	0.1693
Density * Hydroperiod	1	0.031	0.031	0.010	0.9208
Density * Habitat Size	1	0.006	0.006	0.002	0.9651
Hydroperiod * Habitat Size	1	0.523	0.523	0.167	0.6854
Density * Hydroperiod * Habitat Size	1	0.756	0.756	0.241	0.6264
Residual	40	125.743	3.144		
TOTAL	*48*	*136.115*		R-Squared = 0.363	

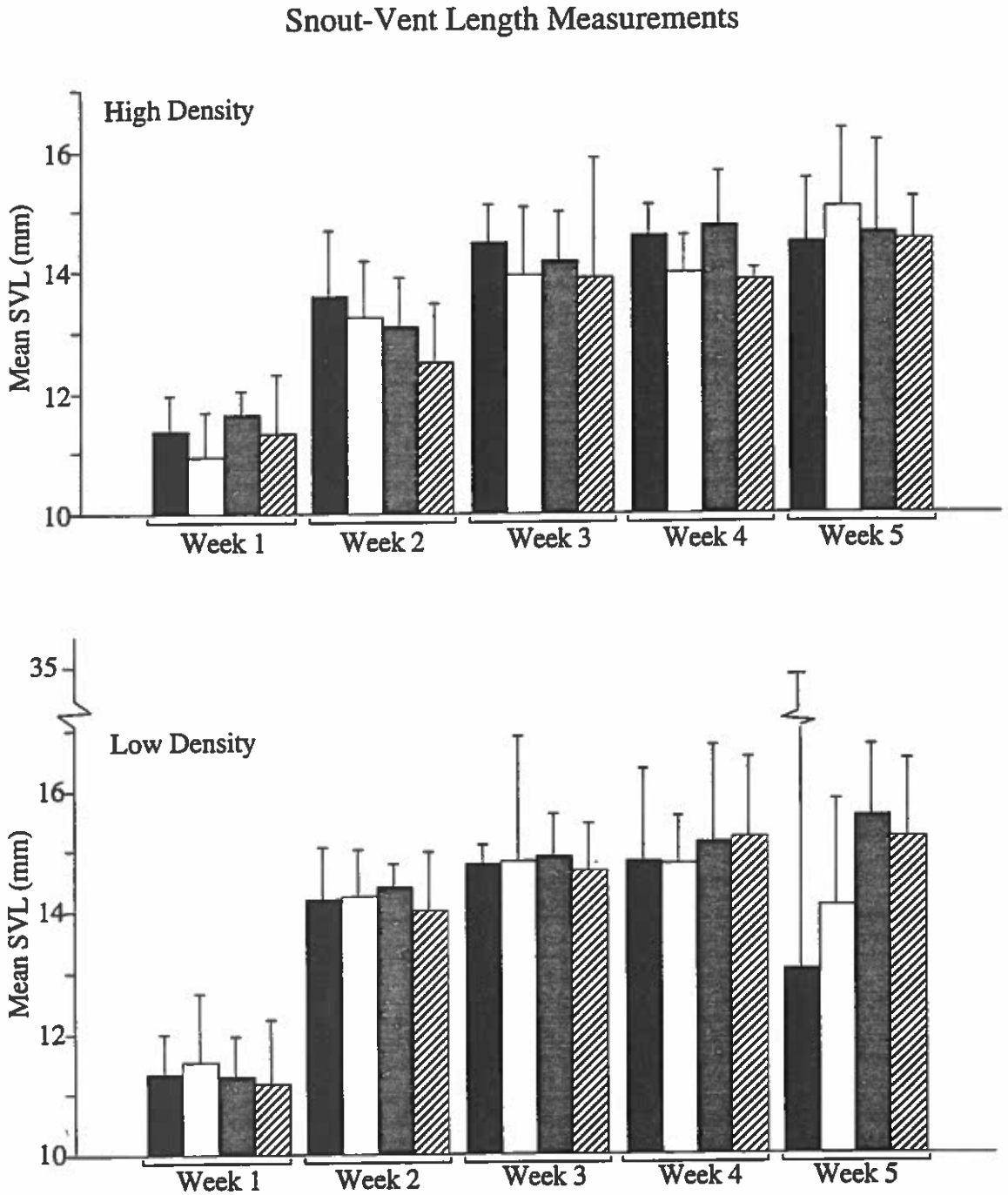
WEEK 5

Response=Mean SVL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	0.339	0.339	0.253	0.6184
Hydroperiod	1	0.716	0.716	0.535	0.4703
Habitat Size	1	0.020	0.020	0.015	0.9026
Density * Hydroperiod	1	5.487	5.487	4.099	0.0519
Density * Habitat Size	1	8.721	8.721	6.515	0.0160
Hydroperiod * Habitat Size	1	2.266	2.266	1.693	0.2031
Density * Hydroperiod * Habitat Size	1	0.297	0.297	0.222	0.6408
Residual	30	40.162	1.399		
TOTAL	*38*	*58.008*		R-Squared = 0.243	

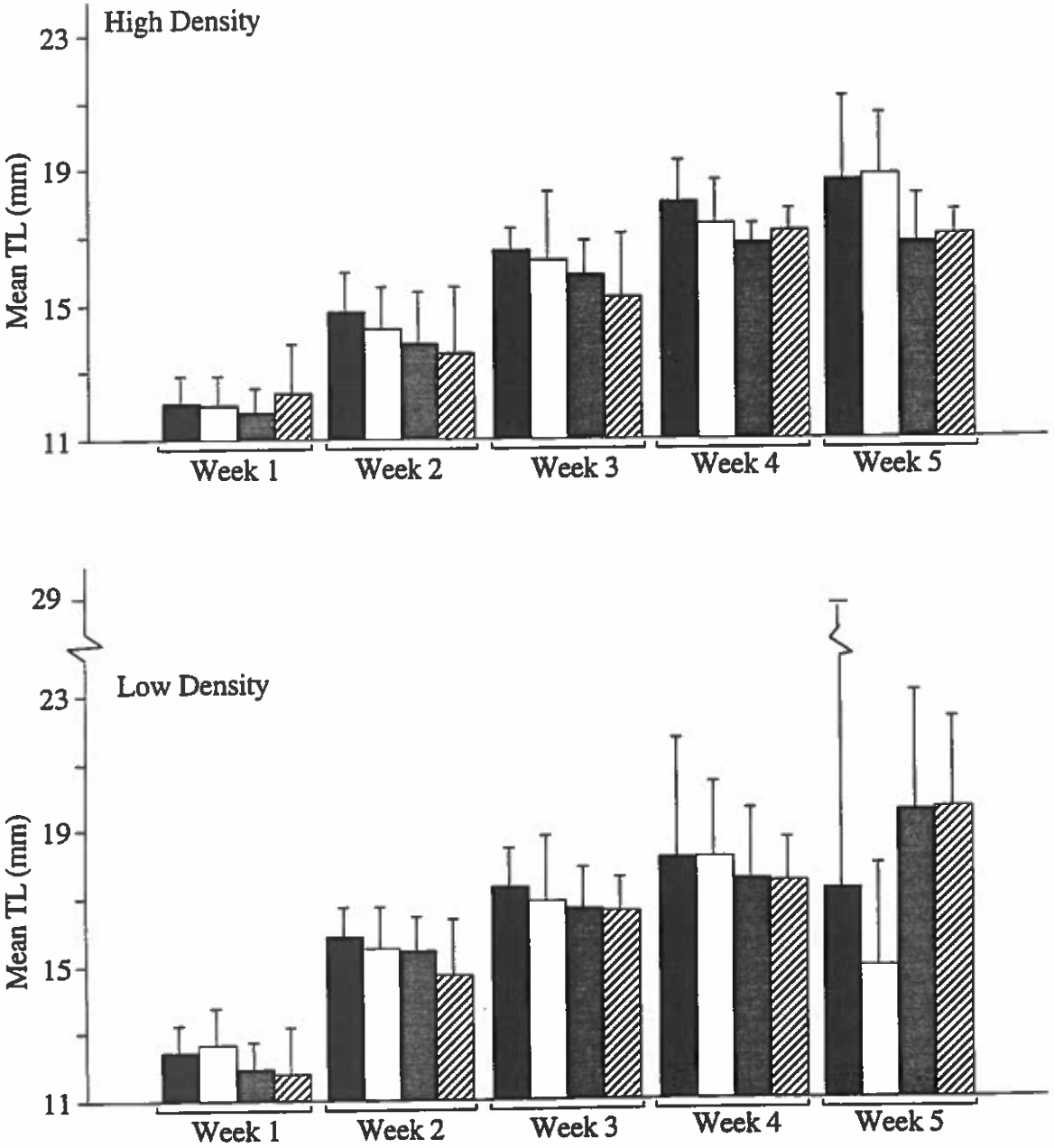
Response=Mean TL:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Density	1	0.006	0.006	0.002	0.9663
Hydroperiod	1	1.881	1.881	0.599	0.4450
Habitat Size	1	5.708	5.708	1.818	0.1877
Density * Hydroperiod	1	3.418	3.418	1.088	0.3052
Density * Habitat Size	1	58.845	58.845	18.738	0.0002
Hydroperiod * Habitat Size	1	3.135	3.135	0.998	0.3257
Density * Hydroperiod * Habitat Size	1	2.664	2.664	0.848	0.3643
Residual	30	94.213	3.140		
TOTAL	*38*	*169.870*		R-Squared = 0.363	



3-way interaction of the 5 weeks of growth data from experiment I (effects on snout-vent length)

Tail Length Measurements



3-way interaction of the 5 weeks of growth data from experiment I (effects on tail length)

APPENDIX E

The regression models presented below are nested analysis. Model 1 is the full models with density, time, hydroperiod and tank as predictors of mass at metamorphosis. Model 2 includes all those trems minus any that included time. By comparing the two models with an F-test (see results) the improtant role of time independant of density was confirmed. The coefficients in the models are not be interpreted individually; rather, it is the comparion of the fit of each of the models which is of interest.

Model 1: Full regression model for explaining mass at metamorphosis using time, density, time^2, density^2, hydroperiod and tank nested within hydroperiod and all their 2-way interactions. * indicates interaction. () indicates nesting. : indicates nested variable with respect to a continuous variable.

Model 1:

*Mass=Time*Hydroperiod(Tank)+Time*Density+Time^2*Hydroperiod(Tank)+Time^2*Density+ Density*Hydroperiod(Tank)+Density^2*Hydroperiod(Tank)+Density^2*Time+Density^2*Time^2*

	Value	Std. Error	t value	Pr(>|t|)
(Intercept)	0.2662	0.0068	39.2900	0.0000
Time	5.12e-05	5.12e-05	1.6190	0.1058
Hydroperiod	6.98e-07	0.0060	-6.0381	0.0000
Density	0.0487	0.0040	12.0602	0.0000
Time^2	6.98e-07	1.51e-07	4.6356	0.0000
Density^2	0.0097	0.0027	3.5420	0.0004
Drying(Tank)	-0.0007	0.0003	-2.2011	0.0280
Non-Drying (Tank)	-2.38e-05	0.0003	-0.0818	0.9348
Time*Hydroperiod	8.037e-05	3.01e-05	2.6669	0.0078
Time*Density	-3.00e-05	2.53e-05	-1.1860	0.2359
Time^2*Hydroperiod	2.48e-07	9.26e-08	2.6834	0.0074
Time^2*Density	-5.33e-07	1.09e-07	-4.8993	0.0000
Density*Hydroperiod	-6.06e-05	0.0039	-0.0154	0.9877
Density^2*Hydroperiod	0.0013	0.0024	0.5589	0.5764
Density^2*Time	-4.84e-05	1.26e-05	-3.8313	0.0001
Density^2*Time^2	-4.94e-08	4.21e-08	-1.1712	0.2418
Drying(Tank):Time	1.35e-07	1.76e-06	0.0765	0.9391
Non-Drying(Tank):Time	-1.91e-06	1.50e-06	-1.2679	0.2051
Drying(Tank):Time^2	1.03e-08	6.93e-09	1.4923	0.1359
Non-Drying(Tank):Time^2	9.73e-10	4.55e-09	0.2136	0.8309
Drying(Tank):Density	-0.0008	0.0002	-3.5139	0.0005
Non-Drying(Tank):Density	-5.65e-05	0.0002	-0.2910	0.7711
Drying(Tank):Density^2	-6.68e-05	0.0001	-0.6518	0.5147
Non-Drying(Tank):Density^2	0.0001	0.0001	1.0642	0.2875

R^2=0.5195

Model 2: Restricted regression model for explaining mass at metamorphosis using density, its square, hydroperiod and tank nested in hydroperiod and all the 2-way interactions possible. Notation as above.

Model 2:

*Mass=Density*Hydroperiod(Tank)+Density^2*Hydroperiod(Tank)*

	Value	Std. Error	t value	Pr(>|t|)
(Intercept)	0.2862	0.0056	50.8022	0.0000
Density	0.0447	0.0034	13.3064	0.0000
Hydroperiod	-0.0281	0.0056	-4.9926	0.0000
Density^2	0.0033	0.0023	1.4592	0.1448
Drying(Tank)	-0.0007	0.0003	-2.3133	0.0209
Non-Drying(Tank)	-0.0004	0.0003	-1.3234	0.1860
Density*Hydroperiod	0.0022	0.0034	0.6568	0.5115
Density^2*Hydroperiod	5.64e-05	0.0023	0.0250	0.9801
Drying(Tank):Density	-0.0006	0.0002	-2.9579	0.0032
Non-Drying(Tank):Density	-0.0001	0.0002	-0.8020	0.4227
Drying(Tank):Density^2	4.03e-05	0.0001	0.4030	0.6870
Non-Drying(Tank):Density^2	0.0005	0.0001	3.6099	0.0003

R^2=0.4581