

AN ABSTRACT OF THE THESIS OF

Erica L. Wildy for the degree of

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Title: Cannibalism in Larvae of the Long-Toed Salamander,

Ambystoma macrodactylum.

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Andrew R. Blaustein

Predator-prey interactions have historically been studied to explain patterns of organization observed in populations and communities. They have also been important in understanding the evolution of antipredator responses in prey and counterstrategies in predators. Despite the breadth of knowledge that exists for heterospecific interactions, relatively few studies have investigated the situation where predator and prey are conspecifics.

Cannibalism has been widely documented within a diversity of animal groups including amphibians. In this thesis, I offer insight into the role of cannibalism in populations of the larval long-toed salamander (*Ambystoma macrodactylum*).

Aggressive behaviors, including cannibalism, have been documented in several Oregon Cascade populations of larval *A. macrodactylum*. Furthermore, some individuals within these populations exhibit a “cannibal” morphology characterized by a disproportionately large head and enlarged vomerine teeth. Through my research, I have attempted to elucidate the adaptive value of aggressive and cannibalistic activity, to examine the behavioral interactions between “cannibal” and “typical” morphs and to determine factors that influence aggression and cannibalism within some populations of larval *A. macrodactylum*.

Larval *A. macrodactylum* commonly inhabit ephemeral environments. Two factors characteristic of these environments, limited food resources and high conspecific densities, appear to influence increased cannibalism in larval *A. macrodactylum*. Alternatively, pond drying alone does not appear to influence aggression. By eating a conspecific diet rather than a heterospecific diet, cannibals benefit from enhanced growth. For individuals living in an ephemeral environment, this may increase the likelihood that the individual will metamorphose before the habitat disappears. As with heterospecific interactions, cannibalistic predators can influence the behavior and life history of their conspecific prey. Typical morphs spatially avoid cannibals and reduce activity in their presence. These antipredator behaviors appear to be a

learned phenomenon rather than an innate condition. Moreover, it appears that the conspecific diet of cannibals, but not their morphology, causes surrounding conspecifics to exhibit a decreased rate of growth and an increased time to metamorphosis.

Cannibalism in Larvae of the Long-Toed Salamander, *Ambystoma
macrodactylum*

by

Erica Lynn Wildy

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Contribution of Authors

Joseph Kiesecker and Douglas Chivers helped with experimental design, maintenance of experiments and statistical analyses for chapter 3 and chapters 3 and 6, respectively. Andy Blaustein helped with interpretation of data and editing for chapters 3, 6 and 7.

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Dedication

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Cannibalism in Larvae of the Long-Toed Salamander, *Ambystoma macrodactylum*

Chapter 1. General Introduction

Predator-prey interactions are of interest to ecologists as a factor contributing to patterns of organization observed in populations and communities. In addition, behavioral ecologists are interested in evolution and diversity of antipredator responses exhibited by prey and counterstrategies exhibited by predators. Despite the breadth of knowledge about these interactions, relatively few studies have investigated the situation where predator and prey are conspecifics (i.e. an individual belonging to the same species). By definition, cannibalism or intraspecific predation, occurs when an animal consumes all or part of a conspecific. Typically these interactions involve an individual feeding on a more vulnerable conspecific, as in a smaller-sized individual or an individual that belongs to a less advanced life stage. Like interspecific predator-prey interactions, cannibalistic interactions contribute to the regulation of population structure and to the evolution of antipredator behavioral responses (Fox, 1975; Polis, 1981; Dong and Polis, 1992).

Here, I present an overview of cannibalistic interactions found within different animal taxa. Specifically, I discuss where cannibalism

has been documented, the potential benefits and costs that may arise as a result of engaging in this behavior, and the factors that may influence and/or facilitate its presence. In my discussion, I will limit the examples of cannibalism I discuss by excluding instances in which individuals consume dead conspecifics or in which infanticide is committed but is not subsequently followed by cannibalism.

Forms of Cannibalism in Animal Populations

Cannibalism has been documented in a wide variety of animal taxa including gastropods (e.g. snails, slugs - Baur, 1992), insects (bees, beetles, mantids - Stevens, 1992; Crespi, 1992; Kukuk, 1992), arachnids (Elgar, 1992), amphibians (Polis and Myers, 1985, Crump, 1992), fish (cichlids, sticklebacks – Smith and Reay, 1991; Fitzgerald and Whoriskey, 1992), birds (kestrels, roadrunners - Stanback and Koenig, 1992) and mammals (rodents, primates - Elwood, 1992; Hiraiwa-Hasegawa, 1992). Depending on the taxon, cannibalistic interactions may take on different forms. For example, adult on adult cannibalism can occur but is generally rare, reported only anecdotally in some birds (i.e. Cawston, 1983; Paullin, 1987), fish (Marconato and Bisazza, 1988) and some insect and gastropod species that engage in sexual cannibalism (see review in Elgar, 1992). This may be because the risk of injury is greater in these instances since predator and prey are

more likely to be of similar sizes and strength. These risks may be reduced in adult/subadult or juvenile cannibalistic interactions because the victims in this case are more vulnerable as a consequence of their less advanced life stage compared to the cannibal. This kind of interaction is commonly seen in birds, rodents, and occasionally in primates (see reviews in Elwood, 1992; Stanback and Koenig, 1992; Hiraiwa-Hasegawa, 1992). In animals having multiphasic life histories (i.e., more than two life stages – egg, larva, pupa, juvenile/subadult, adult) such as amphibians, insects, and gastropods, additional interactions are possible. For example, larval-larval cannibalism has been observed in amphibians (see review in Crump, 1992) and in insects (see review in Stevens, 1992). Adult or juvenile cannibalism on larvae occurs in amphibians and some insects (Crump, 1992; Kukuk, 1992; Steven, 1992). Adults, juveniles or larvae may also eat conspecific eggs. This has been documented in birds, fish, gastropods and certain groups of insects, including bees (see reviews in Fox, 1975; Polis, 1981; Smith and Reay, 1991; Baur, 1992; Fitzgerald and Whoriskey, 1992; Kukuk, 1992; Stanback and Koenig, 1992).

Benefits of Cannibalism

Depending on the taxon and the context in which it occurs, cannibalism can have a variety of benefits. For example, numerous

studies have shown that a conspecific diet can be highly nutritious. Consequently, cannibals eating conspecifics benefit from an amelioration of a variety of life history characteristics including increased survival (Baur, 1990a; Breden and Wade, 1989), enhanced growth (Fiorini, 1988; Baur, 1990a; Crump, 1990; Breden and Wade, 1985, 1989; Wildy et al., 1998; Meffe and Crump, 1987; Elgar and Nash, 1988), accelerated development (Lannoo et al., 1989; Chapman et al., 1999), and improved fecundity (Ho and Dawson, 1966; Hoogland, 1985; Meffe and Crump, 1987; Birkhead et al., 1988; Belles-Isles and Fitzgerald, 1991). For example, Meffe and Crump (1987) demonstrated that mosquitofish fed conspecifics exhibited a greater increase in mass and enhanced fertility (i.e. increased number and accelerated developmental stage of mature embryos and ova) compared to fish fed one of three other heterospecific diets. Lactating female black-tailed prairie dogs, *Cynomys ludovicianus*, that cannibalized unrelated conspecific infants were more likely to wean a litter, have larger litters, and have heavier offspring (Hoogland, 1985). A diet of conspecifics may enhance growth and accelerate development in larval amphibians (Lannoo et al., 1989; Crump, 1990), in some embryonic and hatchling gastropods (Fiorini, 1988; Baur, 1990a) and in larval beetles (Breden and Wade, 1985). Female flour beetles, *Tribolium castaneum*, fed conspecifics exhibited higher fecundity than females that had been fed an alternative diet (Ho and Dawson, 1966).

These benefits to life history are likely explained by the efficiency with which a conspecific meal is digested and converted into tissue. The similarities between predator and prey may increase the efficiency with which nutritional or hormonal matter is incorporated (Gorbman, 1964; Nagai et al., 1971). For example, Nagai et al., (1971) showed that conversion of amino acids to body tissue was enhanced in Japanese toad, *Bufo vulgaris*, tadpoles that were fed conspecifics compared to individuals fed a diet of artificial feed or fish meal.

Consumption of conspecifics can also effectively reduce competition for limited resources. Although this can be difficult to document, it has been suggested as a byproduct of cannibalistic interactions in a number of animal groups. Chimpanzees commonly engage in infanticide and on several occasions, as a result of killing and eating the infants of other females, adults have gained access to novel feeding territories and decreased competition for food (Goodall, 1977; Pusey, 1983). Embryonic sharks will devour less developed siblings *in utero* (Wourms, 1977). Besides gaining a meal, these individuals reduce competition for other food resources, including the egg case in which they are housed (Wourms, 1977). Competition for food can be intense for larval granivorous beetles, *Rhizopertha dominica* (Crombie 1944, 1946) and bark beetles, *Scolytus scolytus* and *S. multistriatus* (Beaver, 1974).

Cannibalism of smaller larvae by larger individuals effectively reduces intraspecific competition.

Intraspecific predation may be employed as part of a reproductive strategy. Some arachnids, including spiders and scorpions (Elgar and Nash, 1988; Polis, 1990), insects (Lawrence, 1992), and nudibranchs (Rutowski, 1983) participate in sexual cannibalism, whereby an individual, usually the female, consumes part or all of a (potential) mate sometime during copulation. Several hypotheses have been generated to explain the adaptiveness of this practice in arachnids and insects.

Buskirk et al., (1984) suggest that this may represent a form of parental investment on the part of the male. This may be particularly true if the prospect of encountering additional mates is low. By being cannibalized, the male provides a nutritional benefit to the female which may directly enhance her weight (Elgar and Nash, 1988) and/or fecundity (Birkhead et al., 1988) and may indirectly affect the health of the offspring he has sired with her. Consequently, the number of offspring he sires through mating with and being cannibalized by one female is more than he would have sired had he attempted to find and mate with other females (Buskirk et al., 1984). An alternative explanation is offered from the perspective of the female. Newman and Elgar (1992) suggest that female cannibalism of males may be a form of female choice. Furthermore, the

nutritional benefits of cannibalizing a male can enhance her fecundity (Elgar and Nash, 1988; Birkhead et al., 1988).

Another role of cannibalism in reproductive behavior is that it can allow an individual to gain access to an otherwise occupied mate.

Female sticklebacks often raid the nests of males and eat the eggs that he has sired with another female (Vickery et al., 1988). In doing so, females are more likely to successfully court that male who is now freed from the responsibility of egg guarding. Furthermore, females raiding a nest were demonstrated to be the first to spawn on the newly reconstructed nest (Fitzgerald and van Havre, 1987).

Group or colonial nesting can facilitate cannibalism of eggs or young offspring by unrelated adults in both birds (e.g. Mumme et al., 1983) and bees (e.g. Sakagami and Maeta, 1982, 1985; Duchateau and Velthuis, 1988, 1989; 1987a,b; Stark et al., 1990; Kukuk, 1992). In consuming the offspring of others, an individual may increase the possibility of successfully raising its own offspring, particularly when an environment is harsh and/or resources are limited. For example, for joint-nesting female acorn woodpeckers, space is limiting. Therefore, a female may eat the eggs of her co-breeding females to ensure that her own eggs are incubated (Mumme et al., 1983). For bees, brooding cells and/or food can be limited. Therefore, joint-nesting or social female carpenter and orchid bees will remove unrelated eggs from brooding cells

or nests and eat them. Subsequently, they deposit their own egg in the nest (Sakagami and Maeta, 1982, 1985, 1987a,b; Garfalo, 1985; Stark et al., 1990).

Cannibalism may also occur in bee populations because of conflicts that arise as a result of the haplodiploid system of sex determination. In social systems of bee organization where reproductive castes are in existence, female workers will be more closely related to their own potential male brood than that produced by their mother, the queen. Consequently, female workers may cannibalize developing male embryos produced by the queen which frees them from the responsibility of caring for them and allows them the opportunity to produce their own sons (Duchateau and Velthuis, 1988, 1989; Kukuk, 1992).

Alternatively, the queen may cannibalize worker eggs to assert her dominance and to increase her own opportunity to produce males (van Doorn and Heringa, 1986; Duchataeau and Velthius, 1988, 1989).

In any given reproductive season, the environment may not be conducive for successful rearing of all offspring produced. For example, climatic conditions may be harsh or resources, like food, may be limited. Under these circumstances, parents may choose to cannibalize some of their young to maximize the chances that the remaining offspring will survive. Male and female burying beetles, *Nicrophorus vespilloides*, lay their eggs on the corpses of small mammals (i.e. mice) and have been

shown to cannibalize a proportion of their eggs to match the number of offspring with the amount of food provided by the corpse (Bartlett, 1987). It has been suggested that cannibalism of offspring by mice (Fuchs, 1981; Perrigo, 1987) and hamsters (Day and Galef, 1977) is a strategy that females continuously employ in response to ongoing variation in availability of food. Younger or weaker offspring appear to be selectively eaten in both rodents (Bronson and Marsteller, 1985) and roadrunners, *Geococcyx californianus* (Omhart, 1973). This is presumably an attempt to preserve quality of offspring at the expense of quantity of offspring (Bronson and Marsteller, 1985). In extremely unfavorable environments, females may opt to cannibalize all of their brood. This strategy would allow a female to recoup her reproductive investment when the likelihood of progeny survival is low (Gandelman and Simon, 1978).

In environments characterized by unpredictable food availability, an alternative to brood reduction is to physiologically synthesize food for offspring. Female mammals and a few birds (e.g. pigeons and penguins) synthesize their own milk for offspring (Anderson 1974). Female parents that are not able to commit to this may instead produce a large brood with the expectation that some offspring, particularly the larger and stronger individuals, may cannibalize their smaller or weaker siblings (Polis, 1981; Anderson, 1974; Mock and Parker, 1997). Walls and Blaustein (1995) offer this “icebox hypothesis” to explain why preferential

cannibalism of smaller siblings by larval spotted salamanders, *Ambystoma maculatum*, can be adaptive. Larvae of this species exploit ephemeral environments and must reach a minimum threshold size for metamorphosis prior to pond drying. The probability of survival for small individuals, particularly toward the end of the season, is low. However, the opportunity to maximize their inclusive fitness still remains. An individual's inclusive fitness is measured by the genes that individual contributes to the next generation along with the genes that individual shares with relatives (i.e. kin) that these relatives contribute to the next generation. Consequently, the inclusive fitness of stunted larvae may be increased by being cannibalized by larger siblings and, thus, improving their chances of survival.

In utero cannibalism by embryonic sandsharks, mackerel and thresher sharks may be a strategy by which females provide nutrition to their developing offspring (Wourms, 1977). Multiple eggs are enclosed within the egg capsule placed in the oviduct, but advanced developing embryos may devour some or all of their slower-developing siblings. The benefit of enhanced nutrition appears to outweigh the loss of fitness associated with cannibalizing siblings (Wourms, 1977). Similarly, early hatching marine prosobranchs (Gastropoda) (Baur, 1992) and stylommatophoran land snails (Burky, 1973; Baur and Baur, 1986)

consume unhatched embryonic siblings, which results in enhanced growth and survival.

It has also been suggested that the production of inviable or underdeveloped eggs, including trophic eggs in insects, (Crespi, 1992), nurse eggs in some marine and terrestrial gastropods (Fiorini, 1988; Smith et al., 1989) and “runt” eggs in birds (Mumme et al., 1983) evolved as a means for females to provide their offspring or other kin with additional food resources without having to sacrifice viable offspring to do so. Some insects (i.e. owlfly - Henry, 1972; coccinellid beetles - Takahashi, 1989; cricket - West and Alexander, 1967) as well as some spiders (Valerio, 1974, 1977) oviposit in areas distantly located from normal juvenile feeding sites or do not have easy access to transportable food for feeding young (Crespi, 1992). Consequently, offspring are provided with trophic eggs on which to feed.

Proximate Causes of Cannibalism

A number of studies have attempted to determine the proximate causes of cannibalism within animal populations. The results of these studies suggest that, genetic differences can sometimes explain variation in cannibalistic behavior between individuals or populations (Hampton and Volpe, 1963; Thiabult, 1974; Englert and Raibley, 1977; Cuthbertson, 1980; Gilbert, 1980; Gould et al., 1980; Dominey and

Blumer, 1984; Berrio et al., 1987; Steven, 1989; Via, 1999). Evidence for a genetic cause for cannibalistic behavior has been found in several different instances. Different strains of the flour beetle, *Tribolium confusum*, exhibit more cannibalism than others and it has been determined that additive genetic effects can predict the frequency of cannibalism exhibited (Steven, 1989). Similarly, extreme variation in the propensity for hatchling land snails to engage in cannibalistic behavior both within and between populations is suspected to be influenced, in part, by differences in interpopulational genetic makeup (Baur and Baur, 1986; Baur, 1987a).

Cannibalistic polyphenisms and/or polymorphisms exist in some populations where some individuals appear to be genetically predisposed behaviorally and/or morphologically to engage in cannibalistic activities. For example, in some populations of threespine sticklebacks, males are more cannibalistic than females (Salfert and Moodie, 1985) while the reverse is true in other populations (Whoriskey and Fitzgerald, 1985a). In populations of herring gulls (Parsons, 1971) and slaty-backed gulls (Watanuki, 1988) some adults were more cannibalistic on chicks than others and were responsible for most of the cannibalism documented. It has also been found that a genetic predisposition exists for the development of the carnivore (i.e. cannibal) morphology (characterized by well-developed jaw muscles, hypertrophied beaks and a larger body size)

in larval frogs of the genus *Scaphiopus* (Hampton and Volpe, 1963; Pomeroy, 1981).

In addition to potential genetic factors, several studies have linked environmental factors with increased cannibalistic activity. Cannibalism is commonly seen in populations of animals that exploit relatively harsh environments, specifically those characterized by low or unpredictable availability of food and/or high conspecific densities (Fox, 1975; Polis, 1981). Thus, a number of studies linking these factors to increased cannibalism have been conducted (i.e. Istock, 1966; Paul and Kupferschmidt, 1975; Svare and Bartke, 1978; Elwood and Ostermeyer, 1984; Dionne, 1985; Hecht and Applebaum, 1988; Bartolotti et al., 1991; Samu et al., 1999). Dionne (1985) experimentally demonstrated that mosquitofish will cannibalize juveniles according to the amount of alternative food available. Reduced food availability, in addition to reduced shelter and increased density, also influenced whether African sharptooth catfish were likely to cannibalize siblings (Hecht and Applebaum, 1988). Both short-term and long-term food deprivation has been demonstrated to increase the prevalence of non-parental consumption of juveniles in gerbils (Elwood and Ostermeyer, 1984a), rats (Paul and Kupferschmidt, 1975) and mice (Svare and Bartke, 1978). Furthermore, as discussed above parents may cannibalize some or all of their developing offspring in food-limited environments (i.e. Omhart,

1973; Day and Galef, 1977; Fuchs, 1981; Bronson and Marsteller, 1985; Bartlett, 1987; Perrigo, 1987; Bortolotti et al., 1991). For example, cannibalism of chicks by parents in American kestrels appears to be negatively correlated with the amount of mammal prey available (Bortolotti et al., 1991).

Increased conspecific densities can effectively reduce per capita food. Consequently a number of studies have linked both variation in density and food with increased cannibalism. Intraspecific predation in the predatory marine slug, *Navanax inermis*, (Paine, 1965) and in hatchling egg cannibalism in the land snail, *Arianta arbustorum* (Baur, 1987a, 1988d) increases with increasing density. Both low food and high densities may contribute to increased cannibalism. Collins and Cheek (1983) found that high conspecific densities promote the production of a cannibal morphology (characterized by a larger, wider head and enlarged vomerine teeth) in larval Arizona tiger salamanders, *Ambystoma tigrinum nebulosum*. Hecht and Applebaum (1988) found that African sharptooth catfish tend to cannibalize siblings more when under conditions of high conspecific density, low food availability and few available refugia.

The availability of vulnerable conspecific prey has also been correlated with the increased prevalence of cannibalism (Polis, 1981; Maret and Collins, 1994). Vulnerability of prey is evident in interactions in which the cannibal is at a more advanced life stage than the prey. For

example, adults freely eat eggs and/or juveniles in gastropods, beetles, amphibians, birds, rodents and primates (see reviews in Fox, 1975; Polis, 1981; Elgar and Crespi, 1992). Furthermore, within-life stage cannibalism is possible if the potential prey is unable to defend itself. For example, during the moulting period, isopods of the species *Saduria entomon* are relatively defenseless. Consequently, they are frequently cannibalized during this time by larger non-moulting conspecifics (Sparrevik, 1999). Cannibalism within a single life stage may be facilitated by high intrapopulation size variation between individuals which can essentially produce a class of vulnerable (i.e. smaller) individuals. Consequently cannibalism of smaller individuals by larger conspecifics may occur. Variation in size within populations may be caused by a variety of factors including the co-occurrence of different aged cohorts (Hopper et al., 1996; Summers, 1999; Samu et al., 1999), genetic differences in growth rates or competitive abilities (i.e. Wilbur and Collins, 1973; Fitzgerald and Whoriskey, 1992), or hatching asynchrony (Beaver, 1974; Mock, 1984; Baur and Baur, 1986; Baur, 1987; Wagner et al., 1987, 1988; Maret and Collins, 1994). Egg cannibalism in the land snail, *Arianta arbustorum*, increases with asynchrony in hatching under both natural and experimental conditions (Baur and Baur, 1986; Baur, 1987a). Samu et al., (1999) found that a large weight ratio between interacting wolf spiders, *Pardosa agrestis*, promoted

cannibalism of smaller individuals by larger ones. Moreover, Maret and Collins (1994) found that larval Arizona tiger salamanders, *Ambystoma tigrinum nebulosum*, were more likely to develop the cannibal morphology when reared in groups of conspecifics exhibiting high size variation and when those larvae were one of the largest individuals in the group.

The location of vulnerable prey can increase the likelihood that cannibals will find those individuals and eat them. For instance, larval ladybeetles, *Harmonia axyridis*, are more likely to cannibalize non-related eggs if those eggs are laid in close proximity to aphid colonies on which larvae normally prey (Osawa, 1989). Cannibalism of eggs by hatchlings in land snails occurs more often when eggs are laid in batches rather than when they are laid singly (Baur, 1992). This is in part due to the abundance and close proximity of potential prey, in the form of unhatched siblings, available to early hatching individuals (Baur, 1988d).

Finally, the genetic relatedness of surrounding conspecifics can determine whether an individual will be likely to exhibit cannibalism. Both larval Arizona tiger salamanders (Pfennig and Collins, 1993) and Southern spadefoot tadpoles, *Scaphiopus multiplicata*, (Pfennig and Frankino, 1997) are less likely to develop the cannibal morphology when reared with siblings than when reared in mixed sibship groups. Similarly, plains spadefoot tadpoles, *Spea bombifrons*, are more likely to

express the carnivore phenotype when raised alone than when raised with siblings (Pfennig and Frankino, 1997). In stylommatophoran land snails, non-cannibalistic species self fertilize and thus lay batches of eggs that are full siblings. In contrast, in species where cannibalism has been identified, individuals multiply mate and consequently lay egg batches consisting of both full and half siblings. It has been suggested that, in this case, cannibalism is more prevalent in a brood that consists of both full and half-siblings, with cannibals preying selectively on their half-siblings (Baur, 1992).

Costs of Cannibalism

Despite its widespread documentation within animal taxa and the variety of potential benefits it may offer, cannibalism comprises only a small proportion of total predator-prey interactions occurring within any given group (Fox, 1975; Polis, 1981; Elgar and Crespi, 1992b). This is likely explained by the potential risks associated with this behavior.

One possible hazard of cannibalism is injury incurred due to struggling conspecific prey (Polis, 1981; Elgar and Crespi, 1992). Of course, this possibility is dependent on the type of cannibalistic interaction occurring. Injury is not expected at all when the cannibal is feeding on eggs. Some potential injury is expected in larval-larval, adult-juvenile or adult-larval interactions. However, in these instances, an

asymmetry between the interacting individuals is typical whereby a larger or stronger predator consumes a smaller or more vulnerable prey. In adult-adult interactions, individuals risk injury in attempting to subdue and consume another adult, particularly if the victim is similar in size to the cannibal and/or if it is fairly healthy, and therefore, potentially resistant. This may explain why these types of interactions are rare.

Another potential cost of cannibalism is the loss of inclusive fitness associated with eating a relative. Eating a relative eliminates the possibility of that relative passing on its genes to the next generation. Consequently, it is not surprising that cannibals often are shown to avoid eating kin (i.e. Fitzgerald and van Havre, 1987; Young, 1988; Nummelin, 1989; Pfennig et al., 1993; Pfennig et al., 1994; Elwood, 1992).

There are instances, however, where kin are preferentially or at least freely eaten by cannibalistic conspecifics (i.e. Omhart, 1973; Day and Galef, 1977; Wourms, 1977; Fuchs, 1981; Hecht and Applebaum, 1988; Osawa, 1989; Breden and Wade, 1989; Walls and Blaustein, 1995; Bronson and Marsteller, 1985; Bartlett, 1987; Perrigo, 1987; Bortolotti et al., 1991). However, the cost of cannibalizing a relative may be offset by the potential gain to the cannibal and/or the low probability of survival of the victims (Eickwort, 1973). This may be particularly true

in environments where the probability of survival for any individual is low due to harsh climatic conditions or low food availability (Baur, 1992; Walls and Blaustein, 1995). For example, Walls and Blaustein (1995) found that cannibalistic larval spotted salamanders preferentially consume smaller-sized kin over non-kin. They suggest that since survival of a larva, particularly smaller individuals, may be low in the temporary habitats which they exploit, preferential cannibalism of siblings may not be costly in terms of indirect fitness for either the cannibal or the victim. Larval gastropods are also subject to possible desiccation and starvation (Baur, 1992). Therefore, cannibalism of unhatched conspecifics by early hatching gastropods can be advantageous since they may gain both a growth and survival advantage over those that do not cannibalize (Baur and Baur, 1986). Parents that practice brood reduction in instances of food limitation can improve the probability of survival and/or maintain the health of their remaining offspring (i.e. Omhart, 1973; Day and Galef, 1977; Gandelman and Simon, 1978; Fuchs, 1981; Bronson and Marsteller, 1985; Bartlett, 1987; Perrigo, 1987).

A third potential cost to cannibals is the risk of exposure to disease through the consumption of infected conspecifics. Some studies have suggested that due to the close genetic constitution of cannibals and their prey, the efficiency with which pathogens are transferred between

individuals is greater (Kiltzman et al., 1984; Schaub et al., 1989; Pfennig et al., 1991) than if predator and prey were heterospecifics (Pfennig et al., 1998). A number of studies have demonstrated that eating infected conspecifics can result in the transmission of disease from victim to predator (i.e. Schaub et al., 1989; Pfennig et al., 1999; Chapman et al., in press). Pfennig et al. (1998) demonstrated that cannibalistic larval tiger salamanders, *Ambystoma tigrinum*, that consumed infected conspecifics, particularly non-kin, were more likely to become infected themselves. Furthermore, in another study individuals consuming infected conspecifics exhibited reduced survival to metamorphosis and reduced growth compared to individuals that were fed healthy conspecifics, healthy heterospecific salamander larvae or diseased heterospecific salamander larvae (Pfennig et al., 1999). The possibility of consuming diseased conspecifics may be avoided if individuals are capable of recognizing and avoiding diseased conspecifics. Indeed, disease avoidance behavior has been identified in some cases (i.e. Kiesecker et al., 1999) and found to be lacking in others (i.e. Pfennig et al., 1999).

Cannibalism can be adaptive in many circumstances, particularly in light of its potential nutritional benefits. However, engaging in this behavior may also be costly. Therefore, the assumption is that where

cannibalism occurs, the benefits of this behavior outweigh the potential costs.

The Long-Toed Salamander, *Ambystoma macrodactylum*, as a Model System

Generally, larval amphibians are an excellent model system for studying questions in behavioral ecology because 1) their natural history is well understood, 2) large numbers of individuals can be obtained for replication, 3) their behavior is easily observed in both the field and in the laboratory, and 4) experimental factors influencing behavior can be manipulated. Specifically, salamanders of the genus *Ambystoma* are excellent organisms for testing hypotheses concerning aggressive behavior. Aggressive behavior, particularly cannibalism, has been widely documented in larval Ambystomatids (Rose and Armentrout, 1976; Lannoo and Bachman, 1984; Walls et al., 1993; Walls and Blaustein, 1995; Polis and Myers, 1985; Crump, 1992; Pfennig, 1997) and numerous studies have unambiguously quantified the effects of aggression in this group of amphibians (Collins and Cheek, 1983; Lannoo et al., 1989; Walls and Roudebush, 1991; Maret and Collins, 1994, 1996; Pfennig et al., 1994; Walls et al., 1993; Walls and Blaustein, 1995; Wildy et al., 1998, 1999).

There are five subspecies of *Ambystoma macrodactylum* and collectively their populations have a range that extends north into British Columbia, south into Northern California and eastward into western Montana. This research involves *A. macrodactylum columbianum*, occurring in the Cascade Mountains of Oregon and *A. m. macrodactylum*, occurring in the Willamette Valley.

Adult *A. macrodactylum* are found in a variety of habitats including lowland forests, alpine meadows, dry woodlands, semiarid sagebrush deserts, and disturbed agricultural areas (Nussbaum et al., 1983; Leonard et al., 1993). During the non-breeding season, adults typically live a subterranean existence. During the breeding season, which may start as early as October in low elevation populations and in May in montane populations, adults can be found under vegetation or woody debris near a particular breeding site. Breeding sites include permanent aquatic habitats, such as lakes and permanent ponds, as well as more ephemeral habitats including temporary ponds and roadside ditches. Females collect spermatophores deposited by males and subsequently lay their eggs in the water. Eggs may be laid singly, in separate clutches or in groups of interconnected clutches. Clutches may contain anywhere from 5 to 100 eggs. Typically single eggs or clutches are attached to vegetation, sticks or rocks present in the water. Depending on the site the larval period may last anywhere from 50 days to 14 months.

Cannibalism of larvae by larger larvae has been documented in several populations of *A. macrodactylum columbianum* (Anderson, 1967; Walls et al., 1993a,b) including populations located in the Three Creek Wilderness Area in Deschutes County, Oregon. In addition to cannibalistic behavior, some individuals possess an alternative morphology characterized primarily by a disproportionately large head and enlarged vomerine teeth (Walls et al., 1993a). This "cannibal" morph is similar to that described for larval tiger salamanders (Powers, 1907; Pierce et al., 1983; Rose and Armentrout, 1984). Larvae not possessing these exaggerated features are referred to as "typical morphs". It is important to note, however, that larvae of this species tend to be aggressive regardless of morphology (Wildy, personal observation). The cannibalistic behavior and, to some degree, the cannibal morphology, has been the focus of my research efforts. Neither the behavior nor the morphology has been observed in populations of *A. macrodactylum macrodactylum* sampled in the Willamette Valley (Benton County) of Oregon.

Previous Investigations of the Significance of Cannibalism in Amphibian Populations

In recent years, the significance of cannibalism within amphibian populations has been investigated. Specifically, studies have examined three main issues:

- 1) the environmental factors that contribute to cannibalism and the morphologies that are sometimes associated with this behavior (i.e. Collins and Cheek, 1983; Kusano et al., 1985; Pfennig and Collins, 1993; Walls et al., 1993a, b; Pfennig et al., 1994; Loeb et al., 1994; Maret and Collins, 1994, 1996; Nishihara, 1996; Hoffman et al., 1999; Pfennig and Frankino, 1997; Walls, 1998; Ziemba and Collins, 1999).
- 2) the type of behavioral interactions that occur in cannibalistic populations (i.e. Crump, 1986; Pfennig et al., 1993; Kats et al., 1994; Walls and Blaustein, 1995; Holomuzki, 1986; Chivers et al., 1997; Pfennig, 1999; Pfennig et al., 1999; Summers, 1999).
- 3) the potential benefits and costs associated with engaging in this behavior (Nagai et al., 1971; Kusano et al., 1985; Lannoo et al., 1989; Pfennig, 1992; Pfennig et al., 1991, 1998, 1999; Crump, 1990).

The species I examined, the long-toed salamander, is one that previously has not received much attention in this area of research.

Much of the previous research on cannibalism in amphibians has focused on various subspecies of tiger salamanders, *A. tigrinum* and spadefoot tadpoles, *Scaphiopus* spp.

Significance of the Current Research

In examining the role of cannibalism in populations of *A. macrodactylum*, I have asked similar questions to those asked in previous investigations. Specifically, I investigated the nutritive benefits associated with cannibalism in these populations, the environmental factors influencing the prevalence of this behavior in these populations, and some behavioral interactions that can occur between cannibals and their conspecific prey. The research presented in this thesis is significant in that it can be used as a basis for comparison with existing studies. Furthermore, the findings of these studies will contribute to a clearer understanding of the role of aggression, and more particularly cannibalism, in amphibian populations.

Thesis Organization

The research presented in this thesis was designed demonstrate the extent to which cannibalism occurs in natural populations of larval

A. macrodactylum (Chapter 2) and to answer three main questions in relation to the significance of cannibalism in these populations

- 1) what are the environmental factors that contribute to cannibalism in some populations of larval long-toed salamanders (Chapters 3 and 4)?
- 2) are there nutritional benefits to engaging in cannibalistic behavior (Chapter 5)? and
- 3) what types of behavioral interactions occur in cannibalistic populations (Chapters 6 and 7)?

In chapters 3 and 4, I focused on determining what factors inherent to the natural habitat exploited by cannibalistic larval long-toed salamander populations, promote aggression, particularly cannibalism. Larvae of this species commonly live in temporary aquatic habitats which typically dry up by the end of the season. In the Three Creeks Wilderness area in Deschutes County, Oregon, where cannibalism and the cannibal morphology have been documented, I have noticed for the past six years that during the second half of the season, larval densities increase as pond drying heightens and per capita food decreases. In addition, I have noticed that larval cannibalism tends to be more prevalent during this time. Consequently, in Chapter 3, employing a 2 x 2 factorial design, I experimentally manipulated two levels of density (low and high) and two levels of food (low and high) to examine the effects that

high densities and low food has on aggression (i.e. biting) and cannibalism in groups of larvae. Furthermore, I also looked at the effect of these factors on size variation and how this, in turn, may affect aggression. Previous studies have suggested that increased within-group size variation can facilitate aggression (Kusano et al. 1985; Nyman et al. 1993; Maret and Collins 1994; Petranka and Thomas 1995).

The field study in Chapter 4 examined the effect of pond drying on aggression. I reared larvae under two levels of pond drying rate (i.e., slow and fast), while keeping density and food level constant. Some studies have shown that pond drying may promote aggression. However, this is likely due to effects associated with pond drying, including increased conspecific densities and/or decreasing per capita food. Whether pond drying alone, without these other factors, affects aggression in amphibians has not been closely examined.

In Chapter 5, I examined one potential benefit of cannibalism in larval long-toed salamanders. Numerous studies have suggested that a conspecific diet can be a highly nutritious meal (Nagai et al., 1971; Hoogland, 1985; Meffe and Crump, 1987; Crump, 1990) resulting in a number of benefits including enhanced growth (Breden and Wade, 1985; Meffe and Crump, 1987; Baur, 1990; Crump, 1990). I offered larvae one of three diets -100% conspecifics (larval long-toed salamanders), 100% heterospecific (Pacific treefrog, *Hyla regilla*, tadpoles) and a 50:50

mix of both diets – and examined the effects of these diets on larval growth.

Chapters 6 and 7 were investigations of interactions between cannibal and typical morphs. Specifically, Chapter 6 built on a previous study (Chivers et al., 1998) which showed that typicals respond to cannibals as though they were predators. Specifically, during behavioral trials, typicals significantly reduced their activity in the presence of cannibals. Furthermore, they spatially avoided the cannibal when given a choice between spending time near a cannibal or typical morph stimulus animal. That study revealed how typical morphs may respond to cannibals in the short term, but does not indicate what happens over a longer period of time. Therefore, I conducted a study in which I raised larvae in the presence of cannibals to determine what long-term effects cannibals may have on typical morph life history. Furthermore, I examined the relative influence of aspects of the cannibal morphology and diet in influencing changes in life history. I raised larvae in the presence of one of four types of stimulus animals: 1) cannibals morphs fed conspecifics 2) cannibal morphs fed *Tubifex* (heterospecific diet), 3) typical morphs fed conspecifics and 4) typical morphs fed *Tubifex*.

Chapter 7 was an investigation into whether naïve animals are capable of recognizing and responding to cannibal morphs as potential predators. Previous experiments (Chivers et al., 1998; Wildy et al., 1999,

Chapter 6) examining interactions between cannibal and typical morph larval long-toed salamanders utilized older, and therefore more experienced larvae as focal subjects. In this study, I used naive (i.e., lab-reared) animals to examine whether larvae innately respond to cannibal morphs as predators or whether it is a learned phenomenon as has been determined in other systems (Magurran 1989; Mathis and Smith 1993a; Chivers et al. 1994b,c, 1996a; Wisenden et al. 1997).

Chapter 2

A Survey of Cannibalism in Natural Populations of Larvae of the Long-Toed Salamander, *Ambystoma macrodactylum*

Erica L. Wildy

Abstract

Cannibalism has been widely documented in amphibians including Ambystomatid salamanders. Larval cannibalism has been documented in one high elevation population of *Ambystoma macrodactylum* and has been anecdotally reported for several other montane populations. The current study examines the prevalence of cannibalism in larval populations of *A. macrodactylum*. Surveys were conducted at sites in both the Willamette Valley and the Cascade Mountains of Oregon. Stomach contents of larval salamanders were collected and identified and the frequency of cannibalism occurring at each site was quantified. Larval size and estimates of size variation were documented within each population.

Cannibalism was not observed in populations of larvae in the Willamette Valley. However, cannibalism occurred with varying frequency in three out of the four montane populations. Cannibals were the largest animals in their populations.

Introduction

The research presented in Chapters 3-7 of this thesis examined the significance of cannibalism in larval long-toed salamanders, *Ambystoma macrodactylum*. Specifically I investigated the potential benefits of cannibalism, factors that may contribute to cannibalism, and behavioral interactions that can occur between cannibals and their conspecific prey. To more fully understand the implications of my research, it is important to document cannibalism in natural populations of *A. macrodactylum*. Indeed, cannibalism has been previously documented in one Oregon Cascade Mountain population (Susan's Pond)(Walls et al., 1993a, b) and anecdotally reported (Anderson 1967; Nussbaum et al. 1983) or observed (Wildy, unpublished data) in a number of others. Furthermore, it has been suggested that an alternative, cannibal morphology exists in at least one montane population in which some individuals possess a disproportionately large head compared to the rest of the body and enlarged vomerine teeth in the roof of the mouth (Walls et al., 1993a).

Although these studies suggest that larval *A. macrodactylum* cannibalize conspecifics in natural montane populations, the scope of these data is limited. Furthermore, this behavior has not been reported for populations of *A. macrodactylum* occurring in the Willamette Valley of Oregon. This chapter describes diet surveys conducted for six populations located in the Willamette Valley (henceforth, Valley) of

Oregon and at four populations located in the Cascade Mountains (henceforth Mountain) of Oregon.

Methods

Willamette Valley Sites

In the Willamette Valley, surveys were conducted at five sites (Elliot Ditch, Tangent Ditch, Farm Pond, Backwoods Pond, and Deep Pond) at two different times between March and June 1998 and at one additional site (Angler Pond) between March and June 1999. Tangent Ditch (411.5 m x 2.3 m) and Elliot Ditch (60.4 m x 2.0 m) are both roadside ditches, located adjacent to agricultural land. Backwoods Pond (Perimeter (P)=140.11m), Farm Pond (P=137.6 m) and Deep Pond (P=70.9 m) are roadside seasonal ponds. Angler's Pond (P=593.7m) is a artificial seasonal pond located at E.E. Wilson Wildlife Refuge located approximately 18 km northeast of Corvallis, Benton County, Oregon. All six are ephemeral sites that are filled by fall rains (beginning Sept – October) and typically dry completely during mid- to late summer. Farm Pond, Backwoods Pond, Deep Pond and Tangent Ditch are located approximately 15 km southwest of Oregon State University in Benton County, Oregon. Because these four sites are within 2 km of one another, larvae inhabiting these areas may not represent genetically distinct populations. However, the two remaining populations, Elliot

Ditch, located 12 km north of these sites, and Angler's Pond, located 21 km north and 10 km north of Elliot ditch are presumed to be genetically distinct from these four other sites and from one another.

Cascade Mountain Sites

Four populations in the Cascade Mountains were surveyed between June and October 1999. Big Lake (P=1003 m), Susan's Pond (P= 403m), Eric's Pond (P=270.7m), and Site A (P=185.8 m) are all fed by snowmelt at the beginning of the season (i.e. May-June). The three ponds are ephemeral, normally drying completely by mid to late September (pers. obs.). However, in 1999, two out of the three typically ephemeral sites (Susan's Pond, Site A; not Eric's Pond) did not completely dry. Big Lake, a much larger site, is perennial.

Surveys

Larval *A. macrodactylum* were collected from each population to assess whether cannibalism occurred at the sites surveyed. Collection methods differed between the Valley and Mountain surveys. In the Valley, each site was divided into lengthwise transects separated by one meter. Sweeps of a 30.5 cm wide D-ring net were made every three meters within each transect. Larvae retrieved during any of these sweeps were transported back to Oregon State University for examination. For

the mountain surveys, 50 animals (exception: 3rd survey, Big Lake - 25 animals were examined) were collected randomly from each site at three different times (week of July 25, 1999; week of August 29, 1999; week of October 3, 1999). A fourth survey of Eric's Pond was conducted during the week of October 24, 1999. Fifty animals were examined at this time. All larvae were examined in the field.

For both Valley and Mountain surveys, the stomach contents of each larva were collected by flushing the stomach of each individual with approximately 10 mL of water with a syringe and a 20-gauge flushing needle. The needle was inserted into the mouth of each animal and the water in the syringe was gently pushed into their gut. This non-lethal method of gut content retrieval is highly effective in yielding most of the contents (approx. 95%) of the larval gut (Holomuzki and Collins, 1987). Stomach contents of each animal was examined and prey species were identified to Order. In early surveys (first surveys for Angler's, Backwoods, Deep, and Farm Ponds in the Willamette Valley and Site A, Eric's Pond, and Big Lake in the Cascade Mountains), gut contents were not collected because of the small size and delicate nature of the larvae. For Valley populations, stomach contents were retrieved only during the second survey and for mountain populations, stomach contents were retrieved on the second and third surveys except for Susan's Pond where stomach contents were retrieved during all three surveys.

The variety of potential prey species available for salamander larvae were assessed from each site as a basis for comparison with stomach contents. This was achieved by performing one-meter sweeps of a 30.5 cm wide D-ring net made at 10 different randomly chosen locations within each pond. In Valley surveys, prey animals were transported to Oregon State University for examination but they were examined in the field for the Cascade Mountain surveys. Prey animals were identified to order and recorded.

In addition to collection of gut contents, larval morphometry was measured, including snout-vent length (SVL), and four measurements potentially indicative of the cannibal morphology: head width at eyes (HWE), head width at jaws (HWJ), head width at gills (HWG), and head length (HL) were taken. Correlations between snout-vent length and each of the head measurements were determined. Finally, the coefficient of variation (CV), a measure of size variation, was calculated for each group of larvae collected.

Results

Valley Populations

Cannibalism was not observed during Valley population surveys. Furthermore, no larvae had consumed vertebrate prey although

H. regilla tadpoles were available at all sites. Larval diets consisted of a variety of invertebrate prey including midges (Diptera), beetles (Coleoptera), isopods (Isopoda), amphipods (Amphipoda), zooplankton (Copepoda, Cladocera), clams (Mollusca) and snails (Gastropoda) (Tables 2.1-2.2).

Since the four measurements of head width/length were highly correlated, HWJ will be used to represent the rest of these measurements. Larvae demonstrated a positive linear relationship between body size and head width (see Figures 2.1-2.6). There appeared to be no trend in the pattern of CV in SVL (Table 2.3).

Table 2.1 Stomach contents of larvae collected from a) Angler's Pond (N = 6) b) Deep Pond (N = 6) c) Backwoods Pond (N = 28) and d) Farm Pond (N = 12)(Willamette Valley) during the second surveys. Data presented are the percentage of total larvae collected that had consumed the prey item listed. Potential prey include beetles (Coleoptera), midges (Diptera), unidentified winged insects (UWI), Zooplankton (Zoop), Scuds (Amphipoda), Isopods (Isopoda), Snails (Gastropoda) and freshwater clams (Mollusca).

	Angler's Pond	Deep Pond	Backwoods Pond	Farm Pond
Vertebrata				
<i>A. macrodactylum</i>	0	0	0	0
<i>H. regilla</i>	0	0	0	0
Aquatic Insecta				
Coleoptera	17	0	4	0
Diptera	33	83	93	42
(UWI)	0	0	4	8
Crustacea				
Zooplankton	0	0	1	33
Amphipoda	17	0	7	8
Isopoda	83	33	82	0
Gastropoda	33	0	36	0
Mollusca	17	83	75	0

Table 2.2 Stomach contents of larvae collected from Elliot Ditch (Willamette Valley) during the a) first (N = 23) and b) second (N = 10) surveys and Tangent Ditch (Willamette Valley) during the c) first (N = 21) and d) second (N = 7) surveys. Data presented are the percentage of total larvae collected that had consumed the prey item listed. Potential prey include beetles (Coleoptera), midges (Diptera), unidentified winged insects (UWI), Zooplankton (Zoop), Scuds (Amphipoda), Isopods (Isopoda), Snails (Gastropoda) and freshwater clams (Mollusca).

	Elliot Ditch		Tangent Ditch	
	Survey 1	Survey 2	Survey 1	Survey 2
Vertebrata				
<i>A. macrodactylum</i>	0	0	0	0
<i>H. regilla</i>	0	0	0	0
Aquatic Insecta				
Coleoptera	17	0	0	0
Diptera	91	30	19	14
(UWI)	4	0	0	0
Crustacea				
Zooplankton	0	0	0	57
Amphipoda	48	50	43	14
Isopoda	0	50	76	86
Gastropoda	4	10	24	43
Mollusca	0	0	0	43

Table 2.3 Descriptive statistics of larval *A. macrodactylum* collected from surveys of the four Willamette Valley sites. Presented here for each site are the total number of larvae surveyed, the mean Snout-Vent Length (SVL) \pm SE for all larvae examined, and the Coefficient of Variation (CV) in SVL for the population.

Site	N	Mean SVL \pm SE (mm)	CV in SVL
Farmer's Pond			
Survey 1	25	14.6 \pm .58	0.20
Survey 2	25	16.9 \pm .59	0.18
Backwoods Pond			
Survey 1	23	10.5 \pm .28	0.13
Survey 2	28	23.4 \pm .67	0.15
Deep Pond			
Survey 1	18	12.9 \pm .28	0.09
Survey 2	6	24.0 \pm 1.24	0.13
Tangent Ditch			
Survey 1	38	15.6 \pm .49	0.19
Survey 2	7	27.3 \pm .95	0.09
Elliot Ditch			
Survey 1	72	13.4 \pm .42	0.27
Survey 2	12	20.5 \pm .83	0.14
Angler Pond			
Survey 1	2	21.4 \pm .05	0.003
Survey 2	6	28.0 \pm 1.8	0.16

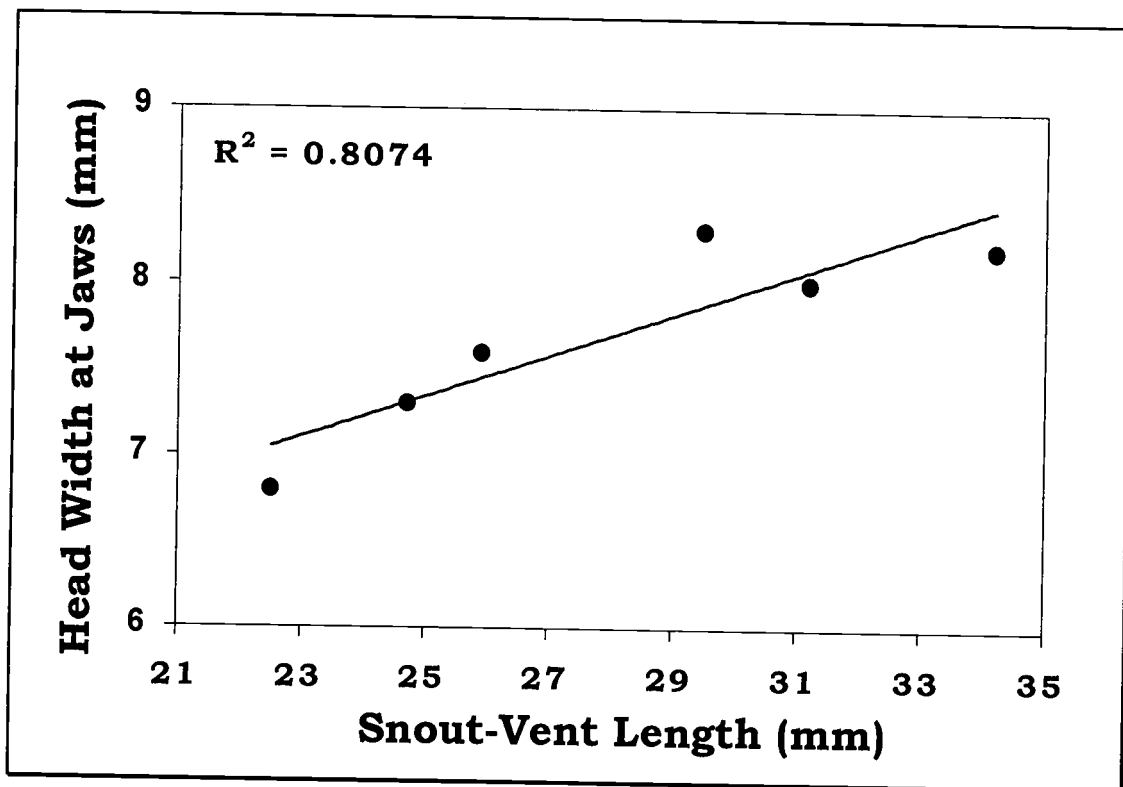


Figure 2.1 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Angler's Pond (Willamette Valley) during the second survey.

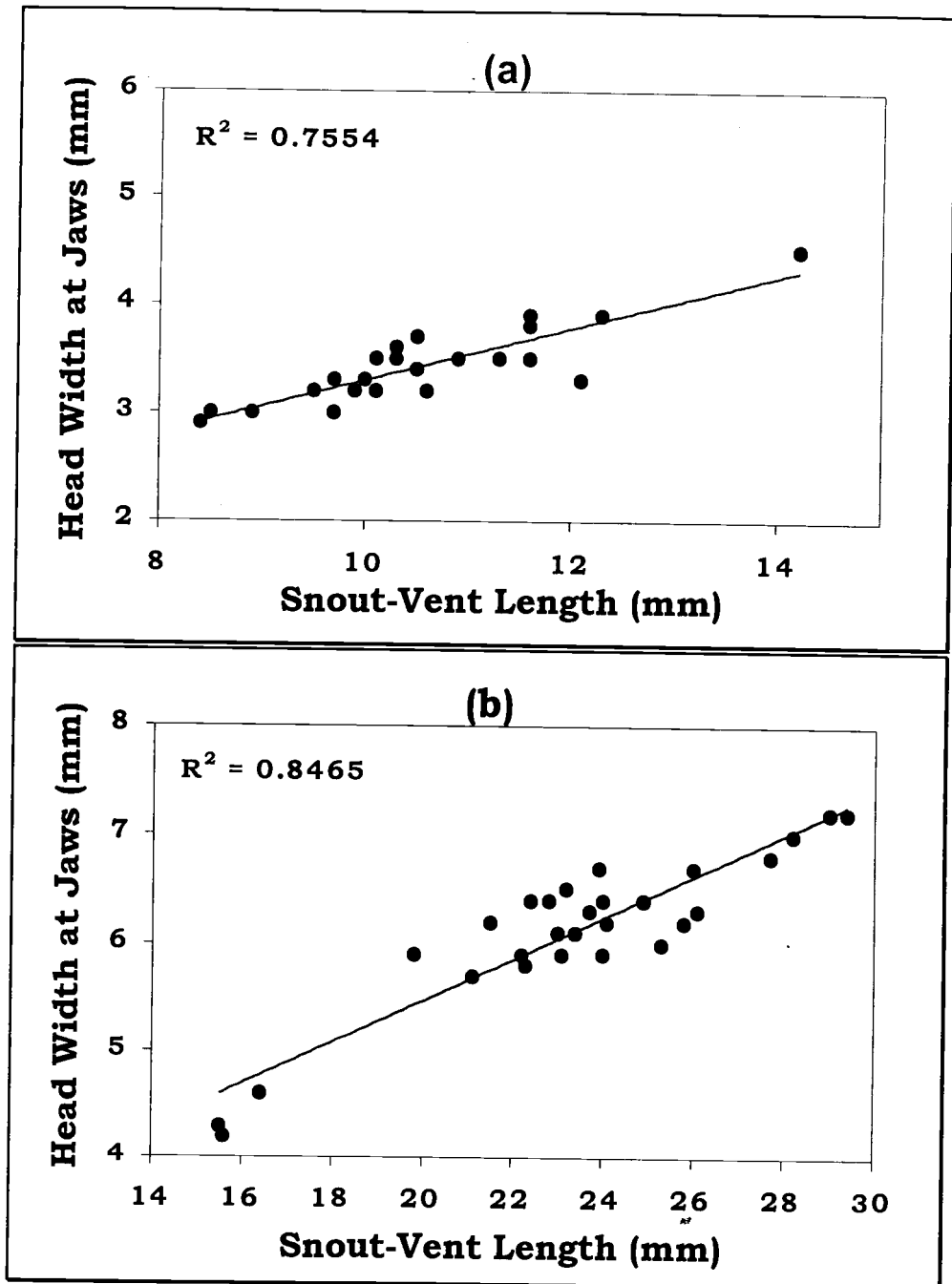


Figure 2.2 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Backwoods Pond (Willamette Valley) during the a) first and b) second surveys.

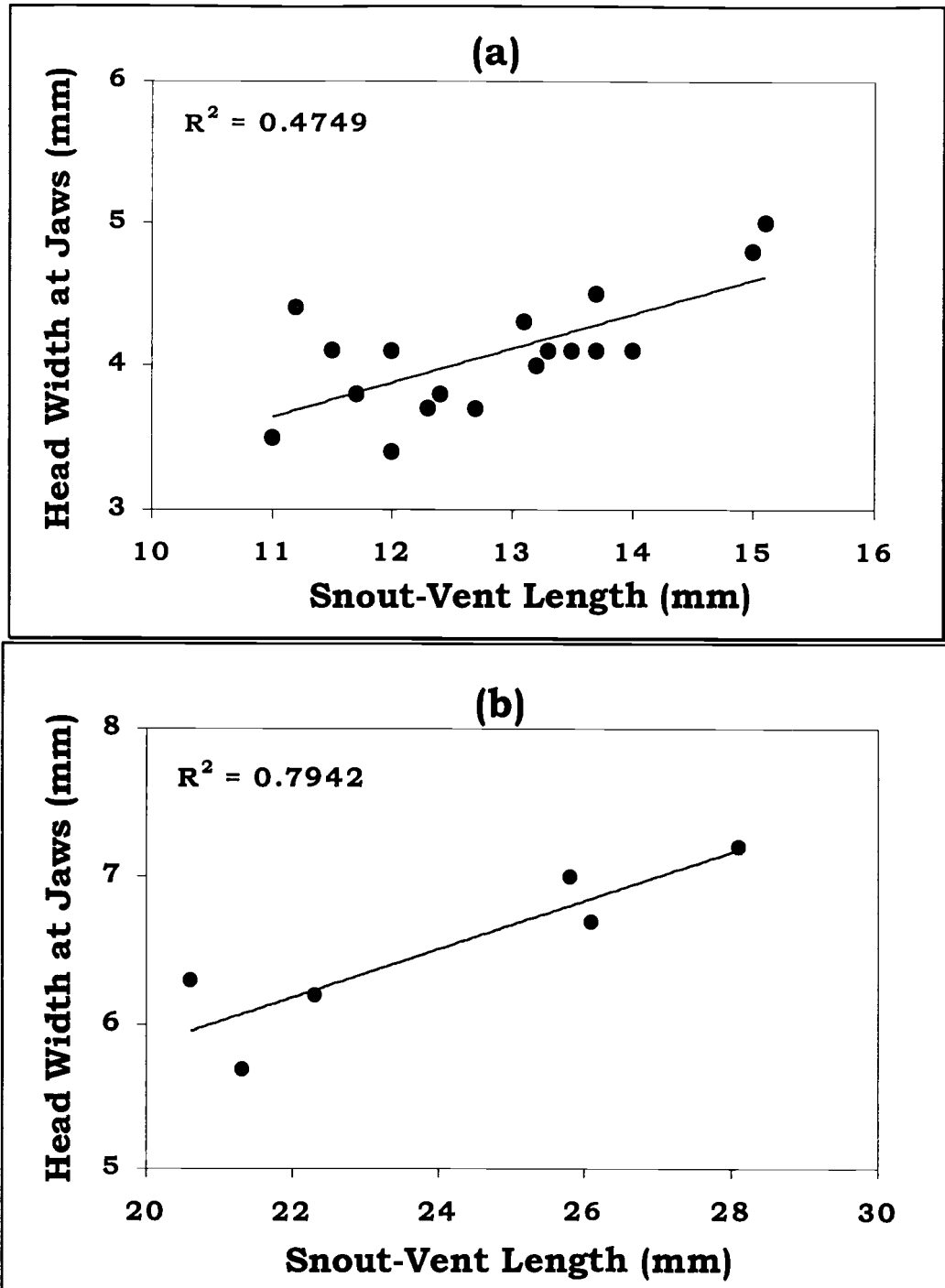


Figure 2.3 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Deep Pond (Willamette Valley) during the a) first and b) second surveys.

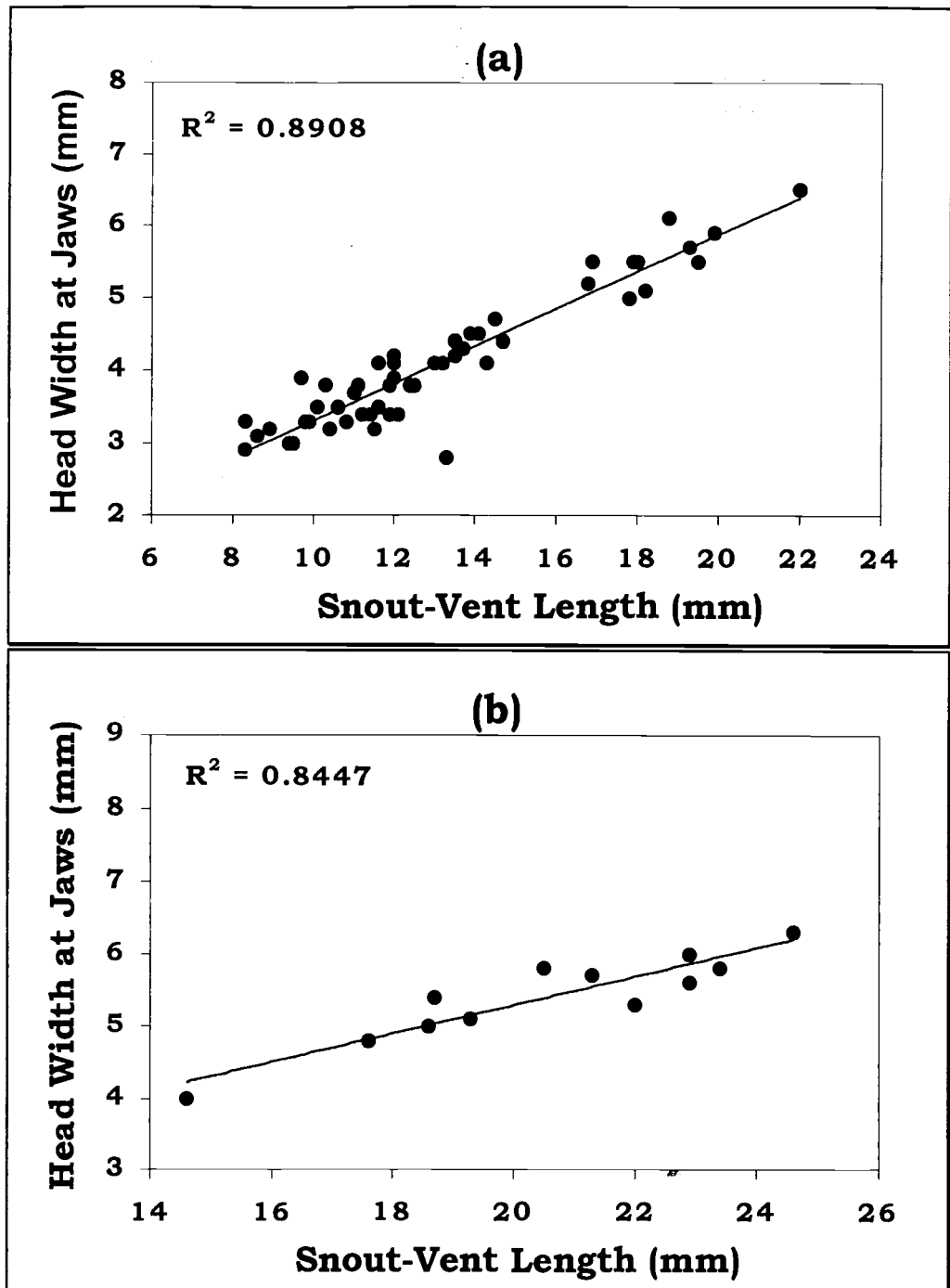


Figure 2.4 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Elliot Ditch (Willamette Valley) during the a) first and b) second surveys.

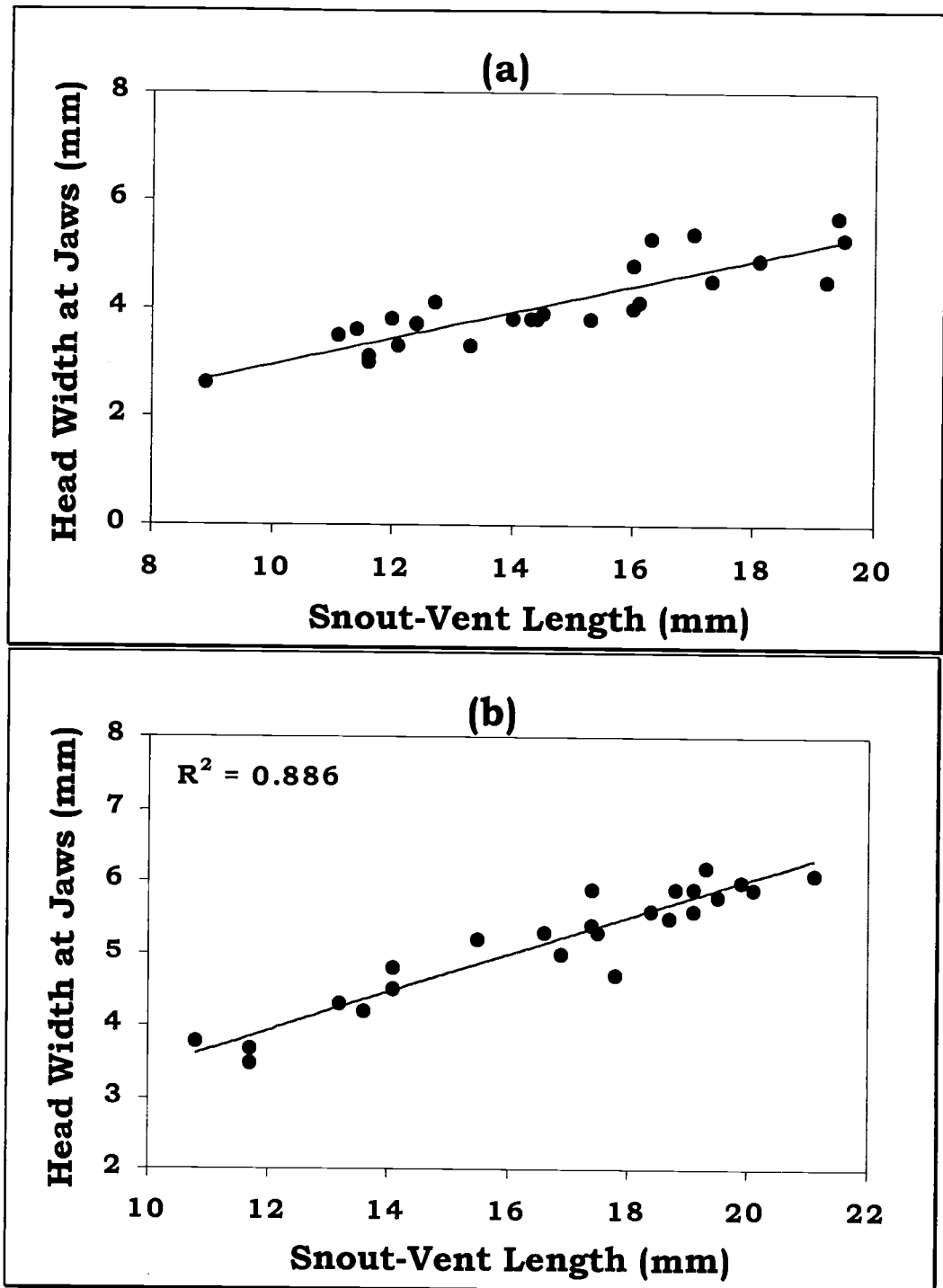


Figure 2.5 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Farm Pond (Willamette Valley) during the a) first and b) second surveys.

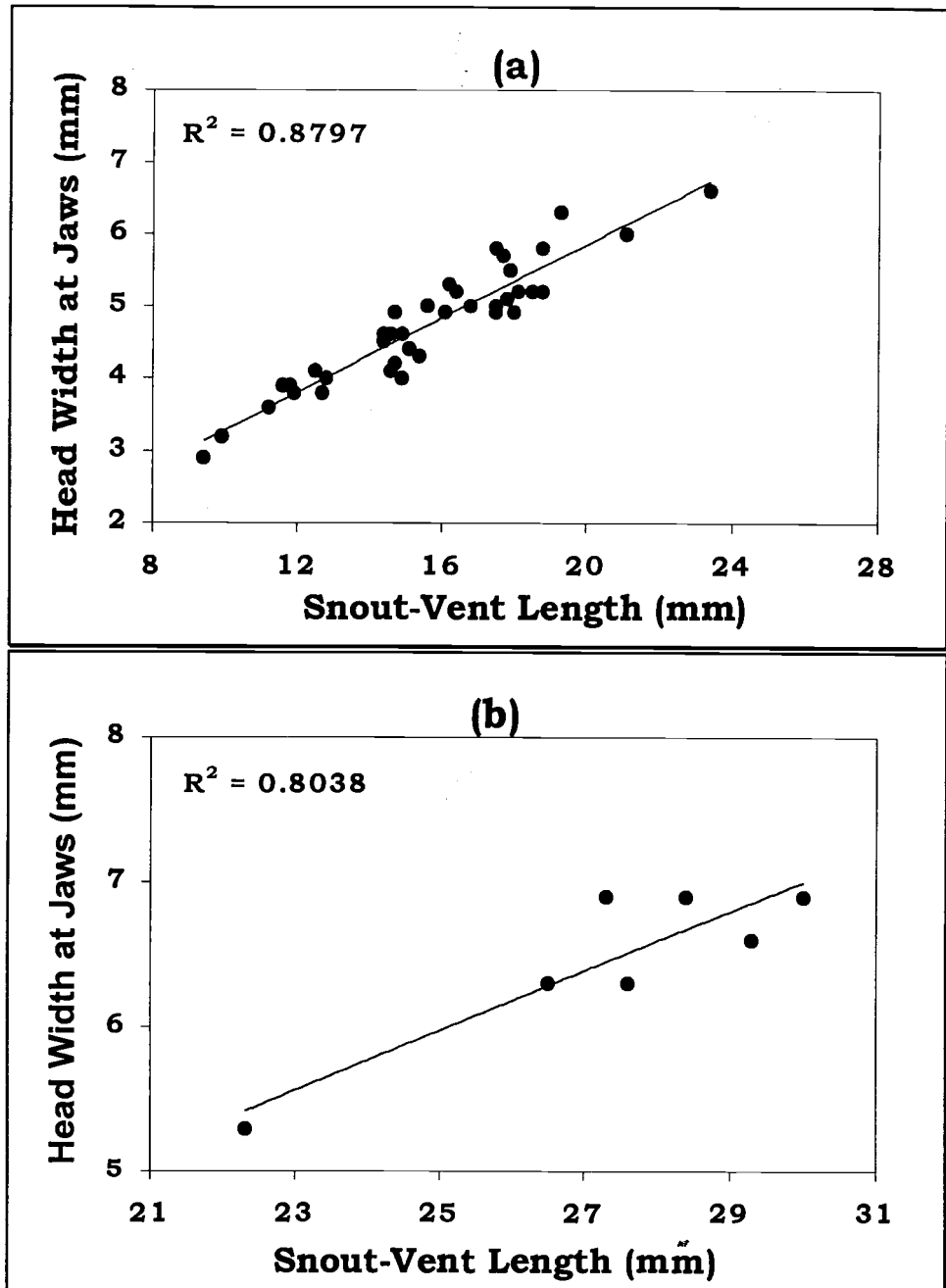


Figure 2.6 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Tangent Ditch (Willamette Valley) during the a) first and b) second surveys.

Mountain Populations

Cannibalism was recorded at 3 of the 4 sites during at least one of the surveys (Tables 2.4-2.5). Cannibalism was observed twice at Susan's Pond, occurring during the first survey with a frequency of 8% and during the third survey with a frequency of 2% (Table 2.4). Cannibalism was not observed at Eric's Pond during the last two scheduled surveys (Table 2.4). However, during a fourth survey, conducted 19 days after the last scheduled surveys were conducted, 20% of larvae surveyed at Eric's Pond were cannibals (Table 2.4). At Site A, 2% of larvae surveyed had eaten a conspecific during the second survey (Table 2.5). No cannibalism was observed at Big Lake (Table 2.5).

Larval *A. macrodactylum* co-occurs with *H. regilla* tadpoles at all four sites and consumption of these tadpoles was also observed at each of these sites. Larvae at Susan's Pond ate tadpoles during the first round of surveys with a frequency of 4% (Table 2.4). During the second round of surveys, the frequency of this behavior was 28% and 2% at Site A and Big Lake, respectively (Table 2.5). During the third round of surveys, 6% percent of larvae had consumed tadpoles at Eric's Pond (Table 2.4). Finally, 4% of larvae at Eric's Pond consumed tadpoles during the last, unscheduled survey (Table 2.4).

The majority of prey items retrieved from the stomachs of larval *A. macrodactylum* were non-amphibian, non-vertebrate prey (Tables 2.4–

2.5). Larvae ate a variety of insects including midges (Diptera), beetles (Coleoptera), mayflies (Ephemeroptera), dragonfly and damselfly larvae (Odonata), and notonectids and water boatman (Hemiptera).

Furthermore, some larvae also had winged insects in their diet. A variety of crustaceans, including fairy shrimp (Anostraca) and zooplankton (i.e. Copepoda, Cladocera) were also eaten as were freshwater clams (Mollusca) and worms (Annelida).

For populations at Big Lake, Site A and Susan's Pond, the CV in SVL was the highest during Survey 2, followed by Survey 3 and then Survey 1. (Table 2.6). For Eric's Pond, CV in SVL steadily increased with each consecutive survey.

Table 2.4 Stomach contents of *A. macrodactylum* larvae collected from Susan's Pond (Cascade Mountains) during the a) first (S₁, N = 42) b) second (S₂, N = 29) and c) third surveys (S₃, N = 50) and Eric's Pond during the d) second (S₂, N = 49), e) third (S₃, N = 50), and f) fourth surveys (S₄, N = 50). Data presented are the percentage of total larvae collected that had consumed the prey item listed. Potential prey include beetles (Coleoptera), midges (Diptera), mayflies (Ephemeroptera), backswimmers or water boatmen (Hemiptera), damselfly or dragonfly larvae (Odonata), unidentified winged insects (UWI), fairy shrimp (Anostraca), Zooplankton freshwater clams (Mollusca), segmented worms (Annelida), *H. regilla* tadpoles (Anura) or other larval *A. macrodactylum* larvae (Urodela).

	Susan's Pond			Eric's Pond		
	S ₁	S ₂	S ₃	S ₂	S ₃	S ₄
Vertebrata						
<i>A. macrodactylum</i>	10	0	2	0	0	22
<i>H. regilla</i>	2	2	0	0	6	4
Aquatic Insecta						
Coleoptera	10	0	0	11	4	10
Diptera	7	46	60	81	56	70
Ephemeroptera	26	0	0	11	0	0
Hemiptera	5	0	0	2	4	0
Odonata	10	0	0	2	10	6
(UWI)	19	0	4	4	12	6
Crustacea						
Anostraca	52	16	6	0	2	0
Zooplankton	7	4	2	98	86	72
Mollusca	0	0	0	0	24	66
Annelida	0	0	0	0	6	4

Table 2.5 Stomach contents of *A. macrodactylum* larvae collected from Site A (Cascade Mountains) during the a) second (N = 15) and b) third (N = 50) surveys and Big Lake during the a) second (N=25) and b) third surveys (N = 25). Data presented are the percentage of total larvae collected that had consumed the prey item listed. Potential prey include beetles (Coleoptera), midges (Diptera), mayflies (Ephemeroptera), backswimmers or water boatmen (Hemiptera), damselfly or dragonfly larvae (Odonata), unidentified winged insects (UWI), fairy shrimp (Anostraca), Zooplankton (Zoop), freshwater clams (Mollusca), segmented worms (Annelida), *H. regilla* tadpoles (Anura) or other larval *A. macrodactylum* larvae (Urodela).

	Site A		Big Lake	
	Survey 1	Survey 2	Survey 1	Survey 2
Vertebrata				
<i>A. macrodactylum</i>	2	0	0	0
<i>H. regilla</i>	33	0	3	0
Aquatic Insecta				
Coleoptera	4	8	0	4
Diptera	17	4	12	84
Ephemeroptera	4	0	0	4
Hemiptera	4	2	0	0
Odonata	15	0	3	0
(UWI)	4	0	0	4
Crustacea				
Anostraca	7	0	64	0
Zooplankton	54	90	0	48
Mollusca	0	70	3	48
Annelida	0	0	0	0

Table 2.6 Descriptive statistics of larval *A. macrodactylum* collected from surveys of the four Cascade Mountain sites. Presented here for each population are the mean Snout-Vent Length (SVL) \pm SE for all larvae, Coefficient of Variation (CV) in SVL, Mean SVL \pm of non-cannibals, Mean SVL \pm SE of cannibals, and Mean SVL \pm SE of larvae that ate *H. regilla* tadpoles. For those surveys where cannibalism and consumption did not occur (not applicable (N/A), the mean SVL is not presented.

Site Survey	Mean SVL \pm SE (mm) of All Larvae (N)	CV in SVL	Mean SVL \pm SE (mm) of Non-cannibals (N)	Mean SVL \pm SE (mm) of Cannibals (N)	Mean SVL \pm SE (mm) of Tadpole Eaters
Big Lake					
Survey 1	13.3 \pm .24 (47)	0.12	13.3 \pm .24 (47)	N/A	N/A
Survey 2	21.4 \pm .70 (50)	0.23	21.2 \pm .67 (49)	N/A	33.8 (1)
Survey 3	24.8 \pm .78 (25)	0.16	24.8 \pm .78 (25)	N/A	N/A
Susan's Pond					
Survey 1	24.7 \pm .48 (50)	0.13	24.57 \pm .51 (46)	26.45 \pm 1.0 (4)	N/A
Survey 2	31.3 \pm .99 (50)	0.22	30.95 \pm .96 (49)	N/A	47.2 (1)
Survey 3	34.1 \pm .75 (50)	0.16	33.91 \pm .73 (49)	45.0 (1)	N/A
Eric's Pond					
Survey 1	10.3 \pm .11 (50)	0.07	10.3 \pm .11 (50)	N/A	N/A
Survey 2	25.8 \pm .56 (50)	0.15	25.8 \pm .56 (50)	N/A	N/A
Survey 3	30.9 \pm .68 (50)	0.16	30.3 \pm .65 (47)	N/A	39.2 \pm .35 (3)
Survey 4	33.5 \pm .16 (50)	0.16	31.5 \pm .76 (37)	39.9 \pm .77 (11)	33.8 \pm 1.25 (2)
Site A					
Survey 1	10.5 \pm .23 (50)	0.16	10.5 \pm .23 (50)	N/A	N/A
Survey 2	28.7 \pm .98 (50)	0.24	26.4 \pm 1.07 (35)	35.3 (1)	34.1 \pm 1.38 (14)
Survey 3	30.3 \pm .81 (50)	0.19	30.3 \pm .81 (50)	N/A	N/A

As demonstrated in the Valley surveys, larvae from the mountain populations demonstrated a positive relationship between SVL and head width (Figures 2.7-2.10). Furthermore, larvae that cannibalized tended to be individuals with the largest body size and widest and longest heads (Figures 2.7a, 2.7c, 2.8a, 2.9c).

Figure 2.7 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Susan's Pond (Cascade Mountains) during the a) first and b) second and c) third surveys. Noncannibals (●), cannibals (Δ), and *H. regilla* tadpole consumers (□) are depicted. The trendline and R^2 values for the noncannibals are also shown.

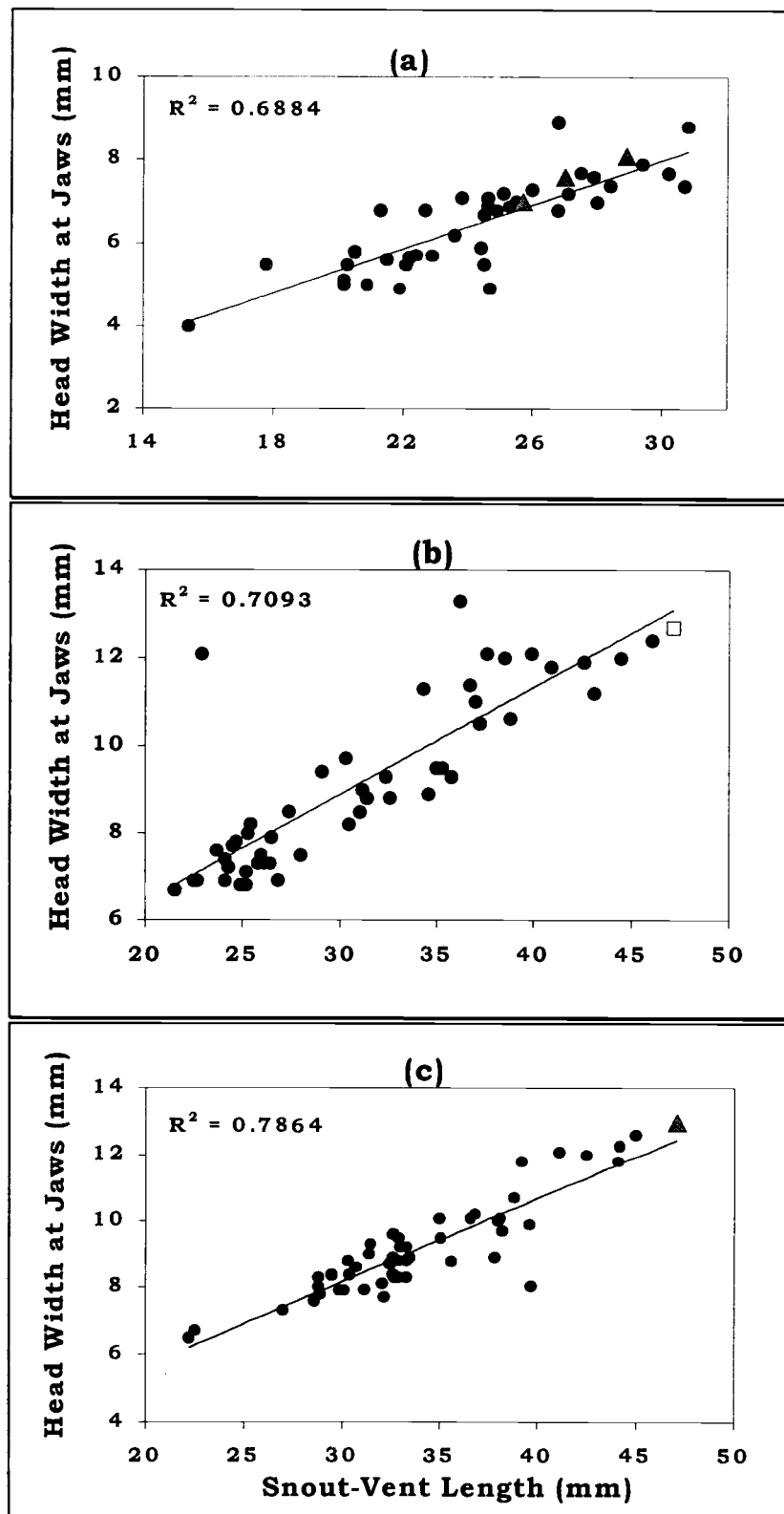


Figure 2.7

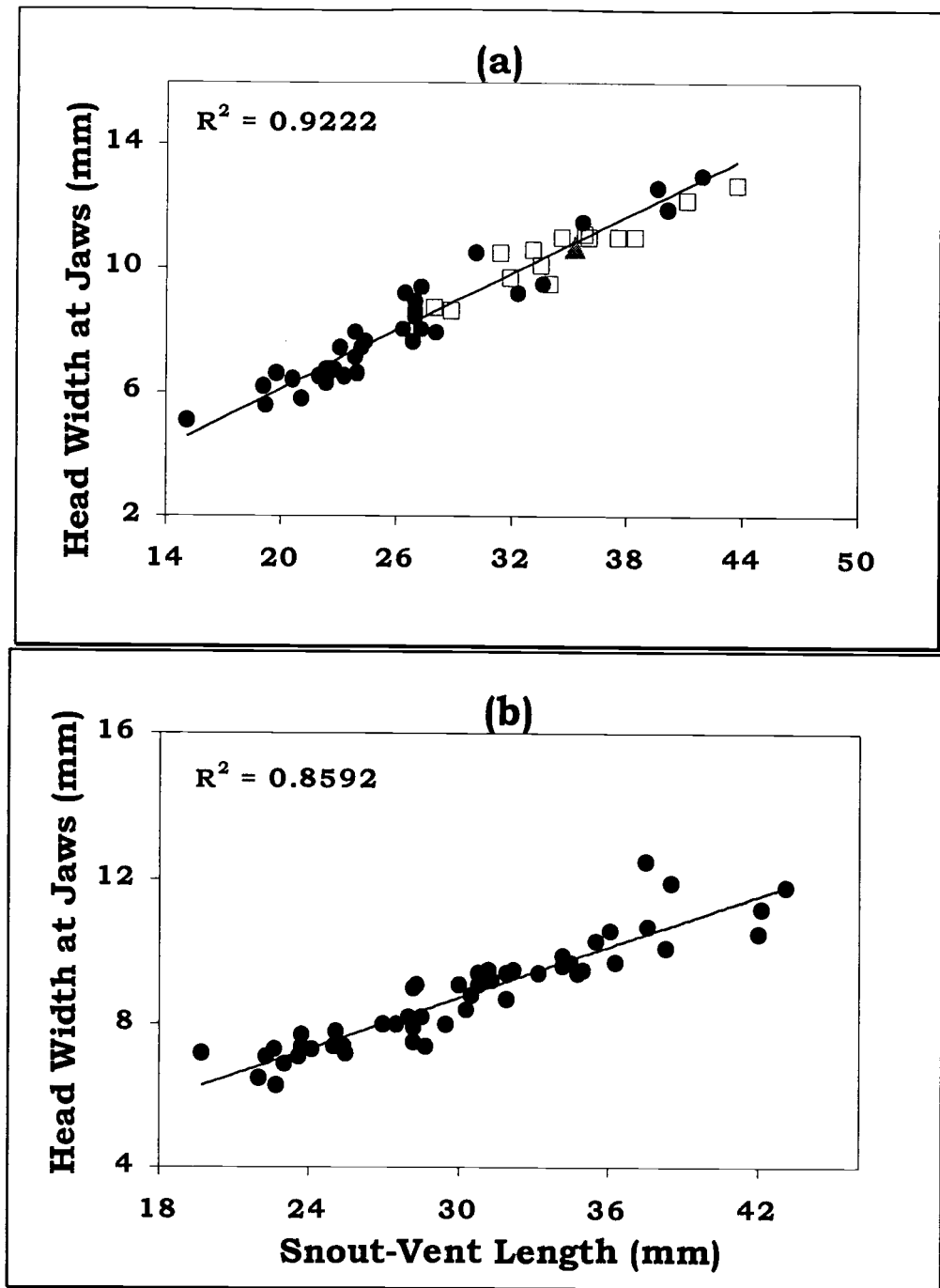


Figure 2.8 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Site A (Cascade Mountains) during the a) second and b) third surveys. Noncannibals (●) and *H. regilla* tadpole consumers (□) are depicted. The trendline and R^2 values for the noncannibals are also shown.

Figure 2.9 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Eric's Pond (Cascade Mountains) during the a) second b) third and c) extra unscheduled surveys. Noncannibals (●), cannibals (Δ) and *H. regilla* tadpole consumers (□) are depicted. The trendline and R^2 values for the noncannibals are also shown.

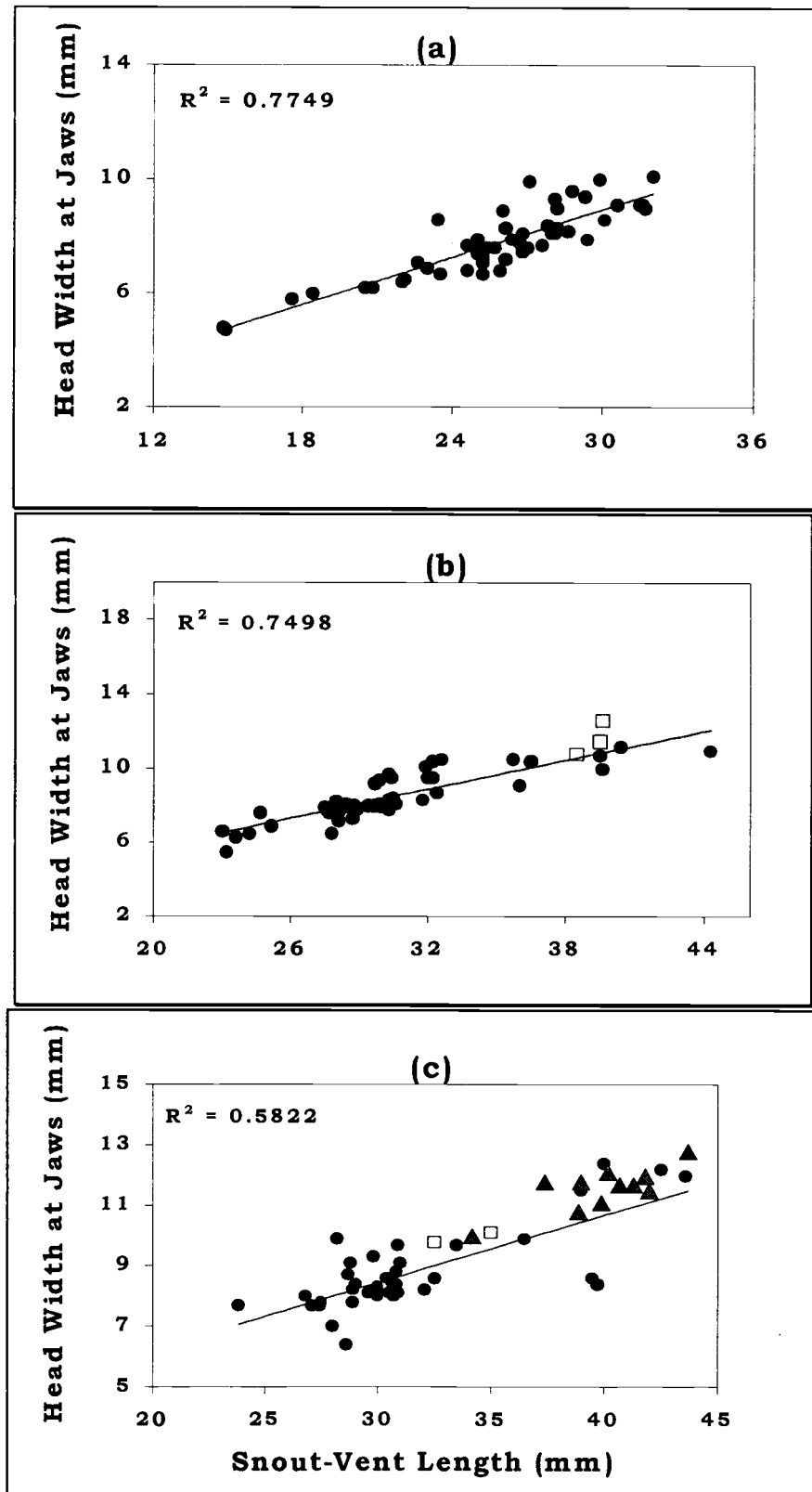


Figure 2.9

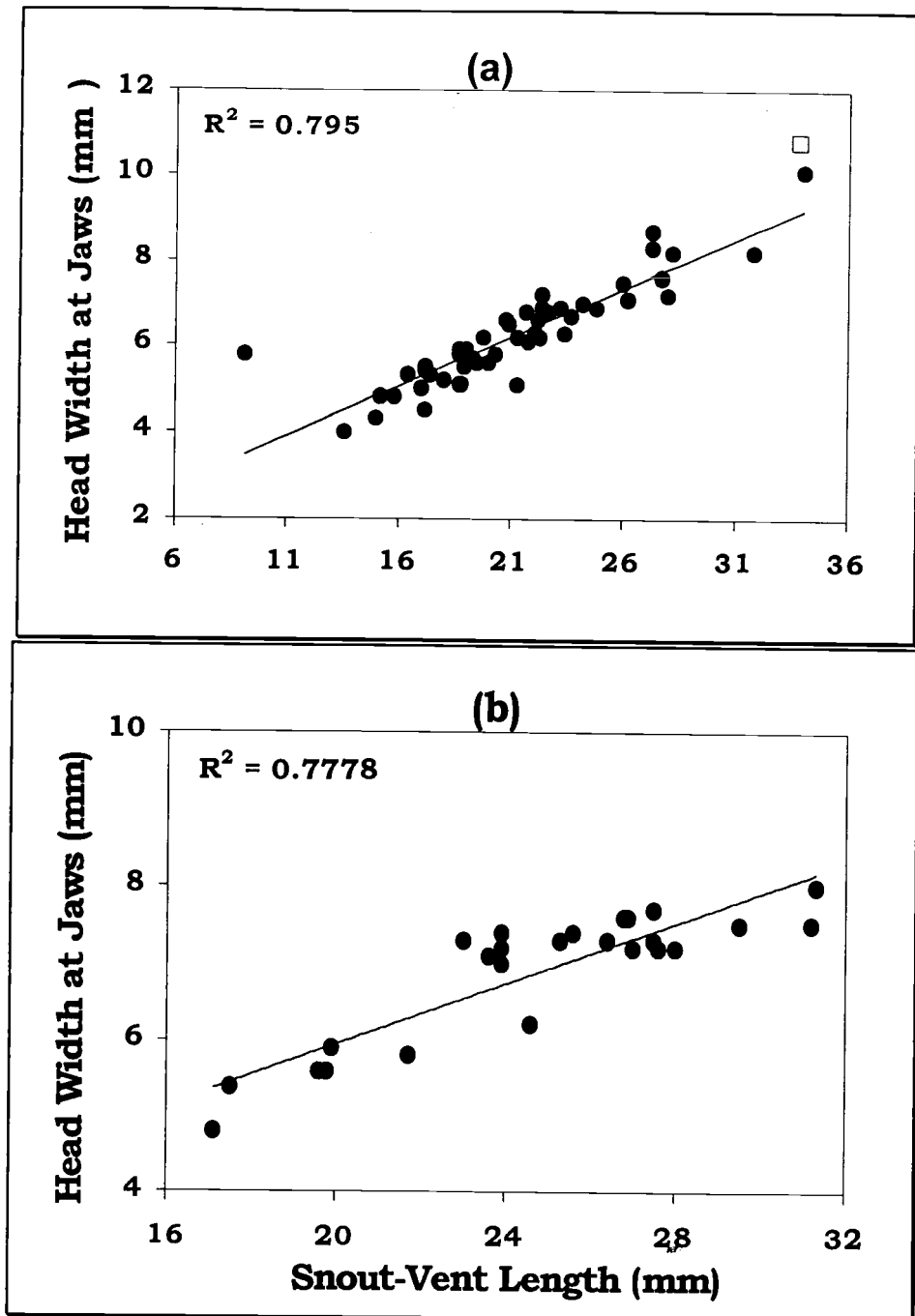


Figure 2.10 Correlation between snout-vent length (mm) and head width (at jaws, mm) of *A. macrodactylum* larvae collected from Big Lake (Cascade Mountains) during the a) second and b) third surveys. Noncannibals (●) and *H. regilla* tadpole consumers (□) are depicted. The trendline and R^2 values for the noncannibals are also shown.

Discussion

Cannibalism was documented at three out of the four Mountain populations and none of the six Valley populations. The lack of cannibalism in Valley sites and the presence of cannibalism in the montane sites may be influenced by variation in any number of abiotic and biotic factors associated with those habitats. For example, based on visual assessments, food seemed more abundant at Valley sites compared to the montane sites. Furthermore, at the montane sites, per capita food appeared to decrease over time while it appeared to remain steady if not to gradually increase over time in the Valley sites (Wildy, personal observation). A decrease in per capita food resources can promote aggression, including cannibalism, in animal populations (Fox, 1975; Polis, 1981; Ducey and Heuer 1991; Walls, 1998). Increased conspecific densities may also promote cannibalism (Collins and Cheek, 1983) and can heighten this behavior in a food-limited environment. Densities of larvae were not quantified during these surveys but were observed to be much lower (< 5 per m^2) in some Valley ponds (i.e., Angler's Pond, Farmers Pond, Backwoods Pond, Deep Pond) compared to montane populations (< 10 per m^2). The combined effects of one or both of these factors may be enough to promote cannibalism in montane populations.

Increased size variation among animals in a population may also facilitate aggression including cannibalism (Maret and Collins, 1994; Kusano et al. 1985; Nyman et al. 1993; Maret and Collins 1994; Petranksa and Thomas 1995). This may be particularly common in high density and food limited environments where some larval amphibians may be able to sequester a disproportionate amount of food early in the larval period due to genetic differences in competitive abilities (Wilbur and Collins, 1973) or by chance. The resulting growth spurt gained early on may offer them an increased ability to catch and consume larger-sized prey including conspecifics and thus increase their competitive advantage for limited resources over conspecifics (Persson 1985; Smith 1990; Walls and Semlitsch, 1991). The growth potential of smaller larvae may be minimized as their foraging efforts are compromised (Smith 1990) perhaps due to being attacked by larger animals (Van Buskirk and Smith 1991; Ziemba and Collins, 1999) and/or due to changes in foraging patterns in response to the presence of larger conspecifics (Chivers et al. 1997; Ziemba and Collins, 1999).

There was only a slight correlation between size variation and the frequency of cannibalism in the current study. At the mountain sites, cannibalism occurred where the coefficient of variation in size was the highest in both Eric's Pond and Site A but not at Susan's Pond.

Additional surveys are needed in which the densities of larval

A. macrodactylum and their potential prey are quantified to determine whether these factors are indeed correlated with the incidence of cannibalism. Other factors, such as rate of pond drying and temperature fluctuation could also be evaluated for their role in influencing increased cannibalism. Furthermore, conducting surveys for more than one year is necessary to begin to determine whether the frequency of cannibalism at a particular site varies from year to year and whether this can be correlated with annual variation in certain abiotic and biotic factors inherent to that site. The current surveys were conducted during a year in which two of the three typically ephemeral montane sites failed to dry. This may have been particularly important in influencing the amount of cannibalism observed during the present surveys since this behavior may be influenced by factors such as larval (Collins and Cheek, 1983; Walls, 1998) and prey density (Loeb et al., 1994), and prey type which are, in turn, influenced by rate of pond drying (Wilbur, 1987; Semlitsch and Wilbur, 1988; Newman, 1989).

A more critical look into those sites where cannibalism is absent may also provide insight into those factors that promote cannibalism. In the current series of surveys cannibalism did not occur at Big Lake. It is possible that cannibalism does occur at this site but was not detected during these surveys. Alternatively, cannibalism may be particularly rare at this site or may not occur at all. This site had the largest

circumference of the four montane sites surveyed and it was by far the deepest. Based on visual assessments, larvae at Big Lake appeared to be less densely clustered than at the other montane sites. As high densities have been demonstrated to promote increased aggression and cannibalism in larval amphibians (e.g. Collins and Cheek 1983; Semlitsch and Reichling 1989; Nishihara 1996), perhaps the minimization of this condition at Big Lake stopped larvae from engaging in this behavior.

Larval size may have also played a role in eliminating the importance of cannibalism at Big Lake. Individuals at this site were generally smaller on average than larvae at the other sites. Gape size is the limiting factor controlling the size of prey larval salamanders can eat. As gape size is directly influenced by larval body size, this may have made it mechanically difficult, if not impossible, for larvae from Big Lake to consume larger-sized prey including conspecifics and other amphibian larvae. In fact, the consumption of a *H. regilla* tadpole was recorded only once (second survey) at this site.

The absence of cannibalism (and tadpole eating) in the Valley populations may be explained by the relatively low larval densities at most of these sites (i.e. Farmers Pond, Deep Pond, Backwoods Pond, Anglers Pond; not necessarily Elliot Ditch, Tangent Ditch) and, perhaps

more importantly (see Chapter 3, this thesis), by the relatively abundant food resources.

In addition, as with larvae at Big Lake, larvae surveyed at the Valley sites were relatively small compared to larvae originating from mountain populations where cannibalism occurred (see Tables 1 and 2) and this may have affected their ability to capture and consume larger sized prey items. Consequently, larval salamanders in valley populations may have not been able to include amphibian larvae in their diets.

The larvae that cannibalized in the Mountain populations appeared to be the largest animals with the largest heads (Figures 2.7a, 2.7c, 2.8a, 2.9c). Presumably, it is the increased gape that accompanies the increased head size that allows cannibals to be so effective at handling and consuming rather large-sized prey (Reilly et al., 1992; Walls et al., 1993a). The larger heads, and corresponding increased gape presumably aid cannibals in their ability to handle and consume these rather large-sized prey animals (Walls et al., 1993a; Reilly et al., 1992). This is further supported by the fact that larvae consuming *H. regilla* tadpoles also tended to be larger in size relative conspecifics that had not eaten amphibian prey (see Figures 2.7b., 2.8a, 2.9b,c, 2.10a). However, this trend was not as extreme as that seen for cannibals.

In both Mountain and Valley populations, measurements for head width at eyes, head width at jaws, head width at gills and head length all

appeared to be positively correlated with their snout-vent length. As larvae increased in body length, measurements of their head width and length increased accordingly. Although separate cannibal and typical morphologies were previously documented at Susan's Pond (Walls et al., 1993a), I did not observe this pattern. If there were separate cannibal and typical morphologies, it would be expected that two subpopulations of larvae would exist where the measurements of head width and length of one group would follow a pattern similar to that depicted on Figures 2.6-2.20, while the other group would have measurements of head width and length that reflect relatively lower values for the same measurements of SVL.

It is possible that during the year in which the current study took place that larvae exhibited only one of the two morphologies. Previous studies examining this phenomenon in tiger salamanders, *Ambystoma tigrinum*, have demonstrated that some sites contain all typical morphs while other sites contain a mixture of both cannibal and typical morphs (Pfennig et al., 1991; Loeb et al., 1994). Furthermore, the frequency of the two morphologies at a given site may from year to year since it can be influenced by environmental factors (Collin and Cheek, 1983; Walls et al., 1993b; Hoffman et al., 1999). Perhaps the morphological profile of larvae at Susan's Pond during the year in which the current study was

conducted was not the same as the year in which the cannibal morph was originally documented by Walls et al. (1993b).

It is also possible that there was not adequate information collected to fully determine whether separate cannibal and typical morphs were actually present. For example, only 4 out of the 5 possible characteristics measured in the original study in which the morph was described were recorded. Walls et al., 1993 found that HWE, HWJ, HWG and HL differed significantly between cannibals and typicals. In addition, they also measured interocular width and found that to be the most important characteristic explaining the difference between cannibals and typicals. Moreover, they found that cannibals had enlarged vomerine teeth. An assessment of this characteristic was not made during my surveys and it is possible that an enlargement of these teeth, characteristic of the cannibal morphology, was present in some animals but was overlooked. This characteristic can be the most important in the classification of Ambystomatid larvae as possessing the cannibal morphology since characteristics of the head morphology can be subject to variation (Walls, 1993 a, b; E. Hoffman, pers. comm).

Despite the questions surrounding the existence of the cannibal morphology in natural populations, cannibalism clearly occurs naturally in some populations of larval *A. macrodactylum*. In the following chapters, I focus primarily on the significance of the behavioral aspect

rather than the morphological aspect of cannibalism in these populations. Moreover, I attempt to address some of the questions raised by these surveys including the environmental factors influence cannibalism in populations of larval *A. macrodactylum* and why cannibals tend to be some of the largest animals in the populations.

Acknowledgements

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Chapter 3

The Effects of Food-Level and Density on Agonistic Behavior in Larval Long-Toed Salamanders, *Ambystoma macrodactylum*

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Abstract

Previous studies have examined abiotic and biotic factors that potentially facilitate agonistic behaviors, including cannibalism. For larval amphibians, the amount of prey consumed and conspecific density have been suggested as important factors influencing biting and cannibalism. In this study, we examined the separate and combined effects of food availability and density on the life history and agonistic interactions of larval long-toed salamanders, *Ambystoma macrodactylum*. We designed a 2 x 2 factorial experiment, in which larvae were raised with either a high or low density of conspecifics and fed either a high or low level of food. We quantified the number of larvae that had received bites to the limb, tail and gill regions, and the percentage of individuals that had been cannibalized. Additionally, we measured survival at, time to and size at metamorphosis. Results indicate that differences in both density and food level influenced all three life history traits measured. Furthermore, differences in food level influenced variation in body size and biting among larvae while both density and food level contributed to differences observed in cannibalism. Larvae fed a lower amount of food demonstrated a higher within-tank variation in snout-vent length. We suggest that this variation in size was a consequence of the domination of limited food resources by a few larvae. Lack of sufficient food may have heightened biting among larvae and combined with high densities,

may have prompted larger individuals to seek an alternative food source in the form of smaller conspecifics.

Introduction

Agonistic behavior in animals can be influenced by a variety of factors including competition for food (Persson, 1988; Drummond and Chavelas, 1989), mates (Ovaska, 1987), and space (Crowley et al., 1987; Ducey and Ritsema, 1988; Mathis, 1990; Walls, 1990). Intraspecific agonistic behaviors, including cannibalism, have been documented in numerous amphibian species (see reviews in Fox, 1975; Polis, 1981; Polis and Meyers, 1985; Crump, 1992; Mathis et al., 1994). In amphibians, agonistic behavior (including cannibalism) may also be influenced by the presence of vulnerable heterospecific (Maret and Collins, 1996) or conspecific prey (Loeb et al., 1994; Maret and Collins, 1994), diet type (Pfennig, 1990; Walls, 1993b), the presence of kin (Walls and Blaustein, 1995; Pfennig and Collins, 1993; Pfennig and Frankino, 1997; Pfennig 1999), food limitation (Semlitsch and Reichling, 1989; Walls, 1998), and high densities of conspecifics (Collins and Cheek, 1983; Semlitsch and Reichling, 1989; Walls, 1998).

Variation in conspecific density and food limitation has been associated with increased biting in amphibians and in the development of morphologies that serve as indicators of aggression (i.e. cannibalistic morphologies) at both the aquatic (Fox, 1975; Polis, 1981; Collins and Cheek, 1983; Crump, 1983; Semlitsch and Reichling, 1989) and terrestrial (Ducey and Heuer, 1991) life history stages. Furthermore, it

has been demonstrated that increased aggression may be influenced by the effects that variation in density and food limitation have on size variation within a population. Increased size variation among individuals within a population may promote increased aggression in larval amphibians (Maret and Collins, 1994; Ziemba and Collins, 1999). It has been suggested that in populations in which the variation in sizes of individuals is high, smaller individuals become more vulnerable to aggression from larger conspecifics (Fox, 1975; Polis, 1981; Dong and Polis, 1992). This situation may be further aggravated by other factors including high densities of conspecifics and/or low food resources.

Aggressive behaviors, in the form of intraspecific biting and cannibalism, has been documented in populations of larval long-toed salamanders, *Ambystoma macrodactylum*, (Anderson, 1967; Nussbaum et al., 1983; Walls et al., 1993a; Wildy et al., 1998; Wildy et al., 1999). Several factors influencing cannibalistic behavior in this species have been explored (Walls, 1993b; Wildy et al., 1998), but have yet to be fully understood. In populations of *Ambystoma macrodactylum*, where cannibalism exists, densities of larvae are relatively high and food resources appear limited (Wildy, Chapter 2). Furthermore, larvae within these populations exhibit a large range in body sizes. To examine how variation in density and food level affect size variation within a population and how this may, in turn, influence agonistic behavior in

this species, we conducted a laboratory experiment in which we manipulated conspecific density and the level of food to which larvae were exposed. Larvae were raised under conditions of either low or high density and low or high food levels.

Methods

We collected *A. macrodactylum* larvae in February 1996 from an ephemeral pond located 26 km east of Corvallis, Benton County, Oregon, U.S.A. We transported larvae to the laboratory and housed them in four 38 L aquaria. These larvae served as our source group. Prior to the experiment, larvae were fed zooplankton ad libitum every other day and were maintained on a 14:10 h light:dark cycle.

Our experiment began on March 12, 1996 and involved a 2 x 2 factorial design, crossing two levels of density with two levels of food. Larvae were raised in 38 L aquaria in one of four treatments: 1) low density/low food, 2) low density/high food, 3) high density/low food, and 4) high density/high food. Initially, each treatment was replicated six times for a total of 24 aquaria. However, one aquarium in the low density/low food treatment was eliminated from the analysis due to the unexplained death of all of the larvae. The final analysis included 23 aquaria. Focal larvae were chosen from the four source aquaria. Larvae were matched for size so that initial differences in size would be minimal.

Density regimes consisted of 10 larvae and 40 larvae for the low and high levels respectively. All larvae were fed zooplankton collected from local ponds every other day for the first four weeks. Animals in the low food treatments were given 2 mL solution of zooplankton per larva for a total of 20 mL in the low density/low food treatments and 80 mL in the high density/low food treatments. Animals in the high food treatments were given 8 mL solution per larva for a total of 80 mL in the low density/high food treatments and 320 mL in the high density/high food treatments. To provide a standard density of zooplankton in each aliquot, we thoroughly stirred the zooplankton solution before each aliquot was extracted. After week five, larvae were fed *Tubifex* worms every other day. Each larva received 10 worms or 40 worms total in the low density/low food and high density/low food treatments respectively (i.e., 1 worm per larva) and 40 worms or 160 worms in the high density/low food and high density/high food treatments, respectively (i.e., 4 worms per larva).

We monitored the experimental aquaria daily and removed dead larvae. The total number of larvae in each aquarium was assessed weekly. Larvae that were missing and that could not be accounted for due to metamorphosis or death were presumed to have been cannibalized. Aquaria were cleaned once per week during which time all larvae were removed. During aquarium cleaning on weeks 3, 5, 7 and 9,

larvae were assessed for injuries due to aggressive intraspecific interactions. Larvae were considered to be recipients of aggressive behavior if they exhibited bites to their limbs, tail and/or gills. We chose to assess these particular characters because they are common sites of damage resulting from conspecific attack in larval salamanders (Semlitsch and Reichling, 1989; E. Wildy, personal observation). Measurements were repeated every other week as opposed to every week, because it allowed time for larvae to initiate regeneration of missing pieces of tail, limbs or gills making it easier for the observer to differentiate between old and new wounds. As test animals reached metamorphosis (i.e. beginning of gill reabsorption) they were removed from their aquarium and their snout-vent length was recorded and time to metamorphosis, in days, was calculated.

The experiment ended after 100 days (June 19, 1996), an amount of time that falls within normal ranges of pond duration inhabited by this species in this region (E. Wildy, personal observation). Larvae that had not completed metamorphosis at this time were considered to have not survived.

Statistical Analysis

Multivariate analysis of variance (MANOVA) was used to examine the effects of density and food level on survival to, time to and size at

metamorphosis of the test animals. After MANOVA, univariate analysis of variance (ANOVA) F tests were used on each response variable to assess which variables were responsible for significant main effects. For each response variable we calculated means for the animals in each tank and used these means for all statistical analyses. Post hoc comparisons (Tukey tests) were used to check for differences between means for the four treatments.

Measurements of within-tank variation in snout-vent length, aggression and cannibalism averaged over weeks 3, 5, 7 and 9 were analyzed using ANOVAs. As a measure of variance in larval size, the coefficient of variation of final snout-vent length was calculated and averaged for each tank. To assess aggression, we averaged the total number of injuries received by each larva throughout the duration of the experiment and averaged the number of injuries per tank. At the end of each two week period, the level of cannibalism within a tank was assessed by calculating the percentage of larvae that were missing and therefore, presumed eaten. We used repeated measures ANOVAs to evaluate the effects of density and food level on these three response variables over time. Tank means for all three measurements were used for all statistical analyses. For all analyses, parametric assumptions were met and, therefore, no data transformations were performed.

Results

As indexed by the CV, larvae responded differently in size, aggression, and cannibalism to levels of density and food (Tables 3.1, 3.2, 3.3). The CV in size, aggression, and cannibalism were highest in the two low food treatments (Figure 3.1). Density did not influence the CV magnitude in size or aggression was significantly related to the amount of cannibalism exhibited between larvae (Table 3.3, Figure 3.1).

The CV in size, aggression, and cannibalism were all affected by time and by interactions between time and density, time and food and time, density, and food (Tables 3.2, 3.3, and 3.4). The change in aggression over time appeared to follow the change in the coefficient of variation over time with animals in the two low food treatments exhibiting the highest size variation and aggression during the last two weeks (i.e., weeks 7 and 9) of sampling (Figures 3.2a,b). Cannibalism was highest in the two low food treatments at the beginning of the experiment but leveled off to relatively low levels in all treatments during the last weeks of sampling (Figure 3.2c).

The results of our MANOVA revealed strong main effects of density and food on larval life history (Table 3.4). In addition, there was a strong interaction between density and food level. Post hoc ANOVAs indicated larval survival and the time required to reach metamorphosis were affected by both food level and density (Table 3.4). Larvae exhibited

reduced survival and took longer to metamorphose in the two low food treatments (Figures 3.3a,b). Higher densities appeared to reduce the time it took for larvae to metamorphose within low food treatments and decreased probability of survival within both low and high food treatments (Figures 3.3a,b).

Table 3.1 Summary Statistics for ANOVA of Total Coefficient of Variation (CV) in Snout-Vent length over weeks 3, 5, 7 and 9 and for Repeated-Measures ANOVA for Coefficient of Variation.

Source of Variation	F	df	p
Total CV			
density	2.898	1	0.105
food	10.142	1	0.005
density X food	0.640	1	0.434
CV Over Time			
Between-Subject Effects			
density	2.890	1	0.105
food	10.156	1	0.005
density X food	0.636	1	0.435
Within-Subject Effects			
time	20.620	3	0.000
time X density	5.498	3	0.002
time X food	4.623	3	0.006
time X density X food	0.486	3	0.693

Table 3.2 Summary Statistics for ANOVA of Total Biting over weeks 3, 5, 7, and 9 and for Repeated-Measures ANOVA for Biting.

Source of Variation	F	df	p
Total Biting			
density	0.571	1	0.459
food	26.044	1	0.000
density X food	2.940	1	0.103
Biting Over Time			
Between-Subject Effects			
density	0.570	1	0.459
food	26.039	1	0.000
density X food	2.940	1	0.103
Within-Subject Effects			
time	22.606	3	0.000
time X density	7.861	3	0.000
time X food	21.445	3	0.000
time X density X food	3.725	3	0.016

Table 3.3 Summary Statistics for ANOVA for Total Cannibalism over weeks 3, 5, 7, and 9 and for Repeated-Measures ANOVA for Cannibalism.

Source of Variation	F	df	p
Total Cannibalism			
density	17.699	1	0.000
food	60.711	1	0.000
density X food	2.177	1	0.156
Cannibalism Over Time			
Between-Subject Effects			0.000
density	17.775	1	0.000
food	61.138	1	0.160
density X food	2.137	1	
Within-Subject Effects			
time	40.356	3	0.000
time X density	6.691	3	0.001
time X food	4.961	3	0.007
time X density X food	3.725	3	0.365

Table 3.4 Results of MANOVA for overall effects of food level and density on time to, survival to and size at metamorphosis for larval *A. macrodactylum* and ANOVAs for each response variable.

	F	df	p
MANOVA			
constant	9040.794	3, 17	0.000
density	9.978	3, 17	0.001
food	171.513	3, 17	0.000
density X food	9.259	3, 17	0.001
ANOVAs			
Survival			
density	19.837	1, 19	0.000
food	46.506	1, 19	0.000
density X food	0.000	1, 19	0.985
Time			
density	9.584	1, 19	0.006
food	65.305	1, 19	0.000
density X food	8.154	1, 19	0.010
SVL			
density	0.001	1, 19	0.971
food	17.546	1, 19	0.000
density X food	2.123	1, 19	0.161

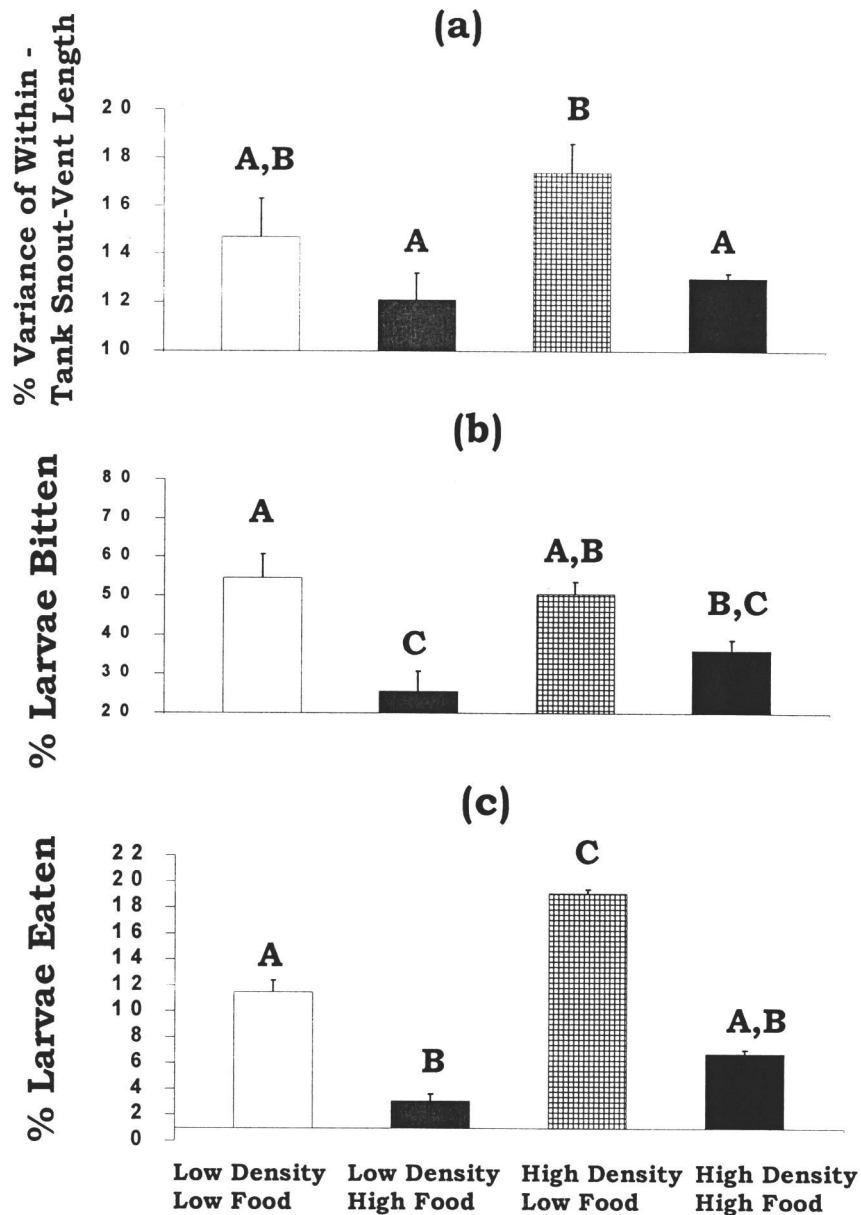


Figure 3.1 Mean (\pm SE) (a) coefficient of variation of snout-vent length, (b) biting (percent larvae bitten) and (c) cannibalism (percent larvae eaten) for *A. macrodactylum* larvae exposed to low and/or high density and food levels. All data represent averages of measurements taken during weeks 3, 5, 7 and 9. If two treatments share the same letter, there is no significant difference.

Figure 3.2. Change in (a) coefficient of variation (CV) in body size, (b) aggression and (c) cannibalism over time for *A. macrodactylum* larvae raised in the low density/low food (-●-), low density/high food (-O-), high density/low food (-▼-) and high density/high food (-▽-) treatments.

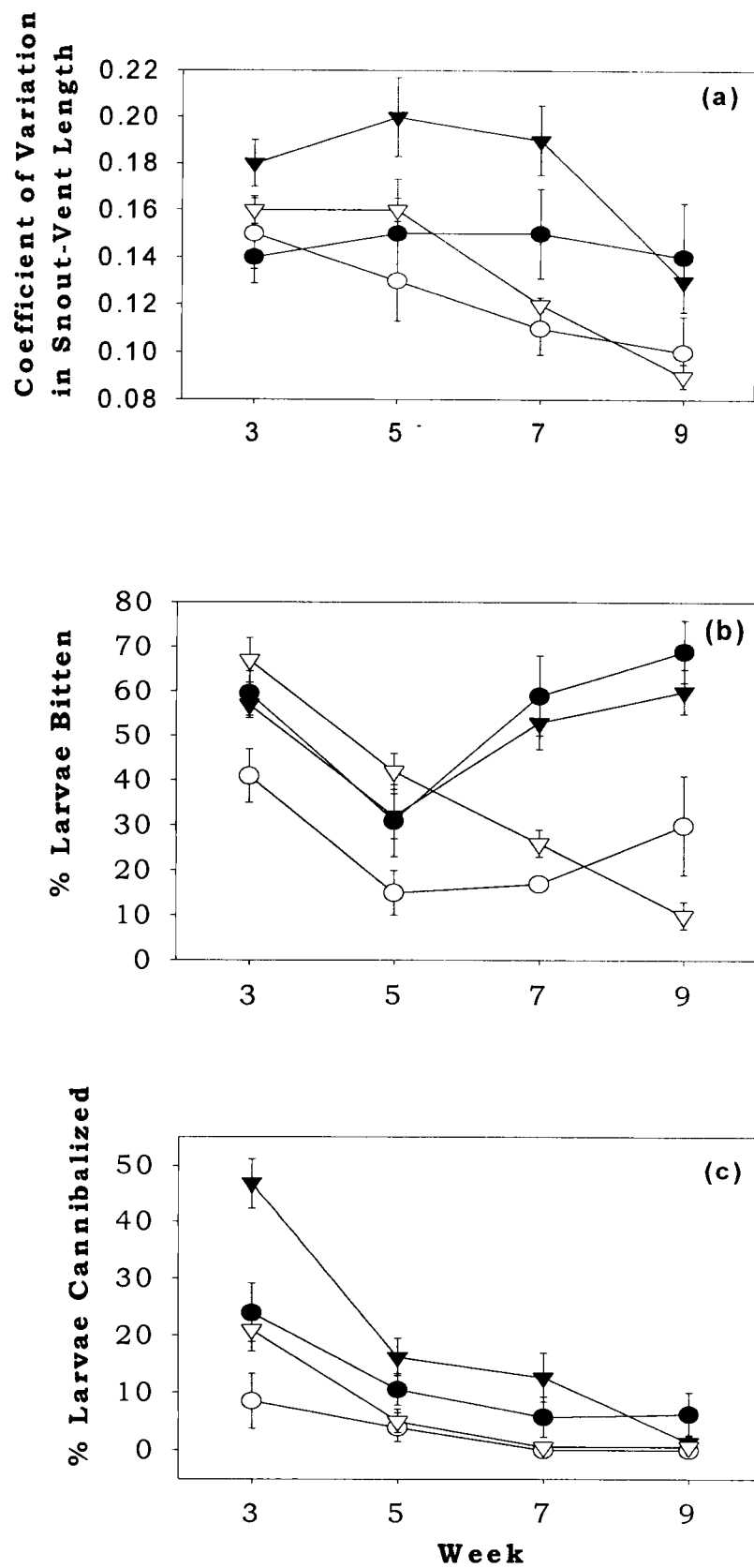


Figure 3.2

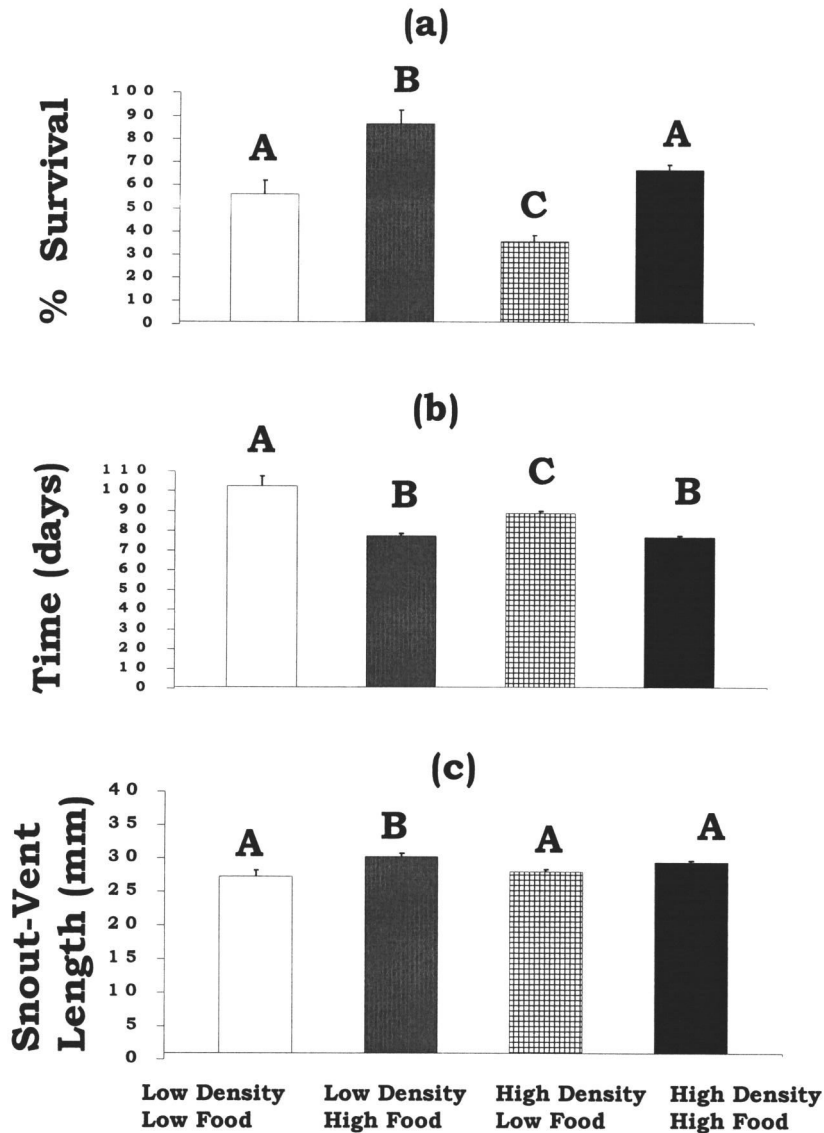


Figure 3.3 Mean (\pm SE) (a) percent survival to, (b) time to (days) and (c) size (millimeters) at metamorphosis for *A. macrodactylum* larvae exposed to low and/or high density and food levels. Letters over bars indicate which treatments are statistically different from one another. If two treatments share the same letter, there is no significant difference.

Size at metamorphosis was slightly influenced by food levels such that larvae in the most benign treatment, low density/high food,

exhibited the greatest mean snout-vent length ($SVL \pm SE = 30.23 \pm 0.52$) by the end of the experiment (Figure 3.3c).

Discussion

Our results indicate that variation in density and food level can influence agonistic behavior in larval long-toed salamanders. Specifically, we found a strong effect of food on both intraspecific biting and cannibalism. Both behaviors were heightened in larvae reared with low food resources. Density also significantly contributed to the increased incidence of cannibalism within a food treatment, with greater frequency at higher densities. This suggests that cannibalism, as opposed to a less extreme form of aggression such as biting, is more likely to occur as the harshness of the environment increases, when the benefits of cannibalism begin to outweigh the costs. These costs may include injury while struggling with the potential victim (Dong and Polis, 1992; Crump, 1992), the inadvertent consumption of a relative (Dong and Polis, 1992) or increased exposure to diseases (Polis, 1981; Pfennig et al. 1991, 1998; but see Pfennig et al., 1999).

The pattern of larval survival in the different treatments is most likely explained by the incidence of biting and cannibalism. Larvae in the two low food treatments exhibited reduced survival compared to animals in the high food treatments. Moreover, within a food treatment,

larvae raised at higher densities did not survive as well. Animals that could not be accounted for were presumably cannibalized as we checked aquaria daily and removed bodies larvae ($n=17$) that had died as a result of some other factor.

We suggest that the frequency with which intraspecific biting and cannibalism occurred within particular treatments was influenced by the variation in body size exhibited among larvae. Variation in size within a population of larval amphibians occurs naturally and may result for several reasons. For example, size differences may be the result of genetic differences between clutches. Alternatively, larvae which are of similar size initially may engage in unequal acquisition of food by chance or due to differences in patterns of resource use, allowing some individuals to become larger faster. This growth advantage early on would allow them to retain a competitive advantage throughout the rest of the larval period. This may be particularly true if the mechanism by which larvae are competing is through interference competition as opposed to exploitative competition (Wilbur and Collins, 1973; Persson, 1985; Smith, 1990; Walls, 1998; Ziemba and Collins, 1999).

We believe that this latter scenario occurred in our study. Larvae in the low food treatments had the highest coefficient of variation in snout-vent length. At the beginning of the experiment, larvae were size-matched. During the experiment, variation in larval size could only have

been the result of genetic differences or unequal food acquisition by larvae within a tank. Genetic differences seem unlikely since larvae were randomly assigned to a treatment and a particular aquarium within that treatment. Therefore variation in size observed was most likely the result of some larvae consuming more food than others over time. This occurred mostly in the low food treatments where some larvae apparently consumed more resources than others causing a significant divergence in size among individuals by the end of the experiment.

Larval injuries resulting from conspecific bites and intraspecific predation were highest in the two low food treatments. These were also the treatments in which coefficient of variation in size was highest. Although we did not record which individuals initiated agonistic interactions, we suspect that larger individuals were the primary aggressors. Larger individuals typically hold a competitive advantage (e.g. Persson, 1985; Smith, 1990; Walls and Semlitsch, 1991) for limited resources, which, in this study, likely contributed to interference competition in the form of increased biting and cannibalism among larvae. The increased time it took larvae on average to metamorphose in the low food treatments can likely be explained by the reduced growth potential of smaller larvae whose foraging efforts were compromised (Smith, 1990) perhaps due to being attacked by larger animals (Van Buskirk and Smith, 1991; Ziemba and Collins, 1999) and/or due to

changes in foraging patterns in response to the presence of larger conspecifics (Chivers et al., 1997; Ziemba and Collins, 1999).

The influence of size variation on aggressive behavior in amphibians has been demonstrated in only a few studies. For example, Maret and Collins (1994) showed that cannibalistic larval Arizona tiger salamanders (*Ambystoma tigrinum nebulosum*) developed under conditions where size variation was high and they were one of the largest animals in a group of conspecifics. They suggested that larval Arizona tiger salamanders may use an assessment of their size relative to surrounding conspecifics as a guide with which they may determine their success as a cannibal. Several other studies have suggested that size variation is important in influencing aggression (e.g. Kusano et al., 1985; Nyman et al., 1993; Maret and Collins, 1994; Petranksa and Thomas, 1995, but see Pfennig and Collins, 1993; Pfennig and Frankino, 1997). An alternative, although less likely explanation of our results is that the low food resources influenced aggression among all larvae regardless of their size relative to other larvae in the same tank."

Lack of food appears to be the main cue influencing biting and cannibalism. The density at which larvae were reared also influenced the incidence of cannibalism, but the effect was not as strong. The greatest amount of cannibalism occurred in the low food treatments. In only a few studies, manipulating both density and food level, has the lack of

food been found to be more important than conspecific density in influencing intraspecific aggression in amphibians (e.g., Ducey and Heuer, 1991). Many more studies suggest that density is the principal factor affecting aggression (e.g., Collins and Cheek, 1983; Semlitsch and Reichling, 1989; Nishihara, 1996). For example, Semlitsch and Reichling (1989) found an effect of higher densities and constant water levels on aggression in larval mole salamanders (*A. talpoideum*) and an inverse relationship between density and larval injury and survival (due to cannibalism and/or vulnerability due to the effects of injury). However, they found no relationship between food level and larval injury or survival. Collins and Cheek (1983) manipulated food level and conspecific density and found that high density, but not low food levels, influenced the production of cannibal morphs in larval Arizona tiger salamanders (*A. t. nebulosum*).

The interaction between density, food level, and size variation has also been previously explored. Walls (1998) showed that food limitation can play a significant role in influencing variation in size but not aggression in larval spotted salamanders (*Ambystoma maculatum*). The size ratio between largest and smallest larva within a tank, suggested as an indicator of the potential for cannibalism, was affected by density and food level treatments. Size ratio between the largest and smallest individuals and the proportion of individuals cannibalized was highest in

high density treatments but food supplementation significantly reduced the amount of cannibalism within these treatments. She found no differences in larval aggression between the three treatments and concluded that density-dependent growth in her study was more likely the result of food limitation than of interference competition.

Our study revealed differences in agonistic behaviors depending on the treatment. Individuals in the low food treatments were significantly more aggressive as suggested by the increased number of injuries sustained by larvae and by the high proportion of larvae cannibalized. This is one of the first studies to show a correlation between low food levels, increased aggression, and cannibalism in larval amphibians. Previous studies have demonstrated a correlation of decreased food availability with increased aggression in terrestrial amphibians (e.g. Ducey and Heuer, 1991) and in other taxa (see reviews in Fox, 1975, Polis, 1981). We suggest that the aggression observed in our study resulted from interference competition due to limited food resources.

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Chapter 4

The Effects of Rate of Hydroperiod Loss on Cannibalism, Growth and Development in Larval Long-Toed Salamanders, *Ambystoma macrodactylum*

Erica L. Wildy

Abstract

There are numerous advantages to living in an ephemeral habitat as demonstrated by the number of amphibian species that exploit these types of environments. However, despite the benefits, there are also costs to living in these habitats. As pond drying intensifies toward the end of the season, densities of larval amphibians may increase, food availability may decrease and the possibility of desiccation intensifies. In some populations, cannibalistic behavior becomes more prevalent under these conditions. This may be the result of attempts by larger larvae to reach the minimum size threshold necessary to metamorphose from their deteriorating habitat. Alternatively, cannibalistic behavior may be just a response to the increased conspecific densities and/or reduced food levels characteristic of drying habitats. One way to determine which scenario is correct is to examine whether cannibalism is expressed under conditions of pond drying alone, in the absence of high densities and low food levels. In this study, I examined the effects of pond drying rate on cannibalism and on life history of larval long-toed salamanders, *Ambystoma macrodactylum*. These animals typically inhabit ephemeral environments and cannibalism has been documented in several populations of this species. I raised groups of larvae in artificial ponds in the field in a fast or slow drying treatment. Cannibalism was not expressed in either of the two treatments. However, larvae

metamorphosed earlier in the fast drying treatments compared to the slow drying treatments. There were no statistically significant differences in survival or size (snout-vent length) at metamorphosis.

Introduction

Many animals exploit temporary environments, including a wide diversity of amphibians (Wilbur, 1980; Crump, 1983; Newman, 1992). There are many advantages to inhabiting such environments, including access to a nutrient rich environment resulting from flooding or snowmelt at the beginning of the season (Wilbur, 1980, 1997) and the absence of larger, predatory species, like fish, that require a more permanent environment (Wilbur, 1980; Semlitsch, 1987a; Schneider and Frost, 1996). Despite these benefits, temporary aquatic habitats can be harsh environments. High densities of conspecifics (Semlitsch, 1987b; Pfennig, 1990), variation in food availability (Semlitsch, 1987b), temperature fluctuations (Semlitsch, 1987b; Newman, 1989; Pfennig, 1990) and desiccation (Crump, 1989; Newman, 1987; Semlitsch, 1987a; Pfennig, 1990) typically characterize these environments. Exposure to any of these factors can be detrimental to an individual's performance and survival. Consequently, amphibians living in these types of habitats demonstrate a wide variety of adaptations including tolerances to high densities, rapid growth rates (Wilbur, 1997), shorter developmental periods (Denver et al., 1997), and the ability to accelerate development in response to a drying habitat (Petranka and Sih, 1987; Newman, 1988b, 1992; Semlitsch and Wilbur, 1988; Crump, 1989; Denver et al., 1998). It is this last response that has been the focus of numerous studies in

recent years (e.g. Petranka and Sih, 1986; Newman, 1988a,b, 1989; Semlitsch and Wilbur, 1988; Crump, 1989; Semlitsch and Reichling, 1989; Rowe and Dunson, 1995; Denver, 1997; Denver et al., 1998).

Although plasticity in amphibian metamorphosis has been well documented, some or all individuals in a population may fail to metamorphose before their habitat dries (Newman, 1987; Semlitsch, 1987a; Crump, 1989; Semlitsch, 1987a; Pfennig, 1990). In many cases, the ability of an individual to initiate metamorphosis is constrained by its not having reached the necessary size threshold to do so (Wilbur and Collins, 1973). An individual's growth potential can be compromised by a number of factors, particularly in ephemeral environments. For example, the density of animals typically increases as the habitat dries (Wilbur, 1987; Semlitsch and Wilbur, 1988; Newman, 1989; Wildy, personal observation). A number of studies have demonstrated that amphibian larvae raised under crowded conditions generally exhibit a decreased growth rate (Gromko et al., 1973; Dash and Hota, 1980; Smith, 1983; Semlitsch, 1987b; Wilbur, 1987, 1988; Petranka and Sih, 1986; Newman, 1987) and/or a longer time to metamorphosis (Smith, 1983; Petranka and Sih, 1986; Newman, 1987). In some instances growth inhibitors released by larger anurans stunt the growth of smaller conspecifics (Rose, 1960; Gromko et al., 1973; Licht, 1967; Steinwascher, 1978; Semlitsch and Caldwell, 1982; Scott, 1990).

Furthermore, as time passes, both the separate and combined effects of increased densities and the deterioration of the habitat due to pond drying (Pfennig et al., 1991) can lead to a decrease in the amount of available food (Semlitsch, 1987a; Crump, 1992; Newman, 1994). Low per capita food can, in turn, lead to a heightening of competition between conspecifics (Smith, 1983; Petranka and Sih, 1986; Wilbur, 1987; Walls, 1998; Wildy et al., in review) resulting in reduced growth in some individuals (Smith, 1983; Petranka and Sih, 1986; Newman, 1987, 1994). In a drying habitat, a reduction in growth rate or development can indirectly affect an individual's probability of survival by increasing the chance that that individual will not metamorphose before the pond completely dries (Newman, 1987; Semlitsch, 1987a; Wilbur, 1987, 1997; Wildy et al., in review).

Larval long-toed salamanders, *Ambystoma macrodactylum*, are commonly found inhabiting ephemeral habitats, including ponds and roadside ditches (Kezer and Farner, 1955; Anderson, 1967; Leonard et al., 1993; Walls et al., 1993a; Wildy, 1998, 1999; Wildy et al., in review). At some sites in recent years, some individuals have failed to metamorphose before the pond dries and consequently succumbed to desiccation. Although this has not been quantified, results of previous studies suggest that the number of dying larvae is inversely proportional to the length of pond duration (e.g. Semlitsch, 1987a; Wilbur, 1987;

Newman, 1988b; Semlitsch and Wilbur, 1988; Rowe and Dunson, 1995). The faster pond drying occurs, the less time larvae have to grow, reducing the probability that they will be able to metamorphose.

Cannibalism has been documented in several populations of larval *A. macrodactylum* (Walls et al., 1993a; Wildy et al., 1998, 1999; Wildy, unpublished data) and the frequency of this behavior tends to increase toward the second half of the season, corresponding with the acceleration of pond drying (Wildy, personal observation). This behavior may be particularly adaptive in ephemeral ponds because of the density-dependent effects associated with pond drying. Previous studies have shown that increased densities and/or reduced food availability contribute to increased aggression (Semlitsch and Reichling, 1989; Ducey and Heuer, 1991; Van Buskirk and Smith, 1991) particularly cannibalism (Collins and Cheek, 1983; Nishihara, 1996; Wildy et al., in review) in larval amphibians. Cannibals may benefit by using conspecifics as an alternate food source when other resources are limited (Crump, 1983, 1990). Another benefit of cannibalism is that it can reduce intraspecific competitors of the cannibal for existing food resources (Fox, 1975; Polis, 1981; Crump, 1983). Although cannibalism appears to be adaptive in high density and/or food limited environments, it is not clear if cannibalism would be as likely to be expressed in a drying environment in the absence of these factors. In the

current study, after eliminating food limitation as a factor and minimizing effects of density, I examined the effects of rate of pond drying on metamorphic plasticity and the expression of cannibalism in larval *A. macrodactylum*.

Methods

A source group of larval *A. macrodactylum*, approximately two months posthatching, were collected in July 1998 from an ephemeral pond (elevation 1951 m) located approximately 24 km south of Sisters, Deschutes County, Oregon. U.S.A. Larvae were temporarily transported to the laboratory where they were housed individually in 850 mL plastic cups containing approximately 600 ml of dechlorinated tap water. Larvae were fed *Tubifex* worms *ad libitum* every other day and were maintained on a 14:10 h light:dark cycle. Ninety individuals were selected and sorted into nine, size-matched groups of ten individuals. Out of each group of ten, a subgroup of five animals was assigned to one of two paired (see explanation below) treatments: a slow drying habitat (28 days) or a fast drying habitat (42 days). Because this experiment was started in the middle of the season, these amounts of time reflect the number of days remaining to previously recorded drying dates.

One week after being assigned to their treatments, larvae were transported back to the field and placed in mesocosms. Eighteen

mesocosms, consisting of rectangular plastic containers (54 x 30 x 27cm), were floated in a pond located approximately ten km from the natal pond of the test larvae. The mesocosms were arranged in blocks with each block containing one slow drying and one fast drying treatment.. Mesocosms were arranged in two rows in the pond with the first row containing four blocks and placed two meters from the shore and the second row containing five blocks and placed one meter behind the first row (=four meters from shore). Each block of mesocosms was supported by a wooden frame that tightly bordered their collective perimeter and rested under the lips of their tops. These wooden frames were tied to aluminum poles that were driven firmly into the pond bottom. After the mesocosms were situated in the water, larvae were placed into their assigned treatments.

To allow for the inflow and manipulation of pondwater levels within the mesocosms, a grid of holes was drilled in each of the widthwise ends. The number of rows of holes created corresponded with the number of weeks over which pond drying would occur. For the slow drying treatment, six rows (=six weeks or 42 days) of five holes were created, with each column measuring 6 cm apart and each row measuring 3.5 cm apart. For the fast drying treatment, four rows (=four weeks or 28 days) of five holes were created with each column measuring 6 cm apart and each row measuring 6 cm apart. To minimize any difference in waterflow

or any other factors associated with that between treatments, an extra two rows of five holes were drilled below the fourth row in the fast drying treatment. This allowed for the number of holes in both treatments to be equal (30 on each end of the container). For both treatments, the top row was started five cm from the top of the container. To keep macroinvertebrates from entering the containers through the drilled holes, mesh screen was placed over the grid. In addition, a piece of mesh screen was clipped across the top of each mesocosm to prevent any predators or other debris from entering through the top.

During the experiment, the water level within mesocosms was manipulated by inserting screws through the outermost holes (corresponding to the first and fifth column) of the appropriate row and allowing them to rest on the wooden frame surrounding the mesocosms. During the first week, the water volume was the same 22.5 cm (= 43.5 L) in mesocosms for both treatments but diverged during the following weeks. At the beginning of the first week, screws were inserted in the first row and during the second week, screws were inserted through holes in the second row, and so on. This effectively lowered the water level in the slow drying treatment by 3.5 cm (= 6.5 L) per week and by 6 cm (= 12.5 L) per week in the fast drying treatment.

Every other day, the test larvae were fed an equal amount of *Tubifex* and any missing, and presumably cannibalized, larvae were

counted. Larvae were maintained under these conditions until they metamorphosed or their habitat "dried". For the fast and slow treatments, 5 cm (= 7 L) remained in the mesocosms during the last week (4th week and 6th week, respectively). By the end of that week, any larvae not metamorphosing were considered to have "dried" with the pond. Metamorphosing larvae were removed and measurements of snout-vent length (SVL) and time to metamorphosis were recorded. Larvae not metamorphosing by the end of the experiment were considered to have not survived.

Statistical Analysis

No cannibalism occurred in either of the treatments and therefore this factor was not analyzed. Multivariate analysis of variance (MANOVA) was used to examine the effects of habitat drying rate on survival to, time to, and SVL at metamorphosis of the test animals. After MANOVA, univariate analysis of variance (ANOVA) F tests were used to assess which variables were responsible for significant main effects. For each response variable I calculated averages for groups of animals in each mesocosm and used these means for all statistical analyses. Finally, to determine if size was a factor in influencing ability of larvae to metamorphose, I examined differences in SVL between larvae that

metamorphosed before the end of the experiment and those that did not using a t test.

Results

MANOVA revealed a strong main effect of pond drying rate on survival to, time to and size at metamorphosis (Table 4.1, $p=0.010$). Post hoc ANOVAs indicated that there was a trend in time to metamorphosis (Table 4.1; $p=0.06$). Larvae in the fast drying treatment tended to metamorphose earlier than larvae in the slow drying treatments (Figure 4.1a). There was no statistically significant difference in survival to or SVL at metamorphosis between individuals in the two treatments (Table 4.1, Figures 4.1b,c). In the fast drying treatments, 84% of the larvae metamorphosed while 95% of the larvae metamorphosed in the slow drying treatment. Larvae in the fast drying treatments metamorphosed at an average of SVL of 34.99 ± 0.27 (SE) mm while larvae in the slow drying treatments exhibited an average SVL of 35.53 ± 0.33 (SE) mm at metamorphosis. Moreover, there was no significant difference in size between larvae that metamorphosed before the end of the experiment and those that did not ($t = 0.260$, $df = 8.3$, $p=0.801$).

Table 4.1 Results of MANOVA for overall effects of hydroperiod on snout-vent length (SVL) at, time to, and survival to metamorphosis for larval *A. macrodactylum* and ANOVAs for each response variable.

	F	df	p
MANOVA			
Constant	16728.84	3, 14	0.000
Rate of Pond Drying	5.635	3, 14	0.010
ANOVAs			
Rate of Pond Drying			
SVL	1.617	1, 16	0.222
Time	3.947	1, 16	0.064
Survival	2.654	1, 16	0.123

Figure 4.1 Mean (\pm SE) a) time (in days) to metamorphosis b) percentage survival to metamorphosis and c) snout-vent length (in mm) at metamorphosis for *A. macrodactylum* larvae exposed to fast and slow pond drying rates. Letters over bars indicate which treatments are statistically different from one another. If treatments do not share the same letter, there is a statistically significant difference.

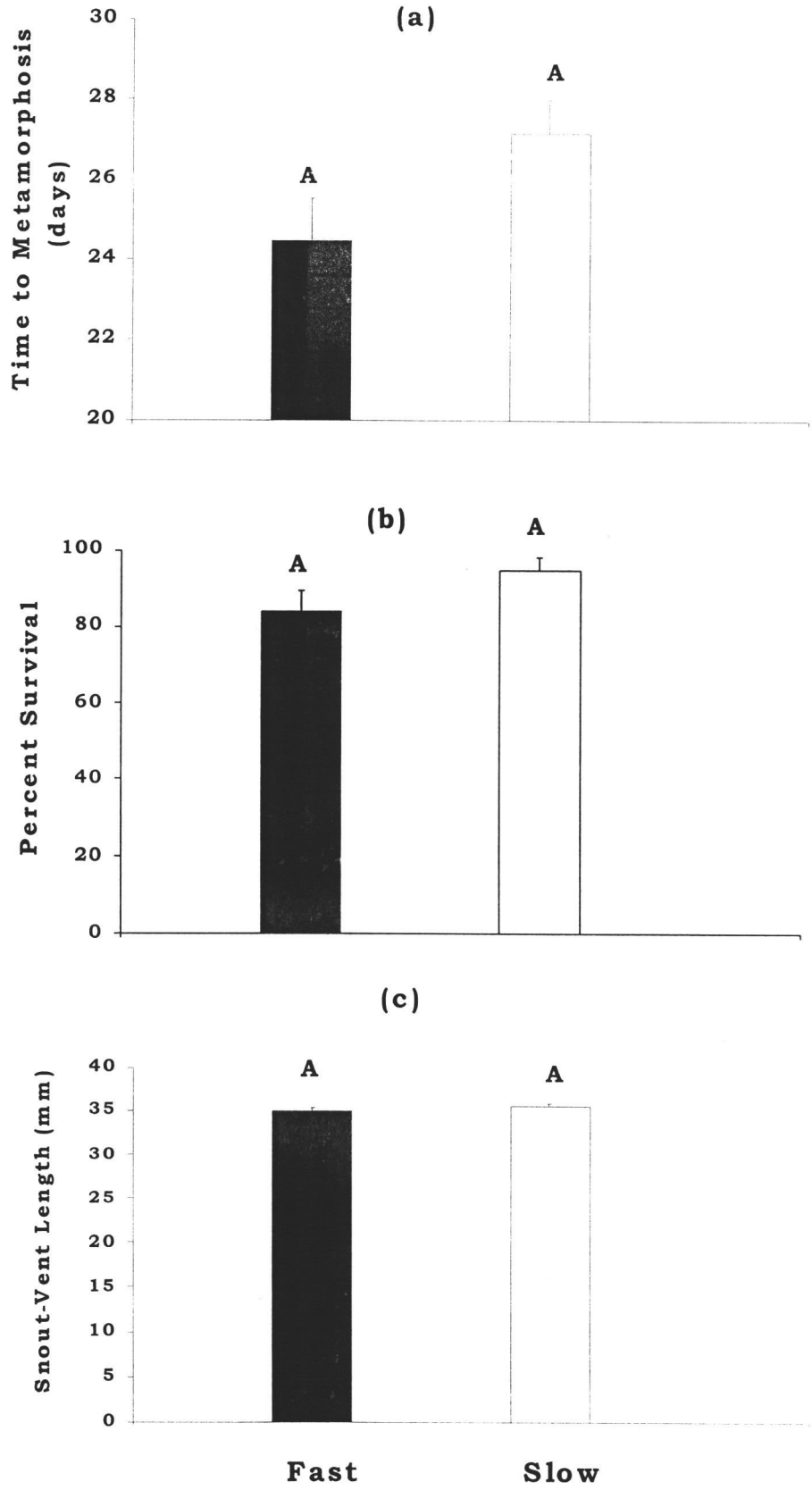


Figure 4.1

Discussion

Larval *A. macrodactylum* did not engage in cannibalism in either the fast drying or the slow drying treatments. The absence of this behavior may be explained by several factors. First, larvae within mesocosms had initially been size-matched and even at the end of the experiment, the average coefficient of variation in size within mesocosms was low (0.050 ± 0.005 (SE) for slow drying treatments and $0.044 \pm .007$ (SE) for fast drying treatments). This alone could have prevented cannibalism from occurring since it would have been mechanically difficult, if not impossible, for a larva to swallow a similar sized conspecific. Typically, cannibals feed on smaller conspecifics (Maret and Collins, 1994; Wildy, Ch. 3). Furthermore, size differences between individuals can facilitate cannibalism under the right conditions (Smith, 1983; Kusano et al., 1985; Nyman et al., 1993; Maret and Collins, 1994; Petranksa and Thomas 1995; Wildy et al., in review a). It is possible that larvae were exhibiting aggression toward one another in the form of biting. The stress of a drying environment may have prompted individuals to become more aggressive toward a conspecific despite not being able to consume it. However, whether individual larvae exhibited evidence of being bitten was not quantified in this study.

An alternative explanation for the absence of cannibalism is that larvae may not have been prompted to engage in this behavior.

Cannibalism may have been beneficial in providing small larvae with a much needed growth spurt to reach the size threshold necessary to initiate metamorphosis. However, the number of metamorphosing individuals was high in both treatments. This demonstrates that most larvae had already reached the minimum size necessary to initiate metamorphosis. In fact, larvae that did not initiate metamorphosis were not different in size from those that did, implying that inadequate size was not an issue.

A third explanation is that the cues associated with pond drying that would normally promote larval cannibalism were absent from our treatment conditions. In nature, as pond drying occurs, food levels can decrease (Semlitsch, 1987a; Crump, 1992; Newman, 1994; Wildy, personal observation) while conspecific density increases (Wilbur, 1987; Semlitsch and Wilbur, 1988; Newman, 1989; Wildy, personal observation). Increased density and/or decreased food levels can promote aggression (Semlitsch and Reichling, 1989; Ducey and Heuer, 1991; Van Buskirk and Smith, 1991), including cannibalism (Collins and Cheek, 1983; Nishihara, 1996; Walls, 1998; Wildy et al., Ch. 3) in larval amphibians. In the current study, the density of larvae in the mesocosms may have increased over time due to the decreasing water volume but the effect may not have been as strong as is found in nature. In nature, as pond drying occurs, water loss is accompanied by a

reduction in volume and a reduction in surface area. In our study, water loss was accompanied by a reduction in volume but not a reduction in surface area. Furthermore, as larval *A. macrodactylum* typically spend more time on the pond bottom rather than swimming within the water column (Wildy, personal observation), factors that might normally change over time as density increases, including an increase in conspecific interactions and a reduction in swimming area, would have changed only minimally in our study. Food limitation was also not a factor in this study. Food level was kept constant as larvae were fed the same amount of *Tubifex* throughout the experiment. In fact, since some larvae metamorphosed and were removed from their mesocosm, food levels for the remaining individuals would have increased.

Although cannibalism did not occur, larval *A. macrodactylum* exhibited plasticity in their ability to metamorphose under different drying regimes. Individuals in the fast drying treatment tended to metamorphose earlier than larvae in the slow drying treatment. This has been observed many times in other studies examining the flexibility of amphibian metamorphosis in response to habitat drying rate (Petranka and Sih, 1986; Wilbur, 1987; Semlitsch and Wilbur, 1988; Newman, 1988b, 1989, 1992; Crump, 1989; Semlitsch et al., 1990; Pfennig et al., 1991; Denver et al., 1998). In the current study, larvae metamorphosed in the absence of food limitation and under limited

effects of density. The cues that influenced metamorphosis in larvae are difficult to determine because several factors were probably altered as water level began decreasing. In nature the thermal profiles of drying ponds change (Newman, 1989) and as pond drying occurs, the average temperature of the water is expected to gradually increase, prompting metamorphosis in larval amphibians. However, this could not have been the case in our study since the temperature of the water between treatments both within and between blocks was essentially equal throughout the experiment. This was most likely due to the fact that the water inside the mesocosms was continuously fed via inflow from the pond in which they were floated.

Alternatively, larvae may have responded to one or a combination of cues associated with the decreasing water levels, other than food, density, or temperature. Possibilities include increased concentrations of salt or waste compounds, decreased oxygen availability, decreased water volume, or increased proximity to the water surface (Semlitsch, 1987b; Pfennig, 1990; Denver, 1997; Denver et al., 1998). In a previous study, it was determined that elevated temperatures, concentration of compounds and increased physical interactions between conspecifics could not explain accelerated development and early metamorphosis in *Scaphiopus hammondi* tadpoles (Denver et al., 1998). Denver et al., (1998) suggested that among other possible explanations, the effects of a

restriction in swimming volume on foraging contributed to early metamorphosis in these amphibians.

Metamorphosing earlier to escape a drying habitat can be critical for survival, but in many instances, amphibians sacrifice size for earlier metamorphosis (Semlitsch, 1987b; Newman, 1988b, 1989; Crump, 1989a; Pfennig et al., 1991). For example, Semlitsch (1987b) found that larval *A. talpoideum* raised in a drying treatment exhibited a reduced snout-vent length compared to individuals raised in a constant water treatment. Similarly, Crump (1989) found that *Hyla pseudopuma* tadpoles raised in a drying treatment metamorphosed at a smaller size than siblings raised in constant deep water. Metamorphosing at a smaller size may have significant effects on an individual's success in the terrestrial environment including a reduced chance of surviving to first reproduction, reaching reproductive maturity later and at smaller sizes and being less successful at avoiding predation and procuring food (i.e. Berven and Gill, 1983; Smith, 1987; Semlitsch et al., 1988; Woodward et al., 1988; Crump, 1989a; Berven, 1990; John-Alder and Morin, 1990; Nyman et al., 1993; Scott, 1994).

In contrast to the studies mentioned above, larval *A. macrodactylum* did not exhibit differences in size at metamorphosis in the current study. However, we suspect that our failure to see these differences could be explained by our experimental design. For example,

the fast drying and slow drying treatments only differed by 14 days and water loss of 6 L per week for four weeks. This relatively small difference in drying rate in conjunction with larvae being size matched within mesocosms may not have allowed for differences in growth or developmental rates to be expressed by larvae in the two treatments. Furthermore, water temperatures between treatments did not differ. This may have minimized growth differences between treatments. Temperature can have a significant influence on the growth and developmental rates of larval amphibians (Petranka 1984; Newman, 1989; but see Denver et al., 1998).

The current design would be improved by not size-matching test animals within mesocosms. Furthermore, test animals should be exposed to treatment conditions over a longer period of time. If eggs or newly hatched larvae were randomly assigned to treatments and raised under these conditions for a longer period of time, the likelihood of observing treatment-dependent differences in life history in larvae would increase. These differences would likely be due to the influence the genetic differences of individual larvae would have on their physiological (growth, developmental rate), and morphological (i.e. competitive ability) traits.

In the current study, we found that larval *A. macrodactylum* are plastic in their ability to metamorphose under different short term pond

drying regimes. Furthermore, larvae based timing of metamorphosis on cues other than food limitation, density or temperature. The plasticity in metamorphosis in response to different drying regimes seems to contrast with observations in recent years. At the end of the last few seasons, hundreds of desiccated larvae have been found in some

A. macrodactylum populations, including the site from which larvae used in this experiment originated (Wildy, personal observation). It is possible that these larvae were unable to metamorphose due to some factor not addressed in our study. For example, in nature, as pond drying occurs, a reduction in per capita food resources due to increasingly high densities may result in the stunted growth of some larvae (Newman, 1994) such that they are unable to achieve the necessary size threshold for metamorphosis (Wilbur and Collins, 1973). Future studies are needed to address what factors prevent some larvae from metamorphosing from a drying environment despite their demonstrated ability to do so in a controlled one.

Additional studies could further address the response of cannibalistic populations to desiccation. It has already been determined that factors associated with drying environments, including high densities (Collins and Cheek, 1983; Wildy et al., 1998) and/or low food (Wildy et al., 1998) can influence aggression, including cannibalism, in larval salamanders. However, the effects of pond drying and food

limitation on expression of this and other aggressive behaviors have not been thoroughly explored.

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Chapter 5

Cannibalism Enhances Growth in Larval Long-Toed Salamanders, (*Ambystoma macrodactylum*)

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Introduction

Intraspecific predation (=cannibalism) has been widely documented within numerous animal populations (Elgar and Crespi, 1992). Several studies have explored possible selective forces behind the existence of cannibalism, including reduction in competition, enhanced reproductive success and better nutrition (Fox, 1975; Polis, 1981; Meffe and Crump, 1987; Elgar and Crespi, 1992). In amphibians, several studies have documented the occurrence of and mechanisms surrounding intraspecific predation (e.g. Chivers et al., in press; Collins and Cheek, 1983; Polis and Meyers, 1985; Lannoo et al., 1990; Crump, 1992; Nyman et al., 1993; Pfennig et al., 1993; Walls et al., 1993a, 1993b; Maret and Collins, 1994, 1996; Pfennig and Sherman, 1994; Kiesecker et al., 1996). Cannibalism in amphibians has been observed within and between all life stages but encounters between larvae are the most frequently documented type of interaction (Crump, 1992).

Although intraspecific predation has been well documented in amphibians, it is not ubiquitous. One explanation for this is that there are potential costs associated with cannibalistic behavior. For example, intraspecific predators may be injured by resistant prey (Crump, 1992). Second, cannibals may reduce their individual fitness through consumption of related individuals. Third, cannibals may risk disease and death through consumption of individuals infected with pathogens

(Pfennig et al., 1991). Potential risks associated with intraspecific predation suggest that where it exists, benefits of consuming conspecifics outweigh potential costs.

Several potential advantages may be accrued by amphibians exhibiting cannibalistic behavior. One possible benefit is enhanced nutrition (Nagai et al., 1971; Crump, 1990). The similarities between predator and prey may increase the efficiency with which nutritional or hormonal matter is incorporated (Gorbman, 1964; Nagai et al., 1971; Crump, 1986; Lannoo et al., 1989). Diets of higher nutritional value may result in accelerated growth and development (Lannoo et al., 1989; Crump, 1990) which, in turn, may have further implications for success in both the larval and terrestrial stages (Salthe and Duellman, 1973; Collins, 1975; Howard, 1980; Berven, 1981, 1990; Brodie and Formanowicz, 1983; Travis, 1983; Berven and Gill, 1983; Crump, 1984; Smith, 1987; Semlitsch et al., 1988; Woodward et al., 1988; John-Alder and Morin, 1990; Scott, 1990; Kiesecker, 1996). Nevertheless, few experimental tests have addressed the question of how intraspecific predation may affect growth in amphibians. In particular this question has not been studied in salamanders.

In this study we experimentally assessed the influence of intraspecific predation on growth within larval long-toed salamanders, *Ambystoma macrodactylum* from a high altitude ephemeral pond.

Hydroperiod at that site slowly decreases over four to five months, during which time food resources become limited and larval density increases. Cannibalism has been observed to occur naturally within this population (Walls et al., 1993a; Wildy et al., personal observation). We tested the hypothesis that consumption of conspecifics enhances growth. We used mass as an indication of growth and predicted that larvae consuming a diet of conspecifics would experience enhanced growth compared to larvae consuming a heterospecific diet.

Methods

We collected *A. macrodactylum* larvae and *Hyla regilla*, Pacific treefrog, tadpoles from two separate high-altitude ephemeral ponds (1951 m) located approximately 24 km south of Sisters, Deschutes County, Oregon. *Hyla regilla* tadpoles, which are potential prey for *A. macrodactylum* larvae (personal observation), were collected on 26 June 1996 and salamander larvae were collected on 2 September 1996. Larvae were housed in 850 ml plastic cups containing approximately 600 ml of dechlorinated tap water in which they remained for the duration of the experiment. Prior to testing they were maintained on a diet of *H. regilla* tadpoles and *Tubifex*. Tadpoles were housed in a 38 L aquarium filled with dechlorinated tap water and fed ground rabbit chow *ad libitum*. Both larvae and tadpoles were maintained on a 14:10 h light: dark cycle.

We divided 45 larvae into 15 groups of three animals. Within each group, larvae were size matched to within 0.01 grams and offered, twice a week, one of three diets: conspecific diet - consisting of 100% *A. macrodactylum* larvae; tadpole diet - consisting of 100% *H. regilla* tadpoles; mixed diet - consisting of 1:1 mix of larvae and tadpoles. For each feeding, all food was preweighed and matched for mass (totaling one gram per week) such that all larvae, both within and between groups, received the same amount of food. The larvae were allowed to grow for three weeks. The experiment was ended three days after the final feeding at which time the mass of each animal was recorded.

Statistical Analysis

We tested for a difference in change in mass with an analysis of covariance (ANCOVA) when the main effect was diet and the covariate was initial mass. Post hoc comparisons (Tukey test) were performed to test for differences between means among the treatment groups.

Results

Differences in initial mass were not significant ($p = 0.947$) among treatments and therefore did not contribute to changes in final mass (see Table 5.1). However, diet did have a significant effect on change in mass ($p < 0.005$). Larvae fed the conspecific and mixed diets demonstrated a

net gain in mass while larvae fed the tadpole diet exhibited a slight weight gain in mass while larvae fed the tadpole diet exhibited a slight weight loss (see Fig. 5.1). Post hoc comparisons indicated that larvae fed conspecifics gained significantly more mass by the end of the experiment than larvae fed the tadpole diet ($p = 0.001$) and the mixed diet ($p = 0.034$). There was no significant difference between the change in mass of individuals fed the tadpole diet and the mixed diet ($p = 0.326$).

Table 5.1 ANCOVA results on the effects of diet and initial size on net change in mass in long-toed salamander larvae.

FACTOR	d.f.	F-ratio	p value
diet	2, 41	8.418	0.001
COFACTOR			
initial mass	1, 41	0.004	0.947

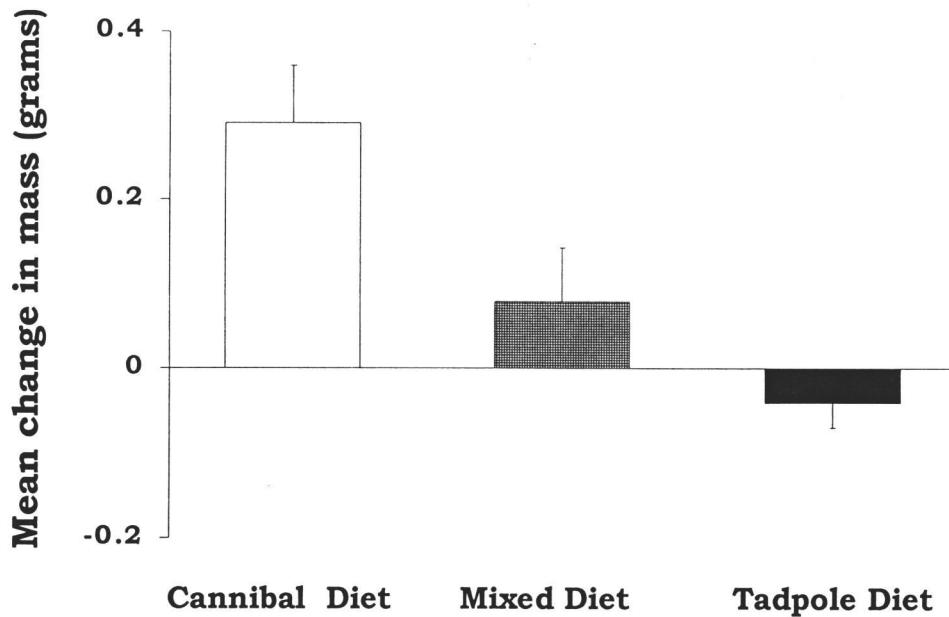


Figure 5.1 Mean (\pm SE) change in mass (grams) for *A. macrodactylum* larvae fed the cannibal, tadpole and mixed diets (see text for details).

Discussion

Our results show that *A. macrodactylum* larvae experience a greater net gain in mass if they consume conspecifics, as opposed to heterospecifics, as the principal component of their diet. We matched larval diets for mass but not for energy content or other dietary components (e.g., specific amino acids or micronutrients). Matching for energy content would not necessarily match for other dietary components including amino acids and various micronutrients, which may also be important factors in larval growth and development. These

dietary factors may be more easily incorporated by a predator and therefore have more pronounced effects on that predator if the prey animal is of the same species. For example, larvae consumed by conspecific predators may contain essential amino acids (Nagai et al., 1971; Crump, 1986) or growth factors (Gorbman, 1964; Lannoo et al., 1989) which may be readily assimilated by the consumer resulting in an increase in growth rate. A similar effect may not occur in larvae consuming a heterospecific.

Crump (1990) conducted a study that controlled for either mass or energy of content of diet. When controlling for food mass, *Hyla pseudopuma* tadpoles fed a conspecific diet (50% *H. pseudopuma* + 50% commercial food) weighed significantly more on average than those fed heterospecific (tadpole) diets (50% *Ololygon staufferi* + 50% commercial food or 50% *Osteopilus septentrionalis* + 50% commercial food). When controlling for energy content, tadpoles offered the conspecific diet weighed significantly more than tadpoles fed the heterospecific diet. Crump (1990) concluded that diets matched for either mass or energy content resulted in higher growth for individuals fed conspecifics.

Our experiment simply addressed the outcome of similar sized *A. macrodactylum* larvae consuming an equal mass of conspecifics versus heterospecifics. We used *H. regilla* tadpoles and

A. macrodactylum larvae as food because these two species represent the main vertebrate food sources for *A. macrodactylum* larvae where they were collected (unpublished data). However, aquatic insects (e.g. *Notonecta*) are also eaten.

Cannibalistic animals risk the possibility of injury or even death on any encounter with conspecific prey. A cannibal may incur injuries from a conspecific defending itself. It has been suggested that cannibalism would be most advantageous at the point in the life history of an animal when nutritional benefits of such behavior is as high or higher than age-specific mortality (Eickwort, 1973). Depending on the costs and benefits of cannibalism, it may be the worth the risk of injury or death (Fox, 1975; Polis, 1981; Polis and Meyers, 1985; Crump, 1992).

Larvae living in ephemeral habitats are subject to relatively harsh conditions, including high densities of conspecifics, low food availability, temperature fluctuations and desiccation. Such unpredictable conditions can make extension of the larval period (i.e. until the pond dries up) maladaptive. Instead, there may be a strong selection pressure for larvae to escape the instability of their harsh environment by metamorphosing sooner (Wilbur and Collins, 1973; Newman, 1988, 1989; Semlitsch and Wilbur, 1988; Crump, 1989). Lannoo et al. (1989) have demonstrated that cannibalism can decrease time to metamorphosis in cannibalistic larval *A. t. tigrinum*. This, in turn, may

result in smaller size at metamorphosis (Newman, 1988, 1989; Crump, 1989) unless the larvae can increase growth rates while decreasing the length of the growth period.

Cannibalism may be one mechanism by which larvae can maximize growth rates while minimizing time spent in the larval stage.

Consumption of a more highly nutritional diet (i.e., conspecifics) could lead to accelerated growth rate (Lannoo et al., 1989; Crump, 1990).

Larvae experiencing an accelerated rate of growth may attain larger sizes relatively quickly. Larger larvae may reach a size refuge from aquatic predators (Brodie and Formanowicz, 1983; Travis, 1983; Crump, 1984) including other salamander larvae.

There are a number of post-metamorphic advantages of larger size. For example, size at metamorphosis has been shown to increase postmetamorphic success. Larger metamorphic amphibians may have a greater chance of surviving to first reproduction (Berven and Gill, 1983; Berven, 1990; Nyman et al., 1993) and reaching reproductive maturity sooner (Collins, 1975; Semlitsch et al., 1988; Smith, 1987) and at larger sizes (Smith, 1987; Semlitsch et al., 1988; Berven, 1990). Larger female anurans may have overall higher fecundity (Salthe and Duellman, 1973; Berven, 1981; Berven and Gill, 1983; Semlitsch, 1987) and larger male anurans may attract more mates than smaller ones (Howard, 1980; Berven, 1981). Larger juvenile amphibians may also be more successful

at avoiding predation (Woodward et al., 1988; Crump, 1989; John-Alder and Morin, 1990; Kiesecker, 1996) and procuring food (Crump, 1989; Kiesecker, 1996).

In conclusion, cannibalism of one larval amphibian by another can have the immediate effect of providing a meal in an environment where food availability may be unpredictable. A byproduct of this may be that larvae are receiving a more nutritious meal than if they had consumed a heterospecific. We demonstrated that consuming conspecifics as the main prey item increases growth rates, which can potentially have many benefits for larvae while they are still in their aquatic environment. Accelerated growth rates of larvae may have strong implications for success in the terrestrial environment.

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Chapter 6

Shifts in Life History Traits as a Response To Cannibalism in Larval Long-Toed Salamanders (*Ambystoma macrodactylum*)

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Abstract

Larval long-toed salamanders (*Ambystoma macrodactylum*) exhibit a trophic polymorphism, whereby some individuals ("cannibal morphs") possess a cannibalistic morphology and others ("typical morphs") do not. In previous studies, typical larvae exhibited antipredator behavior in response to chemical cues of cannibal morphs. Furthermore, the intensity of antipredator behavior of typical morphs was elevated when the cannibal was fed conspecifics compared to when the cannibal was fed a different diet. In this experiment we examined the potential influence of cannibalism on life history characteristics of typical larvae. Using a 2 x 2 factorial design, crossing morphology with diet, we exposed typical larvae to one of four types of stimulus animals: cannibal morphs fed a conspecific diet, cannibal morphs fed a heterospecific diet (i.e. *Tubifex*), typical morphs fed a conspecific diet and typical morphs fed a heterospecific diet. Our results indicate that diet of the stimulus animals, but not morphology, affected life history characteristics of test larvae. Specifically, test larvae exposed to stimulus animals fed a conspecific diet exhibited a slower growth rate and an increase in the time taken reach to reach metamorphosis. These changes in life history may represent a cost of antipredator behavior.

Introduction

A wide diversity of antipredator responses has evolved among prey animals. These antipredatory mechanisms can include changes in morphology (Havel and Dodson, 1984; Havel, 1987; Brönmark and Miner, 1992; McCollum and Van Buskirk, 1996; Stabell and Lwin, 1997), physiology (e.g. Wassersug, 1971; Smith, 1982), and behavior (e.g. Lima and Dill, 1990; Chivers et al., 1997). Behavioral responses to predators may include an increase in the use of shelter, shifts in microhabitat use, reductions in movement, and increased group cohesion (Sih, 1987; Lima and Dill, 1990; Chivers and Smith, 1998; Kats and Dill, 1998). For example, Sih (1986) found that mosquito larvae (*Culex pipiens*) reduce their activity when exposed to the insect predator *Notonecta undulata*. Alternatively, Chivers et al. (1995) found that brook stickleback increased schooling in response to the presence of predatory northern pike. Although each of these behaviors may shield an individual from predation, a tradeoff commonly arises between avoiding a predator and performing other activities, including foraging (Sih, 1992). This tradeoff can lead to a variety of indirect effects on long-term life history traits of the prey.

There are many studies in which long-term effects of predators on their potential prey have been documented (e.g. Minchella and Loverde, 1981; Werner et al., 1983; Dodson and Havel, 1988; Reznick et al., 1990;

Wilbur and Fauth, 1990; Skelly, 1992; Ball and Baker, 1996). Werner et al. (1983) found that the presence of predatory largemouth bass (*Micropterus salmoides*) led to a decrease in the growth rate of small bluegill sunfish (*Leopomis macrochirus*). A study by Dodson and Havel (1988) indicated that *Daphnia pulex* exposed to the non-lethal presence of the invertebrate predator, *Notonecta undulata*, exhibited a reduction in adult body size and a shorter developmental time in juveniles. Skelly (1992) showed that gray treefrog (*Hyla versicolor*) tadpoles exposed to the non-lethal presence of larval tiger salamanders (*Ambystoma tigrinum*) experienced a reduction in their growth and developmental rate. Other examples of long-term effects of predators on amphibian life history are provided by Van Buskirk (1988), Wilbur and Fauth (1990) and Skelly and Werner (1990).

Intraspecific predation (cannibalism) is common in numerous taxa, including amphibians (Fox, 1975; Polis, 1981; Smith and Reay, 1991; Crump, 1992; Elgar and Crespi, 1992; Wildy et al., 1998). Despite the widespread occurrence of cannibalism, there is little information on how intraspecific predation influences long-term life history traits of conspecific prey. In this study we explored the possibility that cues of cannibalistic conspecifics may influence life history characteristics of larval long-toed salamanders (*Ambystoma macrodactylum*). Larvae of this species exhibit a trophic polymorphism, whereby some individuals have a

cannibalistic morphology (i.e. a disproportionately large head and hypertrophied vomerine teeth) (Walls et al., 1993a; b). Larvae lacking cannibalistic characteristics are referred to as typical morphs.

Chivers et al. (1997) documented that typical morph *A. macrodactylum* larvae responded to chemical cues but not visual cues of cannibal morphs with antipredator behavior. Specifically, they avoided cannibals and showed a reduction in activity in their presence. In this experiment, we used larval *A. macrodactylum* as both predator and prey and tested whether the morphology of the predator, its diet type (i.e. conspecifics or heterospecifics), or an interaction between the two, influence long-term life history characteristics of their conspecific prey. Specifically, we looked at how growth rate, time taken to reach metamorphosis and mass at metamorphosis are influenced by intraspecific predators.

Methods

We collected long-toed salamander larvae in July 1996 from a high altitude (elevation 1951 m) ephemeral pond located approximately 24 km south of Sisters, Deschutes County, Oregon. U.S.A. We transported them to the laboratory and housed individual larvae in 850 ml plastic cups containing approximately 600 ml of dechlorinated tap water. Prior

to the experiment, larvae were fed *Tubifex ad libitum* every other day and were maintained on a 14:10 h light:dark cycle.

We created experimental chambers using fiberglass mesh screen to divide glass test aquaria (50 x 25 x 30 cm) into five sections. A central area, measuring 10 x 25 cm was surrounded by four sections each measuring 12.5 x 20 cm. Within these aquaria, we raised typical morph larvae in the presence of one of four types of stimulus animals: (1) cannibal morphs fed a conspecific diet, (2) cannibal morphs fed a heterospecific diet (i.e. *Tubifex*), (3) typical morphs fed a conspecific diet and (4) typical morphs fed a heterospecific diet. (We used the criteria of Walls et al. (1993a; b) to identify individuals with the typical and cannibal morphology). Within each aquarium, a single typical morph test larva was placed in each of the four peripheral sections. Depending on the treatment, one of the four types of stimulus larvae was placed in the central section.

Aquaria were arranged in blocks with each block containing one of the four treatment types. This design was replicated seven times for a total 112 animals housed in 28 aquaria. Within each block, both the stimulus animals (cannibal morphs and typical morphs) and the test animals were matched for size. The mean mass and standard error of the stimulus animals was 33.2 ± 1.3 g, while that of the test animals was 26.6 ± 2.0 g.

Throughout the experiment, test larvae were fed *Tubifex ad libitum* every other day. Stimulus animals were fed *ad libitum* twice per week with either *Tubifex* or larval *A. macrodactylum*. (Feeder larvae were always smaller than stimulus larvae). Aquaria were cleaned once per week. Aquaria were initially filled to a depth of 27.5 cm. Beginning at week 6, the water level in the tanks was lowered by 2.5 cm per week to simulate natural pond drying. We began dropping the water level prior to metamorphosis of any test larvae.

We monitored the experimental aquaria daily. All test animals that reached metamorphosis (i.e., beginning of gill reabsorption) were removed from the test chambers and weighed. If a stimulus animal transformed prior to the end of the experiment, we replaced it with other stimulus animals that were the same size and had the same morphology and diet. For each test animal, we calculated growth rate by dividing the difference between initial and final mass by the number of days from the beginning of the experiment to metamorphosis. For each response variable (see below) we calculated tank means for the four animals in each tank and used these means for all statistical analyses.

Our data conformed to assumptions of parametric statistics. Therefore, we used a multivariate analysis of variance (MANOVA) to examine the effects of morphology and diet of the stimulus animals on life history traits of the test animals. After MANOVA, we used univariate

analysis of variance (ANOVA) on each response variable to assess which variables were responsible for significant main effects.

Results

MANOVA revealed that there was an overall effect of the treatment conditions on the life history parameters that we measured (See Table 6.1). Subsequent ANOVAs showed that growth rate and time taken to reach metamorphosis, but not mass at metamorphosis was influenced by the diet of the stimulus animals (Table 6.1, Figure 6.1). Neither growth rate, time taken to reach metamorphosis or mass at metamorphosis was influenced by the morphology of the stimulus animals (Table 6.1, Figure 6.1). There were no significant interactions between morphology and diet (Table 6.1, Figure 6.1).

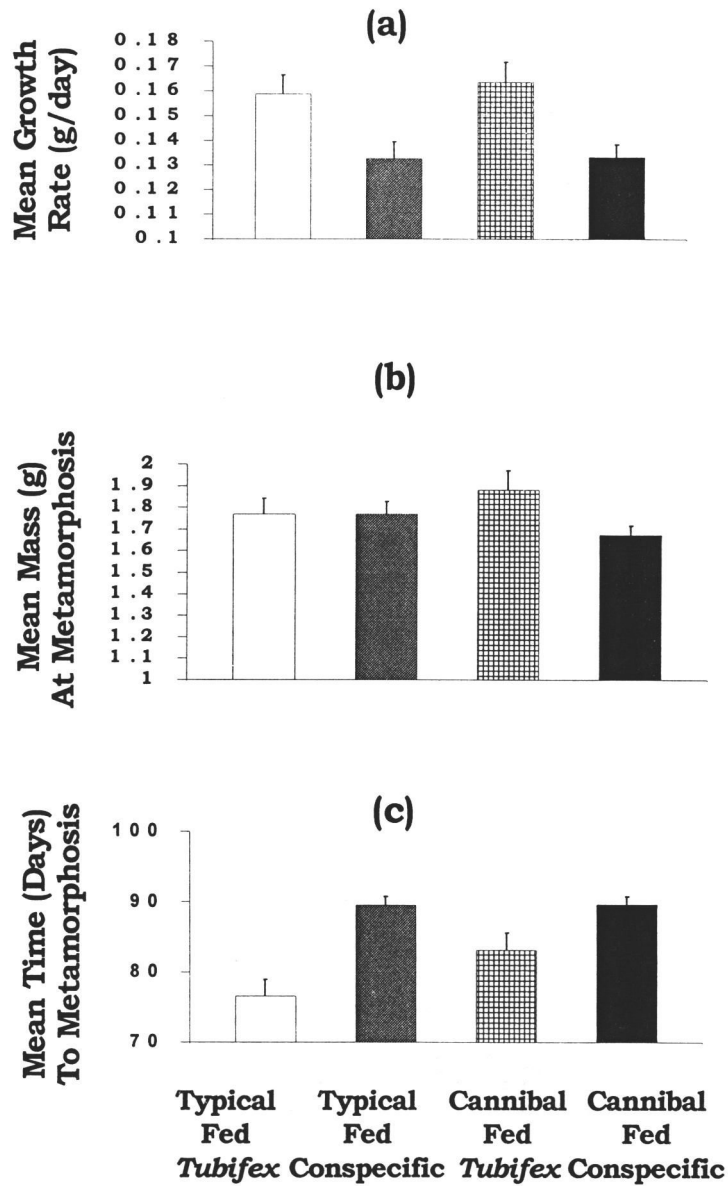


Figure 6.1 a) Mean (\pm SE) growth rate (grams per day) for *A. macrodactylum* test larvae. b) Mean (\pm SE) mass (grams) at metamorphosis. c) Mean (\pm SE) time (days) to metamorphosis.

Table 6.1 Results of MANOVA for overall effects of stimulus types (diet and morphology) on life history traits and ANOVAs for each response variable. Response variables are growth rate (rate), time taken to reach metamorphosis (time) and mass at metamorphosis (mass). Degrees of freedom are 3, 16 for MANOVAs and 1, 18 for ANOVAs.

	Source	F	P
<hr/>			
MANOVA			
	Constant	4418.570	<0.001
	Diet	26.827	<0.001
	Morph	1.926	0.166
	Diet x Morphology	0.801	0.301
ANOVAs			
Rate			
	Diet	15.550	<0.001
	Morphology	0.149	0.704
	Diet X Morphology	0.075	0.787
Time			
	Diet	25.239	<0.001
	Morphology	2.976	0.102
	Diet X Morphology	3.006	0.100
Mass			
	Diet	2.024	0.172
	Morphology	0.009	0.925
	Diet X Morphology	2.162	0.159
<hr/>			

Discussion

Larval long-toed salamanders exhibited significant changes in life history characteristics in response to conspecifics that had cannibalized. Larvae exhibited a significantly slower growth rate and an increased time taken to reach metamorphosis when exposed to cues from stimulus animals fed a diet of conspecifics as opposed to heterospecifics.

Previous studies support the idea that the diet of a potential predator can influence both short-term and long-term responses in potential prey (see reviews in Chivers and Smith, 1998 and Kats and Dill, 1998). For example, Wilson and Lefcort (1993) found that red-legged frog tadpoles reduced activity when exposed to chemical cues from newts fed a diet of red-legged frog tadpoles but not when they were fed insects. A more long-term response was demonstrated by Crowl and Covich (1990) who showed that stream snails (*Physella virgata*) exhibited faster growth and a delay in reproduction in response to cues from predatory crayfish feeding on other stream snails. In our study, we show that larval *A. macrodactylum* experience an increased time to metamorphosis and a decreased growth rate when exposed to other larval *A. macrodactylum* that have been fed a diet of conspecifics but not when they have been fed a diet of *Tubifex*. These results are particularly interesting because predator and prey belong to the same species. In non-cannibalistic populations, conspecifics may engage in competitive and/or reproductive interactions. For cannibalistic populations, predator-prey interactions may also be important.

In our study, there was no effect of the morphology of the stimulus animals on any of the life history variables that we measured. Test animals responded to the stimulus animals in the same manner regardless of whether or not the stimulus animals had a cannibal

morphology. These results differ somewhat from our past behavioral studies (Chivers et al., 1997).

Previously we found that typical morph long-toed salamander larvae exhibited antipredatory behavior in response to chemical cues of conspecific stimulus animals possessing a cannibalistic morphology. This response occurred not only when the cannibal morphs were fed a conspecific diet (as in the present study) but also when they were fed *Tubifex*. We believe that the differences can be explained in the following way. In the behavioral experiments, the typical larvae exhibited an immediate behavioral response when presented with a predatory threat from a cannibal. In this instance, we only observed the behavior of the test animals for a short duration (20 min). Theory predicts that the antipredator response of the test animals to the cannibal should diminish over time if the cannibal does not attack (Lima and Dill, 1990). In the present experiment, the test animals had a long period of time to assess the potential risk posed by the cannibal. In this case, the test animals responded to the cannibals only when the cannibals acted cannibalistic (i.e. when they had consumed a conspecific diet). This interpretation is supported by examining the responses of test larvae to typical morph stimulus animals. Test animals responded to typical morph stimulus animals when the stimulus animals acted cannibalistic

(i.e. had a conspecific diet), but not when the stimulus animals did not (i.e. had a *Tubifex* diet).

Long-term life history shifts may result as byproducts of antipredatory behavior (e.g. Dodson and Havel, 1988; Skelly and Werner, 1990; Skelly, 1992; Ball and Baker, 1996) or may represent facultative alterations in life history (e.g. Minchella and Loverde, 1981; Crowl and Covich, 1990; Wilbur and Fauth, 1990) or a combination of both (e.g. Skelly and Werner, 1990). We suspect that the differences in life history traits that we observed may be a consequence or by-product of the behavioral responses of the test larvae. Chivers et al. (1997) documented that antipredator responses of larval long-toed salamanders to cues of cannibals included reduced movement and spatially avoiding the area near the cannibal. In this study, test larvae exposed to cannibals or typicals fed conspecifics may have demonstrated similar behavioral responses. These behavioral responses likely conflicted with foraging. In our experiment, all test larvae were fed *ad libitum* every other day. We did not attempt to determine if there were differences in the amount of food eaten by test larvae in the different treatments.

In our experiment, we observed that test animals exhibited a reduction in growth rate and a reduction in the time taken to reach metamorphosis in response to stimulus animals that were fed conspecifics over *Tubifex*. We did not observe any differences in the final

mass larvae reached at metamorphosis. Other amphibians, including American toads (*Bufo americanus*), have been shown to metamorphose at a smaller size in response to the non-lethal presence of predators (Skelly and Werner, 1990).

We suggest that the responses exhibited by larvae in this study were mediated by chemical cues. Previously, we found that larval *A. macrodactylum* demonstrated antipredator behavior when exposed to both the chemical and visual cues of the cannibal morphs or chemical cues only, but not when exposed only to visual cues (Chivers et al., 1997). Moreover, the intensity of this antipredator behavior to chemical cues appeared to be elevated when the cannibal morphs were fed a diet of conspecifics (prior to behavioral trials) compared to when the cannibals were fed a diet of *Tubifex*. Therefore, we concluded that larvae were primarily using information from chemical cues to assess their surroundings. Similarly, in the current study, shifts in life history parameters demonstrated by larvae were most likely a response to chemical cues arising as a byproduct of the cannibal diet that the stimulus larvae had consumed.

Even though we have no evidence that larval long-toed salamanders use visual or tactile cues to recognize predatory cues, we cannot completely rule out this possibility. It is possible that these cues could have been used by larvae in our experiment. The fiberglass mesh

screen separating the test larvae from the stimulus larva allowed visual and tactile as well as chemical cues to be exchanged between the test and stimulus larvae. Test larvae could see the stimulus larva in the act of eating and detect any movement associated with this. Visually detecting a stimulus larva consuming a conspecific could have contributed to the shifts in life history observed.

It is not uncommon for adult long-toed salamanders to lay their eggs in temporary environments (Leonard et al., 1993; Wildy, personal observation). Consequently, it is critical for larvae developing in these habitats to metamorphose before the habitat completely dries. In our experiment, we observed that larval salamanders exhibited a reduction in growth rate and an increase in time taken to reach metamorphosis in response to the threat of cannibalism. These responses may be particularly costly in ephemeral environments. The pond may dry prior to the larvae reaching metamorphosis.

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Chapter 7

Evidence for Learned Recognition of Alarm Cues in Larval Long-Toed Salamanders, *Ambystoma macrodactylum*

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Abstract

The ability of prey to detect predators and respond accordingly is critical to their survival. The use of chemical cues by animals in predator detection has been widely documented. In many cases, predator recognition is facilitated by the release of alarm cues from conspecific victims. Alarm cues elicit antipredator behavior in many species, which can reduce their risk of being attacked. It has been previously demonstrated that adult long-toed salamanders, *Ambystoma macrodactylum*, exhibit an alarm response to chemical cues from injured conspecifics. However, whether this response exists in the larval stage of this species and whether it is an innate or a learned condition is unknown. In the current study, we examined the alarm response of naive (i.e. lab-reared) larval long-toed salamanders. We conducted a series of behavioral trials during which we quantified the level of activity and spatial avoidance of hungry and satiated focal larvae to water conditioned by an injured conspecific, a cannibal that had recently been fed a conspecific or a noncannibal that was recently fed a diet of *Tubifex* worms. Focal larvae neither reduced their activity nor spatially avoided the area of the stimulus in either treatment when satiated and exhibited increased activity toward the cannibal stimulus when hungry. We regard this latter behavior as a feeding response. These results suggest that an antipredator response in naive larvae is absent. Therefore, we conducted

an additional experiment examining whether larvae can learn to exhibit antipredator behavior in response to cues from cannibalized conspecifics. We exposed larvae to visual, chemical and tactile cues of conspecifics that were fed a conspecific (experienced) or a heterospecific diet (naive treatment) and subsequently tested the response of these larvae to cannibals and noncannibal stimulus animals simultaneously in behavioral trials. Experienced larvae significantly reduced their activity compared to naive larvae in response to chemical cues of cannibals that had recently consumed conspecifics. This suggests that the antipredator response may be learned in at least some populations of this species. Furthermore, we suggest that this behavior may be a response to alarm cues that have been released by consumed conspecifics and that have labeled the cannibal.

Introduction

The ability of prey to recognize areas of high predation risk and respond accordingly is of critical importance to their survival (Lima and Dill, 1990). The use of chemical cues by prey to detect predators has been widely documented (see reviews in Weldon, 1990; Dodson et al. 1994; Chivers and Smith 1998; Kats and Dill 1998). Although information provided through other means (e.g. visual, tactile cues) may aid prey in locating predators, chemical cues may be particularly useful for individuals that are nocturnal (Chivers et al., 1996a), living in highly complex or turbid environments (Magurran, 1989; Chivers et al., 1996a) or when dealing with cryptic predators or predators adopting a sit-and-wait strategy (Mathis and Smith 1993a; Chivers et al., 1996a).

Prey locating a predator chemically may be responding to one of several cues. They may be reacting to the smell of the predator (i.e. Holomuzki, 1986; Petranksa et al., 1987; Elliot et al., 1993; Kats et al., 1994; Chivers et al., 1995; Chivers et al., 1997a). This may be due to an innate mechanism which causes them to regard an animal of a particular type as a potential predator. Alternatively, an animal may have had prior experience with a predator and has learned to avoid it.

Previous studies have shown that naive animals may not exhibit antipredator behavior to some potential predators (e.g. Magurran 1989; Mathis and Smith 1993a; Chivers and Smith 1994 b,c; Chivers et al.

1996a; Wisenden et al., 1997). However, when these naive animals are exposed to chemical cues from injured or consumed conspecifics in conjunction with predatory cues, previously unresponsive animals may respond with antipredator behavior (Magurran, 1989; Mathis and Smith, 1993a; Chivers and Smith 1994b,c; Chivers et al., 1996a; Wisenden et al., 1997).

The presence of an alarm response has been documented in a wide variety of taxa (see reviews in Dodson et al., 1994; Chivers and Smith, 1998) including insects (Sih, 1986; Chivers et al., 1996a; Wisenden et al., 1997), gastropods (Appleton and Palmer 1988; Rittschhof et al., 1992); crayfish (Hazlett, 1994); echinoderms (Parker and Schulman, 1986; Lawrence, 1991), and fish (Smith, 1989, 1992; Mathis and Smith, 1993a; Smith 1992). Typically, alarm substances like Schreckstoff in ostariophysian fishes (Smith, 1986, 1992) or anthopleurine in sea anemones (Howe and Harris 1978), are released when an animal is damaged as may occur during an attack from a predator. Indeed, the presence of conspecific alarm cue elicits an antipredator response from some animals (i.e. Crowl and Covich 1990; Mathis and Smith 1992; Wilson and Lefcort 1993; Chivers and Smith 1994a; Chivers et al. 1996b, 1997b). Furthermore, it has been demonstrated that a predator can be chemically labeled with the alarm substance released by the consumed prey (i.e. Chivers et al., 1996a;

Mathis and Smith 1993, a,c; Wilson and Lefcort,1993; Wisenden et al., 1997) prompting conspecifics of that prey to respond with antipredator behavior. Over time, this may facilitate learned predator recognition such that prey respond to the predator cues alone.

Although numerous investigations of behavioral responses of prey to the diet of heterospecific predators have been conducted, relatively few studies have focused on the behavioral interactions between cannibals and their conspecific prey. In the current study we examined the response that an amphibian prey has to cues from cannibalized conspecifics. Cannibalism has been documented in several populations of larval long-toed salamanders, *Ambystoma macrodactylum* (Anderson, 1967; Nussbaum et al., 1983; Walls et al., 1993a, b; Chivers et al., 1997b; Wildy et al.,1998, 1999; Wildy, unpublished data). Furthermore, larvae of this species exhibit a trophic polymorphism, whereby some individuals have an alternative (i.e. cannibalistic) morphology characterized by a disproportionately large head and hypertrophied vomerine teeth (Walls et al. 1993a).~ Typical morphs lack these characteristics.

Results from a previous study (Wildy et al., 1999) revealed that larval long-toed salamanders exhibit shifts in certain life history traits (i.e. increased time to metamorphosis, decreased growth rate) when raised with stimulus larvae that are being fed a diet of conspecifics. The

authors suggested that these shifts in life history are likely due to individuals exhibiting antipredatory behavior (i.e. reduced foraging) in response to the cues released by the cannibalized conspecifics. It is possible that the recognition of and subsequent antipredator behavior directed toward these cues is an innate response. However, the larvae used in this study were collected as older individuals and an alternative explanation may be that these individuals had some prior experience with cannibalism and may have learned to respond with antipredator behavior to cues associated with this. We explored this question by examining the antipredator response of naïve animals.

Materials and Methods

Eggs of long-toed salamanders were collected in May 1998 from an ephemeral pond (elevation 1951 m) located approximately 24 km south of Sisters, Deschutes County, Oregon, U.S.A. Eggs were transported to the laboratory and housed individually in plastic cups containing approximately 600 ml of dechlorinated tap water. After hatching, larvae were maintained on brine shrimp (*Artemia*) and later on *Tubifex* worms ad libitum every other day. The water in the cups was changed three times a week when larvae were fed *Artemia* and once per week when their diet was switched to *Tubifex*. Larvae were maintained on a 14:10 h light:dark cycle and were allowed to grow under these conditions for four

months. Individuals from this source population were used as focal animals in the three experiments described below, with individuals not used in more than one behavioral trial.

Experiment 1

Behavioral trials were conducted in which focal larvae were exposed to one of three pairs of stimuli: 1) water exposed to a cannibal that had recently consumed a conspecific vs. plain tap water 2) water exposed to a noncannibal that had recently been fed a heterospecific diet (i.e. *Tubifex* worms) vs. plain tap water or 3) water exposed to an injured conspecific vs. plain tap water. Each trial was conducted in an aquarium (90cm x 38cm x 30 cm) filled to a height of 4 cm with dechlorinated tap water. Fiberglass screening divided this chamber into three sections: two smaller stimulus sections located on either end of the aquarium (in which water from stimulus animals or plain water was placed) and one larger, centrally-located testing section in which the behavior of test larvae in response to the stimuli was observed. The stimulus sections were designed such that only chemical cues could reach the focal larva. Each stimulus section was defined by two 2.5-cm long opaque plastic barriers with a 5-cm long mesh screen attached to one end. The two partitions were mounted 1 cm apart with the mesh portions of the partitions mounted on opposite sides of the aquarium.

This design allowed water and any chemical cues to move between the end cages and the middle portion of the tank, while visual cues were blocked.

During each trial, the time spent in the section of the testing chamber near each stimulus and activity level were quantified as a measure of antipredator behavior for each focal larva. Both of these behaviors are well documented responses of larval salamanders to the risk of predation (e.g. Holomuzki 1986; Semlitsch 1987; Sih and Kats 1991, 1994; Chivers et al. 1997a). To determine where larvae spent their time, lines were drawn on the side of the aquarium facing the observer. This divided the testing section into three main subsections: one area of choice for each stimulus (or side of the aquarium) and one middle “no choice” area. During each trial, the amount of time that a focal larva spent in each of these sections was recorded using a stopwatch. To quantify activity level, a grid of 18 squares, each measuring 9.5 X 9 cm, was drawn on the floor of the testing area. The grid was made up of 27 lines and the observer estimated the activity level of a focal larva by counting the number of gridlines crossed during the trial.

A total of thirty-three trials were conducted with eleven salamanders tested individually under each treatment condition. Each trial lasted for ten minutes, preceded by a five-minute habituation time.

Between trials, the testing chamber was thoroughly rinsed and filled with dechlorinated tap water.

For all trials, the treatment type used during a trial and the end cage to which it was added were randomly selected. Before the beginning of each trial, 30 mL of stimulus (treatment) water and 30 mL of dechlorinated (control) tap water were collected in a 60 cc syringe and introduced into the appropriate stimulus section of the test chamber. Cannibal cues and noncannibal cues were randomly collected from one of two stimulus animals. The sizes of the two cannibal and noncannibal stimulus animals were all within 1.2 mm of one another with cannibals averaging $29.1 \text{ mm} \pm 0.10 \text{ (SE)}$ and non-cannibals averaging $29.3 \text{ mm} \pm 0.60 \text{ (SE)}$ in snout-vent length. Twelve hours before the beginning of the first trial, cannibals were fed one conspecific each while noncannibals were fed *Tubifex* worms. These diets were matched for mass such that the amount of *Tubifex* fed to noncannibals matched the mass of the conspecifics fed to the cannibals. Larvae consumed their diets within thirty minutes and were then rinsed and placed in 26 X 17 X 12 cm containers filled with 3.5 L of dechlorinated tap water. Stimulus animals continued to condition this water until the end of the last trial.

The injured cue was newly prepared for each trial to maintain freshness of the cue. Larvae used to prepare this cue averaged $33.4 \pm 0.17 \text{ mm}$ in size (N=12). Five minutes before the beginning of a trial in

which injured cue was used, a stimulus larva was rinsed and approximately 1 mm of the tip of the tail was removed. The tail piece was cut into three smaller pieces and then both the larva and the homogenized tail pieces were placed in a 26 X 17 X 12 cm container filled with 3.5 L of water.

We stopped feeding focal larvae 58 hours before the beginning of the first trial. Individuals were allowed to habituate for five minutes in an enclosure placed in the middle of the testing section. This habituation enclosure, measuring 10cm X 10cm X 7cm, consisted of an opaque plastic enclosure, the sides of which were removed and replaced with fiberglass mesh screening. This enclosure allowed chemical cues from the stimulus animals to reach the focal larva housed inside but did not allow the focal larva to freely move about the testing section. Repeated dye trials indicated that cues from the stimulus sections required approximately two minutes to reach the center of the testing section.

Experiment 2

Focal larvae in Experiment 1 increased their activity in response to cannibal cues which we interpreted to be a feeding response since it was not consistent with the suite of antipredator behaviors known for this species. To test whether the hunger level of focal larvae may have

affected their behavior, we conducted a second experiment. The design of the experiment and preparation of stimuli was the same as described in Experiment 1. However, focal larvae used in these trials had been fed 10 h before the beginning of the first trial and all focal larvae used in this experiment were tested no more than 25 h after they were last fed. We used the same stimulus animals as were used in Experiment 1.

Experiment 3

This experiment was conducted to examine if prior exposure to cannibalism would result in antipredator responses on subsequent exposure to those cues. The cannibalism-naïve larvae used in this experiment were selected from the laboratory-raised source population. Twenty-four larvae were chosen and assigned to be focal animals in either a "naïve" or an "experienced" treatment. Twelve focal larvae were assigned to each treatment and for both treatments, larvae were placed temporarily in pre-trial conditioning glass test aquaria (50 x 25 x 30 cm) that were divided by fiberglass mesh screen into five sections: a central stimulus section (10 x 25 cm) surrounded by four test sections each measuring 12.5 x 20 cm. There were three conditioning aquaria for each treatment for a total of six aquaria.

In each aquarium, four focal larvae belonging to the same treatment were placed individually into one of the four peripheral testing

sections. All focal larvae were size matched within and between aquaria so that all but one larva was within 1.7 mm snout-vent length of all others (that one larva was 2.7 mm smaller than the largest larva).

Each central section held a stimulus animal consisting of either a noncannibal maintained on a diet of *Tubifex* (naive treatment) or a cannibal maintained on a diet of conspecifics (experienced treatment). There was one stimulus animal for each aquarium for a total of three noncannibals and three cannibals. Stimulus animals were size-matched so that one stimulus animal belonging to one treatment was approximately the same size as one stimulus animal belonging to the other treatment.

Focal larvae were raised with their respective stimulus animals for 24 hours. During that time all focal larvae were fed *Tubifex* ad libitum for one hour. At the end of the 24 hour period, larvae within a treatment were rearranged between aquaria such that all focal larvae were in a different aquarium and were exposed to a different stimulus animal and to at least two (out of three) new focal animal tank-mates. During the next 24 hours, stimulus animals were fed their respective diets. These diets were matched for mass such that the amount of *Tubifex* fed to a naive-treatment stimulus animal matched the mass of the conspecific fed to the experienced-treatment stimulus animal with which it was size-matched. While stimulus animals were feeding, surrounding focal larvae

received visual, chemical and possibly tactile cues associated with this activity through the mesh screen.

Behavioral trials were conducted in the same experimental chamber used in the first two experiments. Four new stimulus larvae, two cannibals and two noncannibals, were used for these trials. Cannibals averaged $31.0 \text{ mm} \pm .50 \text{ (SE)}$ in snout-vent length and were within 1.5 mm of one another while noncannibals averaged $31.1 \text{ mm} \pm .55 \text{ (SE)}$ snout-vent length and were within 1.6 mm of one another. All stimulus animals were fed 11 hours before the beginning of the first trial. Cannibals were fed one conspecific each while noncannibals were fed *Tubifex* worms. These diets were matched for mass such that the amount of *Tubifex* fed to noncannibals matched the mass of conspecifics fed to the cannibals.

All behavioral trials were conducted in one day with the last trial ending 10 h after the beginning of the first trial. Trials were conducted in an identical manner to those in Experiments 1 and 2 except that before the beginning of each trial, the actual stimulus animals rather than water treated by a stimulus animal was placed in the stimulus section of the testing chamber. One of the two cannibals and one of the two noncannibals were randomly chosen and randomly assigned to one of the stimulus sections of the testing chamber. During each trial we observed activity level and spatial avoidance of each focal larva.

Statistical Analysis

In all three experiments, we used a binomial test to compare the number of trials in which larvae spent less than 50% of their time on the treatment (Experiments 1 and 2) or cannibal side (Experiment 3) of the testing chamber. We used a Kruskal-Wallis one-way analysis of variance with non-parametric multiple comparisons in Experiments 1 and 2 and a Mann Whitney U test in Experiment 3 to compare the activity level (i.e. number of line crosses) between treatments.

Results

Experiment 1

There was a significant effect of treatment on activity level (KW=6.18, $p=0.045$) when larvae were hungry. Larvae exposed to cues from cannibals exhibited increased activity compared to larvae exposed to either cues from injured conspecifics or noncannibals (Fig. 7.1). Larvae did not show a preference for spending time near a particular side of the tank when exposed to cues from either cannibals, injured conspecifics, or noncannibals. In each case, the number of trials in which larvae spent greater than 50% of their time on the treatment side of the testing chamber was not significantly different from the number of times larvae spent on the water side (Table 7.1).

Table 7.1: Number of trials in which test animals chose the treatment over the control sides of the experimental chamber (see text for details; p values are based on one-tailed binomial tests).

Experiment	Side chosen	No. of trials	Side chosen	No. of trials	p
1. Hungry	cannibal	6	water	6	1.0
	injured	9	water	3	0.15
	noncannibal	4	water	8	0.39
2. Satiated	cannibal	6	water	6	1.0
	injured	5	water	7	0.77
	noncannibal	6	water	6	1.0
3. Naive/ Experienced	cannibal	4	noncannibal	7	0.55

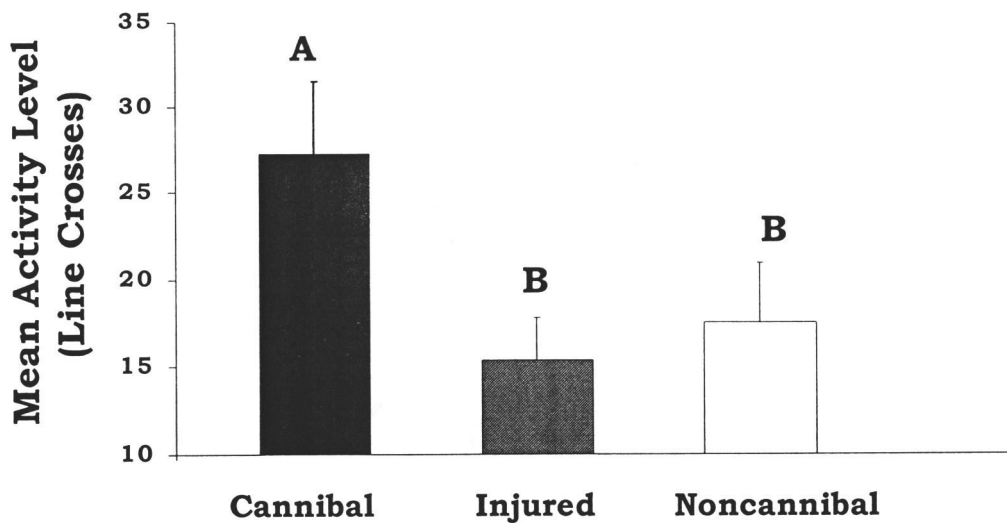


Figure 7.1 Mean (\pm SE) number of line crosses made by focal larvae during behavioral trials in Experiment 1. Hungry (i.e. not been fed for between 58 and 73.5 hours before the trial in which they were used) focal larvae were exposed to chemical cues from a cannibal that had recently been fed a conspecific diet, a noncannibal that had recently been fed a diet of *Tubifex* worms or an injured conspecific. Treatment bars that share the same letter are not statistically different from one another.

Experiment 2

There was no effect of treatment on activity level when focal animals were satiated (KW=0.941, $p = 0.63$). Larvae exposed to the cannibal, injured or noncannibal treatments showed no significant difference in the number of line crosses (Fig. 7.2). In addition, larvae did not show a preference for spending time near a particular side of the aquaria when exposed to cannibal, injured conspecific, or noncannibal cues. In all three cases, the number of trials in which larvae spent

greater than 50% of their time on the treatment side of the testing chamber was not significantly different from the number of times larvae spent on the water side (Table 7.1).

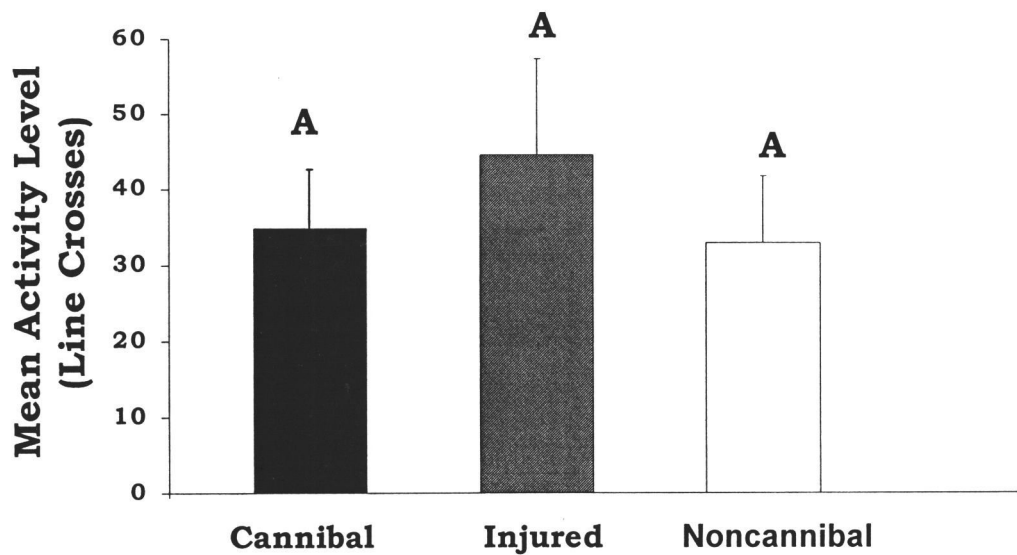


Figure 7.2 Mean (\pm SE) number of line crosses made by focal larvae during behavioral trials in Experiment 2. Satiated (i.e. fed between 10 and 25 hours before the trial in which they were used) focal larvae were exposed to chemical cues from a cannibal that had recently been fed a conspecific diet, a noncannibal that had recently been fed a diet of *Tubifex* worms and an injured conspecific. Treatment bars that share the same letter are not statistically different from one another.

Experiment 3

There was a significant effect of treatment on activity level ($U = 92.5$, $p = .036$). Experienced larvae reduced their activity in response to the stimulus animals significantly more so than naive animals (Fig. 7.3). Neither experienced nor naive larvae showed a preference for spending time near a particular stimulus animal. The number of trials in either treatment in which larvae spent greater than 50% of their time on the cannibal side of the testing chamber was not significantly different from the number of times larvae spent on the noncannibal side (Table 7.1).

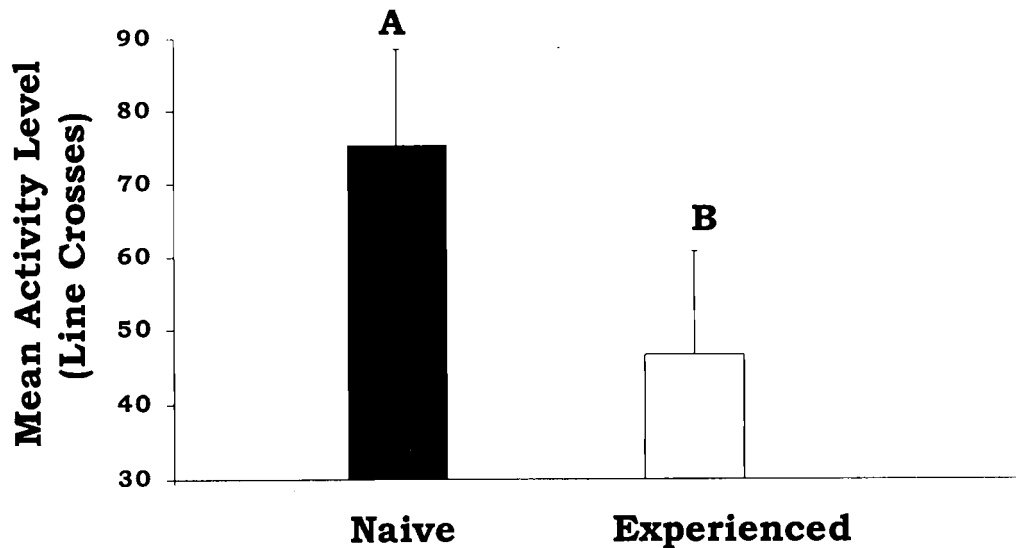


Figure 7.3. Mean (\pm SE) number of line crosses made by focal larvae during behavioral trials in Experiment 3. Initially naive focal larvae that had been previously exposed to visual, chemical and tactile cues from either a cannibal consuming a conspecific (experienced) or a noncannibal consuming *Tubifex* worms (naive) were exposed to chemical cues from a cannibal that had recently cannibalized and a noncannibal that had recently been fed a diet of *Tubifex* worms.

Discussion

Our results suggest that naive larval long-toed salamanders do not respond with antipredator behavior to cues from either injured or consumed conspecifics. In that first two experiments, naive larvae that were either hungry or satiated did not exhibit a reduction in activity or spatial avoidance when exposed to either of these cues. In fact, hungry larvae tended to increase their activity

in response to cues from cannibals. We interpreted this to be a feeding response directed at cues from cannibalized conspecifics. This has been suggested in previous studies when larval amphibians have increased activity in response to an injured conspecific (e.g. Hokit and Blaustein, 1996).

Although naïve larvae did not respond with antipredator behavior to chemical cues from conspecifics that had been cannibalized, results from the third experiment suggest that individuals can learn to recognize and exhibit an antipredator response to these cues. Experienced larvae that were exposed once to cues from a conspecific in the act of cannibalizing subsequently exhibited a significant reduction in activity compared to naïve larvae in the presence of chemical cues from another cannibal that had recently cannibalized. A reduction in activity is one of several behaviors typically characterizing an antipredator response in larval amphibians (Chivers and Smith, 1998; Kats and Dill, 1998; Sih, 1987). In nature, the ability of larvae to detect cues associated with predation (i.e. predatory cues, alarm cues released by conspecifics) and to respond with an appropriate antipredator response may protect those individuals from being eaten. In the present study, reducing activity in response to chemical cues of a cannibal may allow larval long-toed salamanders to remain undetected, and thus uneaten, by that predator.

Although experienced larvae did exhibit a reduction in activity, larvae did not spatially avoid the side of the testing chamber where the cannibal stimulus animal was housed. A similar result was found in a previous study (Chivers et al., 1997a). In that study, larval long-toed salamanders both spatially avoided and reduced activity in the presence of cannibals when they were provided with visual, chemical and tactile cues. However, when focal larvae were provided with only chemical cues, they exhibited a reduction in activity level but they failed to spatially avoid the side of the testing chamber housing the cannibals. The authors concluded that although chemical cues may alert a larva to the presence of a conspecific predator, visual cues may also be needed to help the individual identify the specific location of the predator. In the current study, our testing chamber was designed such that only chemical cues from the stimulus cages were allowed to reach the focal animal in the center testing arena. It is possible that, although chemical cues were adequate to reveal the presence of cannibalized conspecifics to focal larvae as suggested by the corresponding reduction in activity, visual cues may have been required to allow larvae to pinpoint the origin of those cues and subsequently avoid that area.

The results of this study show that naive larvae can learn to exhibit antipredator behavior in response to chemical cues of a cannibal recently fed a conspecific diet. We go further in offering the possibility

that what focal larvae were actually responding to were alarm cues that were released by their cannibalized conspecifics and that subsequently labeled the cannibal. Furthermore, we suggest that the antipredator behavior exhibited by focal larvae was an alarm response. Indeed, adults of this species have been found to possess an alarm response. Chivers et al. (1996b) showed that adult long-toed salamanders demonstrated spatial avoidance of conspecific alarm cue when given a choice between spending time on a paper towel moistened with either conspecific alarm cue or water. The presence of an alarm response has also been documented in other amphibians particularly within the families Bufonidae (Hrbacek, 1950; Pfeiffer, 1974; Hews and Blaustein, 1985; Hews 1988), Ranidae (Hews and Blaustein, 1985; Wilson and Lefcort, 1993; but see Hokit and Blaustein, 1996), Salamandridae (Marvin and Hutchison, 1995), and Plethodontidae (Chivers et al., 1997a).

If larval long-toed salamanders are exhibiting an alarm response in the current study, our results suggest that, unlike in some other systems, this response is learned rather than innate. The lack of an innate response here may be a populational phenomenon. Perhaps a high predation risk is needed in a population to foster the development of a genetically based response to alarm cues. This is not necessarily the case in many populations of larval long-toed salamanders. In fact, the larvae used in this study originated from a pond similar to others found

in that region in that it is relatively devoid of large aquatic heterospecific predators presumably due to the temporary nature of the habitat. In addition, larvae escape predation from smaller invertebrate predators through a size refuge achieved early in development (Wildy personal observation). For most of the larval stage, one of the few sources of predatory threat appears to come from cannibalism, the frequency of which can vary (2-20% of animals cannibalizing at any one time) depending on the year or time of the season (Wildy personal observation).

Numerous studies have shown that a predator can be chemically labeled by alarm substances released by the prey it consumed and consequently elicit antipredator behavior in conspecifics of that prey (Hews 1988; Crowl and Covich 1990; Mathis & Smith 1993 a,c; Wilson & Lefcort 1993; Chivers et al. 1996a). Moreover, it has been shown that an association between conspecific alarm cues and predator cues may facilitate acquired predator recognition in naïve animals (i.e. Magurran 1989; Mathis & Smith 1993a; Chivers et al. 1994b,c; Chivers et al. 1996a; Wisenden et al. 1997). In these studies, naïve animals did not initially respond to cues from a predator. However, after exposure to a predator that had either consumed a conspecific or when cues from the predator were paired with alarm cue from a conspecific, focal animals responded with antipredator behavior when subsequently exposed to predator cues only. For example, Magurran (1989) showed that after one

exposure to a combination of chemical cues from either a native or non-native potential predator and conspecific alarm cue, previously naïve European minnows increased schooling and shelter use in response to the predator odor alone.

A similar scenario may occur in at least some populations of larval long-toed salamanders. Chivers et al. (1997a) showed that older (12 weeks posthatching), wild caught larval long-toed salamanders exhibited antipredator behavior to conspecifics possessing the cannibal morphology but not to conspecifics possessing the typical morphology. Furthermore, larvae exhibited this antipredator behavior both when the diet of the cannibal morphs consisted of conspecifics and in a separate experiment when it did not. It appears that larval long-toed salamanders recognize and respond to cannibal morphs as potential predators regardless of their diet. Based on the results of the current study, we suspect that this is most likely a learned response facilitated by prey associating cues from cannibalized conspecifics with cues from cannibal morphs (assuming that cannibal morphs are chemically distinguishable from typical morphs).

In cannibalistic populations, the ability of individuals to discriminate between conspecifics that represent a predatory threat and those that do not is key. This would be particularly true for animals living in close spatial proximity and under high conspecific density as is

the case for many amphibians including larval long-toed salamanders (Wildy, unpublished data). In cases where some animals are more likely to cannibalize than others, avoiding all conspecifics would be inefficient. However, by responding to the presence of alarm substance with antipredator behavior, prey may reduce the chance that they are detected by a predator by avoiding those areas where a conspecific was recently injured or consumed (Chivers and Smith 1994a; Mathis and Smith 1992). Here we demonstrate that naive larvae can learn to recognize cues from cannibalized conspecifics and, in turn, will exhibit antipredator behavior. We suspect that this behavior is an alarm response.

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Chapter 8. General Conclusions

Recently, a number of studies have examined the importance of cannibalism in animal populations. As with other predator-prey interactions, cannibalistic behavior may play a significant role in population regulation and may influence the morphology, life history, and behavior of both predators and prey (Fox, 1975; Polis, 1981; Elgar and Crespi, 1992). For some species, a considerable amount of information concerning the role of cannibalism within populations has already been established. However, with other species, relatively little is known about cannibalism as is the case for larval long-toed salamanders, *Ambystoma macrodactylum*. Prior to the research presented in this thesis, some anecdotal reports of cannibalism had been made for some *A. macrodactylum* populations (Anderson, 1967). Moreover, only two empirical studies examining cannibalism within this species had been conducted. One study documented cannibalistic behavior and the cannibal morphology in one population of this species (Walls et al., 1993a) while the other investigated the effect that diet type had on the induction of the cannibal morphology (Walls et al., 1993b). In this thesis, I have offered further insight into the role of cannibalism in *A. macrodactylum*.

In Chapter 2, I presented the results of surveys conducted in several Oregon Willamette Valley and Oregon Cascade Mountain

populations of larval *A. macrodactylum*. Cannibalism occurred with some frequency in the Cascade Mountains of Oregon but was not observed in Willamette Valley populations. At any given site, larvae that had cannibalized tended to have some of the largest body sizes and widest heads. This suggests that larger *A. macrodactylum* eat smaller *A. macrodactylum*. Moreover, cannibals may be as large as they are, at least in part, because they are consuming a highly nutritious meal in the form of conspecifics.

During the surveys, I also identified abiotic and biotic factors associated that may promote cannibalism at those sites. For example, in two out of the three populations where cannibalism was documented, it was observed when size variation in the population was the highest. High densities of larvae and limited food resources also were correlated with cannibalistic activity.

During the surveys, I only identified correlations between certain environmental factors and the incidence of cannibalism. In Chapters 3 and 4, I went further by experimentally manipulating several of these factors to investigate a causal relationship between them and larval salamander aggression.

In Chapter 3, I examined the effects of high densities and low food levels on the prevalence of biting and cannibalism in larval

A. macrodactylum. Both of these factors have been linked with increased aggression in amphibian populations (e.g. Collins and Cheek 1983; Crump 1983; Semlitsch and Reichling 1989; Ducey and Heuer 1991; Walls, 1998). I demonstrated that low food levels, more so than high density, significantly influenced intraspecific biting and cannibalism among groups of larval *A. macrodactylum*.

Moreover, in this study, the amount of aggression exhibited was directly proportional to the amount of group size variation present. These data parallel the findings of previous studies suggesting that increased size variation can influence increased aggression (i.e. Kusano et al. 1985; Nyman et al. 1993; Maret and Collins 1994; Petranka and Thomas 1995). A future study would need to concentrate on the direct manipulation of size variation within groups of larvae such that a causal relationship between that factor and increased biting and cannibalism can be established.

In Chapter 4, I investigated the influence that the rate of hydroperiod decrease, without the corresponding changes in of density and food levels, has on cannibalistic activity. Cannibalism was not observed in either fast drying or slow drying conditions. This suggests that cannibalism is not simply a response to pond drying itself but is a response to the limited food levels and the increased size variation that sometimes accompanies pond drying.

In ephemeral habitats, as pond drying occurs, larvae are faced with the risk of desiccation. Indeed, high mortality due to desiccation has been documented in populations of larval amphibians (Semlitsch, 1987a; Wilbur, 1987; Newman, 1988b; Semlitsch and Wilbur, 1988; Rowe and Dunson, 1995), including in populations of larval *A. macrodactylum* (Wildy et al., 1998, 1999). In Chapter 5, I demonstrated that larvae exhibit enhanced growth when fed a diet of conspecifics. The added growth exhibited by cannibals may minimize their risk of desiccation by increasing the likelihood that they will reach the size threshold necessary to metamorphose (Wilbur and Collins, 1973) before the pond completely dries. Furthermore, since larger larval size is translated to larger metamorphic size, individuals may accrue further benefits as a terrestrial metamorph including increased prey availability (Crump, 1989; Kiesecker, 1996), enhanced ability to escape predators (Woodward et al., 1988; Crump, 1989; John-Alder and Morin, 1990; Kiesecker, 1996) and decreased time to sexual maturity (Collins, 1975; Semlitsch et al., 1988; Smith, 1987).

Besides examining factors that may promote cannibalism in larval populations and investigating the potential benefits of engaging in this behavior, I studied the influence that cannibals have on the behavior and life history of potential conspecific prey. Previous studies have demonstrated that amphibians have the ability to recognize

heterospecific predators and will demonstrate antipredator behavior in their presence (e.g. Sih, 1987; Lima and Dill, 1990; Chivers and Smith, 1998; Kats and Dill, 1998). Since cannibals function as predators, it might be expected that noncannibalistic conspecifics would respond to them as they would to a heterospecific predator. Previous studies have shown that larval amphibians exhibit antipredator responses in the presence of cannibalistic conspecifics (Holomuzki, 1986; Kats et al., 1994). Chivers et al. (1998) demonstrated that typical morph *A. macrodactylum* larvae spatially avoid cannibalistic conspecifics and exhibit reduced activity in their presence. In Chapter 6, I demonstrated that the antipredator response exhibited by typical morphs may translate into further, life historical, consequences for these individuals. Compared to typical morphs that had been exposed to larval *A. macrodactylum* that had consumed a heterospecific diet, typical morph larvae that were exposed to larval *A. macrodactylum* that had consumed conspecifics exhibited a reduced growth rate and took longer to metamorphose. As this species typically inhabits ephemeral environments, this increased time taken to metamorphose could decrease their likelihood of survival for individuals taking longer to metamorphose, particularly at the end of the season.

In Chapter 7, I investigated whether the antipredator response of larvae to cannibals is a learned phenomenon. Previous studies indicated

that experienced larval *A. macrodactylum* (i.e. collected from the field as older animals) recognize cannibals and respond to them with antipredator behavior (Chivers et al., 1997a). Naive (i.e. lab-raised) animals did not respond to the odor of stimulus *A. macrodactylum* that had been fed conspecifics. However, test larvae that had been previously exposed to a cannibal consuming a conspecific exhibited reduced activity in the presence of the odor of stimulus *A. macrodactylum* that had been fed conspecifics. Perhaps an inherent antipredator response to conspecifics possessing the cannibal morphology or to the odor of a consumed conspecific has not developed in typical larvae because of the potential annual variability with which cannibal morphs and/or the frequency of cannibalism appear in a population. The results of previous studies suggest that the frequency of cannibalism and the development of the cannibal morph for populations of larval *A. macrodactylum* is likely to be influenced by one or more environmental factors (Walls et al., 1993b; Wildy et al., in review). The frequency of cannibalism of a given population may be directly proportional to the variability in these environmental factors. High variability in both of these factors may result in only weak selection on the ability to recognize and respond to cannibals and cues from cannibalized conspecifics.

There is much more to be studied concerning the role of cannibalism and other forms of aggression in populations of larval

A. macrodactylum. In Chapter 2, I suggested that cannibalism is more common in some populations of this species than others. A more extensive survey of populations throughout the entire range of larval *A. macrodactylum* populations is needed to identify how widespread cannibalism and the cannibal morphology is and what factors are most important in influencing their existence in certain populations.

Additional questions that were prompted by the research presented in this thesis and that need to be addressed in future studies are:

(Chapter 2)

- a) Would the cannibal morph be better described as a “large prey morph”?

The consumption of *H. regilla* tadpoles was observed at most of the sites where cannibalism was observed. Perhaps the increased gape and hypertrophied vomerine teeth characteristic of this morph facilitate the consumption of any relatively large prey item and not just conspecifics. Reilly et al. (1992) found this to be true for larval *A. tigrinum*.

- b) Do the structural features of the cannibal morph readily observed in larvae carry over to the terrestrial stage?

For cannibals, the enhanced growth resulting from the consumption of conspecifics does carry over into the terrestrial stage. Therefore, it is also possible that certain physical features characteristic

of the cannibal morphology, particularly the enlarged head size with corresponding increased gape, will also carry over to the metamorphic stage. This may have a number of implications for life in the terrestrial stage including access to a wider diversity (i.e. larger sized) prey and enhanced defensive and/or aggressive abilities (in terrestrial salamanders, biting can be used in aggressive encounters with conspecifics).

(Chapters 3 and 4)

- a) What other factors besides conspecific density, food resources, and population size variation influence cannibalism in populations of larval *A. macrodactylum*?

The presence of disease (Pfennig et al., 1994, 1998, 1999), the relatedness of surrounding individuals (Pfennig et al., 1993, 1994; Walls and Blaustein, 1995) and the availability of more vulnerable prey (Loeb et al., 1994) have all been suggested to influence the prevalence of cannibalism in other populations of cannibalistic amphibians. Perhaps these factors are also important in influencing cannibalistic activity in larval *A. macrodactylum* populations.

(Chapter 5)

- a) Are there other benefits to cannibalism besides enhanced growth?

Lannoo et al. (1989) showed that larval *A. tigrinum* that cannibalized exhibited reduced time to metamorphosis. This may be

particularly significant for larvae inhabiting temporary habitats. In addition, reduced competition has been suggested to be a possible byproduct of cannibalistic behavior (Fox 1975; Polis 1981). Indeed, this may have significant implications for populations in food limited environments.

b) What are the possible costs larval *A. macrodactylum* engaging in cannibalism?

One potential hazard of cannibalism in the increased spread of disease. The presence of pathogens and disease in amphibian populations has been widely documented (WorthyLake and Hovingh 1989; Sessions and Ruth 1990; Pfennig et al. 1991; Blaustein et al. 1994; Kiesecker and Blaustein 1995, 1997; Jancovich et al. 1997). Some studies have suggested that due to the close genetic constitution of cannibals and their prey, the efficiency with which pathogens are transferred between cannibalistic individuals is greater (Kiltzman et al. 1984; Schaub et al. 1989; Pfennig et al. 1991) than if predator and prey were heterospecifics (Pfennig et al. 1998). Other studies go further in providing evidence that certain pathogens are more easily transmitted between kin (Shykoff and Schmid-Hempel 1991; Black 1994) rather than between non-kin. Pfennig et al. (1999) showed that cannibalistic larval tiger salamanders, *A. tigrinum*, do not necessarily avoid consuming diseased conspecifics while Pfennig et al. (1998) found that cannibals

that did consume diseased conspecifics exhibited decreased survival and reduced growth compared to cannibals that ate diseased heterospecifics.

(Chapters 6 and 7)

a) How do cannibal morphs respond to the presence of other cannibal morphs?

Smaller, typical morph *A. macrodactylum* (Chivers et al., 1997) and *A. tigrinum* (Holomuzki, 1986) spatially avoid cannibals and reduce activity in the presence of larger cannibals. However, typical morphs do not have the same reaction to other typical morphs (Chivers et al., 1997). To my knowledge, no study has investigated behavioral interactions between cannibals. It is possible that cannibals will not react with antipredator behavior toward other cannibals because of the similarity in their sizes and thus the decreased likelihood that one could consume the other.

b) Do cannibals avoid eating kin?

Studies examining this questions in congeneric species have yielded a mix of results. Larval *A. tigrinum* have been found to preferentially eat non-kin (Pfennig et al., 1993) while larval *A. opacum* were shown to preferentially consume relatives (Walls and Blaustein, 1995). Larval northwestern salamanders, *A. gracile*, appear to lack kin discriminating abilities (Walls et al. 1996). The variability in kin-directed

behavior in congeners makes examining the behavior in *A. macrodactylum* particularly intriguing.

Together with the results of previous studies and studies presented in this thesis, answers to these questions promise to offer further insight into the complexities of predator/prey interactions. This is particularly true of cannibalistic interactions where two individuals can simultaneously occupy the role of competitor and of predator and prey.

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