

Effects of nonlethal predation and competition on life-history characteristics and behavior of
larval Oregon spotted frog (*Rana pretiosa*) and larval red-legged frog (*Rana aurora*)

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Abstract

Oregon spotted frogs (*Rana pretiosa*) and red-legged frogs (*Rana aurora*) co-occur in the northern portions of their range and use similar larval rearing habitat in southwestern British Columbia and Washington State. I conducted a field mesocosm study to test hypotheses about the effects of inter- and intraspecific competition and nonlethal predation on metamorphic characteristics. Tadpoles were exposed to treatments in the presence and absence of one another, two density levels and to the presence or absence of a predacious odonate larva (*Aeshna palmata*) isolated in an enclosure. I examined the metamorphic responses of both species by evaluating weight at metamorphosis, time to metamorphosis and survival to metamorphosis. Additionally, a laboratory study evaluated behavioral mechanisms potentially responsible for any changes observed in metamorphic characteristics. Results from the four-week laboratory test indicated that both species reduced activity and moved away from the predator in the presence of an enclosed dragonfly larva, thus I expected to see effects on characteristics associated with growth as previous studies have shown. In the field mesocosm study, red-legged frogs exhibited lengthened larval periods and were 12% larger at metamorphosis when exposed to *Aeshna*. In the presence of Oregon spotted frogs, they decreased time to metamorphose by a week and a half, and were 12% larger than those reared alone at metamorphosis. Individuals from high density treatments were 28% smaller than those metamorphosing from low density treatments, suggesting that interspecific competition influences metamorphic characteristics of red-legged frogs. The proportion of tadpoles surviving to metamorphose was very high at over 0.9 in all treatments for red-legged frogs. Although red-legged frogs and Oregon spotted frogs rear

under similar conditions, their responses to experimental manipulations were different. Oregon spotted frogs in treatments with red-legged frogs were an average 14% larger at metamorphosis when a predator was present. However, in treatments where Oregon spotted frogs were alone with a predator, results indicate tadpoles weight at metamorphosis was 26% smaller than those in the absence of *Aeshna*. In addition, Oregon spotted frogs had the lowest survival rate observed in the experiment when alone with the predator. In low density treatments survival was approximately 0.8 while in treatments with Oregon spotted frogs alone in the presence of *Aeshna* was only 0.2. High density treatments caused tadpoles to metamorphose only 11% smaller than from low density tanks. The field mesocosm study demonstrated that the presence of a predacious invertebrate alters metamorphic characteristics of both species likely by changing their foraging behavior. Furthermore, results suggest that Oregon spotted frogs benefit through a facilitative interaction with red-legged frogs in the presence of a predator, however it is not known if similar behavioral adjustments are prevalent in natural populations. Ecological relationships like those discovered in these experiments should be considered when planning long-term conservation strategies for both species.

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Chapter 1. General Introduction: the conservation situation.

Concern over declines of amphibians worldwide has sparked much research in the last decade (Blaustein and Wake 1990, Wake 1991, Blaustein and Wake 1995, Houlihan et al. 2000, Kiesecker et al. 2001, Pounds 2001). Causes of population declines vary depending on human pressures on habitat, species-specific requirements and in many cases are unknown. The uncertain future of some amphibian populations depends on factors ranging from habitat destruction, urbanization, agriculture, pesticides, global climate change, increased ultraviolet-B exposure, competition and predation from introduced species and effects of forestry practices. The complex life cycle of amphibians, depending on both aquatic and terrestrial systems, complicates understanding factors involved in population declines. Species have evolved within a community and habitat to optimize factors associated with growth and survivorship, and the biotic interactions within these communities are important in determining success of individuals. Investigation of inter- and intraspecific relationships in larval rearing habitats may further help to identify possible causes for population declines in specific habitats. The larval period has important implications for population dynamics of amphibians. Low juvenile numbers recruiting to the breeding population has been shown to cause the population to eventually decline in at least one species (*Rana sylvatica*), because the breeding population closely mirrors juvenile recruitment (Berven 1990). The work contained in this thesis investigates the role of inter- and intraspecific interactions as a possible contributing factor to declining populations of the Oregon spotted frog (*Rana pretiosa*, Baird and Girard 1853) and the red-legged frog (*Rana aurora*, Baird and Girard 1852) in southwestern British Columbia.

Conservation situation for Oregon spotted frog

Concern over declining populations of red-legged frogs and Oregon spotted frogs has sparked conservation efforts and research in the Pacific Northwest (McAllister and Leonard 1991, Hayes 1994, Blaustein et al. 1996, McAllister and Leonard 1997, Watson et al. 1998, Adams 1999, Blaustein et al. 1999, Lawler et al. 1999, Watson et al. 2000). *Rana pretiosa* is rare throughout its range and estimates of the historical distribution suggest it has disappeared from 90% of its historic range (Hayes 1994, McAllister and Leonard 1997). In 1996, the spotted frog complex was separated into two subspecies, the Columbia spotted frog (*Rana luteiventris*) and the Oregon spotted frog (*Rana pretiosa*) based on allozyme analysis (Green et al. 1996). Although these sister species are similar in appearance and behavior, it has been determined that they are genetically distinct. Concern has arisen over the persistence of small isolated populations of *R. pretiosa* throughout its range. *Rana pretiosa* occurs at 22 sites in Oregon State, 4 sites in Washington State and 3 sites in southwestern British Columbia.

Rana pretiosa's situation in Canada has only recently come to the attention of scientists and conservationists when the species was rediscovered in the lower mainland in 1997. In November 2000, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) announced an emergency listing of the species as 'endangered'. The most comprehensive studies on the ecology of *R. pretiosa* in Canada date back to the 1970s when Lawrence Licht studied a sympatric population of Oregon spotted frogs and red-legged frogs in southwestern British Columbia (Licht 1971a, 1971b, 1974, 1975, 1986a, 1986b). Much of the life-history and behavioral information for these species in sympatry originates from his work. More recent studies in Washington and Oregon States have focused on habitat associations of adult Oregon spotted frog as well as critical breeding habitat rather than interspecific relationships. Very little information exists on the ecology of Oregon spotted frog larvae and relationships with other

anuran species within larval rearing sites. Efforts are underway in southwestern British Columbia to improve habitat conditions where *R. pretiosa* is currently found and more information on larval communities is needed.

Natural History

These species are similar in appearance, size, and timing of reproduction. Breeding begins early in the year, usually late February or early March soon after the ice melts (Licht 1974). However, important differences do exist between the species. Red-legged frogs lay eggs in water 10-20 cm deep while Oregon spotted frogs deposit eggs in <10 cm depth. Additionally, a unique attribute of Oregon spotted frogs breeding behavior is deposition of egg masses in a communal pile, where 10-75 individual egg masses have been observed (McAllister et al. 1997). In contrast, red-legged frogs lay egg masses singly attached to vegetation (Corkran and Thoms 1996). As waters recede during the larval period, tadpoles of sympatric *R. aurora* and *R. pretiosa* come into contact with each other (pers. obs.). The duration of the larval period for Oregon spotted frog is usually 13-16 weeks, after which tadpoles metamorphose into small froglets 30-33 mm snout-vent length (Licht 1971b). Red-legged frogs metamorphose approximately four weeks earlier than Oregon spotted frog and range between 25-27 mm snout-vent length at metamorphosis (Licht 1971b). The tadpoles of both species feed on algae and detritus, and may also gain nutritional benefit from bacteria growing on these materials (Licht 1974). Both species ingest food by grazing the surface of substrate and are not thought to filter suspended particles from the water column.

Species exploiting the same resources in the same habitat may develop a competitive relationship influencing their growth rates, size at metamorphosis, length of the larval period and potentially larval survival rates. *Rana pretiosa* is sympatric with *R. aurora* only at the northern

portion of their distribution, and does not occur in sympatry in Oregon State (Marc P. Hayes pers. comm.). *Rana aurora* and *R. pretiosa* have likely adapted to avoid competition by differentiation of adult habitat use (Licht 1971b), or other processes. *Rana aurora* moves off into grasslands or nearby forested areas upon reaching metamorphosis while *R. pretiosa* remains in the permanent water body throughout its life cycle. Dependence on the aquatic system renders the Oregon spotted frog vulnerable to hydrologic alterations of wetlands, pesticides and pollutants in water, and to exotic species such as introduced fish, bullfrogs (*Rana catesbeiana*) and green frogs (*Rana clamitans*) that also depend heavily on the aquatic system (Adams 1999).

Information concerning ecological relationships within the larval community is lacking for *R. pretiosa*. Studies in southwestern British Columbia suggest that due to heavy predation both *R. pretiosa* and *R. aurora* have a 1-5% survival rate of individuals from embryo to metamorphosis (Calef 1973, Licht 1974). However, Licht (1974) highlights a key difference observed during one year between the species in the larval phase. Calculations concluded that 10,880 *R. aurora* eggs yielded 9,901 hatchlings and that 7,716 embryos yielded 5,710 *R. pretiosa* hatchlings (Licht 1974). Although these numbers vary from year to year, the difference in hatching success is attributed to *R. pretiosa* laying eggs at the shallow margins of seasonally flooded areas in wetlands and is a common occurrence (Licht 1974). As discussed above, the eggs may be stranded without adequate rain to maintain water levels. Hatchlings were followed to metamorphosis and it was determined through weekly systematic searches that a larger percentage of mortality for *R. pretiosa* occurred at the egg stage, while a greater proportion of *R. aurora* mortality occurred during the tadpole phase (Licht 1974). Licht (1974) comments that conducting accurate larval survival surveys in the field is extremely difficult. He located metamorphs and used mark-recapture techniques to determine juvenile recruitment. In total 187 *R. aurora* and 171 *R. pretiosa* metamorphs were collected. These observations suggest that there

may be morphological or behavioral differences in response to predation between the two species enabling *R. pretiosa* to produce a greater proportion of metamorphs from fewer hatchlings. Additionally, there may be an important relationship between the two species in the presence of predators allowing *R. pretiosa* to show greater survivorship than *R. aurora* during the tadpole phase.

Effects of competition and predation on *R. pretiosa* and *R. aurora*

Ecological theory predicts that competition for resources between ecologically similar species can regulate population structure along with other environmental factors (Wilbur and Collins 1973, Werner 1986, Werner 1992). Research conducted on sympatric species is especially helpful in clarifying the role of interspecific interactions on the landscape and may provide partial solutions to questions surrounding amphibian population dynamics. For example, a study examining the survival of the Columbia spotted frog (*Rana luteiventris*), the closest relative of *R. pretiosa*, and the northern leopard frog (*Rana pipiens*) in five natural ponds concluded that when *R. luteiventris* reared in the same pond as *R. pipiens*, it gradually disappeared and juveniles failed to disperse to neighboring ponds (Dumas 1964). Both species are heavily dependent on the aquatic environment, and interspecific interactions may explain the mechanism behind declining numbers of *R. luteiventris* shown in the study. Mortality rates were disproportionately greater for *R. luteiventris*, and after three years the only remaining spotted frogs found in surveys consisted of three adult males. However, large numbers of *R. pipiens* tadpoles and adults were observed in the ponds. The study suggests that the spotted frog complex is either an inferior competitor, predation pressure is heavier on this species than other similar native species or both (Dumas 1964).

Interspecific interactions may also help to explain mechanisms responsible for the decline of populations due to the introduction of exotic species into native systems. The effects of competition and predation on *R. aurora* from introduced bullfrogs (*Rana catesbeiana*) illustrate negative influences including alteration of natural behavior, reduction in size at metamorphosis, and reduced survival of juveniles within the population (Kiesecker and Blaustein 1998). Inter- and intraspecific relationships between similar species can create a situation where one species performs less well in the presence of the other, observed through changes in life-history characteristics or survival rates (Wilbur 1987).

The role of predation in most ecological research concentrates on direct effects of animals dying when predators eat them. Decreasing numbers of individuals in a habitat reduces competition in many systems. However, predators also exert a nonlethal impact on animals by altering behavior that may influence important life-history characteristics and individual fitness. I define 'nonlethal effects' as changes in behavior or life-history characteristics when prey is in the presence of a predator that cannot physically harm them. Although the predator is not physically injuring the prey, its presence causes alterations in the behavior of the prey, which can affect life-history characteristics or individual fitness. Many studies of larval amphibian communities have documented the impact of nonlethal effects due to predators (Skelly and Werner 1990, Figiel and Semlitsch 1991, Peacor and Werner 1997, Anholt and Werner 1998, Van Buskirk and Yurewicz 1998, Laurila and Kujasalo 1999, Relyea 2000). These studies suggest that a complete understanding and more accurate scientific prediction of the role of predation in ecological communities must account for nonlethal influences as well as the direct impacts of predation.

This thesis tested experimentally inter- and intraspecific interactions observed within larval communities of *R. aurora* and *R. pretiosa* in the presence and absence of a nonlethal

odonate predator (Chapter 2). The effects of nonlethal predation and competition were experimentally evaluated simultaneously to determine responses in metamorphic characteristics of individuals. In addition, another experiment investigated behavioral predator avoidance mechanisms potentially responsible for these interactions (Chapter 3). Finally, the importance of these studies to the conservation of Oregon spotted frogs and red-legged frogs in southwestern British Columbia are summarized and discussed in Chapter 4.

Chapter 2. Experimental evaluation of nonlethal predation and competition on life-history characteristics of larval Oregon spotted frog and red-legged frog.

Introduction

A community of interacting species can be described by direct predator-prey and consumer-resource relationships in the form of a food web. However, representing such communities based simply on food web interactions can oversimplify the influence that individual species have on each other through more subtle mechanisms. For example, the effects of behavioral interactions among species, facilitative interactions and mutualisms are difficult to represent in food webs but may be equally important in structuring animal communities and the processes that regulate their populations. The direct effects of density, competition and predation are well documented in many systems (Calef 1973, Smith 1983, Morin 1986, Kiesecker and Blaustein 1997, Kupferberg 1997, Lawler et al. 1999, Morey and Reznick 2001). Recent attention has been given to indirect interactions of species within food webs (Hart 1992, Werner and McPeck 1994, Schoener and Spiller 1999). Identification of these indirect effects in natural systems requires detailed exploration of specific components. Predictive theory regarding how indirect effects influence community structure and life-history characteristics of species continues to develop through investigation of predator-specific and prey-specific responses to various factors observed under natural conditions thereby uncovering common patterns (Anholt et al. 2000, Relyea 2001a, Relyea 2001b, Relyea 2001c).

Indirect effects in a community are often mediated through specific behavioral traits of the species involved in the presence of a competitor, predator or both. When faced with a pressure (e.g. predation), individuals lacking morphological predator defenses commonly alter behavior in an effort to reduce the risk of being eaten. Different types of antipredator behavior have been studied for many species by behavioral ecologists and in some cases are known to

result in indirect effects influencing community dynamics (Werner 1991, Relyea 2000). For example, in a system containing two prey species and a predator, if individuals of one prey express a greater degree of predator vigilance or have other predator induced adaptations for avoiding predator encounters, those individuals may decrease predator detection and increase their likelihood of survival. In this example, growth rates or development may be compromised due to increased effort toward traits reducing predator detection as an additional indirect effect observed in the community. Higher levels of activity may make a predator more likely to attack that individual and less likely to attack alternative prey (Petranka et al. 1987, Skelly 1994). Many species respond behaviorally when they encounter visual or chemical stimuli associated with predators (Sih 1986, Godin and Smith 1994, Kiesecker and Blaustein 1999, Belden et al. 2000). Understanding these behaviors and the potential resulting indirect effects contributes to an enhanced understanding of the community.

Tadpole communities provide excellent models for examining competition and the effect of predation on inter- and intraspecific relationships. They are easy to collect in large quantities as eggs or young tadpoles, survive well under laboratory conditions and have evolved in complex communities. Additionally, experimental pond communities can be created with fairly complete community structure including complex sets of interactions between species or environmental factors. Anti-predator behavior is well documented in larval anurans, and some studies suggest that changes in behavior may have large impacts on community dynamics (Werner 1991, Horat and Semlitsch 1994, Laurila and Kujasalo 1999, Relyea 2000). Community ecology theory predicts that interactions between competitors, predators, wetland hydroperiod, as well as other environmental factors in a specific habitat combine to determine individual fitness. Tadpoles are presented with an essential trade-off during their larval period that is influenced by factors mentioned above. If they metamorphose early, individuals benefit by escaping predation or other

pressures in the aquatic habitat, but consequently are small at metamorphosis and potentially less fit than individuals metamorphosing larger. Even though they escape aquatic predators, their small size may make them less able to escape terrestrial or semi-aquatic predators (Werner 1986). Alternatively, tadpoles with extended larval periods often metamorphose at larger sizes, but are subjected to many pressures in the larval rearing habitat including potentially to a degrading environment. Models predicting the timing of metamorphosis for amphibians show the relative growth rate in a specific habitat is the main parameter influencing this trade-off (Wilbur and Collins 1973, Rowe and Ludwig 1991, Werner 1991). The relative growth rate may increase under factors such as increased or more nutritious food resources, low levels of competition, and low pressure from predators.

Role of competition, predation and density

Understanding the influence of predation in natural communities is complicated because predation reduces the density of individuals and potentially alters levels of inter- and intraspecific competition. Mortality due to predation frees up resources for surviving tadpoles. These processes (predation and density-dependent competition for food) work together to determine the structure of the community, abundance and fitness of individuals. In larval anuran communities, high density of individuals increases competition and results in reduced size at metamorphosis (Licht 1967, Brockelman 1969, Morin 1986, Wilbur 1987). However, the presence of a predator also influences the foraging behavior, activity, and ultimately metamorphic characteristics of larval anurans. While ecological theory has traditionally concentrated on the lethal effect of predation (mortality), the nonlethal component (alteration of behavior) must be evaluated to understand the community (Lima 1998). An experiment designed to compare the impact of nonlethal predation (caged odonate larvae) with the effects of gradually reduced densities

intended to simulate natural predation, documented that the reduction of density caused the greatest effect at the end of the larval period when tadpoles were intensely competing for scarce resources (Van Buskirk and Yurewicz 1998). However, tadpoles were most influenced in early development by the nonlethal presence of the predator. Even though predacious odonates did not physically confront tadpoles, antipredator behavior in the form of lowered activity levels was recorded. This study further underscores the need to understand behavioral responses to a predator in communities with more than one species where competitive relationships may be altered due to behaviors induced by a predator (Van Buskirk and Yurewicz 1998). Species-specific knowledge of predator-induced behaviors is critical to a full understanding of community dynamics.

It has been suggested that under natural conditions competition in larval anuran communities has a minimal role due to abundant resources and the mobility of tadpoles (Hayes and Jennings 1986). Furthermore, predation likely reduces the effects of competition observed in natural settings by inflicting high mortality during early larval development (Licht 1974). However, the role of competition is important in evaluating indirect effects (Werner 1991), and understanding the competitive ability of species within a species assemblage may help to explain habitat preferences or other important species-specific characteristics. Individuals of one species faced with competition from another species in their environment may express a change in competitive ability when faced with pressures from a predator and the competing species simultaneously. A study evaluating two sizes of bullfrogs (*Rana catesbeiana*) and small green frogs (*Rana clamitans*) found that the competitive relationship was changed when a dragonfly larva (*Anax*) was introduced to the prey community (Werner and Anholt 1996). Similarly, studies examining interactions between tadpoles and predatory salamanders (Morin 1986) as well as interactions in a community of tadpoles, fish and snails have demonstrated that the

introduction of a predator alters competitive ability between sympatric tadpole species (Lefcort et al. 1999). These studies indicate that competition and predation must be studied together to disentangle proximate causes of performance within specific communities of larval anurans.

Importance of weight at metamorphosis, larval period and survival to larval anurans

Metrics typically used to evaluate the influence of competition and predation in tadpole communities include size at metamorphosis, length of the larval period and proportion of the larval cohort surviving to metamorphosis. Although the latter factor has clear ecological importance for the individual, it has also been demonstrated that increased survival within a cohort relates directly to numbers in the breeding population in subsequent years (Berven 1990). If several years of poor juvenile recruitment are experienced, the breeding population declines as those cohorts begin to reach reproductive age. Weight at metamorphosis and timing of metamorphosis may also exert control over survivorship of individuals at subsequent life-history stages. In fact, weight at metamorphosis has been correlated with better juvenile survival and larger body size at age of first reproduction (Berven 1990, Smith 1987, Goater 1994, Morey and Reznick 2001). It should be expected that individual tadpole behavior and foraging activity has evolved to optimize both weight at metamorphosis and timing of metamorphosis. An individual tadpole must emerge at a size large enough to capture invertebrate prey, but soon enough to optimize fat storage for surviving the first winter of life.

The importance of cohort survival rate at the population level was demonstrated by a seven-year study on wood frogs (*Rana sylvatica*). Berven (1990) found that the size of the breeding population fluctuated with variation in juvenile survival. Juvenile recruitment varied by a factor of 100 and consequently the adult populations varied by a factor of 10. A study examining subsequent survival of juvenile wood frogs that metamorphosed from larval

populations of varying density determined that individuals emerging at larger sizes had a greater probability of surviving to reproductive age (Berven 1990). Timing of metamorphosis also had a significant effect on juvenile survival, as early emerging individuals were larger and survived to the next year at a higher rate than smaller individuals within the same cohort. Greater size at metamorphosis provided extended benefits through the rest of the life cycle through greater egg production as adults (Berven 1990). Similar results were documented in larval spadefoot toads where individuals metamorphosing at larger sizes had greater survival rates (*Spea hammondi*) (Morey and Reznick 2001). A three-year study of chorus frogs (*Pseudacris triseriata*) demonstrated that smaller sized and later metamorphosing individuals delayed sexual maturity by a year (Smith 1987). Adult body size in this population of chorus frogs declined significantly with increased length of the larval period and a higher proportion of frogs classified as large at metamorphosis were recaptured the following year suggesting that survival of larger frogs was greater. Additionally, benefits are shown at reproductive maturity because female body size and clutch size are correlated and a large body size likely will improve male mating success for treefrogs (Smith 1987). Results suggest that for several species of anurans size at metamorphosis and length of the larval period provide good predictors of future reproductive success in a population.

Use of mesocosms in examining community interactions

An excellent method for evaluating the effects of predation, competition, and density is the use of mesocosms to simulate larval rearing conditions. Use of experimental tanks to represent a rearing site compromises the realism of natural ponds or wetlands, but allows control of many extraneous factors of unknown effects and provides opportunity for replication thereby increasing statistical inference (Wilbur 1989, Resetarits and Fauth 1999). Creation of realistic

larval rearing habitat is possible through an understanding of the animal's habitat preferences and food requirements, and provides a useful tool for evaluating community dynamics.

In this experiment, I tested several hypotheses about how metamorphic characteristics of each species would be affected by the presence of a predator, competitor, and two different densities. I posited that *R. aurora* would be a superior competitor due to the shorter larval period and more rapid development (Licht 1974), but that it would metamorphose relatively later and at a smaller weight when competing with *R. pretiosa* at high densities. I also expected the presence of *R. aurora* to have a negative impact on *R. pretiosa* through increased interspecific competition during the early larval development phase. Due to increased competition from a faster developing species, I predicted the presence of *R. aurora* would delay metamorphosis of *R. pretiosa* and cause smaller weights at metamorphosis. I expected both species to respond to the presence of a typical predator by metamorphosing earlier and at smaller weights in accordance with models of amphibian metamorphosis (Wilbur and Collins 1973, Werner 1986). Evaluating the interspecific relationship between these species and a typical predator will provide a more complete understanding of the larval community that may aid in reintroducing *R. pretiosa* to restored habitat within its historic range.

Methods

Study site

I conducted an experiment examining ecological relationships between *R. aurora*, *R. pretiosa* and a typical predacious odonate (*Aeshna palmata*) in Vancouver, British Columbia, Canada during the spring and summer of 2000. The University of British Columbia (UBC)

maintains a research area at its South Campus with large open fields providing an excellent location for this outdoor artificial pond study. The entire field is fenced and access is restricted.

Eggs were collected from Beaver Creek, Thurston County, Washington, with the permission of the Washington State Department of Fish and Wildlife (WSDFW). Egg masses were not collected from sites in Canada due to the ongoing evaluation of the species' status, a desire to leave the natural populations undisturbed, and estimated low numbers of adults in the breeding population. Oregon spotted frog breeding phenology has been carefully monitored for several years in Washington State and researchers have specific information about oviposition sites and wetland hydroperiod (McAllister and Leonard 1997, Watson et al. 1998, McAllister et al. 1999, Watson et al. 2000). The site in Washington State was carefully monitored and egg masses were collected only after it was certain that they would desiccate due to receding waters. Consequently, the removal of egg masses should not have affected recruitment of juveniles to the Beaver Creek population. Nine egg masses of *R. pretiosa* and twelve egg masses of *R. aurora* were transported to UBC where they were hatched in separate containers at 14°C in the laboratory.

Experimental tanks

Larval rearing ponds were simulated using 1136-L fiberglass Rubbermaid brand livestock watering tanks equipped to represent larval habitat of *R. pretiosa* and *R. aurora*. Tanks measured 2.5 m in diameter, and a permeable 1 mm mesh mosquito net divided each tank in half in order to increase replication of treatments. A double layer of netting was sealed to the sides of the cattle tanks with silicone sealant to provide extra protection against nets failing and animals moving between sides. A total of 18 tanks (36 sides) were arranged in a grid of three rows containing 6 tanks each evenly spaced with 1 m distance between each tank. Tanks were oriented to ensure

similar solar exposure to replicate sides. To simulate a shallow area in the ponds, a piece of clear fiberglass roofing rested on a concrete brick 10 cm below the water surface over a portion of each side. All tanks were fitted with an L-shaped standpipe that allowed the water to rise no higher than 57 cm in the tanks during rain events. Tanks were covered with fiberglass window screening attached to a wooden frame preventing amphibians from entering or exiting the ponds and excluding oviposition of predatory insects. An additional layer of coarse mesh plastic construction fencing was attached to the covers to ensure that larger mammalian or avian predators could not break through the cover into the ponds.

On February 26, the tanks were filled with well water and 150g of dried leaf material was added to each half of the tank. Leaf material consisting mainly of red-alder (*Alnus rubra*) and big leaf maple (*Acer macrophyllum*) was collected as it fell during the fall of 1999, dried and stored over the winter. Additionally, 25g of crushed rabbit chow was spread throughout each tank providing a nutrient base for phytoplankton and young tadpoles until algae became established. On March 2, each tank was inoculated with 10-L of pond water filtered through a 64µm filter to exclude large zooplankton and invertebrates. Pond water was collected from man-made ponds at UBC's South Campus. Over the subsequent two weeks, water was exchanged between tanks in order to ensure similar algae community development across the experiment.

When tadpoles began foraging and reached approximately Gosner stage 26 (Gosner 1960), they were added to the cattle tanks on April 14. Treatments and the number of individuals added to each tank are shown in Table 2.1. Location of treatments within the matrix was designated at random, but the same treatment was placed on either side of the netting within a tank. Tadpole densities were established at approximately 0.10 and 0.20 individuals per liter for low and high density treatments respectively. These densities are consistent and within the ranges observed in field observations of larger tadpoles (McAllister (WDFWS) pers. comm.,

Calef 1973). The number of tanks did not allow for a complete factorial design as shown in Table 2.1, and the layout of the experiment is shown in Figure 2.1. Tadpoles were selected at random from hatching containers to ensure mixing of sibling groups amongst treatments. Total wet mass of a group of individuals placed in each tank was recorded for each species. The tadpoles were released in the tank and allowed to acclimate for seven days before initiating predator treatments.

Nonlethal predators

Odonate larvae such as the paddle-tailed darner (*Aeshna palmata*) are common in wetlands inhabited by sympatric *R. aurora* and *R. pretiosa* (McAllister and Leonard 1997). Darners are voracious predators of tadpoles and many studies indicate that predation by these invertebrates can be substantial during early development of tadpoles (Calef 1973, Skelly 1994, Anholt and Werner 1998, Van Buskirk and Yurewicz 1998). The family Aeshnidae contains the largest mature larvae of all dragonfly species in British Columbia ranging from 33 mm to 47 mm total length. The paddle-tailed darner spends almost one year in the larval phase before metamorphosing to the adult form (Cannings and Stuart 1977). The species is a “sit-and-wait” predator sensing movement of prey items and attacking once the prey nears hiding perches often located in reeds or grasses. Known prey include other dragonfly larvae, tadpoles, invertebrates, and small fish.

Tadpoles were exposed only to the risk of predation from enclosed *Aeshna*, and not direct predation. Previous studies indicate that tadpoles respond to chemical cues released into the water as dragonflies eat tadpoles (Petranka et al. 1987, Werner 1991, Anholt and Werner 1998). In order to expose tadpoles to these cues but protect them from direct predation, predator enclosures were constructed to contain dragonfly larvae. Each side of all tanks contained two

predator enclosures for a total of four per tank. These enclosures were constructed of 10 cm PVC pipe cut into 10 cm lengths and capped with fiberglass window screening secured by rubber bands. Predator enclosures were thoroughly scrubbed and soaked for two weeks before addition to the tanks. A small square of Styrofoam was added to the inside of each enclosure so that it floated at the water surface. A nylon cord attached to a weight held each enclosure in the center of the tank to provide central distribution of the chemical cues throughout the tank. Enclosures were established in all treatments but populated only in predation treatments.

Predator treatments began on April 22, when one odonate larvae was added to each enclosure. Dragonfly larvae were collected from ponds at UBC's South Campus and at the nearby UBC Botanical Garden in final instars of development and held in the laboratory before initiation of the experiment. At the beginning of the experiment, it was difficult to find large larvae, so medium and large sized larvae were spread evenly among the predator treatments. One dragonfly larva was added to each enclosure in predator treatments and fed 1-2 tadpoles two to three times weekly until September 1, when no more large *Aeshna* larvae were available. Empty enclosures in non-predator treatments were removed and replaced during each feeding to ensure equal disturbance among treatments during predator feedings. At the beginning of September, few dragonfly larvae were collected in the ponds at UBC, and predator treatments were ended even though tadpoles remained in the tanks. The cessation of predator treatments at this time is consistent with natural patterns when the majority of *Aeshna* emerge during June and July. For purposes of my experiment, I assumed metamorphic responses to these predators would have been established during the preceding rearing period. The species of tadpole fed to dragonfly larvae were consistent with treatments. In tanks where both species were present, one *R. aurora* tadpole and one *R. pretiosa* tadpole were given to each dragonfly larva. In treatments where species were alone, dragonfly larva were fed 1-2 tadpoles of the same species in that treatment.

Rubberbands on the predator enclosures were changed weekly to ensure they did not fail and allow the dragonfly larva to escape during the experiment. *Aeshna* larvae with swollen wing pads, indicating imminent metamorphosis, were removed from the experiment and replaced. Cages in predator free treatments were removed and replaced at the same time dragonflies were fed to equalize disturbance among treatments.

Collection of metamorphic individuals and data

As tadpoles approached metamorphosis, tanks were checked daily for metamorphs. The first metamorph collected was *R. aurora* emerging on June 20. Metamorphosis was defined as the emergence of at least one forelimb (Gosner stage 42). At this time, metamorphosed individuals were placed in separate plastic cups with 1-2 cm of water, labeled with the date and tank number, and covered with screening to prevent froglets from climbing out of the cups. They were moved to the laboratory to complete tail absorption. While in the lab each frog was weighed at Gosner stage 42, 43, 44, 45 and 46 (metamorphic climax) when the tail was completely absorbed. The period from forelimb emergence to metamorphic climax took approximately one week for all individuals and during this time metamorphs do not eat, so holding them in the lab should not have influenced weight at metamorphosis. *Rana aurora* juveniles were returned to Washington State at the site of egg collection and *R. pretiosa* juveniles were used as part of a trial introduction at UBC's Malcolm Knapp Research Forest.

The artificial pond experiment was terminated on October 15. Few metamorphs had been observed emerging from ponds for several weeks and tadpole activity levels were minimal. Average daily and nighttime temperatures had dropped and it appeared unlikely that remaining tadpoles would metamorphose. The tanks were drained and leaves carefully searched for all

remaining individuals. The remaining tadpoles were identified to species, weighed and their Gosner stage was determined in the laboratory.

Statistical Analysis

In total 1,624 individuals were collected and used to calculate mean values for analysis of treatments in the experiment. Inspection of scatterplots indicated that the distribution of weight at metamorphosis and date at metamorphosis were skewed to the right in a log-normal pattern. In order to approximate a normal distribution necessary to meet the assumptions of analysis of variance (ANOVA), the weight at metamorphosis and larval period data were log transformed prior to analysis. Survival data were recorded as the percentage of metamorphs surviving from the initial number stocked in each tank, i.e., tadpoles collected at the termination of the experiment did not contribute to survival numbers. The survival data were arcsine square root transformed prior to analysis in order to meet normality assumptions for ANOVA. Residuals from preliminary trials were tested for normality using the UNIVARIATE procedure in SAS version 8 (SAS Institute Inc.), and met the assumption of normality required for ANOVA procedures.

The experiment was analyzed in SAS using multiple analysis of variance (MANOVA). However, because the experimental tanks were split in half, the two sides shared water and may have been dependent on one another to some unknown degree. To compare each treatment effect against the appropriate error term (taking into consideration this dependence), nesting of the separate sides of tanks within treatments was necessary to account for sampling error (Underwood 1997). Side nested within treatment effects provided the sampling error and the degrees of freedom were removed from the experimental error against which main effects and interactions possible given the experimental design were tested with MANOVA (Table 2.1).

Analyses were run by species in order to determine the effects of predator (presence/absence), density (high/low) and competitor (presence/absence) on metamorphic characteristics. An interaction of competitor by density was not possible due to the lack of the treatment of high density when the species were alone. Additionally, because this treatment was omitted, a three-way interaction was not possible in analysis. By nesting the sides within main effects, the sampling error was removed from the experimental error used for testing main effects and interactions making the degrees of freedom lower for significance testing. Therefore, the results from this analysis are likely conservative.

Results

Rana aurora and *R. pretiosa* responded differently to experimental treatments despite showing similar breeding times, oviposition sites, and preferences for larval rearing pools. The natural time of metamorphosis for *R. aurora* is near the first of July in southwestern British Columbia and Washington State (Licht 1974). In this experiment *R. aurora* began to metamorphose in late June, similar to natural populations. The first Oregon spotted frog metamorph was collected on July 3, but most individuals did not metamorphose until early August consistent with observations of natural populations (Licht 1974). *Rana pretiosa* began metamorphosing in most treatments at the time when the last *R. aurora* were metamorphosing. The effects of experimental treatments on metamorphic characteristics are presented below.

Temperature

Monitors set to log temperature hourly (HOBO onset) were placed randomly in four tanks at the beginning of the experiment. Examination of temperature data from these tanks indicated that temperature was not significantly different among them and was within the error specification of the equipment ($\pm 0.2^{\circ}\text{C}$). All four tanks showed similar variation in temperature (Table 2.2).

Red-legged frogs

Weight at metamorphosis

The main effects of predation, competition and each density level significantly influenced weight at metamorphosis for *R. aurora* larvae. Mean average weight at metamorphosis per treatment ranged from 0.33 g to 0.51 g in the experiment. Variation in the mean average weight at metamorphosis was small in all treatments (Figure 2.2). Contrary to my initial predictions, *R.*

aurora grew significantly larger in all treatments when in the presence of *Aeshna* ($p = 0.004$, Figure 2.2, Table 2.3a). The average weight of *R. aurora* metamorphs in treatments alone and in the presence of *Aeshna* was 12.2% larger than in the absence of *Aeshna* (Figure 2.2). Similar differences in response to predator exposure were shown in treatments with both species at low and high density for *R. aurora*. Tadpoles at low density metamorphosed 4% larger in the presence of *Aeshna* and those at high density were 15.4% larger at metamorphosis when exposed to *Aeshna*. The presence of a competitor (*R. pretiosa*) also had a significant effect on weight at metamorphosis for *R. aurora* ($p = 0.019$, Figure 2.2, Table 2.3a). Tadpoles at low density with *R. pretiosa* metamorphosed 12.2% larger than those alone (also at low density) in the absence of *Aeshna*, and 4% larger in the presence of *Aeshna*. The MANOVA showed the effect of density on weight at metamorphosis was highly significant ($p < 0.0001$, Figure 2.2, Table 2.3a). The smallest metamorphs were observed in the high density treatments where the predator was absent. These froglets weighed 32.7% smaller than low density treatments in the absence of the predator and 23.5% smaller in the presence of the nonlethal predator. Interactions between predator and density and between predator and competitor were not significant (Table 2.3a).

Length of the larval period

The larval period for *R. aurora* was an average 6.5% longer in treatments containing *Aeshna* ($p = 0.078$, Figure 2.3, Table 2.3b). Individuals in low density treatments (with *R. pretiosa*) metamorphosed earliest followed by low density treatments with *R. aurora* alone 10 days later and finally by high density treatments approximately 10 days afterward. Timing of metamorphosis was also significantly affected by the presence of *R. pretiosa* (competitor) ($p = 0.006$, Table 2.3b). The combined density of tadpoles in low density competitor treatments and treatments with the species alone was identical, yet *R. aurora* reached metamorphosis 11.9% more quickly in the absence of *Aeshna* and 10.1% more quickly in the presence of *Aeshna* when

R. pretiosa was present. The initial density of tadpoles stocked in the tanks produced a highly significant effect on the length of the larval period ($p < 0.0001$, Figure 2.3, Table 2.3b). In treatments without *Aeshna* the length of the larval period was extended by 20.4%, and in the presence of *Aeshna* the larval period was lengthened by 19.2%. There was no significant interaction between predator and density or between predator and competitor (Table 2.3b).

The relationship between weight at metamorphosis and length of the larval period was investigated for all replicates. Results for *R. aurora* are presented in Appendix 1. In general, larger tadpoles metamorphosed later in the larval period, and this trend was similar between all treatments and replicates.

Proportion surviving to metamorphosis

Survival of *R. aurora* was high in all treatments with the lowest survival rate at 0.90 in high density treatments, in the presence of a competitor and a predator. The MANOVA found no significant effects on survival to metamorphosis for *R. aurora*, although in all treatments survival was an average of 5.1% lower when a predator was present (Figure 2.4, Table 2.3c).

Oregon spotted frogs

Weight at metamorphosis

The results for *R. pretiosa* were much more complex and replicates within treatments showed more variability. The MANOVA showed no significant main effects or interactions for *R. pretiosa* on weight at metamorphosis (Table 2.4a). In treatments where *R. aurora* was present, *R. pretiosa* metamorphosed an average 13.8% larger in the presence of *Aeshna* (Figure 2.5). However, in treatments where *R. pretiosa* was alone, the pattern was reversed (Figure 2.5). In fact, the largest tadpoles in the absence of *R. aurora* emerged from treatments where *R. pretiosa* developed without *Aeshna*. Tadpoles in non-predator treatments where *R. pretiosa* was

alone metamorphosed 25.6% larger, showing the greatest difference of any comparison made between predator treatments in the experiment. The effect of density was not significant in explaining differences in weight at metamorphosis (Figure 2.5, Table 2.4a). *Rana pretiosa* emerged only 11.1% smaller in the high density treatments without *Aeshna*, and 11% smaller in high density treatments in the presence of the predator than low density treatments (Figure 2.5). There was a large amount of variation in weight at metamorphosis in high density treatments.

Length of the larval period

Rana pretiosa showed variable responses in length of the larval period to experimental manipulations. A significant effect of density was discovered in which high density treatments took approximately two weeks longer to metamorphose than low density treatments ($p = 0.018$, Figure 2.6, Table 2.4b). The largest difference in length of the larval period was shown in treatments where *R. pretiosa* was alone in the presence or absence of *Aeshna* (Figure 2.6). In this treatment, tadpoles in predator treatments took over two weeks (12.9%) longer to emerge than conspecifics in non-predator treatments. At low density (with *R. aurora*), tadpoles emerged at nearly identical times regardless of the presence or absence of a predator. And at high density (with *R. aurora*), tadpoles in treatments without predators took 4 days longer to emerge than tadpoles in predator treatments (Figure 2.6). The effect of predator, competitor and the interaction terms were not significant (Table 2.4b).

The relationship between weight at metamorphosis and length of the larval period was also investigated for *R. pretiosa* (Appendix 2). In many cases, the trade-off between emerging early and at a small size versus later at a large size was not apparent in replicates, presumably because these tanks had very few individuals that reached metamorphosis. However, replicates producing a relatively large number of individuals to metamorphosis showed earlier emerging individuals to be smaller in weight at metamorphosis than those metamorphosing later.

Proportion surviving to metamorphosis

The proportion of individuals surviving to metamorphosis was much lower in the experiment for *R. pretiosa* than for *R. aurora*. *Rana pretiosa* survived best at a proportion of 0.82 observed at low density with *R. aurora* (Figure 2.7). The effect of a competitor had a nearly significant effect on survival to metamorphosis for *R. pretiosa* ($p = 0.072$, Table 2.4c). In low density treatments tadpole survival rate was 24.6% higher in the absence of *Aeshna* and 73.5% higher in the presence of *Aeshna* when *R. aurora* was present. It should be noted, that at the time *R. pretiosa* was metamorphosing there were very few *R. aurora* remaining in the low density tanks and this may have influenced the competitive interaction. Figure 2.8 shows the cumulative emergence timing for both species, and indicates that the mean emergence time for *R. aurora* was earlier than *R. pretiosa* by 44 days. The presence of a predator did not have a significant effect on survival to metamorphosis (Table 2.4c). When *R. pretiosa* was alone, exposure to the predator decreased the survival rate by 58.7%. But when the species were together at high or low density the predator increased survival by an average of 8% (Figure 2.7). The survival at high density was significantly lower than in low density treatments ($p = 0.013$, Figure 2.7, Table 2.4c), with only approximately 0.30 of the tadpoles reaching metamorphic climax. One replicate in both high density treatments (*Aeshna* present and absent) had no individuals reaching metamorphosis. When compared to low density treatments, survival rates were reduced by 53.6% in the absence of *Aeshna* and by 60.5% when the predator was present at high density (Figure 2.8).

Remaining Oregon spotted frog tadpoles

The experiment was terminated in mid-October when no more metamorphs emerged from the tanks for one-week prior. However, when ponds were drained it was determined that 208 *R.*

pretiosa tadpoles remained alive in the tanks at various stages of development. Although these individuals are not included in analysis of responses of metamorphic characteristics to experimental treatments, it was important to investigate patterns that might emerge from individuals remaining as tadpoles. When tanks were drained no *R. aurora* remained, i.e. all individuals of this species had either metamorphosed or died.

Figure 2.9 shows the proportion of the original population stocked in the tank remaining as tadpoles when the tanks were drained. The highest proportion of animals remaining appeared in low density treatments without a predator when *R. aurora* was present, with 35% of all animals initially placed in this treatment remaining alive in the tanks upon termination of the experiment. This treatment also contained the largest number of individuals completing metamorphosis (see previous section). A proportion of approximately 0.25 of all animals placed in high density treatments remained in predator free tanks and 0.11 remained in treatments containing *Aeshna*. However, in tanks where *R. pretiosa* was alone, the proportion remaining alive in the tanks was higher in the treatment with predators by approximately 6% than the treatment without *Aeshna*. The ANOVA showed no significant effects or interactions for the tadpoles remaining at the end of the experiment (Table 2.5).

Investigation of total number of *R. pretiosa* alive at the termination of the experiment is presented in Figure 2.10. *Rana pretiosa* survived in lower proportions in the presence of *Aeshna* in all treatments (Figure 2.10). Results indicate that survival for the species was highest in low density treatments with *R. aurora* at approximately 0.85 survival rate. When *R. pretiosa* was alone, larval survival was reduced by 14% when exposed to a predator. An ANOVA showed that the presence of a competitor significantly influenced overall survival of *R. pretiosa* ($p = 0.030$, Table 2.6). And in high density treatments with *R. aurora*, the presence of *Aeshna* reduced total survival by 16%. The effect of density significantly reduced total survival of *R. pretiosa* in the

experiment ($p = 0.014$, Figure 2.10, Table 2.6). Interaction terms did not contribute to explaining combined tadpoles and metamorph survival results (Table 2.6). Although examining the results of total metamorphosed individuals and tadpoles remaining was important, including unmetamorphosed individuals did not change the patterns observed in results for survival rates of *R. pretiosa* and underscored the importance of *R. aurora* in influencing metamorphic characteristics of *R. pretiosa*.

Discussion

This experiment demonstrates alterations in metamorphic characteristics that are important to larval anuran populations due to the presence of a predator, a competitor, or both. My experimental design allowed examination of the ecological relationship between *R. aurora* and *R. pretiosa* to determine if a competitive relationship exists and how this relationship might change in the nonlethal presence of a typical larval predator such as *Aeshna palmata*. Weight at metamorphosis has been correlated with higher juvenile survival and larger body size at reproductive maturity in wood frogs (*Rana sylvatica*) (Berven 1990). Timing of metamorphosis is also crucial for individual fitness in larval amphibian populations (Smith 1987, Berven 1990). Tadpoles must balance the trade-off between foraging requirements and risk of predation in order to optimize size at metamorphosis (Wilbur and Collins 1973). If the larval period extends too long, tadpoles face emerging at an inopportune time and may lack time during mild months of the summer to increase fat storage for the coming winter, and additionally increase the length of exposure to aquatic predators such as dragonfly larvae.

Weight at metamorphosis and time to metamorphosis

The metamorphic characteristics of weight at metamorphosis and time to metamorphosis are highly correlated in many anuran species (Pfennig et al. 1991), and are interpreted together in this discussion. Surprisingly, replicates showed little variation in weight at metamorphosis and length of the larval period. Both species exhibited a similar pattern of emerging later and at larger sizes in the presence of nonlethal *Aeshna*. However, most studies have found that larval amphibians metamorphose at a smaller size and earlier when exposed to a predator (Wilbur and Fauth 1990, Werner and McPeck 1994, Anholt and Werner 1995, Kiesecker and Blaustein 1998,

Lawler et al. 1999). In addition to individuals metamorphosing larger, mean length of the larval period in this experiment was extended under the risk of predation.

One explanation for the difference between my results and the results of other researchers is that due to reduced activity levels from perceived risk of predation, the rate of development was delayed. A laboratory experiment discussed in detail in Chapter 3 of this thesis indicates that both *R. aurora* and *R. pretiosa* reduced activity and moved away from *Aeshna*. Other studies have determined that chemical stimuli from predators cause a reduction in activity of prey species and movement away from a predator (Lawler 1989, Semlitsch and Reyer 1992, Horat and Semlitsch 1994, Lardner 1998, Relyea 2000). The presence of *Aeshna* was shown experimentally to alter foraging rates and may have slowed development of individuals causing them to extend their larval period. Therefore, it is possible that tadpoles in the predator treatments metamorphosed later due to delayed development even though growth may not have been compromised. Laurila and Kujasalo (1999) found that development of *Rana temporaria* tadpoles was slowed in the presence of *Anax* and that consequently individuals in predator treatments emerged larger and later at metamorphosis. While this poses a satisfying explanation, it is inconsistent with the idea of trade-offs between the risk of predation and weight at metamorphosis in the same manner that empirically developed models for amphibian metamorphosis suggest (Wilbur and Collins 1973, Werner 1986).

Alternatively, it is possible that feeding dragonfly larvae had a fertilizing effect increasing algal productivity in predator tanks and leading individuals to metamorphose at larger sizes. However, the magnitude of effects varied between competitor treatments and the response was not observed in all treatments (e.g. *R. pretiosa* alone). Furthermore, no differences in algal growth were noted during the experiment in tanks containing predator (pers. obs.). Finally, lower survival rates of *R. pretiosa* and *R. aurora* in the presence of a predator may reduce

competition through fewer individuals surviving and competing for resources. Individuals in these treatments may benefit by reduction in competition and therefore metamorphose larger. Reduced competition for resources has been shown to increase weight at metamorphosis for many species (Brockelman 1969, Wilbur and Fauth 1990, Griffiths 1991).

Several laboratory studies have reported results for weight at metamorphosis and time to metamorphosis similar to those recorded in this experiment. As noted above, the common frog (*Rana temporaria*) was exposed to the presence of a predator (*Anax*) in the laboratory, they took longer to develop and were slightly larger at metamorphosis. The results indicated that an odonate predator delayed tadpole development, but did not affect growth rate, causing the tadpoles to reach metamorphosis at a slightly larger size (Laurila and Kujasalo 1999). A similar result was found with the same species when presence of a predator was crossed with food availability. *Rana temporaria* metamorphosed at significantly larger sizes and also had a longer larval period in the nonlethal presence of a predatory fish (Niecieza 2000). Additionally, a study of *Rana arvalis* in the presence or absence of nonlethal *Dytiscus* (Coleoptera) demonstrated no difference in predicted size at metamorphosis between predator treatments (Lardner 1998). These studies suggest that for some species, the effects of the nonlethal presence of a predator on size at metamorphosis and length of the larval period is difficult to predict. Both *R. temporaria* and *R. pretiosa* are considered aquatic anurans, while *R. aurora* is a semi-aquatic anuran. Results from my experiment and previous research suggest that at least these two species classified as 'aquatic species' are less likely to follow traditional models for amphibian metamorphosis (e.g. Werner 1986) and that species-specific characteristics are important to the outcome.

My data shows a positive relationship between weight at metamorphosis and length of the larval period for *R. aurora* and for *R. pretiosa*, when enough individuals metamorphosed to

provide this relationship. Previous studies indicate that a positive relationship between these variables is found when food is not limiting in a habitat and that a negative relationship is documented when food is limiting or in a deteriorating environment (Pfennig et al. 1991, Tejedo and Reques 1992, Reques and Tejedo 1995). It is unlikely that *R. aurora* tadpoles within a single treatment were exposed to deteriorating algal resources in the tanks as shown by the strong positive relationship between weight at metamorphosis and length of the larval period. Tadpoles remaining in tanks for longer periods of time were released from interspecific competition as earlier developing individuals metamorphosed. High density treatments exhibited smaller weights at metamorphosis indicating an increase in competition for available resources. These treatments showed a positive relationship as well suggesting that the environment was not deteriorating and tadpoles were benefiting from extended larval periods by consuming more resources (Wilbur and Collins 1973). However, the relationship for some replicates of *R. pretiosa* could not be predicted significantly or in some replicates were negatively related. For this species, it is possible that food limitation or lack of appropriate algal resources in the experimental setting played a role in influencing metamorphic characteristics. Alford and Harris (1988) suggest that slower developing individuals metamorphosing at smaller sizes than expected is a response to a crowded environment and deteriorating habitat. *Rana pretiosa* tadpoles may have experienced a deteriorating environment where algal resources were not as abundant for individuals metamorphosing later and thus showed a negative relationship between weight at metamorphosis and length of the larval period.

An interesting result discovered in the experiment shows *R. pretiosa* had opposite patterns regarding weight at metamorphosis when alone versus with *R. aurora*. The increased weight at metamorphosis and better survival suggests a facilitative effect of *R. pretiosa* by *R. aurora* in the presence of a nonlethal predator. The nonlethal effect of predation may be

ameliorated when *R. aurora* is reared in the same habitat. Most experimental evidence for facilitative interactions in animal communities suggests that the presence of one species benefits another by improving habitat characteristics (Chandler and Fleeger 1987, Thompson et al. 1991). Possible mechanisms for a facilitative interaction between the species are discussed in detail below.

Larval survivorship

Under the same experimental conditions, the survival to metamorphosis of *R. aurora* and *R. pretiosa* showed different results. In all treatments, *R. aurora* survived in extremely high proportions to metamorphosis. *Rana pretiosa* experienced lower survivorship to metamorphosis in the experiment suggesting that they are a more vulnerable species or require more specialized larval rearing conditions. Many studies demonstrate that increased competition reduces larval survival and weight at metamorphosis (Licht 1967, Brockelman 1969, Wilbur 1982, Morin 1986, Wilbur 1987, Alford and Harris 1988, Griffiths 1991). Inter- and intraspecific competition for resources is high in species that remain in the aquatic environment for extended periods of time (Licht 1967). In treatments where *R. pretiosa* was alone, only 0.20 of the original number survived to metamorphosis when *Aeshna* was present, while 0.82 survived to metamorphosis with *R. aurora* present in the absence of *Aeshna*. These results show that larval survival rates in the presence of *Aeshna* were increased by the presence of *R. aurora*. This response again suggests a facilitative effect of *R. pretiosa* by *R. aurora* that served to increase larval survival. Additional support for the beneficial effect of *R. aurora* on *R. pretiosa* comes from high density treatments where approximately 0.30 survived to metamorphosis. The mechanism for this response is unknown and was not fully explained by the laboratory behavioral study (Chapter 3). Potential mechanisms for a facilitative interaction are discussed below. The high larval survival

for *R. aurora* under the same conditions when compared to *R. pretiosa* also highlights an important difference between these species to experimental conditions, and suggests high vulnerability of *R. pretiosa* during the larval phase to environmental factors such as available algal resources.

Mechanisms for Facilitation

Results regarding weight at metamorphosis and larval survival suggest that a facilitative interaction may exist between *R. aurora* and *R. pretiosa*, allowing *R. pretiosa* to metamorphose at larger weights and increased survival rates to metamorphosis in the presence of *Aeshna*. The mechanism responsible for this facilitative interaction is unknown, however I will suggest some possible hypotheses that could cause such a result based in part on a laboratory behavioral experiment discussed in Chapter 3, observations in the tanks, and support from the literature.

First, a facilitative interaction is possible due to *R. aurora* reducing activity proportionally more than *R. pretiosa* in the presence of *Aeshna* (see Chapter 3). The decreased foraging activity of *R. aurora* in these treatments may benefit *R. pretiosa* by reducing interspecific competition and allowing individuals to consume more resources. Tadpoles could exhibit larger weights at metamorphosis and increased larval survivorship under these conditions. Several studies indicate that a reduction in interspecific competition through indirect interactions increases size at metamorphosis (Werner 1991, Relyea 2000).

Secondly, *R. pretiosa* may benefit by the presence of *R. aurora* through reduction of interference competition. The greater proportional reduction of *R. aurora* activity in the presence of *Aeshna* suggests that physical contact between tadpoles will decrease. *Rana pretiosa* may be startled less frequently by *R. aurora* from foraging activity in the presence of *Aeshna* and therefore exhibit greater success in metamorphic characteristics.

Thirdly, a facilitative interaction may be mediated through aggregations of tadpoles under pressure from a predator. When presented with chemical cues from a predatory garter snake (*Thamnophis sirtalis*), the Pacific treefrog (*Hyla regilla*) and the western toad (*Bufo boreas*) increased aggregation behavior (De Vito et al. 1998, De Vito et al. 1999). Aggregations of between 1,000 and 1,500 *Rana luteiventris* tadpoles have been observed under natural conditions (Carpenter 1953), and *R. pretiosa* tadpoles aggregate in communal groups within the experimental tanks (pers. obs.) possibly in response to the threat of predation from *Aeshna*. More active *R. aurora* larvae may encourage increased activity of aggregates of *R. pretiosa*. The laboratory study in which activity of the species together was evaluated suggests that *R. pretiosa* may be slightly more active when both *R. aurora* and the predator are present (see Chapter 3). If *R. pretiosa* does increase activity in the presence of *R. aurora* increased weight at metamorphosis would result. This hypothesis is supported by evidence from a study of American toads (*Bufo americanus*) that determined a strong correlation between activity and growth rate in the presence of an invertebrate predator (Skelly and Werner 1990).

Finally, studies in lake communities have shown that certain species digest food quickly, which increases nutrient turnover in the environment (Vanni and Findlay 1990, Vanni and Layne 1997). When digesting material, *R. aurora* may increase algal productivity in the mesocosm. *Rana pretiosa*, which remains in the tanks for approximately one month longer, would benefit from this increase in algal productivity and show increased success in metamorphic characteristics. Wilbur (1987) found a positive effect of *Rana utricularia* on *Scaphiopus holbrooki* and suggested that the facilitative interaction was due to *R. utricularia* scraping periphyton off the tank walls and increasing nutrient productivity in the tanks. Additionally, the nutritional value of tadpoles feces, although lower in quality than algal resources, has been shown to increase size at metamorphosis of *Rana catesbeiana* tadpoles (Steinwascher 1978). The faster

developing *R. aurora* tadpoles produce fecal material in the tank providing an available and energy efficient food source for the later metamorphosing *R. pretiosa* to utilize. Tadpoles took refuge from the odonate predator in the leaf litter (pers. obs.) and may find this material an abundant and 'safe' food resource under conditions where they alter spatial proximity to avoid the predator (see Chapter 3).

Conclusions

The interspecific relationship between *R. aurora* and *R. pretiosa* is complex and cannot be easily explained even by an experiment where many stochastic environmental variables are held constant and the community structure is simplified. The species although existing in the same larval rearing habitat maintain different requirements upon reaching metamorphosis. Metamorphosis delineates the time when *R. aurora* juveniles become terrestrial and escape predation in the aquatic realm. However, *R. pretiosa* juveniles remain in the water body after metamorphosis and may be exposed to some of the same predators throughout their life cycle (Licht 1974). Therefore, the trade-off discussed in amphibian models between metamorphosing smaller but more quickly or remaining in the aquatic habitat for longer periods of time and metamorphosing larger may not be as important to this species. Individuals used in this experiment originated from a sympatric population of *R. pretiosa* and *R. aurora* and have evolved in the presence of one another. The longer developmental period of *R. pretiosa* allows *R. aurora* to become the superior competitor in the larval community. *Rana aurora* is able to grow larger and metamorphose earlier when released from intraspecific competition as illustrated in low density treatments with *R. pretiosa*. Additionally, *R. pretiosa* benefits from the presence of *R. aurora* under the threat of predation. The increased weight at metamorphosis and

proportion of the cohort surviving to metamorphosis indicate that a facilitative interaction appears in this simple three species system.

The results from this study demonstrate that the risk of predation and interspecific interactions influence the metamorphic characteristics of both *R. aurora* and *R. pretiosa*. Under natural conditions, both species would be exposed to a wide variety of predators and studies have shown that antipredator responses to different predators are species-specific (Relyea 2001a, Relyea 2001b). In a natural setting, interactions become more complex with the addition of predators as well as other potential competitors. When additional competitors such as the Pacific treefrog (*Hyla regilla*), the northwestern salamander (*Ambystoma gracile*), and the roughskin newt (*Taricha granulosa*) and predators such as leeches, predacious diving beetles (*Dytiscus*), and fish are incorporated in the community during the larval period, interactions become more complex and a facilitative interaction may be more difficult to identify. Regardless, the influence of nonlethal predation on *R. aurora* and *R. pretiosa* and the interspecific relationship between the species affects factors associated with metamorphic success and should be considered in determining dynamics within the larval community where the species are sympatric.

Table 2.1. Experimental design for tadpole and predator manipulations.

Treatments						
<i>Aeshna palmata</i>	Absent	Present	Absent	Present	Absent	Present
Density	Low	Low	Low	Low	High	High
Competitor	Absent	Absent	Present	Present	Present	Present
# Individuals	50	50	50	50	100	100
Replicates	4	4	4	4	6	6

Table 2.2. Mean, maximum and minimum temperatures from tanks with continuous temperature monitors.

<i>Tank #</i>	<i>Mean (°C)</i>	<i>S.E.</i>	<i>Maximum (°C)</i>	<i>Minimum (°C)</i>
2	17.81	0.059	29.25	8.9
5	17.98	0.059	29.35	8.8
10	17.96	0.058	28.85	9.05
15	18.11	0.059	28.84	8.73

Table 2.3. MANOVA results for a) weight at metamorphosis, b) length of the larval period, and c) proportion of original number stocked reaching metamorphosis for *R. aurora*. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

a)

Source	d.f.	F	P
Predator	1	11.48	0.004*
Competitor	1	6.78	0.019*
Density	1	98.38	<0.0001*
Pred*density	1	2.05	0.171
Pred*competitor	1	0.99	0.335
Side	1	0.73	0.405
Side (predator)	1	0.04	0.848
Side (competitor)	1	1.77	0.202
Side (density)	1	1.47	0.243
Side (pred*den)	1	1.80	0.199
Side (pred*comp)	1	0.48	0.497
Error	16		

b)

Source	d.f.	F	P
Predator	1	3.55	0.078
Competitor	1	10.21	0.006*
Density	1	44.81	<0.0001*
Pred*density	1	0.03	0.858
Pred*competitor	1	0.03	0.871
Side	1	0.23	0.639
Side (predator)	1	0.00	0.970
Side (competitor)	1	0.02	0.899
Side (density)	1	0.29	0.600
Side (pred*den)	1	0.08	0.780
Side (pred*comp)	1	0.03	0.867
Error	16		

c)

Source	d.f.	F	P
Predator	1	0.29	0.595
Competitor	1	0.12	0.733
Density	1	2.31	0.148
Pred*density	1	0.10	0.750
Pred*competitor	1	1.17	0.296
Side	1	3.77	0.070
Side (predator)	1	1.14	0.302
Side (competitor)	1	1.48	0.241
Side (density)	1	0.96	0.343
Side (pred*den)	1	0.56	0.466
Side (pred*comp)	1	0.04	0.837
Error	16		

Table 2.4. MANOVA results for a) weights at metamorphosis, b) length of the larval period, and c) proportion of original number stocked reaching metamorphosis for *R. pretiosa*. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

a)

Source	d.f.	F	P
Predator	1	0.17	0.685
Competitor	1	0.08	0.782
Density	1	1.24	0.287
Pred*density	1	0.00	0.982
Pred*competitor	1	3.09	0.104
Side	1	0.02	0.897
Side (predator)	1	0.18	0.677
Side (competitor)	1	0.08	0.787
Side (density)	1	0.12	0.732
Side (pred*den)	1	0.20	0.663
Side (pred*comp)	1	0.25	0.629
Error	12		

b)

Source	d.f.	F	P
Predator	1	2.87	0.116
Competitor	1	0.97	0.344
Density	1	7.44	0.018*
Pred*density	1	0.94	0.353
Pred*competitor	1	2.85	0.117
Side	1	0.98	0.342
Side (predator)	1	1.03	0.329
Side (competitor)	1	0.29	0.599
Side (density)	1	2.32	0.154
Side (pred*den)	1	0.01	0.915
Side (pred*comp)	1	0.43	0.524
Error	12		

c)

Source	d.f.	F	P
Predator	1	0.67	0.424
Competitor	1	3.72	0.072
Density	1	7.72	0.013*
Pred*density	1	0.08	0.776
Pred*competitor	1	1.02	0.327
Side	1	0.00	0.945
Side (predator)	1	0.12	0.736
Side (competitor)	1	0.05	0.832
Side (density)	1	0.00	0.996
Side (pred*den)	1	0.02	0.893
Side (pred*comp)	1	0.24	0.629
Error	16		

Table 2.5. ANOVA results for proportion of *R. pretiosa* tadpoles remaining in tanks out of the original number stocked. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

Source	d.f.	F	P
Predator	1	0.38	0.550
Competitor	1	0.31	0.558
Density	1	0.04	0.850
Pred*density	1	1.11	0.315
Pred*competitor	1	4.37	0.061
Side (predator)	1	0.32	0.584
Side (competitor)	1	0.04	0.846
Side (density)	1	0.02	0.901
Side (pred*den)	1	0.09	0.786
Side (pred*comp)	1	0.00	0.982
Error	11		

Table 2.6. ANOVA results for total tadpoles and metamorphic individuals alive in tanks at termination of experiment for *R. pretiosa*. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

Source	d.f.	F	P
Predator	1	1.41	0.252
Competitor	1	5.65	0.030*
Density	1	7.55	0.014*
Pred*density	1	0.34	0.566
Pred*competitor	1	0.19	0.668
Side (predator)	1	0.00	0.969
Side (competitor)	1	0.01	0.930
Side (density)	1	0.00	0.988
Side (pred*den)	1	0.02	0.891
Side (pred*comp)	1	0.34	0.571
Side	1	0.00	0.968
Error	16		

Table 2.5. ANOVA results for proportion of *R. pretiosa* tadpoles remaining in tanks out of the original number stocked. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

<i>Source</i>	<i>df.</i>	<i>F</i>	<i>P</i>
Predator	1	0.38	0.550
Competitor	1	0.31	0.558
Density	1	0.04	0.850
Pred*density	1	1.11	0.315
Pred*competitor	1	4.37	0.061
Side (predator)	1	0.32	0.584
Side (competitor)	1	0.04	0.846
Side (density)	1	0.02	0.901
Side (pred*den)	1	0.09	0.786
Side (pred*comp)	1	0.00	0.982
Error	11		

Table 2.6. ANOVA results for total tadpoles and metamorphic individuals alive in tanks at termination of experiment for *R. pretiosa*. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

<i>Source</i>	<i>df.</i>	<i>F</i>	<i>P</i>
Predator	1	1.41	0.252
Competitor	1	5.65	0.030*
Density	1	7.55	0.014*
Pred*density	1	0.34	0.566
Pred*competitor	1	0.19	0.668
Side (predator)	1	0.00	0.969
Side (competitor)	1	0.01	0.930
Side (density)	1	0.00	0.988
Side (pred*den)	1	0.02	0.891
Side (pred*comp)	1	0.34	0.571
Side	1	0.00	0.968
Error	16		

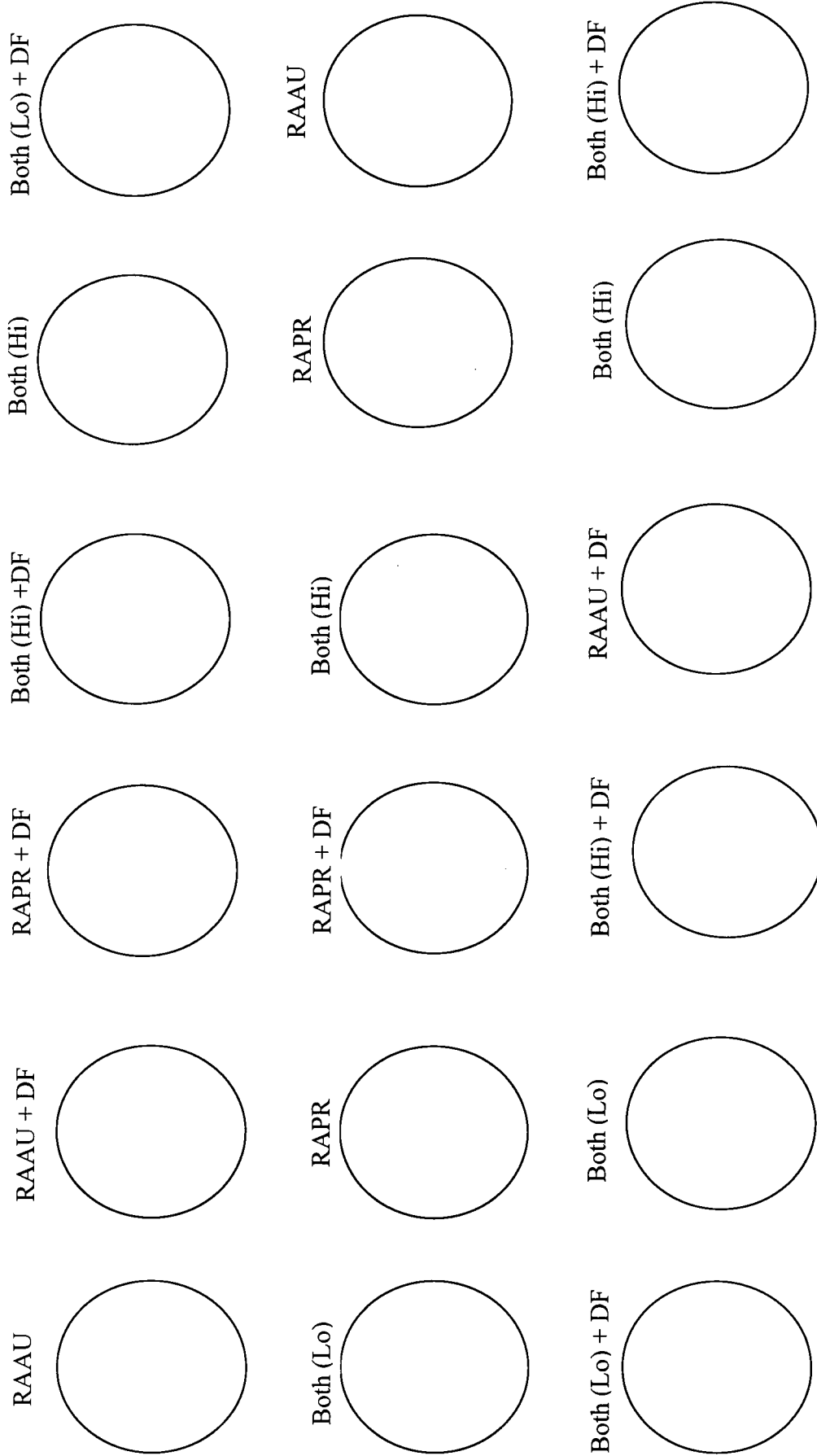


Figure 2.1. Layout of mesocosms and treatments associated with each. Red-legged frog (RAAU), Oregon spotted frog (RAPR), and both species together (Both), dragonfly larva present (DF). (note: each tank was divided in half and the same treatment was placed on either side of the tank.)

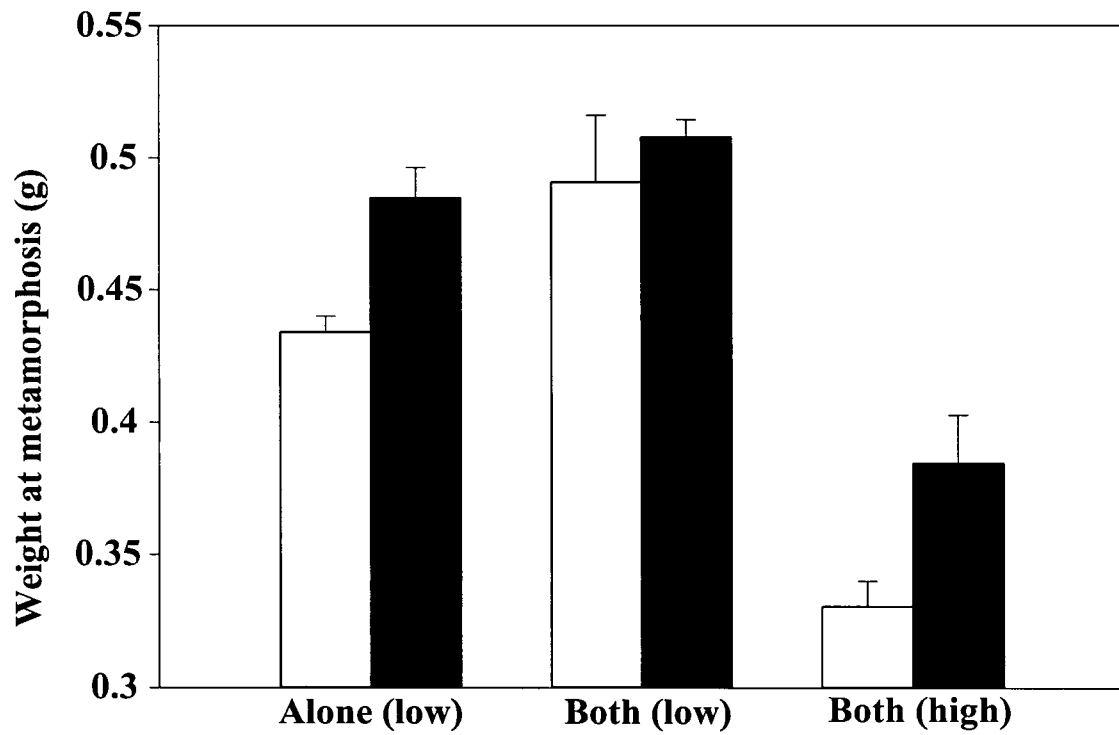


Figure 2.2. Average weight at metamorphosis for *R. aurora* in the presence (solid bars) and absence (open bars) of *Aeshna*. Histogram bars are treatment means (± 1 SE) for replicates.

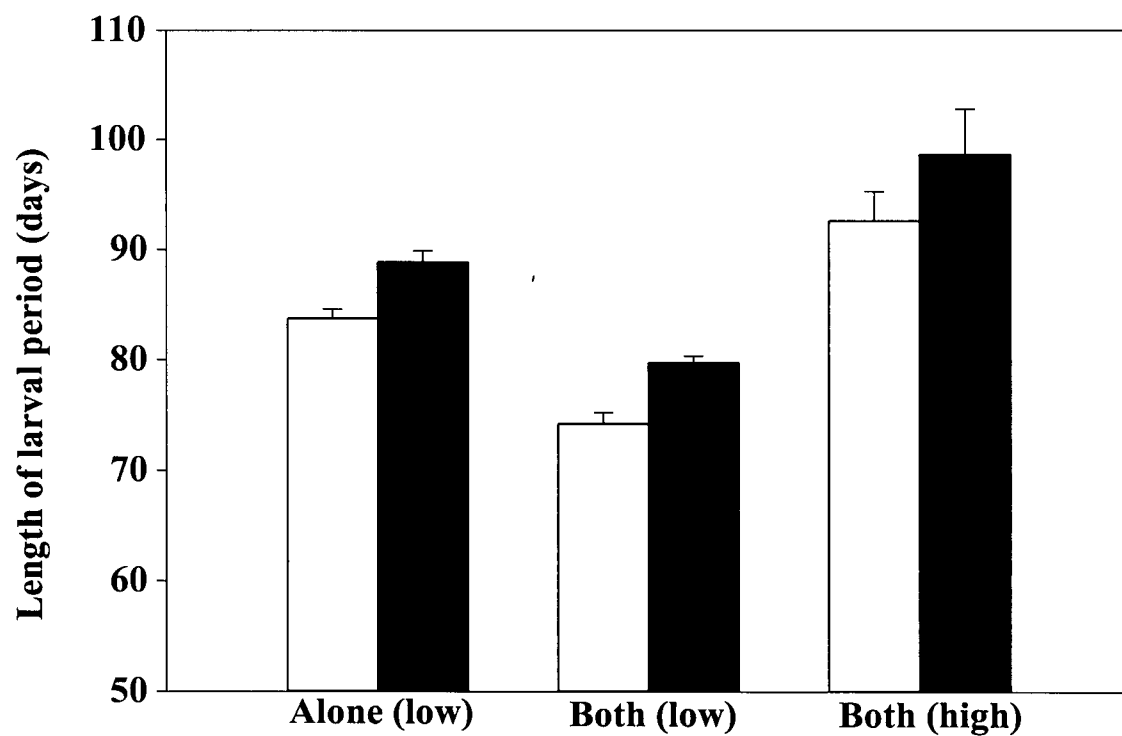


Figure 2.3. Average length of the larval period for *R. aurora* in the presence (solid bars) and absence (open bars) of *Aeshna*. Histogram bars are treatment means (± 1 SE) for replicates.

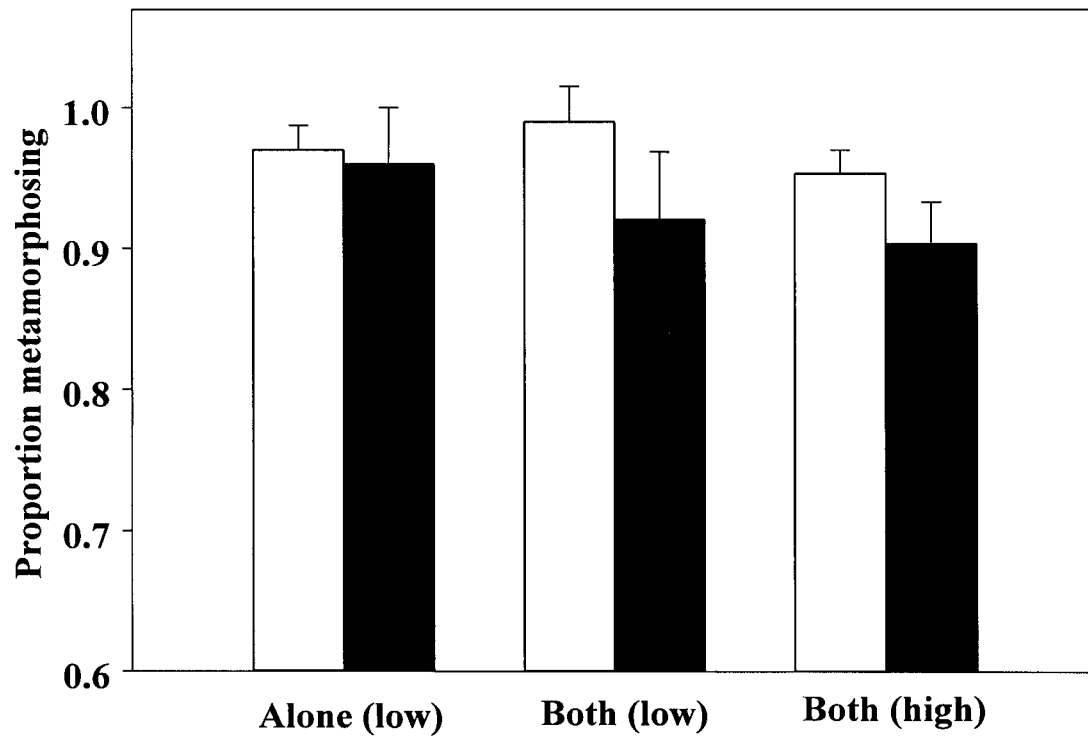


Figure 2.4. Proportion of original number stocked surviving to metamorphosis for *R. aurora* in the presence (solid bars) and absence (open bars) of *Aeshna*. Histogram bars are treatment means (± 1 SE) for replicates.

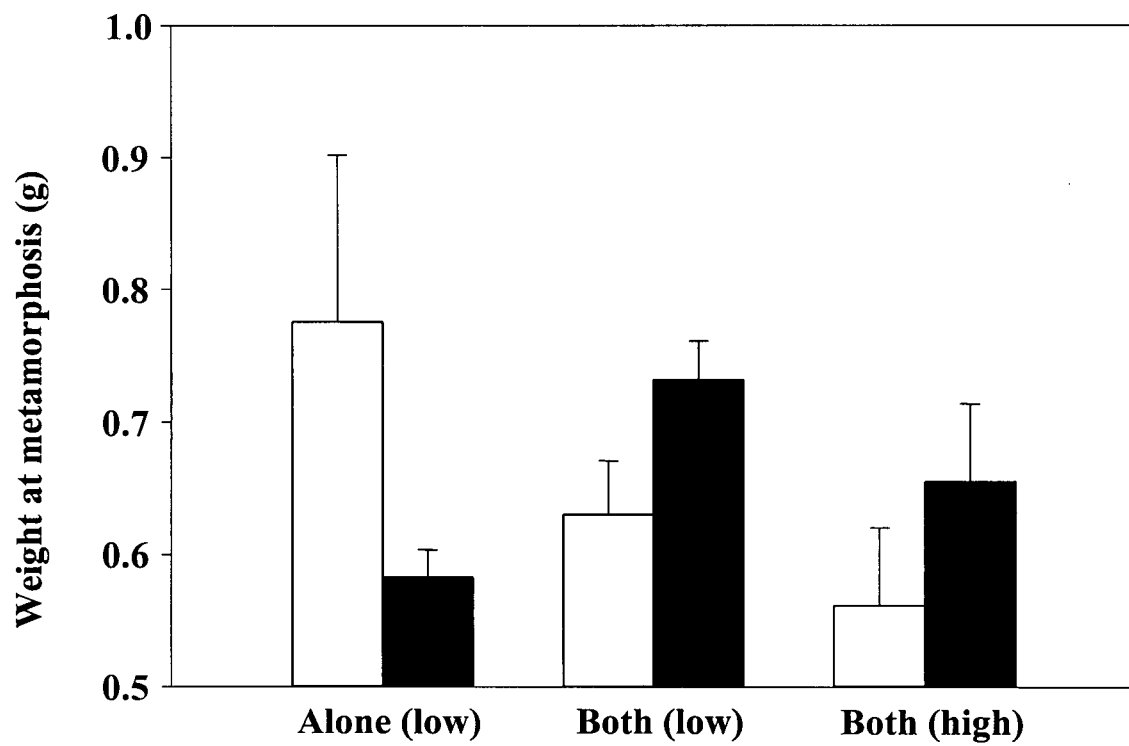


Figure 2.5. Average weight at metamorphosis for *R. pretiosa* in the presence (solid bars) and absence (open bars) of *Aeshna*. Histogram bars are treatment means (± 1 SE) for replicates.

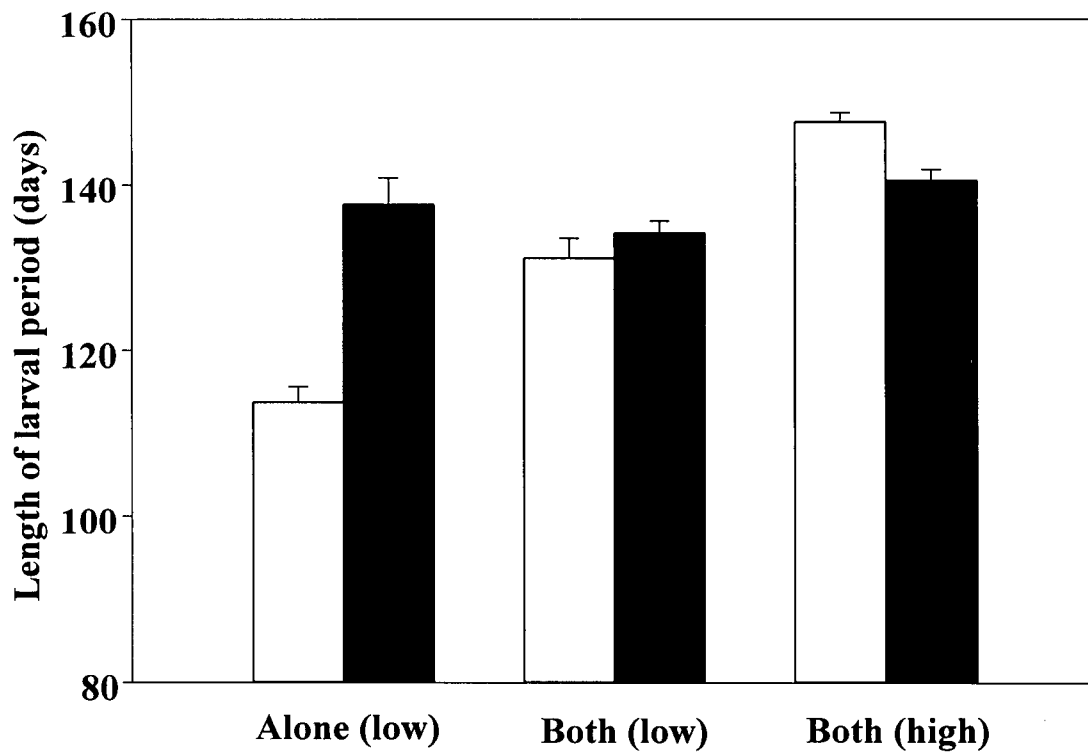


Figure 2.6. Average length of the larval period for *R. pretiosa* in the presence (solid bars) and absence (open bars) of *Aeshna*. Histogram bars are treatment means (± 1 SE) for replicates.

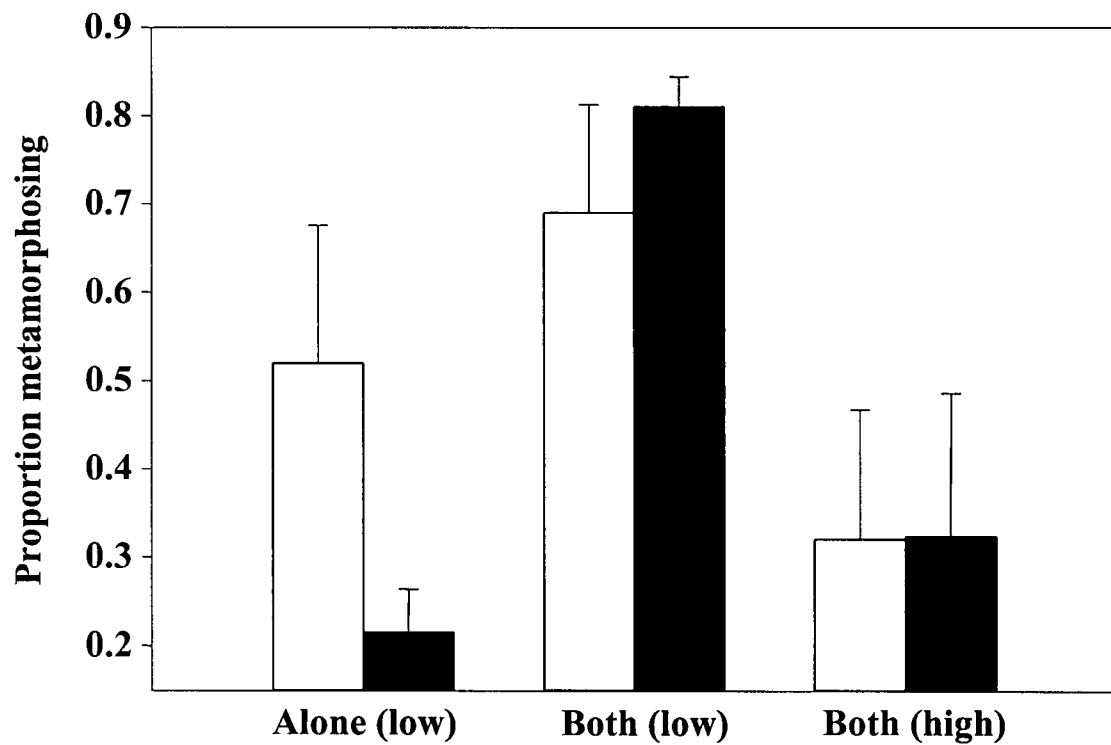


Figure 2.7. Proportion of original number stocked surviving to metamorphosis for *R. pretiosa* in the presence (solid bars) and absence (open bars) of *Aeshna*. Histogram bars are treatment averages (± 1 SE) for replicates.

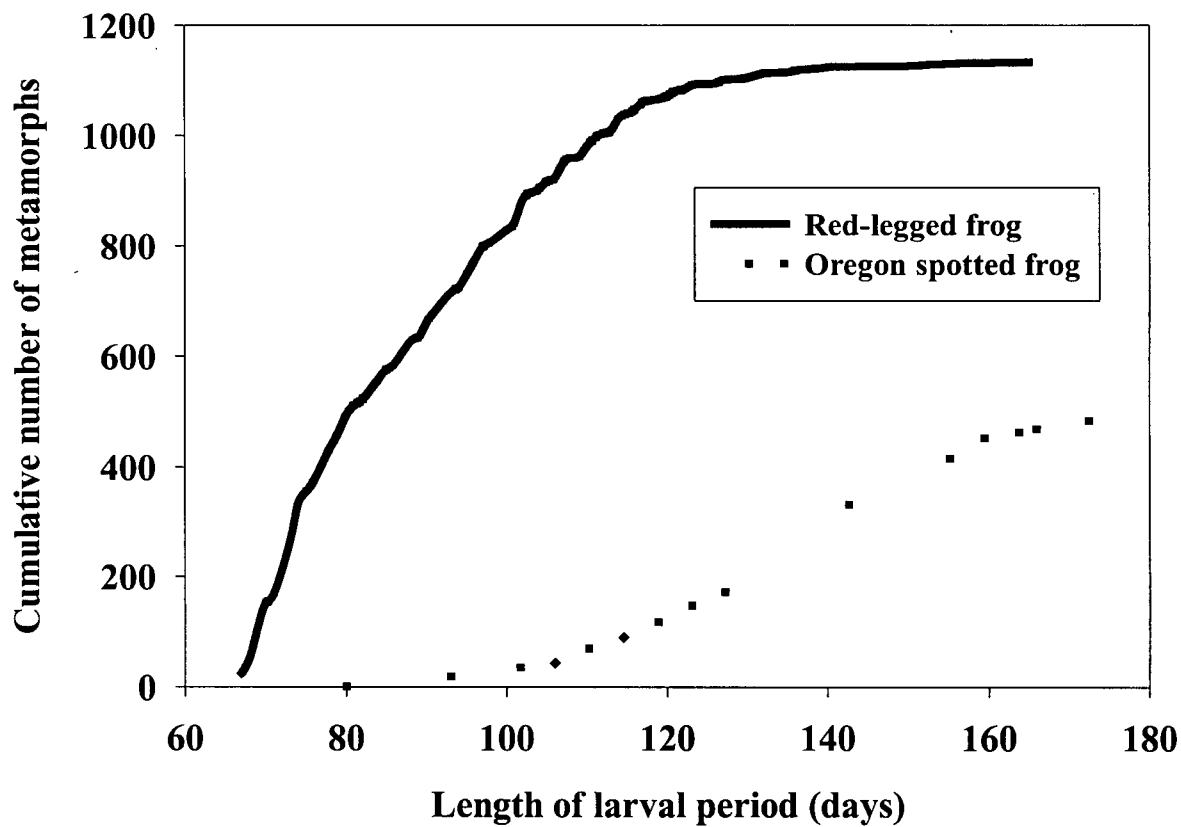


Figure 2.8. Cumulative number of metamorphs collected from all treatments. The solid line represents *R. aurora* and dotted line represents *R. pretiosa*. Both species began with an equivalent number ($n = 1400$) of tadpoles at the start of the experiment.

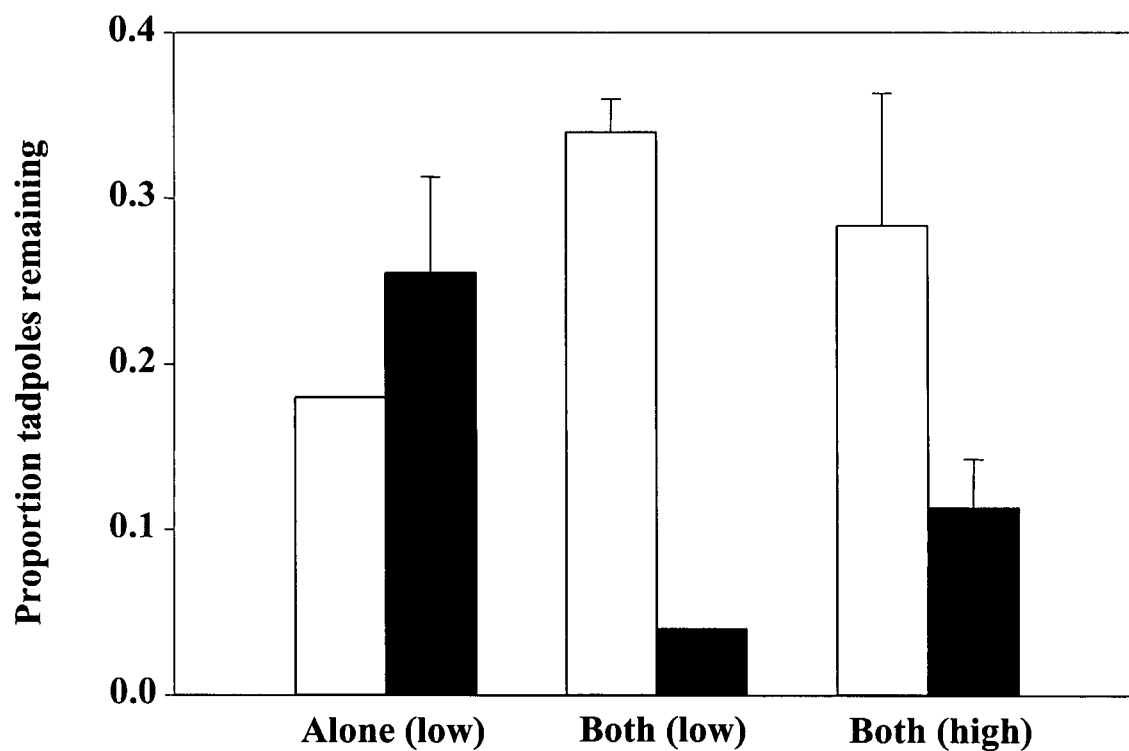


Figure 2.9. Proportion of all *R. pretiosa* tadpoles alive at termination of the experiment as tadpoles in the presence (solid bars) and absence (open bars) of *Aeshna*. Histogram bars are treatment averages (± 1 SE) for replicates. Histograms with no error bars are for a single replicate only.

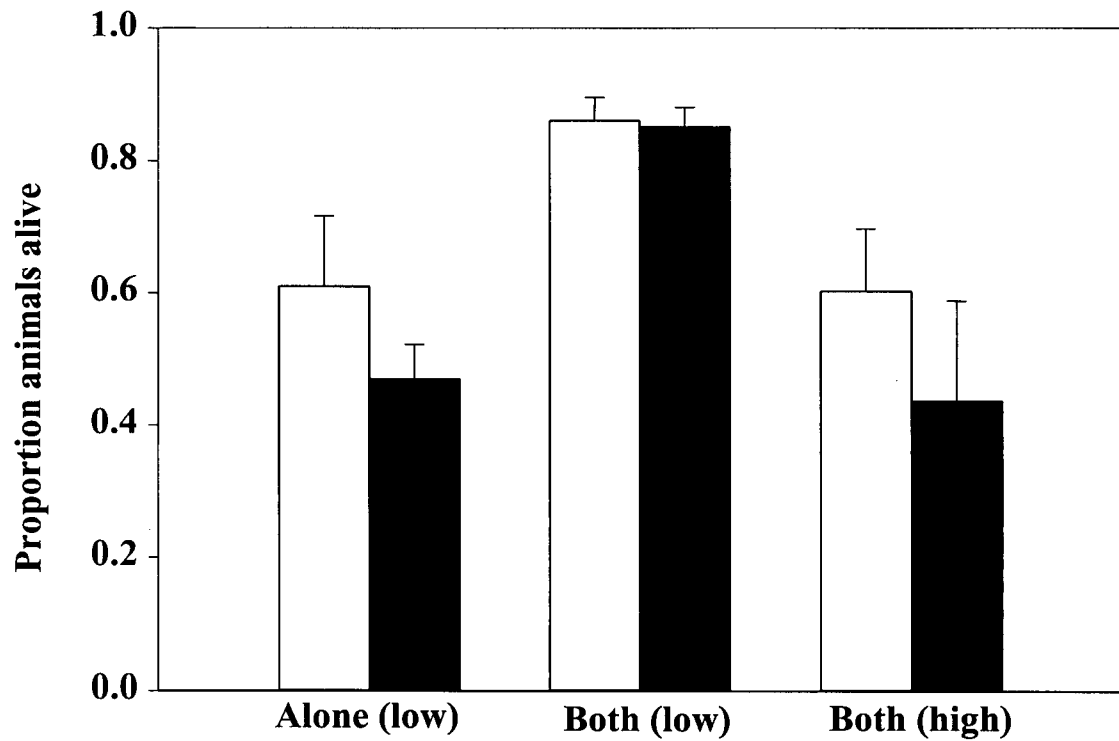


Figure 2.10. Proportion of total *R. pretiosa* tadpoles alive at termination of the experiment as tadpoles or metamorphic individuals in the presence (solid bars) and absence (open bars) of *Aeshna*. Histogram bars are treatment averages (± 1 SE) for replicates.

Chapter 3. Behavioral response of red-legged frog tadpoles and Oregon spotted frog tadpoles to the nonlethal presence of an invertebrate predator.

Introduction

The behavioral response of organisms to predators, competitors, and other environmental variables can influence community dynamics (Werner et al. 1983, Cooper 1984, Stamp and Bowers 1991, Wooster and Sih 1995, Eklov 2000). Identification of the mechanisms responsible for interspecific relationships leads to a better understanding and ability to predict community processes. Species-specific behavior can also help to predict fitness of animals to varying abiotic and biotic factors.

Theory suggests that many animals face a trade-off between foraging and avoiding predation within their habitat. To compensate for the threat of predation animals exhibit specific behavior that reduces their risk of being eaten (Lima et al. 1985). Larval anurans provide an excellent model system in which to evaluate this trade-off in aquatic habitat as they adapt readily to laboratory conditions and community composition can be realistically recreated. An individual tadpole must balance foraging activity with antipredator behavior in order to optimize size at metamorphosis, a characteristic shown to correspond directly to the probability of survival (Wilbur and Collins 1973, Berven 1990). The trade-off between balancing energy between growth while maintaining antipredator responses may compromise important metamorphic characteristics of the individual tadpole. Studies show that higher activity in larval anurans, e.g. when food is scarce, results in increased detection by predators (Semlitsch and Reyer 1992, Skelly 1994). Therefore, understanding the behavior of species in the presence of abiotic and

biotic pressures such as predation may help to explain interspecific relationships in a community and success of tadpole cohorts.

Activity levels are influenced by many factors in the larval environment including the risk of predation. In an experiment using *Rana esculenta* and *Rana lessonae* together, both species reduced activity in response to a predator and increased activity in response to food limitation (Horat and Semlitsch 1994). A similar pattern has been shown in other species of tadpoles (Anholt et al. 1996, Anholt and Werner 2000), guppies (Godin and Smith 1988), and stream invertebrates (Kohler and McPeck 1989). When food resources are scarce, tadpoles may increase foraging activity to avoid starvation and in doing so increase the risk of detection by a predator. Larval amphibians face many such trade-offs during the vulnerable period before reaching metamorphosis and a large amount of mortality results during this phase (Calef 1973, Licht 1974). These studies reveal that larval amphibians can adaptively alter behavior to respond to the differing degrees of threats they face. If the threat of being eaten is more apparent than the threat of starving, the tadpoles reduce foraging activity. Similar responses of tadpoles responding to varying degrees of threat have been shown with lowered water levels crossed by threat of predation (Laurila and Kujasalo 1999), and the threat of trematode parasitism crossed with the presence of a predator (Thiemann and Wassersug 2000).

The addition of a competing species into the system may place additional pressure on the focal species if prey species compete for resources or space. In fact, the presence of a predator has been shown to alter the behavior of competitive prey species thus changing the competitive interaction (Werner 1991, Relyea 2000). A study examining larval bullfrogs (*Rana catesbeiana*) and larval green frogs (*Rana clamitans*) found that in the absence of a nonlethal odonate predator both species exhibited equal competitive abilities by growing at the same rate. However, the presence of an odonate larva suppressed green frog growth rates more heavily allowing bullfrogs

to become the dominant species in the community. The change in growth rate was attributed to a greater proportional reduction in activity of green frogs when the predator was present (Werner 1991). The reduction in activity of green frogs allowed larval bullfrogs to capitalize on the lowered activity of their competitor and consume more resources to grow at an accelerated rate in the presence of a predator.

Alterations in behavioral responses to factors discussed above may have consequential effects on metamorphic characteristics. Behavior may alter growth rates or other factors associated with individual fitness. Many studies have shown that a decrease in activity leads to smaller size at metamorphosis in larval anurans (Skelly and Werner 1990, Werner 1991, Kiesecker and Blaustein 1998). Size at metamorphosis is an important metamorphic characteristic for anurans because individuals that metamorphose at larger sizes have greater survival to the following year and become larger adults that lay more eggs (Smith 1987, Berven 1990, Morey and Reznick 2001). Behavioral responses to the variety of pressures associated with the larval environment are important in determining the fitness of individuals and potentially to the entire population (Berven 1990).

Studies of natural populations indicate that predation during the early phase of the larval period is a major source of mortality for anuran species. In Oregon spotted frog (*Rana pretiosa*) and red-legged frogs (*Rana aurora*) where an estimated 1-5% of the tadpoles survive to metamorphosis, the pressure from predation is large (Calef 1973, Licht 1974). Larval *R. pretiosa* and *R. aurora* coexist at the northern portion of the Oregon spotted frog distribution in southwestern British Columbia and Washington State and use similar larval rearing habitat. *Rana pretiosa* is classified as endangered in southwestern British Columbia with an estimated cumulative population of fewer than 300 individuals remaining in three isolated breeding populations. Efforts are underway to restore habitat conditions favorable to *R. pretiosa* as well

as to restore habitat in other areas where populations have disappeared for possible reintroductions. For this reason, understanding the response of larval *R. pretiosa* to potential predators and interspecific interactions with *R. aurora* may shed light on this community and suggest possible effects on metamorphic characteristics ultimately influencing population dynamics. Understanding basic ecological relationships between these species and a typical predator may help in planning recovery efforts and possible reintroductions for *R. pretiosa*. Larval dragonflies are common predators of tadpoles and found in the wetlands inhabited by sympatric populations of *R. aurora* and *R. pretiosa* (Licht 1974). The predator selected for use in this experiment was the paddle-tailed damner (*Aeshna palmata*) due to its wide range overlapping with *R. pretiosa* (see Chapter 2). I investigated the following questions in the context of the larger mesocosm study discussed in Chapter 2. First, how do *R. aurora* and *R. pretiosa* alter behavior in response to a typical predator? Second, do these species respond differently to a presence of the predator? And third, does a typical predator alter the growth rates of *R. aurora* and *R. pretiosa*?

Methods

Egg masses of *R. pretiosa* and *R. aurora* were collected from Beaver Creek, Thurston County, Washington, and transported to UBC with the permission of the Washington State Department of Fish and Wildlife. *Rana pretiosa* egg masses were collected from areas where eggs clearly would be lost to desiccation as waters receded; therefore impact to the natural population was minimal. In total, tadpoles were hatched from nine different *R. pretiosa* egg masses and twelve different *R. aurora* egg masses in the laboratory and held at a temperature of 14°C until May 11, when the experiment was initiated.

The experimental design was a completely randomized design with the presence or absence of the predator (*Aeshna palmata*) crossed with each species alone and together (Table 3.1). The experimental design included four replicates of each treatment: 1) *R. aurora* with no predator, 2) *R. aurora* with *Aeshna palmata*, 3) *R. pretiosa* with no predator, 4) *R. pretiosa* with *Aeshna palmata*, 5) both species with no predator, and 6) both species with *Aeshna palmata*.

The experiment was performed in an isolated laboratory, and a blind was constructed with black plastic around the entire setup to reduce disturbance when I entered the room. A total of twenty-four 40-L aquaria (50.8 cm x 25.4 cm) were filled to a depth of 15 cm using water from a pond located on UBC's South Campus. The water used in this experiment came from a pond that contained *Aeshna palmata* as well as many other species of dragonfly larvae, and other predatory invertebrates. This man-made pond was part of another long-term experiment and has been kept free of fish since it was constructed in 1991. Any differences observed in behavior are therefore conservative, because the chemical cues emitted from feeding dragonflies influence these tadpoles (Petranka et al. 1987).

Black plastic covered each tank on the back and sides to ensure that individuals between tanks were not responding to movement from neighboring tanks. The front of each aquarium remained open so that I could observe tadpole behavior from a distance. Lights were suspended within the lab to equally illuminate all tanks on a 16:8 day:night timer. All tanks were marked at the midpoint with a piece of masking tape so I could quickly discern the location of tadpoles relative to the predator. Predator enclosures were constructed of a 10x10-cm bag of mosquito netting with a coiled wire inside providing an open area for the dragonfly larva to move freely. The enclosure was placed randomly at one end of each aquarium (including empty enclosures in the non-predator treatments).

All tadpoles were at approximately Gosner stage 26 (Gosner 1960) when they were added to experimental treatments and the experiment was initiated. One hundred and twenty tadpoles of each species were selected at random from hatching containers to be equal in size. These tadpoles were then assigned to an experimental aquarium at a constant density of 10 individuals per tank. In treatments with both species, five individuals of each species were placed in the tank. Tadpoles that died during the first week of the experiment were removed and replaced with similar sized individuals (3 *R. pretiosa*). After the start of the second week, tadpoles that died were removed but not replaced (in total, 5 *R. aurora* and 5 *R. pretiosa*, excluding the initial 3 *R. pretiosa* that were replaced).

Tadpoles were added to the tanks on May 11, and allowed to acclimate for three days before predators were added to enclosures. During the experiment, dragonfly larvae (*Aeshna palmata*) were collected from the South Campus ponds and fed tadpoles of both species until their addition to predator cages. Dragonfly larvae were carefully monitored and removed as they approached later stages of their development and began to initiate metamorphosis. The water in aquaria was changed weekly, tadpoles were weighed as a group and odonate larvae were fed one tadpole according to experimental treatments.

Tadpoles were fed *ad libitum* a 3:1 mixture of *Xenopus* tadpole food (Carolina Biological Supply, Burlington, NC, USA) and TetraMin fish flakes crushed to a powder. Therefore, tadpoles in this experiment were never under food limiting circumstances. Observations consisting of number of tadpoles swimming and number of tadpoles on the predator side of the aquarium were initiated the day after a single dragonfly larva was placed in each mesh cage designated as predator treatments. These records were taken twice daily on four days each week over the four-week duration of the experiment. Care was taken to move slowly and to move in a manner that shadows were not cast into the aquaria. I stood in front of a bank of aquaria for one

minute before observations were taken to allow tadpoles to resume normal behavior on the chance that they had been disturbed. To eliminate observer bias, I made all observations for the experiment. Temperature of each tank was also taken after all behavioral observations were recorded to ensure equality among treatments and replicates. In total 33 observations (in general 4weeks x 4 days x 2 periods, one extra observation was added) of both activity levels and spatial distribution were recorded per aquarium. The experiment was terminated after four weeks and tadpoles completed metamorphosis in the lab.

Statistical Analysis

Observations for number of active tadpoles and number to tadpoles near the predator were converted to proportions in each aquaria for analysis. The data were tested for normality and met the assumption, therefore data was not arcsine square root transformed prior to analysis. Weekly averages of behavioral responses for single species treatments were analyzed using a two-by-two repeated measures analysis of variance (rmANOVA) for the fixed effects of predator and species and the interaction between these factors. All analyses were conducted using the PROC GLM procedure in SAS (version 8.0). A two-by-two rmANOVA was also conducted on the treatments where the species were together for the fixed effects of predator and competitor as well as the interaction term. Average weekly weight of each species was analyzed using two-way rmANOVAs testing in the first for effects of predator, species, and the interaction and in the second, predator, competitor, and the interaction.

Results

Temperature

Temperature was not significantly different in any of the treatments throughout the experiment. The temperature ranged from 16.2°C to 19.6°C, with an average temperature of 18.6°C. The largest difference detected during any one measurement was 1.2°C, with an average difference among tanks of 0.5°C at any one time temperature measurements were recorded. Table 3.2 shows the mean temperature per treatment over the experiment.

Activity levels (species alone)

Rana aurora was significantly more active than *R. pretiosa* in the absence of the nonlethal (caged) odonate predator ($p = 0.006$, Figure 3.1, Table 3.3). *Rana aurora* tadpoles were active 36.5% of the time, while activity level of *R. pretiosa* treatments was slightly lower at 25.5%. In the presence of *Aeshna*, *R. aurora* tadpoles reduced activity proportionally more than *R. pretiosa*, and the rmANOVA found a significant interaction between species and predator ($p = 0.011$, Figure 3.1, Table 3.3). The effect of the predator's presence on both species was highly significant causing both species to reduce activity levels ($p < 0.0001$, Figure 3.1, Table 3.3).

Rana aurora reduced activity over all four weeks of the experiment between 3.3 times and 5.3 times in the presence of *Aeshna*, but no significant trend was shown (Figure 3.2). Over the course of the experiment *R. aurora* tadpoles in predator treatments gradually became more active. The within subjects effect from the rmANOVA indicated that the effect of time was significant in determining tadpole activity level ($p = 0.001$, Figure 3.2, Table 3.3). *Rana pretiosa* tadpoles experienced a 3.2 fold reduction in activity in the presence of *Aeshna*. When the four weeks were examined separately, activity of *R. pretiosa* tadpoles was consistently reduced in the presence of the predator between 2.2 times and 4.2 times (Figure 3.3). Activity levels for *R.*

pretiosa gradually increased over the experiment in treatments containing *Aeshna*. However, larvae in treatments containing a dragonfly were always significantly less active than treatments without dragonfly larvae ($p < 0.0001$, Figure 3.3, Table 3.3). The within subjects effect from the rmANOVA indicated that the effect of time was significant in determining activity levels ($p = 0.001$, Table 3.3).

Activity levels (both species)

Activity observations when both species were present were calculated using averages of five individuals of each species and compared to tanks containing 10 individuals of one species in order to maintain a constant overall density. The presence of a competitor did not significantly affect the activity levels of either *R. aurora* ($p = 0.326$, Figure 3.4, Table 3.4) or *R. pretiosa* ($p = 0.586$, Figure 3.5, Table 3.5). Both species maintained activity levels at approximately the same level regardless of the presence of a competitor. Exposure to *Aeshna* significantly reduced activity levels for both species ($p < 0.0001$, Table 3.4 and 3.5). The interaction between competitor and predator for *R. aurora*, although not significant ($p = 0.060$, Figure, 3.4, Table 3.4), indicated that when *R. pretiosa* is present, *R. aurora* is slightly less active in the absence of *Aeshna*. Additionally, *R. aurora* reduces activity levels less in the presence of *R. pretiosa* than when alone with *Aeshna* (Figure 3.4). The interaction for competitor and predator was similar for *R. pretiosa*, i.e. they were slightly less active in treatments with *R. aurora* and reduced activity levels to the predator slightly less in the presence of *R. aurora* (Figure 3.5). However, the effect was not significant ($p = 0.176$, Table 3.5).

Spatial proximity to predator (species alone)

Tadpoles of both species showed a significant spatial response to the presence of *Aeshna*. Both *R. aurora* and *R. pretiosa* were evenly distributed in the tank in the absence of the predator (Figure 3.6). Spatial orientation in the presence of the predator was altered so that an average

over the experiment of 26.7% *R. aurora* tadpoles were located on the predator half of the aquarium and 25.9% of *R. pretiosa* tadpoles were located near the predator. The presence of the predator was highly significant in influencing spatial proximity to *Aeshna* ($p < 0.0001$, Figure 3.6, Table 3.6). Both species responded to *Aeshna* in the same manner ($p = 0.872$, Figure 3.6, Table 3.6), and the number of individuals on the predator side of the aquarium was reduced by one half when *Aeshna* was present (Figure 3.6). The rmANOVA showed no interaction between species and predator ($p = 0.886$, Table 3.6).

Rana aurora and *R. pretiosa* larvae maintained an even distribution of tadpoles in treatments without a dragonfly larva for all four weeks of the experiment (Figure 3.7, 3.8). In predator treatments, the tadpoles of both species gradually decreased avoidance of the dragonfly larva as the weeks progressed. The within subjects effect from the rmANOVA indicated that the effect of time was significant ($p < 0.0001$, Table 3.6). Additionally, a significant time by species interaction was shown in the within subjects effect indicating that the species responded differently to the predator over time ($p = 0.004$, Table 3.6). Spatial proximity of *R. aurora* to the predator was reduced between 1.9 and 2.1 fold during the four weeks of the experiment (Figure 3.7). Similarly, *R. pretiosa* tadpoles avoided *Aeshna* between 1.6 and 3.0 times more frequently over the four week period (Figure 3.8).

Spatial proximity to predator (both species)

The major response determined in the analysis with both species present was a significant difference between predator and non-predator treatments for both *R. aurora* and *R. pretiosa*. Significantly more individuals were opposite the predator when exposed to the presence of *Aeshna* ($p < 0.0001$, Table 3.7, 3.8) as seen for treatments where the species were alone. The presence of *R. pretiosa* larvae did not impact the spatial distribution of *R. aurora* tadpoles to the predator ($p = 0.215$, Figure 3.9, Table 3.7). A similar result was shown for *R. pretiosa*

distribution where individuals behaved nearly identically in treatments in the presence or absence of a competitor (Figure 3.10). Data indicate a slight increased proximity for *R. pretiosa* to the predator in the presence of *R. aurora* when *Aeshna* is both present and absent ($p = 0.081$, Figure 3.10, Table 3.8). The interaction term between predator and competitor was not significant for either *R. aurora* or *R. pretiosa* (Tables 3.7, 3.8).

Average weekly weight

The weight of tadpoles within an aquarium was recorded as a group when water was changed weekly, consequently individuals were not followed throughout the course of the experiment. A one-way ANOVA showed that *R. aurora* tadpoles were significantly heavier than *R. pretiosa* tadpoles when the experiment began ($p = 0.041$, Table 3.9). An average weight for each tank was calculated weekly and used to calculate means used in rmANOVA analysis. The rmANOVA for average weekly weights for treatments with *R. aurora* and *R. pretiosa* alone suggests that *R. aurora* maintained significantly higher average weekly weights throughout the experiment ($p = 0.001$, Figure 3.11, Table 3.10). The presence of *Aeshna* had a nearly significant effect on average weights in treatments with the species alone ($p = 0.054$, Table 3.10), causing tadpoles to have slightly greater weights. The within subject effect showed significant effects of time ($p < 0.0001$, Table 3.10) and an interaction of time and species ($p < 0.0001$, Table 3.10). This result suggests that *R. aurora* increased average weekly weight more quickly than *R. pretiosa* over time (Figure 3.11). Additionally, the presence of a competitor did not have significant effects on average weekly weights of *R. aurora* or *R. pretiosa* (Table 3.11). Tadpoles in treatments where they were alone versus with a competitor showed the same average weekly weights (Figure 3.12, 3.13).

Discussion

Reduction in activity and movement away from a predator are common antipredator responses in larval anurans (Lawler 1989, Semlitsch and Reyer 1992, Anholt and Werner 1995). Skelly (1994) found that larval wood frogs (*Rana sylvatica*) treated with a solution of tricaine methanosulphate to anesthetize them were less frequently eaten by dragonfly larvae (*Anax*) than untreated tadpoles. A reduction in activity therefore decreases the probability of detection by a predator. However, antipredator behavior is typically accompanied by effects on life-history characteristics or changes in morphological features of the tadpole (Skelly and Werner 1990, Werner 1991, Van Buskirk et al. 1997, Van Buskirk and McCollum 1999). The results of this experiment suggest that the presence of a nonlethal predator in the laboratory significantly influenced the behavior of tadpoles of both *R. aurora* and *R. pretiosa*, but did not significantly influence their average weekly weights during early larval development. However, developmental stage was not assessed and may have been altered by the presence of *Aeshna* even though average weekly weight was not. A study examining the common frog (*Rana temporaria*) found that a predacious invertebrate did not influence growth rates, but did have an effect on developmental stages of tadpoles in predator treatments (Laurila and Kujasalo 1999), suggesting that a nonlethal predator may affect development of tadpoles without altering weights. In this experiment, both species maintained similar weekly average weights in the presence and absence of the competitor. Analysis suggested a tendency for both species to reduce activity slightly less in the presence of one another when *Aeshna* was present. The increase of activity as weeks progressed may be attributed to tadpoles reaching a size threshold when invertebrate predator's ability to catch them decreases (Semlitsch 1990).

Tadpoles responded to *Aeshna* by moving to the opposite side of the aquarium and by reducing activity. These results are consistent with the pattern seen in systems with other larval

ranids and larval toads (Skelly and Werner 1990, Werner 1991, Anholt et al. 1996). In the absence of the predator, tadpoles were evenly distributed around the tank as expected. Behavioral changes did not depend on whether the treatment contained a single species or both species. However, neither *R. aurora* nor *R. pretiosa* produced results suggesting domination of the competitive interaction. It is possible that the level of food provided throughout the experiment remained high enough to preclude any competitive interactions in the results for both spatial distribution and activity levels.

Activity was significantly reduced in this experiment when a nonlethal predator was present. This finding is consistent with ecological theory suggesting when prey is faced with the knowledge that a predator is near, they are more likely to alter behavior to compensate for this threat (Lima 1998). *Rana pretiosa* tadpoles were less active than *R. aurora* tadpoles when a predator was absent. However, *R. aurora* reduced their activity by 10% more than *R. pretiosa* in the presence of *Aeshna*. The disproportional reduction in activity levels of *R. aurora* caused both species to have similar activity levels in the presence of a predator. In a system with free ranging predators, the reduction of activity in *R. aurora* to nearly the same level as *R. pretiosa* would be highly advantageous. By keeping an activity level similar to the other prey species in the system, both *R. pretiosa* tadpoles and *R. aurora* tadpoles would be detected and eaten at the same rate by dragonfly larvae in a natural system.

In wetland systems, *R. pretiosa* are frequently captured in shallow areas at the base of vegetation while *R. aurora* can be found swimming in deeper waters through ponds as well as near the margins (pers. obs.). Vegetation is also a preferred hunting perch for species such as *Aeshna palmata* that are classified as “sit-and-wait” predators (Cannings and Stuart 1977). *Rana pretiosa* may have adapted to be less active to compensate for closer proximity to invertebrate predators. Indeed studies indicate that less active species have greater survival in the presence of

a predator (Azevedo-Ramos et al. 1992, Skelly 1994), and additionally that patterns on the tail are an adaptation to the presence of invertebrate predators and provide camouflage making tadpoles more cryptic (McCollum and Leimberger 1997). *Rana pretiosa* larvae have numerous speckles on their tails (Corkran and Thoms 1996) which may help to camouflage the tadpoles from invertebrate predators in natural habitat. Anti-predator behavior in two closely related species, *Rana lessonae* and *Rana esculenta* showed a similar activity pattern to those produced in this experiment. *Rana lessonae* typically is associated with shallow edges while *R. esculenta* is found in deeper, benthic habitat. *Rana lessonae* was less active in general than *R. esculenta* in the presence of a predatory dragonfly larva (Semlitsch and Reyer 1992). Choices in habitat likely have created adaptations in these species to avoid the predators to which they are most exposed. *Rana pretiosa* may be less active because it is typically found in shallow areas where encounters with invertebrate predators may be high.

Crowding of tadpoles into a smaller area may result in increased competition for space and have a negative impact on growth simply due to many individuals vying for space and food resources in close proximity (Wilbur 1982). However, no impacts on average weekly weights over four weeks were shown between predator treatments. The fact that no differences were observed again supports the notion that food was not limiting to the tadpoles in this experiment and that they may have compensated for lower activity by foraging or digesting material more efficiently in the presence of the predator. However, many similar studies have found that growth rates are significantly impacted by the presence of a predator (Skelly and Werner 1990, Werner 1991). Although average weekly weights were not shown to be different during the four weeks of the experiment, I cannot assume that all tadpoles would have metamorphosed at the same weight. Even though tadpoles did not exhibit differences in average weekly weights, the tadpoles in the predator treatments may have shown a response in length of the larval period or

weight at metamorphosis had the experiment continued to metamorphic climax due to delayed development (Laurila and Kujasalo 1999). Most individuals were between Gosner stage 38-41 when the experiment was terminated (Gosner 1960), so the majority of the larval development period was covered under approximations of weekly weights.

The behavioral responses of both species to the nonlethal presence of *Aeshna* may help to explain effects observed on metamorphic characteristics in the artificial pond study (Chapter 2). The disproportionate reduction of activity for *R. aurora* in the presence of *Aeshna* may free resources for *R. pretiosa* enabling them to metamorphose at larger sizes. The lack of significant differences detected when a species was alone versus in the presence of a competitor may be due to the low number of individuals used to calculate mean values (i.e. 5 of each species when together), or alternatively indicate that food resources were extremely high in the experiment which may not be true in the mesocosm setting. Both *R. aurora* and *R. pretiosa* were slightly more active in the presence of a competitor when *Aeshna* was present suggesting that an interaction between the species is possible when exposed to *Aeshna*.

Anti-predator behavior is important to vulnerable animals such as tadpoles that balance the need to forage against the risk of predation from a wide variety of predators. The results of my experiment emphasize the importance of the presence of a predator in regulating behavior of larval anurans. Behavioral adjustments in proximity of larval anurans to predators suggest that prey will adjust microhabitat location to avoid predators. These shifts in habitat could increase inter- and intraspecific competition within larval communities. Additionally, tadpoles failing to respond behaviorally to the presence of a predator or a competitor may experience greater mortality during the larval phase (Skelly 1994). *Rana aurora* and *R. pretiosa* show a strong behavioral response to an invertebrate predator, which may result in alteration of important life-

history characteristics associated with increased juvenile survival and ultimately reproductive potential.

Table 3.1. Experimental design of laboratory experiment. (RAAU = red-legged frog, RAPR = Oregon spotted frog).

Species and (# individuals)	Predator (present=P, absent=A)	# of replicates
RAAU (10)	P	4
RAAU (10)	A	4
RAPR (10)	P	4
RAPR (10)	A	4
RAAU (5), RAPR (5)	P	4
RAAU (5), RAPR (5)	A	4

Table 3.2. Mean temperature per treatment over the experiment. RAAU = red-legged frog and RAPR = Oregon spotted frog.

<i>Treatment</i>	<i>Mean temperature(°C)</i>	<i>S.E.</i>
RAAU no predator	18.67	0.08
RAPR predator	18.57	0.08
RAAU predator	18.61	0.08
Both no predator	18.64	0.08
Both predator	18.71	0.08
RAPR no predator	18.68	0.08

Table 3.3. Results from repeated measures ANOVA for the fixed effects of species and predator on tadpole activity during the experiment. Mean values used in analysis are from treatments with *R. aurora* and *R. pretiosa* alone. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

<i>Source</i>	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between subject			
Species	1	5.32	.006*
Predator	1	149.46	<0.0001*
Species*predator	1	1.2	.011*
Error	12		
Within subject			
Time	3	6.66	0.001*
Time*predator	3	1.03	0.392
Time*species	3	0.59	0.624
Time*spp*pred	3	1.43	0.249
Error (time)	36		

Table 3.4. Repeated measures ANOVA for the fixed effects of competitor and predator on the activity levels of *R. aurora* tadpoles. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

<i>Source</i>	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between subject			
Competitor	1	1.05	0.326
Predator	1	92.55	<0.0001*
Comp*pred	1	4.31	0.060
Error	12		
Within subject			
Time	3	12.68	<0.0001*
Time*comp	3	2.25	0.099
Time*pred	3	2.62	0.066
Time*comp*pred	3	1.08	0.370
Error (time)	36		

Table 3.5. Repeated measures ANOVA for the fixed effects of competitor and predator on the activity levels of *R. pretiosa* tadpoles. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

<i>Source</i>	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between subject			
Competitor	1	0.31	0.586
Predator	1	104.16	<0.0001*
Comp*pred	1	2.07	0.176
Error	12		
Within subject			
Time	3	6.17	0.002*
Time*comp	3	1.16	0.338
Time*pred	3	0.78	0.513
Time*comp*pred	3	1.18	0.331
Error (time)	36		

Table 3.6. Results from repeated measures ANOVA for the fixed effects of species and predator on tadpole spatial distribution during the experiment. Mean values used in analysis are from treatments with *R. aurora* and *R. pretiosa* alone. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

<i>Source</i>	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between subject			
Species	1	0.03	0.872
Predator	1	102.12	<0.0001*
Species*predator	1	0.02	0.886
Error	12		
Within subject			
Time	3	9.33	0.0001*
Time*predator	3	1.70	0.184
Time*species	3	5.26	0.004*
Time*spp*pred	3	2.06	0.123
Error (time)	36		

Table 3.7. Repeated measures ANOVA for the fixed effects of competitor and predator on the spatial distribution of *R. aurora* tadpoles. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

<i>Source</i>	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between subject			
Competitor	1	1.72	0.215
Predator	1	160.38	<0.0001*
Comp*pred	1	0.85	0.375
Error	12		
Within subject			
Time	3	1.85	0.156
Time*comp	3	1.03	0.392
Time*pred	3	3.89	0.166
Time*comp*pred	3	2.02	0.129
Error (time)	36		

Table 3.8. Repeated measures ANOVA for the fixed effects of competitor and predator on the spatial distribution of *R. pretiosa* tadpoles. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

<i>Source</i>	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between subject			
Competitor	1	3.63	0.081
Predator	1	126.61	<0.0001*
Comp*pred	1	0.09	0.776
Error	12		
Within subject			
Time	3	7.51	0.001*
Time*comp	3	4.38	0.010*
Time*pred	3	2.45	0.080
Time*comp*pred	3	0.94	0.433
Error (time)	36		

Table 3.9. One-way ANOVA for effect of species on initial average weekly weight measurement. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

Source	d.f.	F	P
Species	1	5.04	0.041*
Error	15		

Table 3.10. Repeated measures ANOVA for the fixed effects of species and predator on average weekly weights. Mean values used in analysis are from treatments with *R. aurora* and *R. pretiosa* alone. Significant P-values at $\alpha \leq 0.05$ are indicated by *.

Source	d.f.	F	P
Between subject			
Predator	1	4.56	0.054
Species	1	20.67	0.001*
Pred*species	1	1.18	0.299
Error	12		
Within subject			
Time	4	3845.77	<0.0001*
Time*species	4	11.71	<0.0001*
Time*pred	4	3.84	0.028*
Time*spp*pred	4	0.47	0.660
Error (time)	48		

Table 3.11. Repeated measures ANOVA for the effect of competitor and predator on the average weekly weights of *R. aurora* tadpoles. Significant P-values values at $\alpha \leq 0.05$ are indicated by *.

Source	d.f.	F	P
Between subject			
Competitor	1	0.08	0.782
Predator	1	0.10	0.755
Comp*pred	1	0.00	0.960
Error	12		
Within subject			
Time	4	869.63	<.0001*
Time*comp	4	0.40	0.647
Time*pred	4	0.34	0.681
Time*comp*pred	4	0.17	0.813
Error	48		

Table 3.12. Repeated measures ANOVA for the effect of competitor and predator on the average weekly weights of *R. pretiosa* tadpoles. Significant P-values values at $\alpha \leq 0.05$ are indicated by *.

<i>Source</i>	<i>d.f.</i>	<i>F</i>	<i>P</i>
Between subject			
Competitor	1	0.79	0.393
Predator	1	0.27	0.614
Comp*pred	1	1.16	0.302
Error	12		
Within subject			
Time	4	468.80	<.0001*
Time*comp	4	0.17	0.803
Time*pred	4	0.14	0.825
Time*comp*pred	4	0.77	0.454
Error	48		

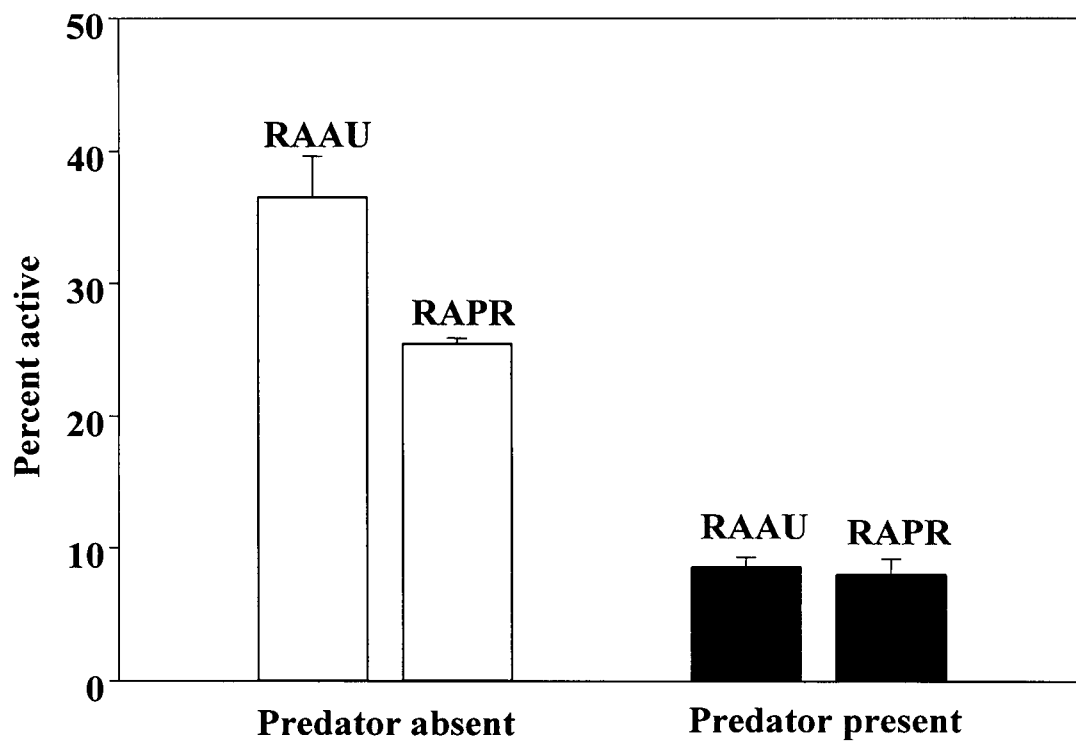


Figure 3.1. Summary of percent activity in treatments with and without *Aeshna* for *R. aurora* (RAAU) and *R. pretiosa* (RAPR). Histograms are mean values calculated over the duration of the experiment (± 1 S.E., $n = 4$).

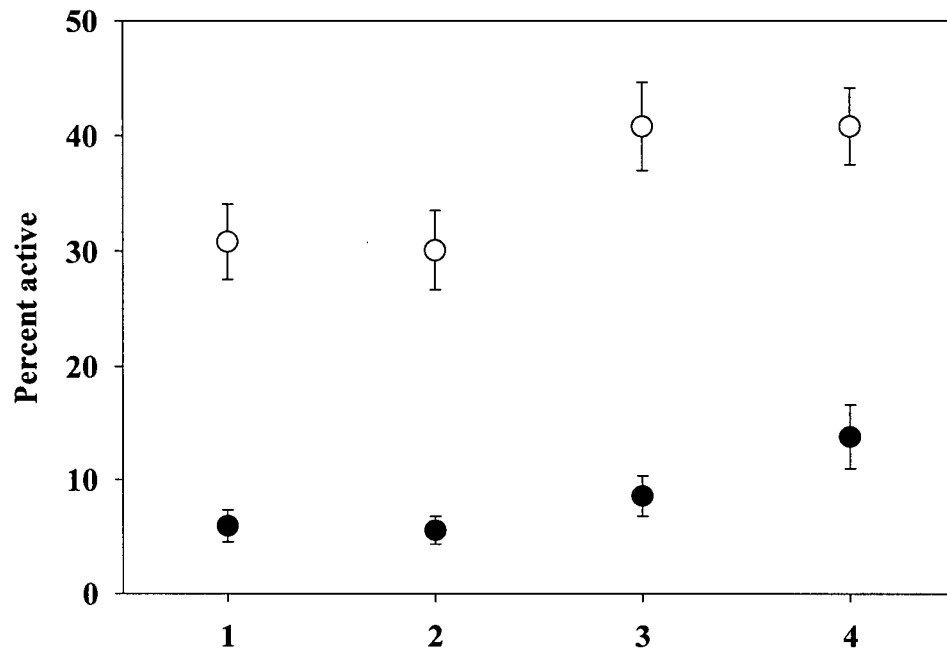


Figure 3.2. Mean percent larvae of *R. aurora* tadpoles active in treatments in the presence and absence of *Aeshna* by week of the experiment. Open circles are treatments without *Aeshna* and filled circles represent treatments with *Aeshna*. Points are means (± 1 SE) for the four replicate aquaria in each treatment.

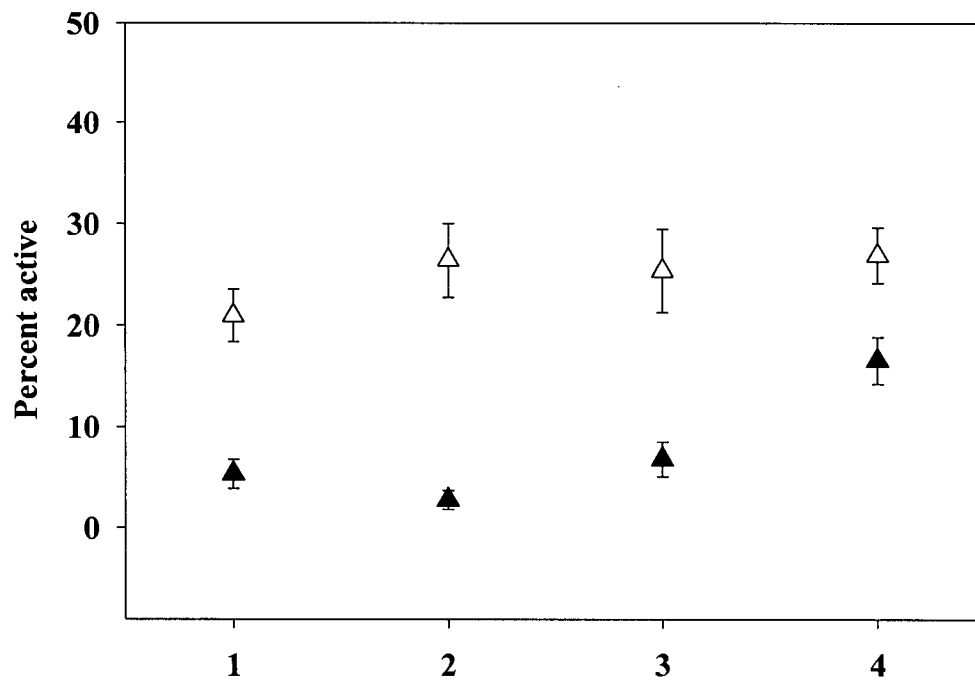


Figure 3.3. Mean percent larvae of *R. pretiosa* tadpoles near the predator in treatments in the presence and absence of *Aeshna* by week of the experiment. Open triangles are treatments without *Aeshna* and filled triangles represent treatments with *Aeshna*. Points are means (± 1 SE) for the four replicate aquaria in each treatment.

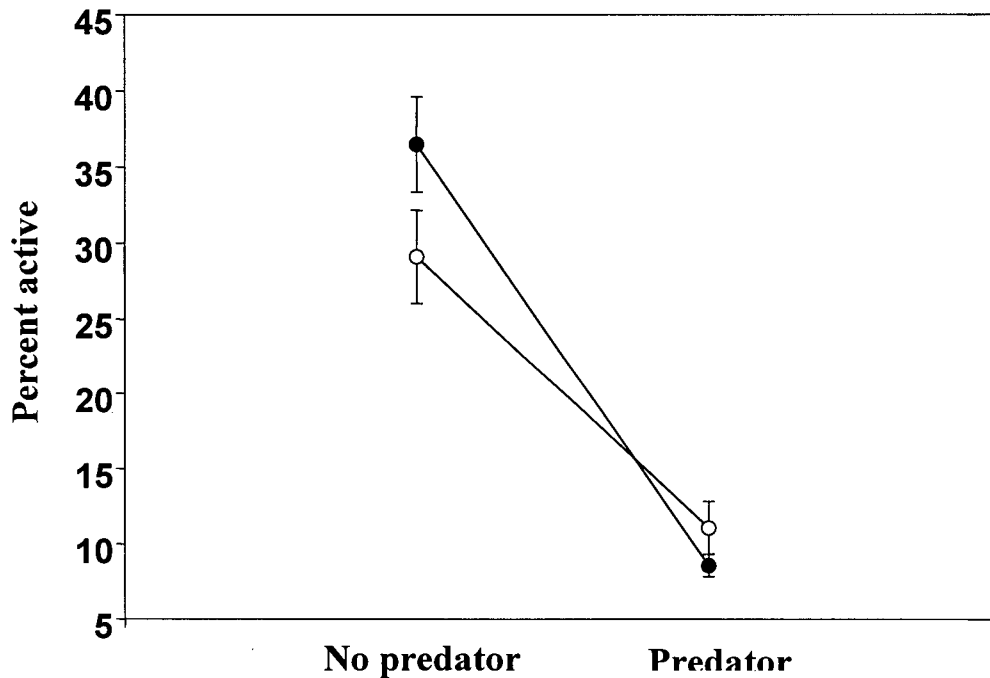


Figure 3.4. Mean percent larvae of *R. aurora* tadpoles active in treatments in the presence and absence of *Aeshna* for single species treatments (filled circles) and treatments containing both species (open circles). Points are mean values (± 1 SE) for the four replicate aquaria in each treatment.

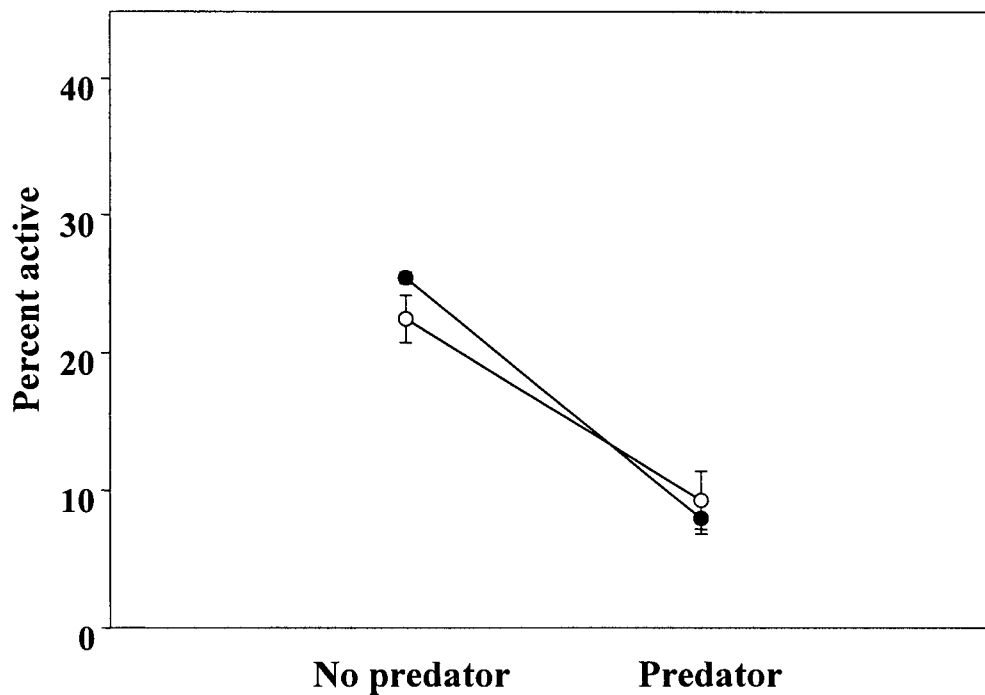


Figure 3.5. Mean percent larvae of *R. pretiosa* tadpoles active in treatments in the presence and absence of *Aeshna* for single species treatments (filled circles) and treatments containing both species (open circles). Points are mean values (± 1 SE) for the four replicate aquaria in each treatment.

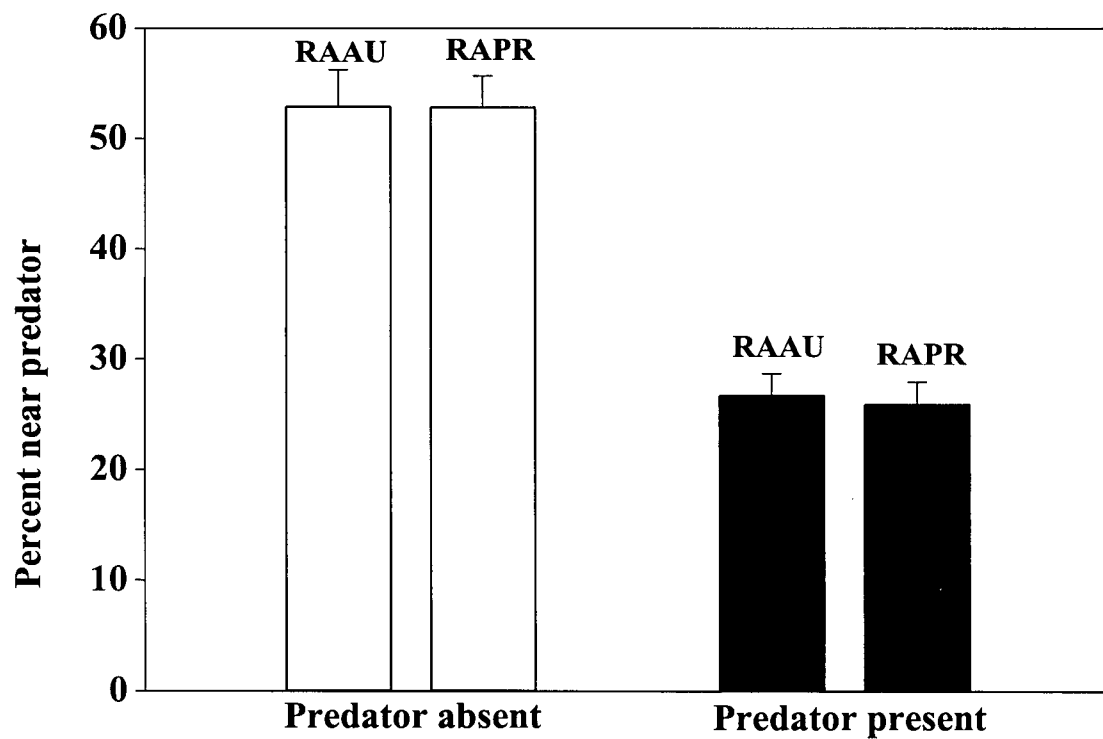


Figure 3.6. Summary of percent larvae near the predator in treatments with and without dragonfly larva for *R. aurora* (RAAU) and *R. pretiosa* (RAPR). Histograms are mean values calculated over the duration of the experiment (± 1 SE, $n = 4$).

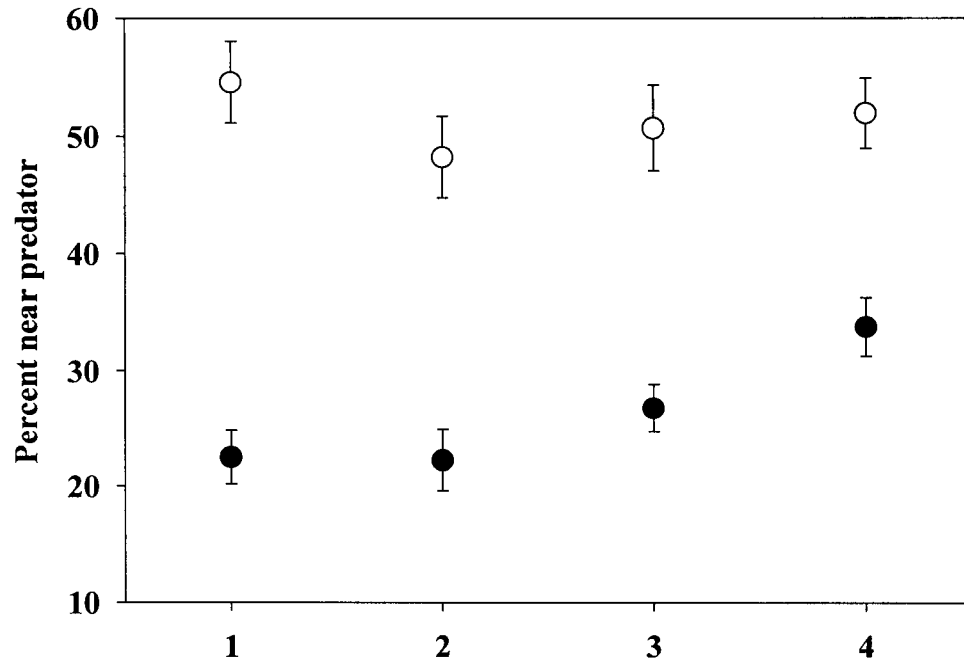


Figure 3.7. Mean percent larvae of *R. aurora* tadpoles near the predator in treatments in the presence and absence of *Aeshna* by week of the experiment. Upper points are treatments without *Aeshna* and lower points represent treatments with *Aeshna*. Points are means (± 1 SE) for the four replicate aquaria in each treatment.

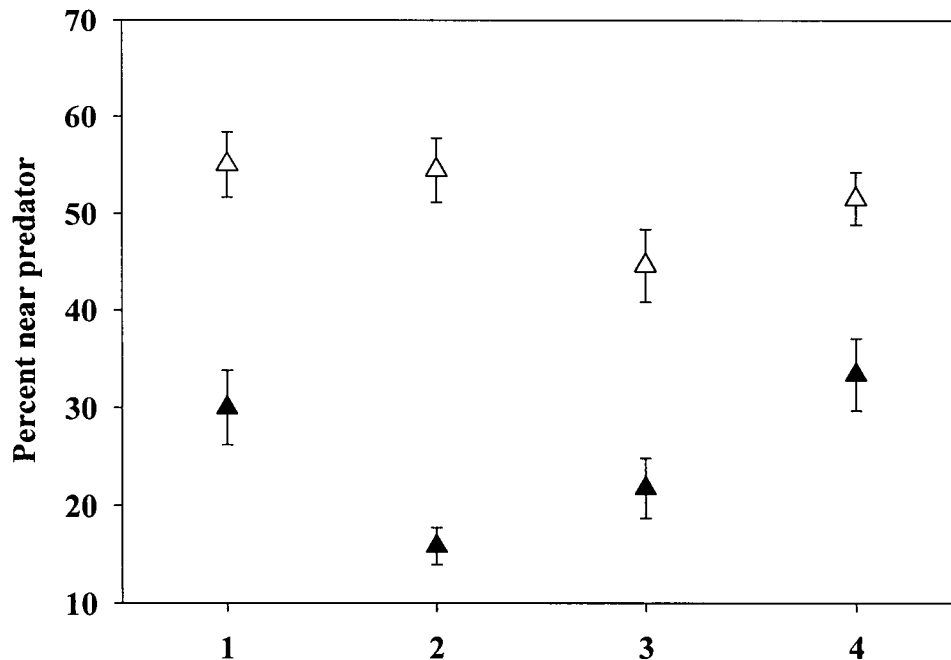


Figure 3.8. Mean percent larvae of *R. pretiosa* tadpoles near the predator in treatments in the presence and absence of *Aeshna* by week of the experiment. Upper points are treatments without *Aeshna* and lower points represent treatments with *Aeshna*. Points are means (± 1 SE) for the four replicate aquaria in each treatment.

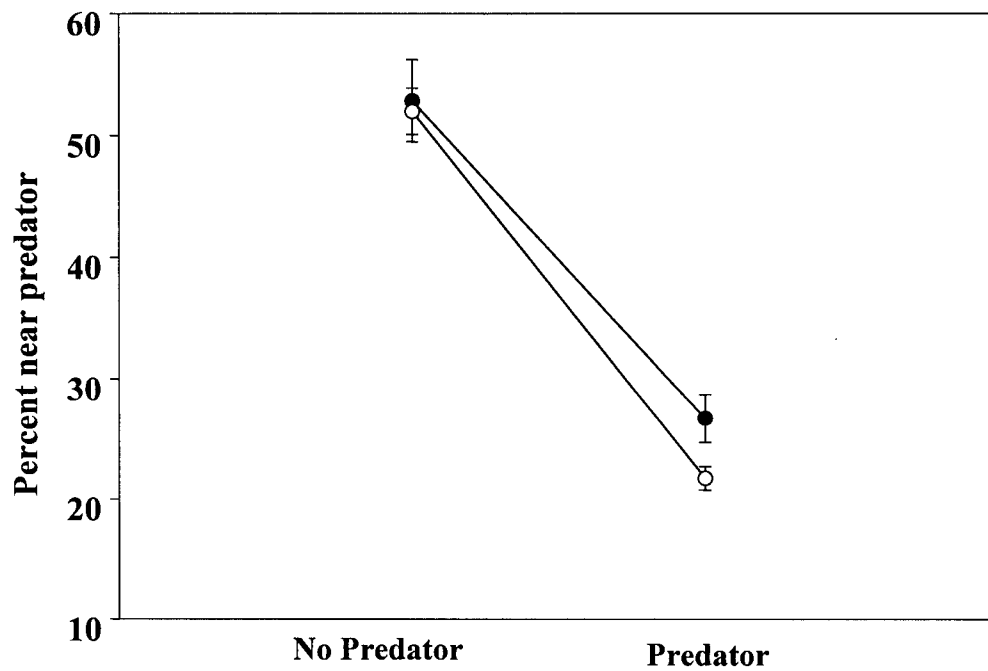


Figure 3.9. Mean percent larvae of *R. aurora* tadpoles near the predator in treatments in the presence and absence of *Aeshna* for single species treatments (filled circles) and treatments containing both species (open circles). Points are mean values (± 1 SE) for the four replicate aquaria in each treatment.

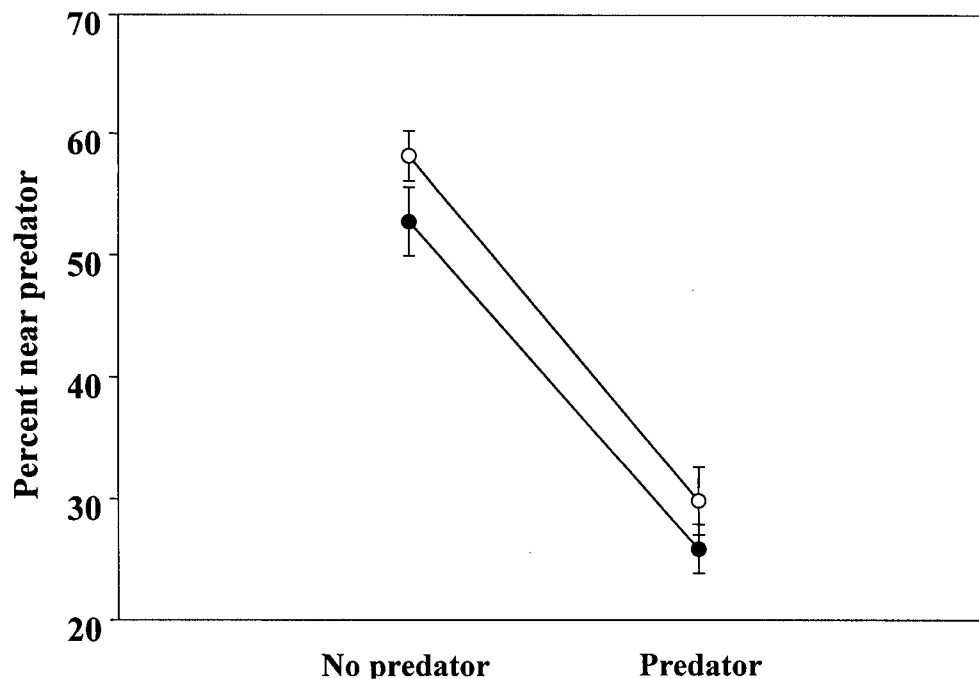


Figure 3.10. Mean percent larvae of *R. pretiosa* tadpoles near the predator in treatments in the presence and absence of *Aeshna* for single species treatments (filled circles) and treatments containing both species (open circles). Points are mean values (± 1 SE) for the four replicate aquaria in each treatment.

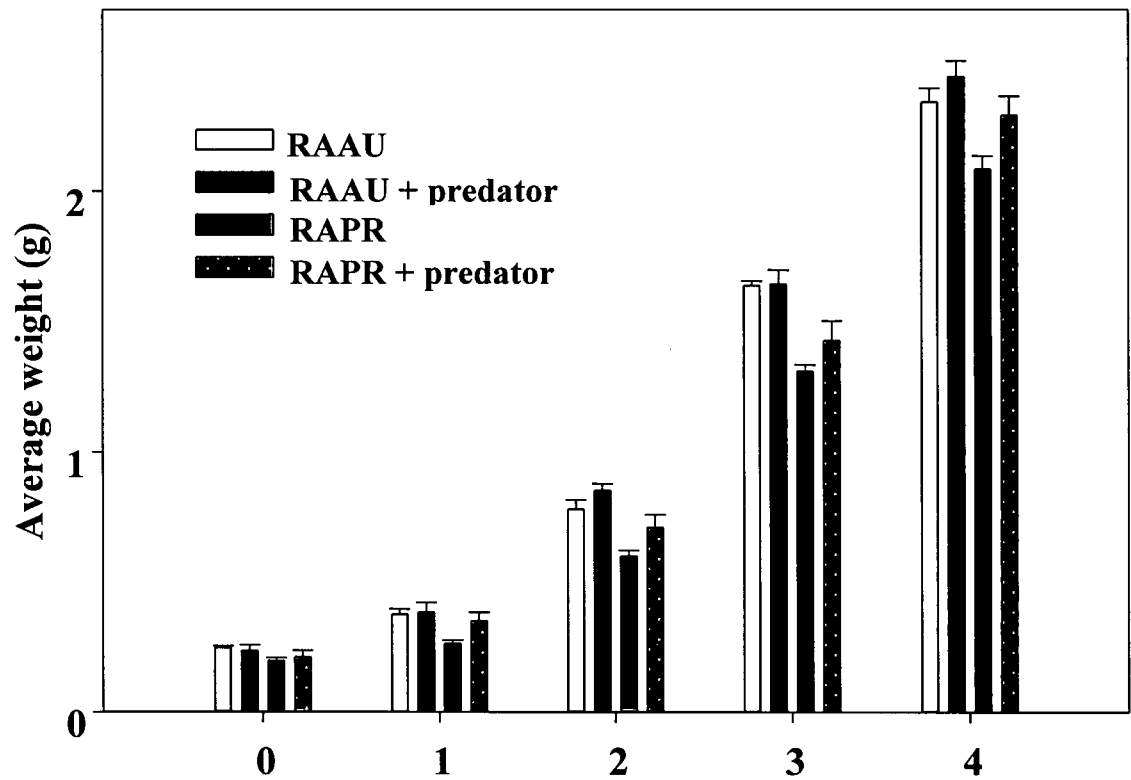


Figure 3.11. Mean weekly weights of *R. aurora* (RAAU) and *R. pretiosa* (RAPR) tadpoles in treatments with the species alone. Histograms are mean values (± 1 SE) for the four replicate aquaria in each treatment.

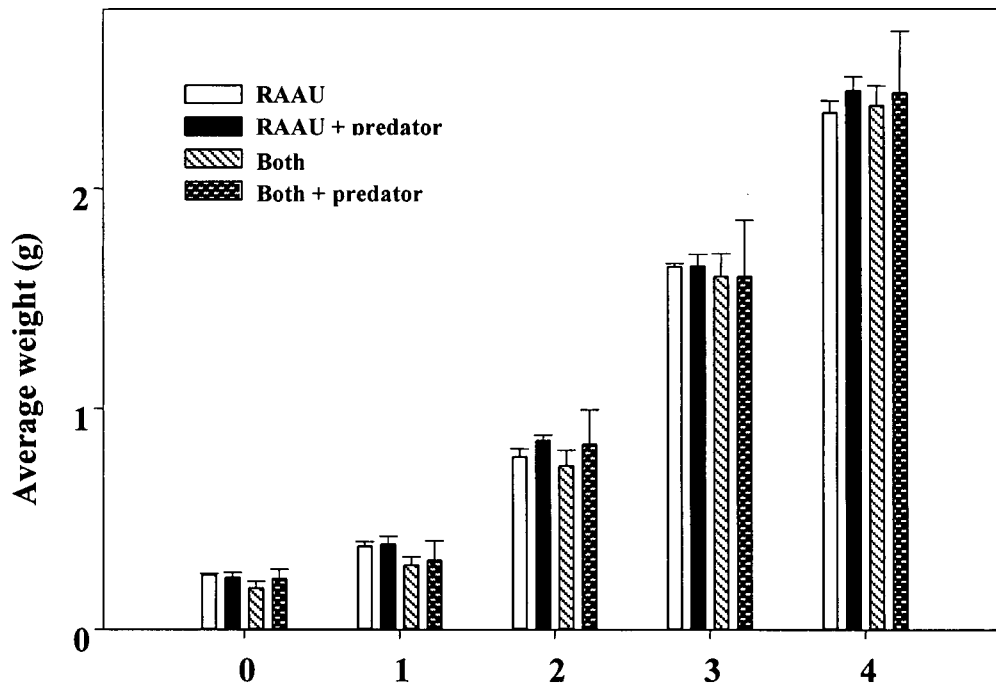


Figure 3.12. Mean weekly weights of *R. aurora* tadpoles in treatments alone (RAAU) and with *R. pretiosa* (Both). Histograms are mean values (± 1 SE) for the four replicate aquaria in each treatment.

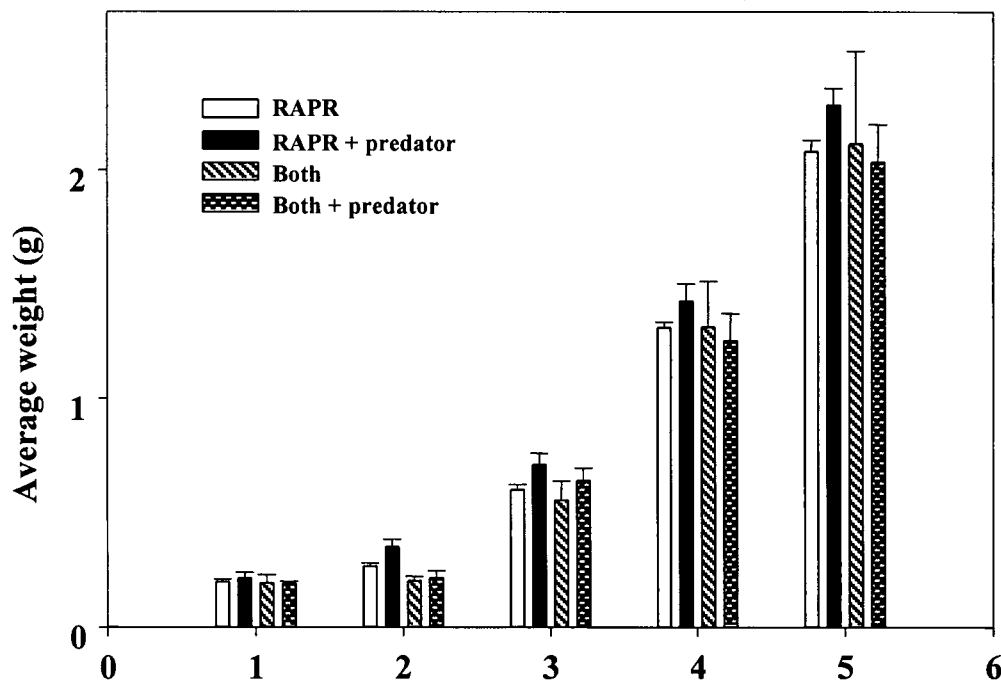


Figure 3.13. Mean weekly growth rates of *R. pretiosa* tadpoles in treatments alone (RAPR) and with *R. aurora* (Both). Histograms are mean values (± 1 SE) for the four replicate aquaria in each treatment.

Chapter 4. Conclusions, conservation considerations for Oregon spotted frogs and red-legged frogs, and direction for future research.

Concern has arisen in the conservation and scientific community during the last decade over the apparent global decline in amphibian populations. Studies have shown that amphibians existing in highland and northern latitudes have been among the most severely affected (Wake 1991). However, the causal mechanism behind the declines has been debated and many studies have been conducted to uncover the reason for these troubling observations in many species of frogs. A variety of subsequent studies have shown effects on amphibians from UV-B exposure (Blaustein et al. 1996, Blaustein et al. 1999, Kiesecker et al. 2001), introduced predators (Tyler et al. 1998a, Tyler et al. 1998b, Knapp and Matthews 2000), competition from introduced species (Kiesecker and Blaustein 1998), pathogens (Kiesecker and Blaustein 1999), climate change (Kiesecker et al. 2001) and habitat degradation (Marco et al. 1999). It is agreed upon that some of these factors produce a cumulative effect that impacts populations and species in different parts of the world to varying degrees, and additionally some factors interact to further complicate the situation.

The Pacific Northwest contains only a small fraction of amphibian species known worldwide. However, based on the total number of species occurring in temperate regions, the Pacific Northwest has the second highest richness of amphibians in the United States and the highest concentration of amphibian species in Canada (Walls et al. 1992). Most amphibians in this region are forest dwellers dependent on ponds, lakes, streams and wetlands for breeding sites. High levels of agricultural development within the Puget Sound Basin and Fraser River Lowlands has degraded water bodies and poses a large threat to the long-term stability of amphibian populations (Adams 1999). Understanding and planning recovery efforts for species

such as the Oregon spotted frog and the red-legged frog in the U.S. and Canada will require a holistic approach accounting for habitat requirements, environmental attributes of water bodies and species interactions. In this thesis I have addressed inter- and intraspecific interactions occurring during the larval period of *R. aurora* and *R. pretiosa*, both species of concern in Canada and the Pacific Northwest of the United States.

I have shown that both inter- and intraspecific competition as well as the risk of predation affects metamorphic characteristics of both Oregon spotted frogs and red-legged frogs. Some ecologists suggest that high mortality during the early larval phase of most tadpole communities lowers the density of individuals and creates a situation where competition is rarely the major driving force in community interactions (Hayes and Jennings 1986). Most evidence for competition comes from experimental enclosure studies (Licht 1967, Brockelman 1969, Morin 1986, Griffiths 1991, Kupferberg 1997). Regardless of the debate over the prevalence of the role of competition in structuring communities, species sharing the same resources must interact within a community. *Rana aurora* and *R. pretiosa* co-occur and potentially compete for resources within larval rearing areas. Therefore, the competitive relationship between species in a community warrants investigation as a potential influencing factor in larval community processes.

My study demonstrated that varying the threat of predation and presence of a competitor in the larval rearing environment of *R. pretiosa* and *R. aurora* alters important metamorphic characteristics. Because each of these traits are associated with improved survival as juveniles and better reproduction as adults it is also likely that competition and predation affect fitness overall. The mechanism likely responsible for changes in metamorphic characteristics of these species is discussed in Chapter 3 of this thesis. Although intensity of antipredator behavior may be dampened in the larger 1000L-mesocosm habitat, these alterations in behavior are likely the

mechanism responsible for differences in metamorphic characteristics between treatments.

Lower activity levels likely contributed to slower development exhibited in this experiment by extended larval periods in predator treatments. Additionally, studies indicate that antipredator behavior is highly dependent on the specific prey and predator involved (Relyea 2001a, Relyea 2001b).

Previous work indicates that the historic range of *R. aurora* and *R. pretiosa* has been severely altered and wetlands degraded (Hayes 1994, Adams 1999). Specific predator-prey relationships for *R. aurora* and *R. pretiosa* should be investigated to determine if a similar relationship exists in the presence of other predators, including trout, bullfrogs (*Rana catesbeiana*), greenfrogs (*Rana clamitans*), garter snakes and mammalian predators. Several studies have shown bullfrogs to have a negative impact on larval and juvenile *R. aurora* (Kiesecker and Blaustein 1997, Lawler et al. 1999). Petranka and Kennedy (1999) found that greenfrog tadpoles reduce survival of wood frog eggs by directly eating freshly laid egg masses. The introduction of non-native fish in California's Sierra Nevada protected parks was correlated with declines of the mountain yellow-legged frog (*Rana muscosa*), a species highly dependent on the aquatic environment (Knapp and Matthews 2000), much like *R. pretiosa*. These studies suggest that future research on *R. pretiosa* and *R. aurora* in the Pacific Northwest and southwestern British Columbia should address impacts of the introduction of these non-native species. Only by understanding the entire community can we attempt to predict how predator-prey relationships will influence population dynamics in the long-term.

The artificial pond experiment indicated that when a predator was present, Oregon spotted frogs performed better in the presence of red-legged frogs than they did alone. The eggs for this experiment were collected from a sympatric population of Oregon spotted frogs and red-legged frogs. Although the breeding time occurs at the same time during the year, *R. pretiosa*

takes longer to metamorphose than *R. aurora*. Under experimental conditions, *R. aurora* has a facilitative effect on *R. pretiosa*. Licht (1974) noted that Oregon spotted frogs produced a larger proportion of metamorphs from a smaller number of hatchlings than did red-legged frogs. Invertebrate predators may be attracted to the greater activity of red-legged frogs and cause greater mortality during the larval development phase than on Oregon spotted frogs. This argument could pose one answer to Licht's observation regarding high mortality of red-legged larvae during the tadpole stage.

Conservation and recovery efforts for these species should account for the fact that *R. pretiosa* performs better in the presence of *R. aurora* (at least in a test of individuals from a sympatric population). In order for conservation efforts to be as successful as possible in Canada, the interaction of these species, as well as other native species should be considered. An important goal of conservation efforts involves reducing the degree of isolation between current populations of Oregon spotted frogs. If new populations within the historic range of *R. pretiosa* were created, recovery efforts should consider that interspecific interactions could perhaps benefit the target species for conservation. For example, by introducing red-legged frogs, the survival of *R. pretiosa* may be improved and the population may have a better likelihood of establishing. Interactions similar to the one discovered in this thesis work may exist with other native species rearing with *R. aurora* and *R. pretiosa*. Species within communities interact in complex manners producing in some instances negative impacts on one another (e.g. predation), but also may have positive effects on each other as shown for *R. aurora* and *R. pretiosa* under the threat of nonlethal predation.

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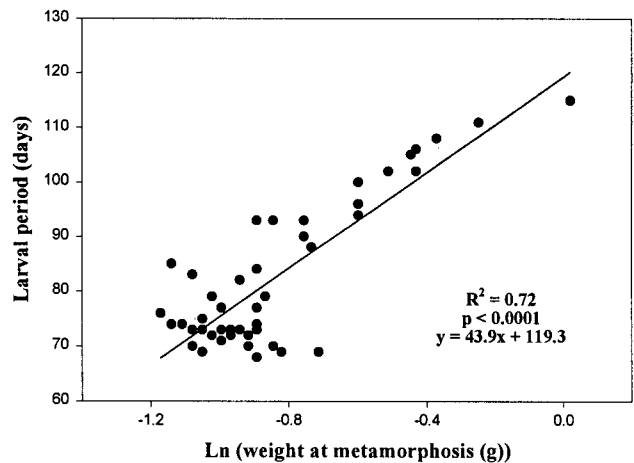
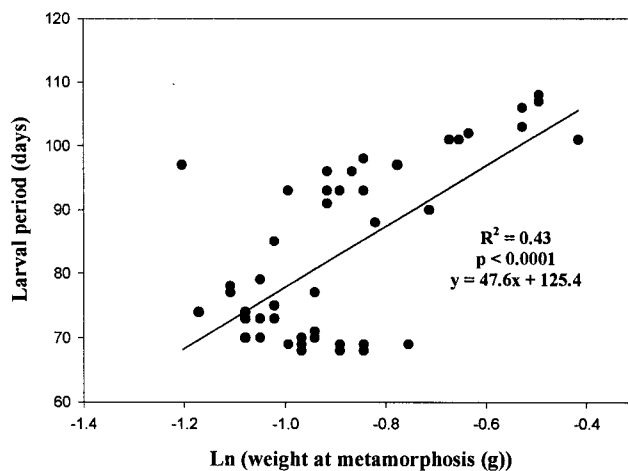
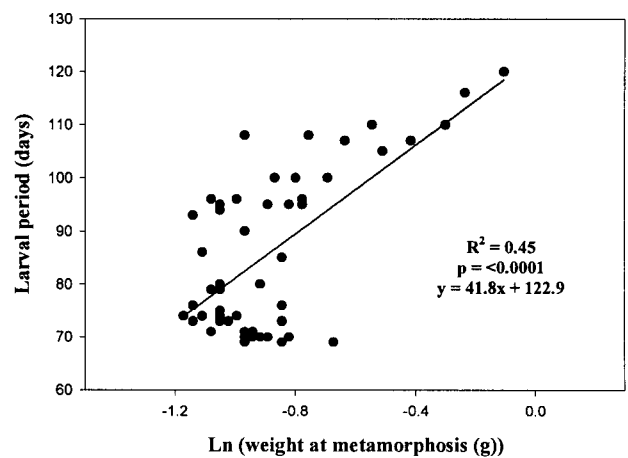
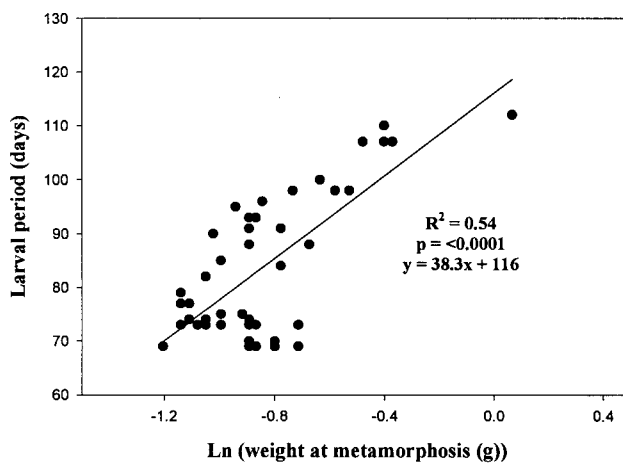
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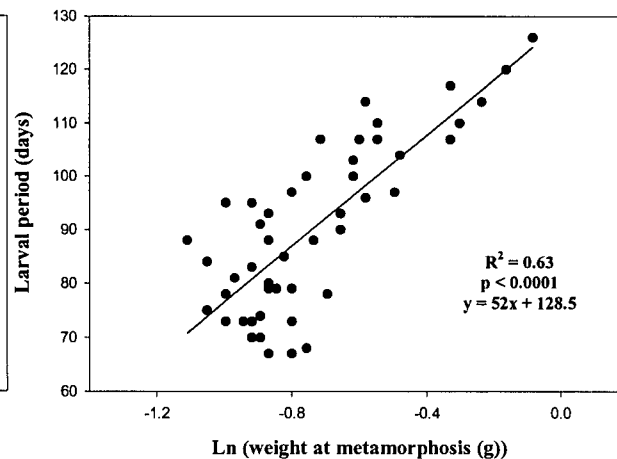
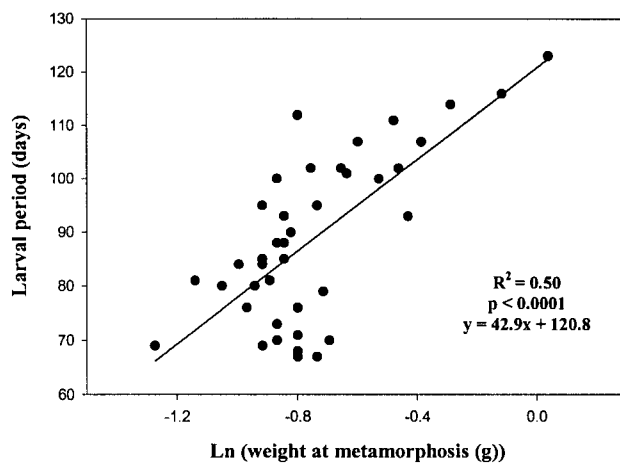
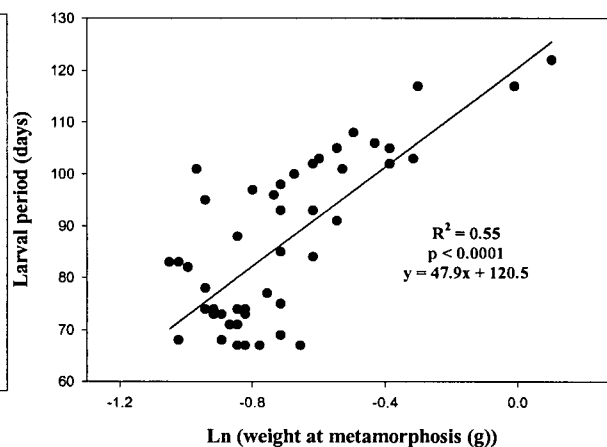
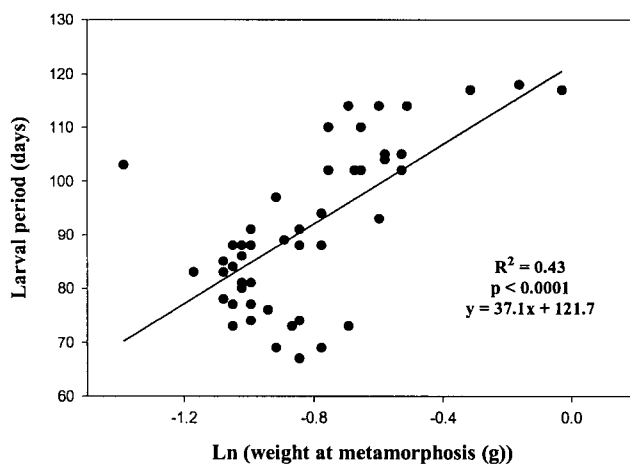
Appendix 1: Relationship between weight at metamorphosis and length of the larval period for *R. aurora*. R^2 values are indicated on each graph.

Red-legged frog alone in the absence of *Aeshna*.



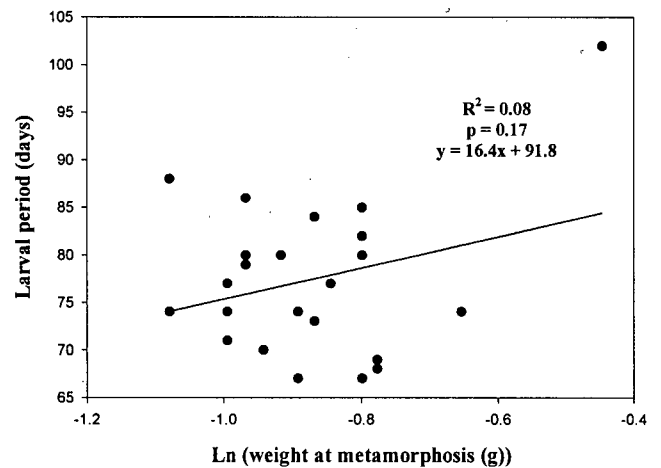
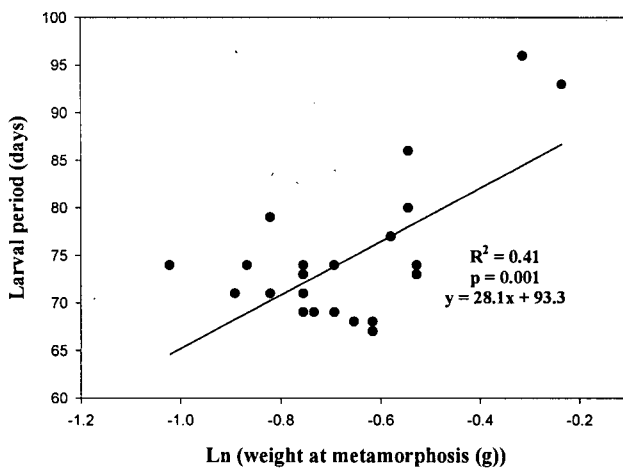
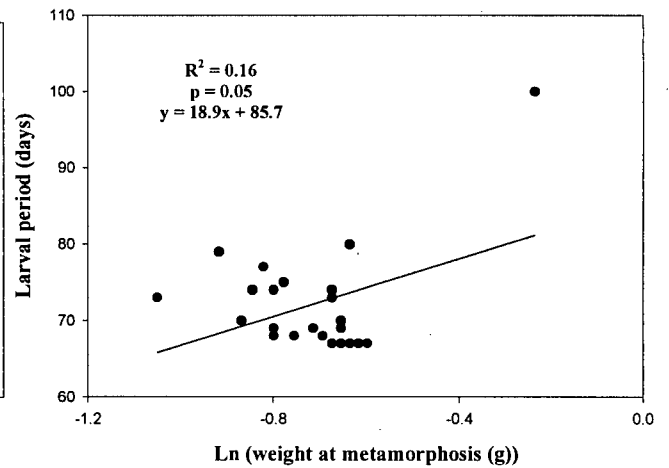
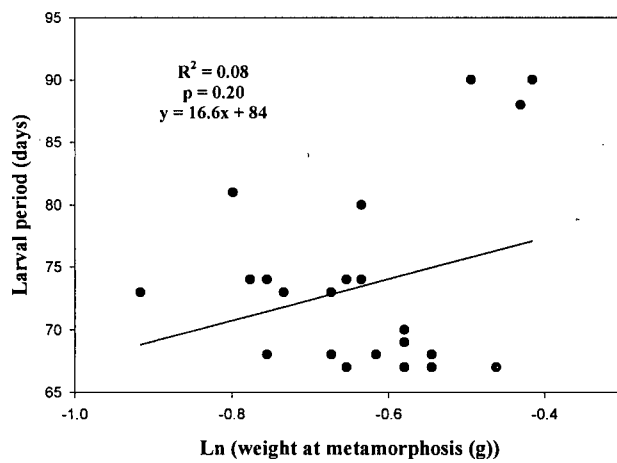
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Red-legged frogs alone in the presence of *Aeshna*.



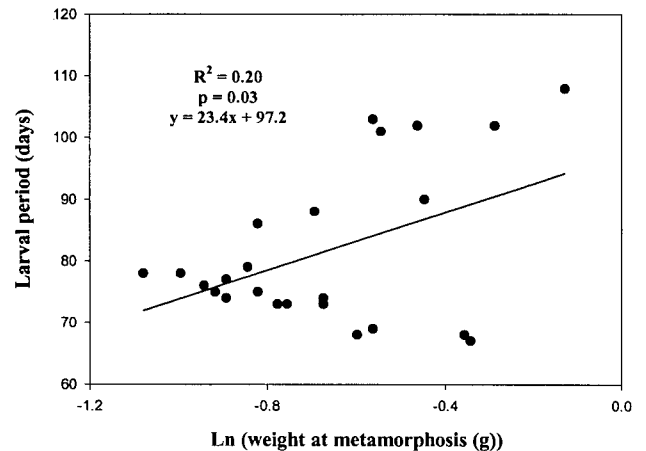
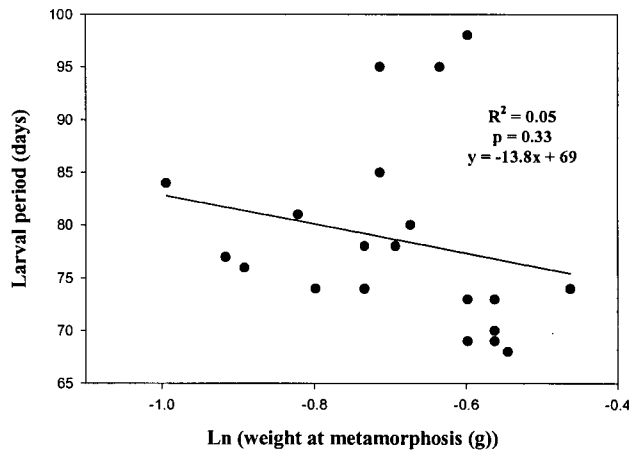
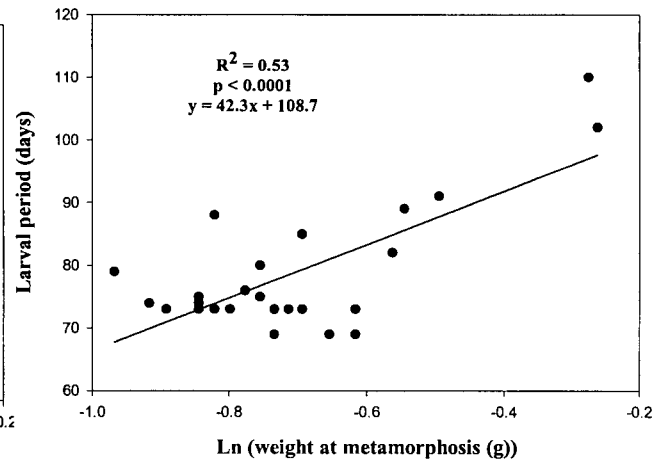
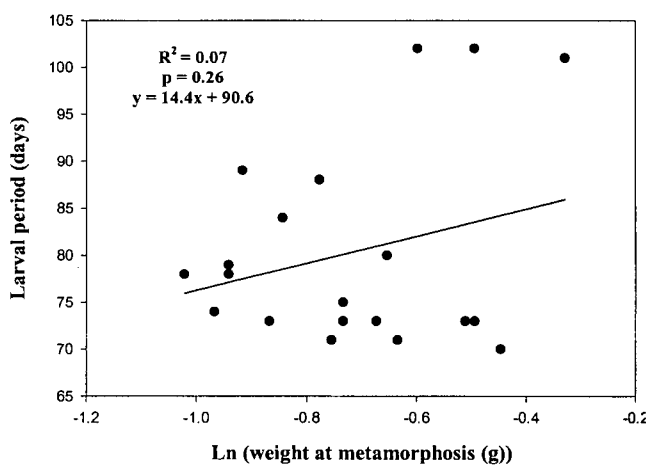
Appendix 1 cont.: Relationship between weight at metamorphosis and length of the larval period for *R. aurora*. R^2 values are indicated on each graph.

Red-legged frogs in the absence of *Aeshna* and presence of Oregon spotted frogs at low density.



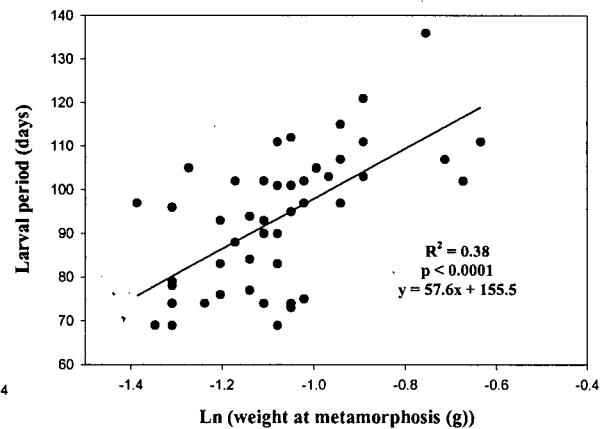
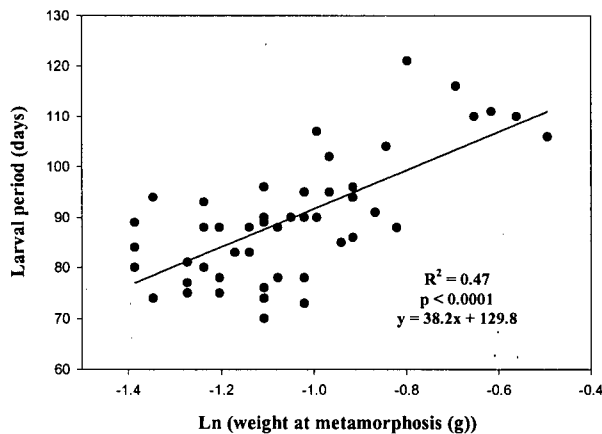
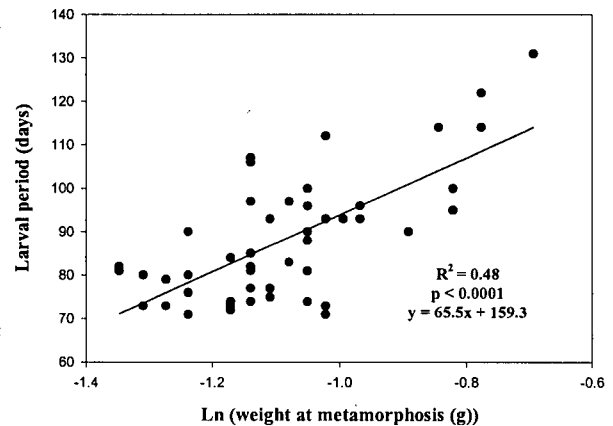
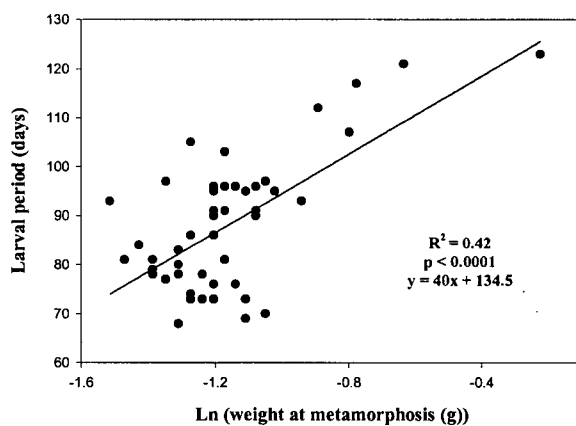
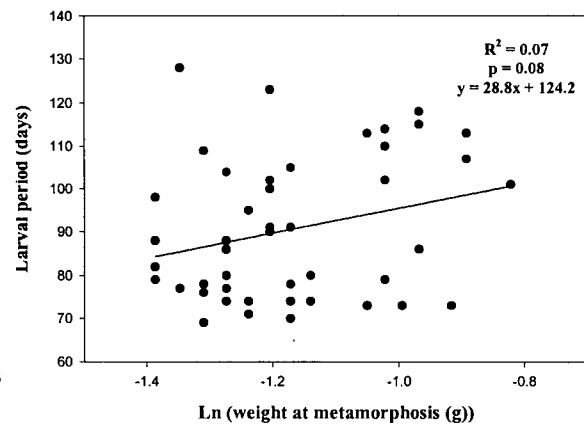
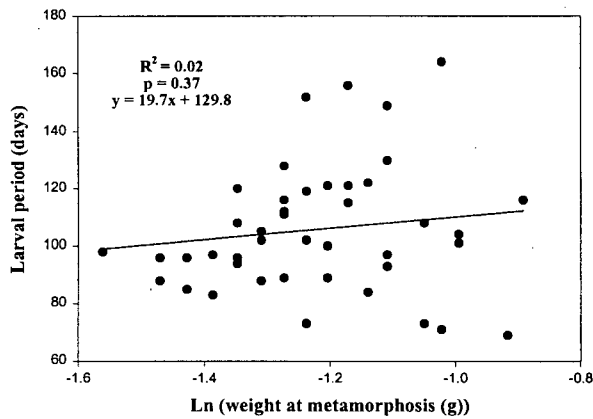
Appendix 1 cont.: Relationship between weight at metamorphosis and length of the larval period for *R. aurora*. R^2 values are indicated on each graph.

Red-legged frogs in the presence of *Aeshna* and the presence of Oregon spotted frogs at low density.



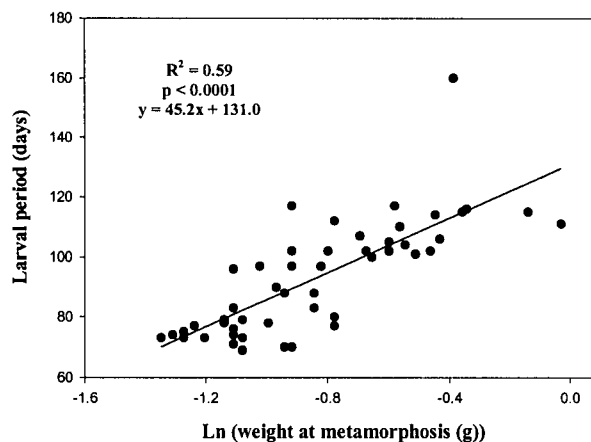
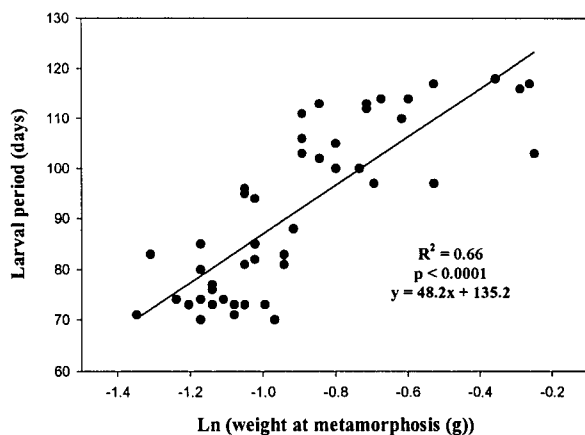
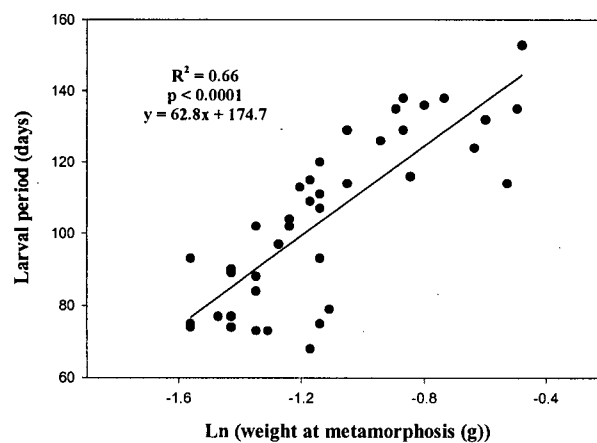
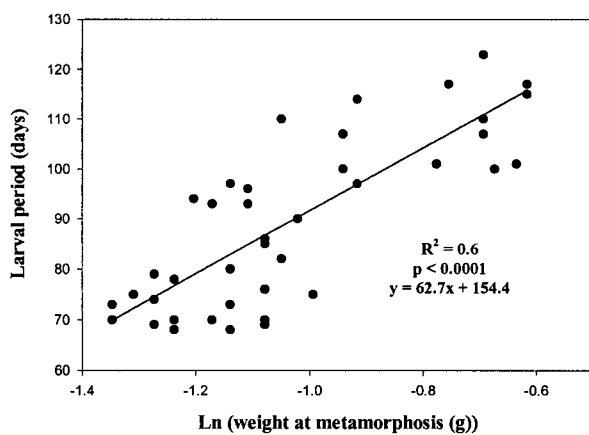
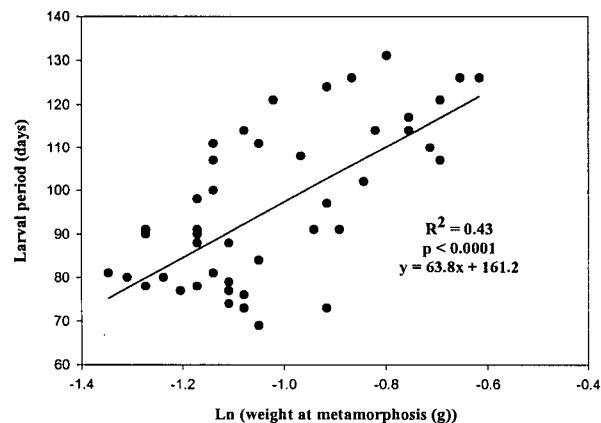
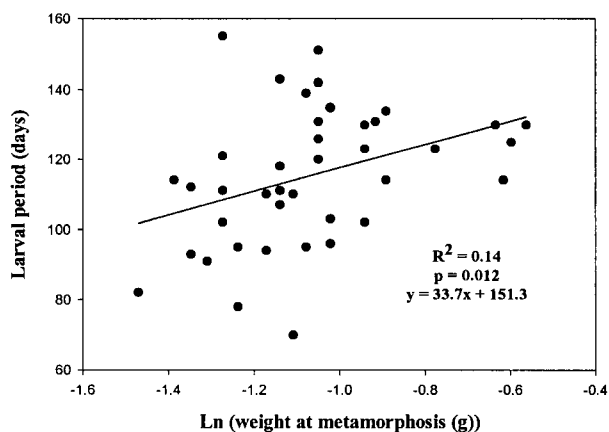
Appendix 1 cont.: Relationship between weight at metamorphosis and length of the larval period for *R. aurora*. R^2 values are indicated on each graph.

Red-legged frogs in the absence of *Aeshna* and presence of Oregon spotted frogs at high density.



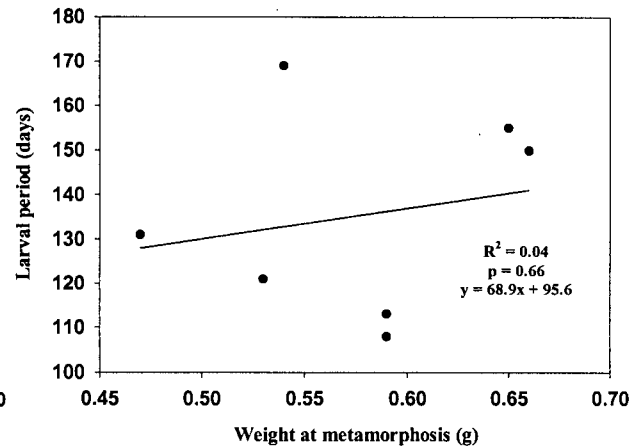
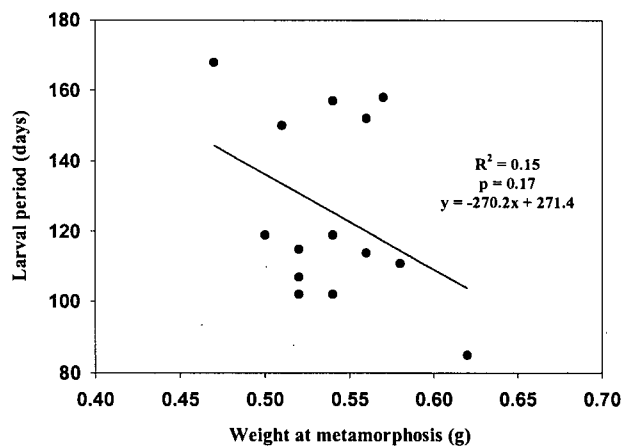
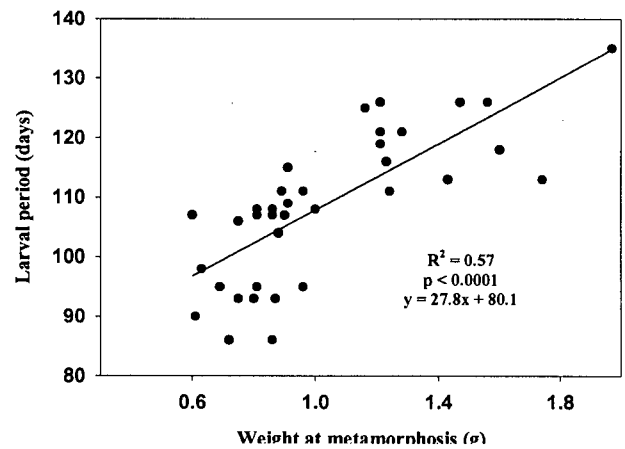
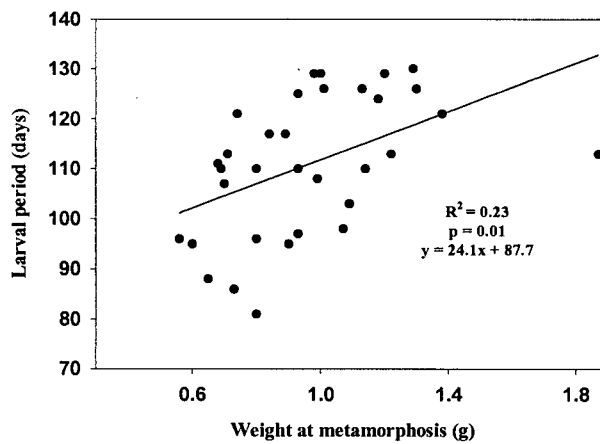
Appendix 1 cont.: Relationship between weight at metamorphosis and length of the larval period for *R. aurora*. R^2 values are indicated on each graph.

Red-legged frogs in the presence of *Aeshna* and presence of Oregon spotted frogs at high density.



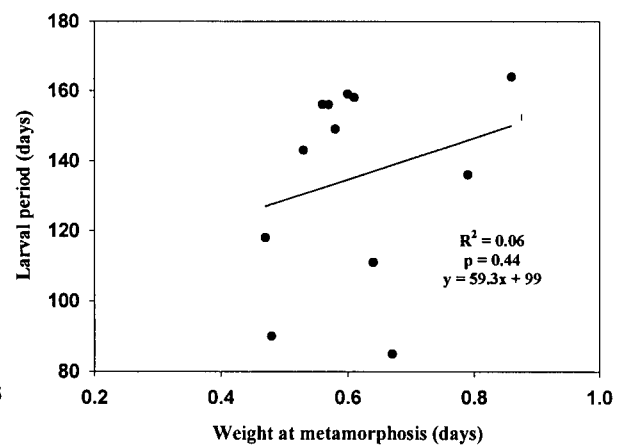
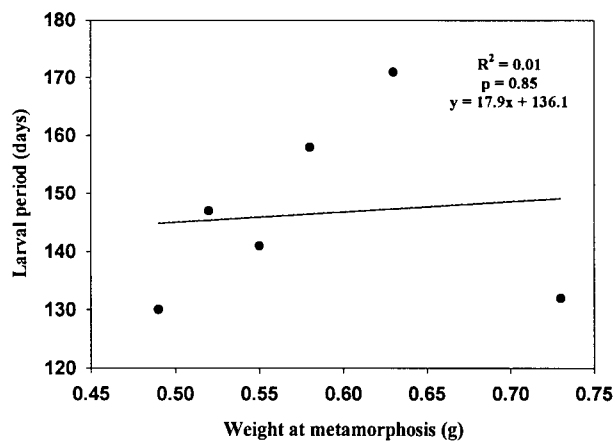
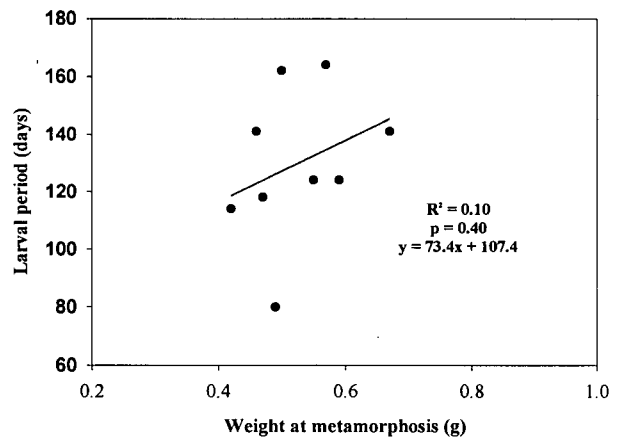
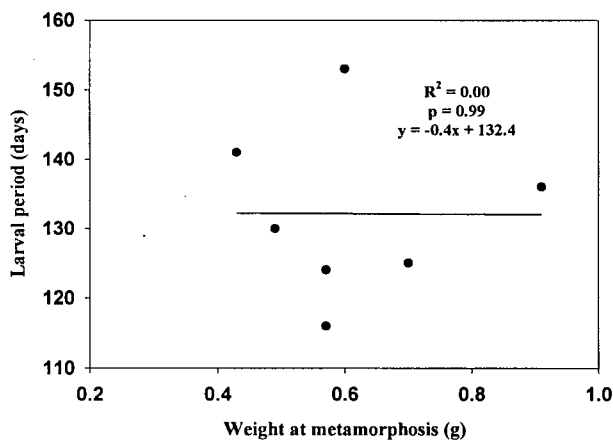
Appendix 2: Relationship of weight at metamorphosis and length of the larval period for *R. pretiosa*. R^2 values are indicated on each graph.

Oregon spotted frog alone in the absence of *Aeshna*.



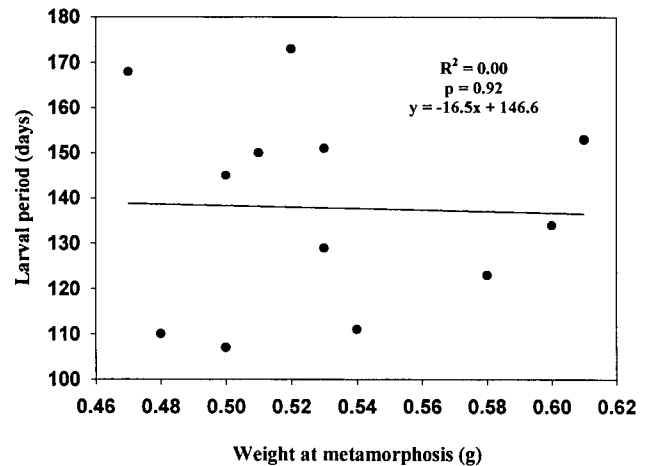
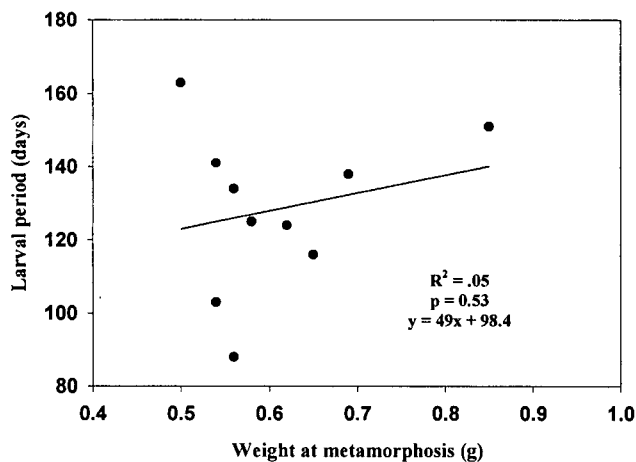
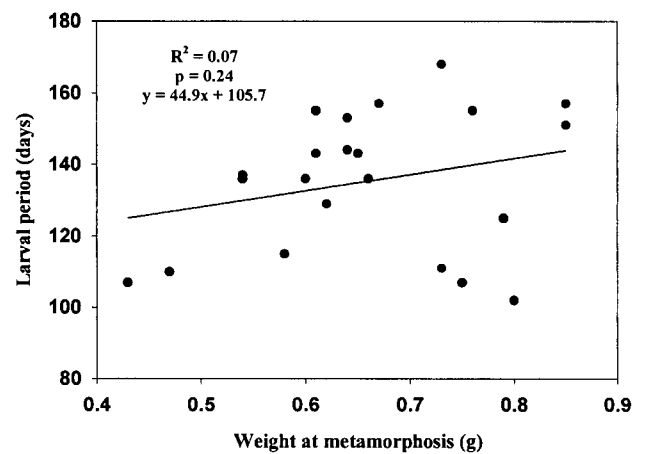
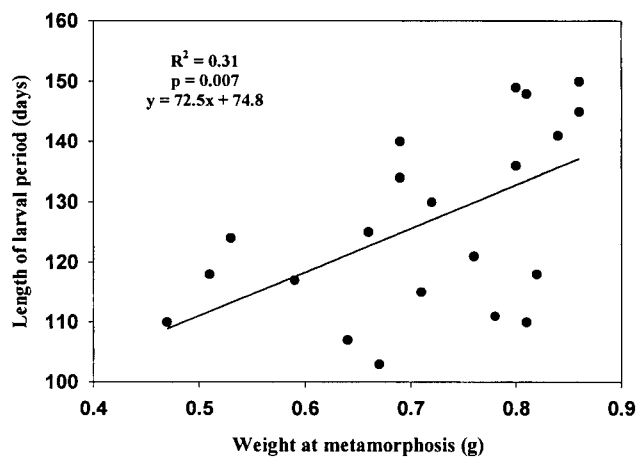
Appendix 2 cont.: Relationship of weight at metamorphosis and length of the larval period for *R. pretiosa*. R^2 values are indicated on each graph.

Oregon spotted frog alone in the presence of *Aeshna*.



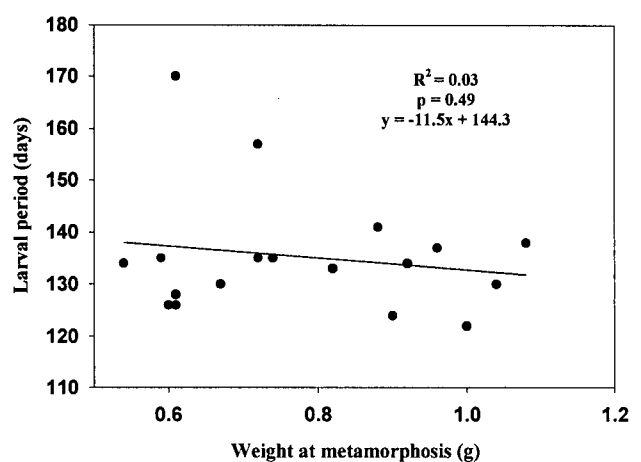
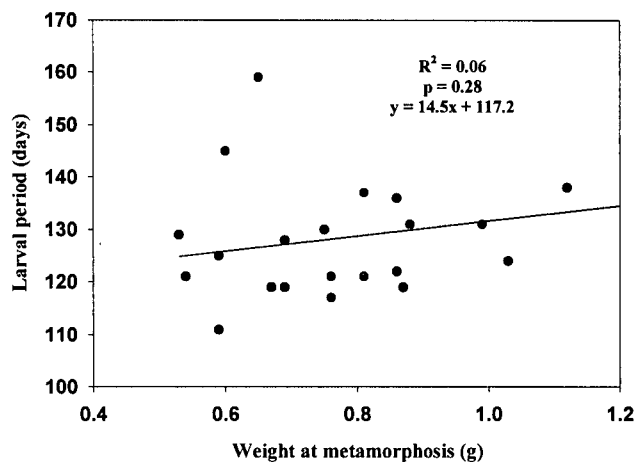
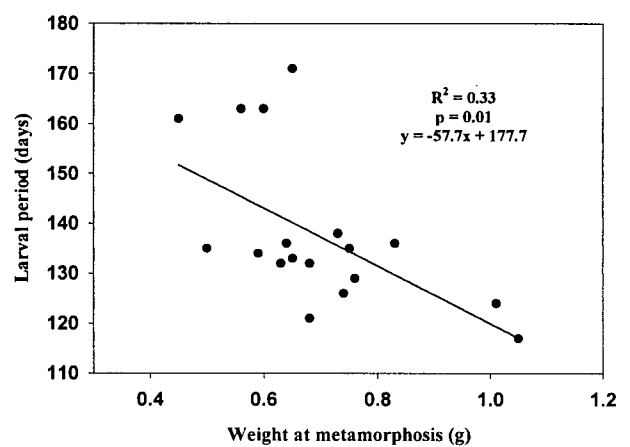
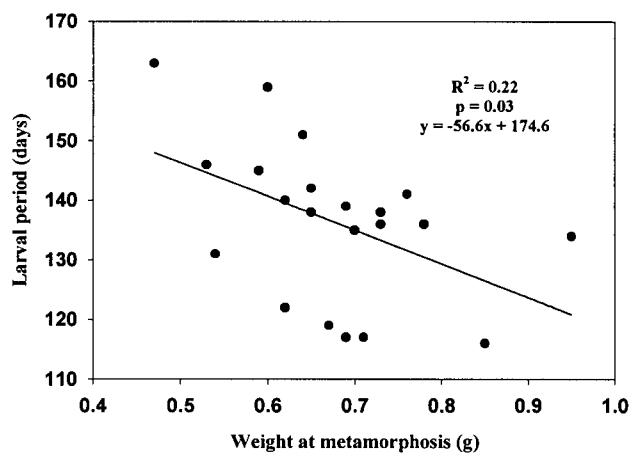
Appendix 2 cont.: Relationship of weight at metamorphosis and length of the larval period for *R. pretiosa*. R^2 values are indicated on each graph.

Oregon spotted frog with red-legged frogs in the absence of *Aeshna* at low density.



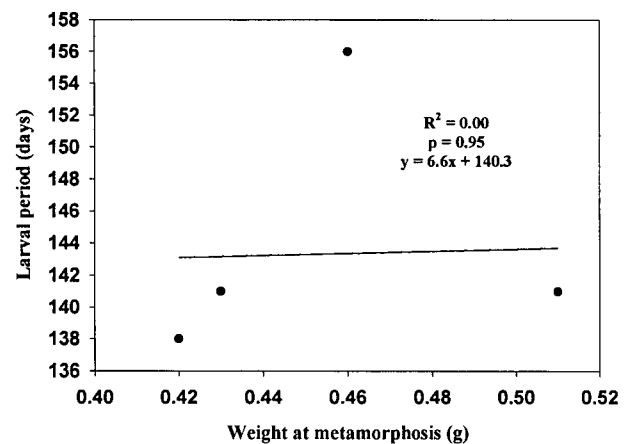
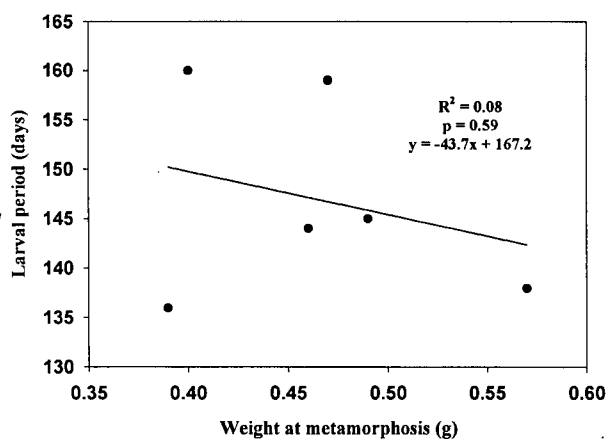
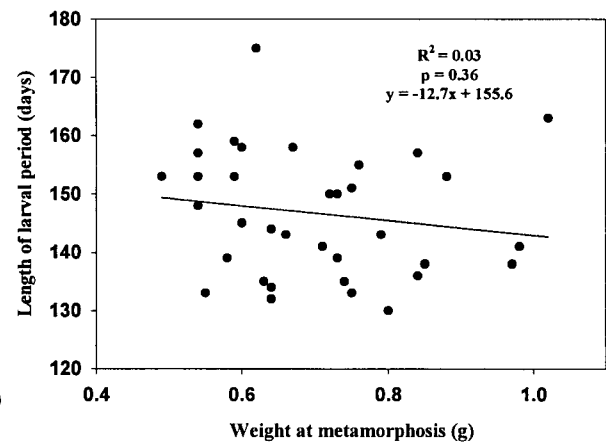
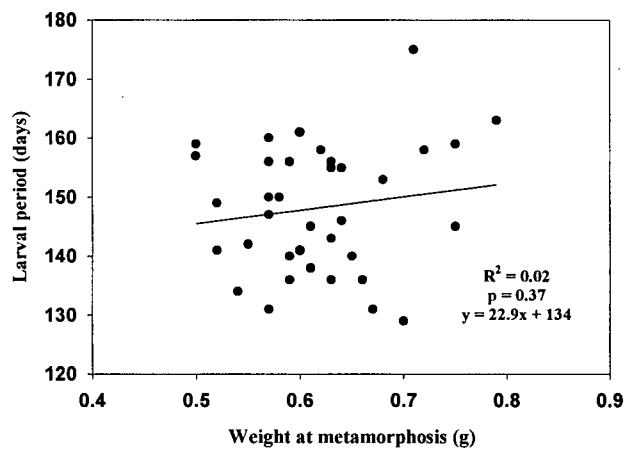
Appendix 2 cont.: Relationship of weight at metamorphosis and length of the larval period for *R. pretiosa*. R^2 values are indicated on each graph.

Oregon spotted frog with red-legged frogs in the presence of *Aeshna* at low density.



Appendix 2 cont.: Relationship of weight at metamorphosis and length of the larval period for *R. pretiosa*. R^2 values are indicated on each graph.

Oregon spotted frog with red-legged frogs in the absence of *Aeshna* at high density.



Appendix 2 cont.: Relationship of weight at metamorphosis and length of the larval period for *R. pretiosa*. R^2 values are indicated on each graph.

Oregon spotted frog with red-legged frogs in the presence of *Aeshna* at high density.

