AN ABSTRACT OF THE THESIS OF

D. Grant Hokit for the degree of Doctor of Philosophy in Zoology presented on August 18, 1994.

Title: Ecological Aspects of Kin Discriminating Behavior With Implications of Functional Value

Abstract approved: Andrew R. Blaustein

To assess the context dependence of kin discriminating behavior, I examined kin-biased aggregation behavior in tadpoles of *R. cascadae* in different ecological conditions. I manipulated food distribution, predator presence, thermal heterogeneity, and relatedness in a multifactorial mesocosm experiment. All four factors interacted to influence tadpole dispersion. My results suggest that kinship is an important factor in aggregation behavior dependent upon ecological conditions.

Kin-biased predator defense mechanisms have been proposed as a possible functional explanation for kin discrimination in anuran larvae. Tadpoles may better cooperate in predator vigilance while in kin groups or release kin specific alarm pheromones when attacked by a predator. I examined predator avoidance and alarm response behavior in tadpoles of the Cascades frog (*Rana cascadae*) and tested whether such behavior is influenced by kinship factors. I found no evidence of an alarm...
response behavior in *R. cascadae*. My results suggest that crushed tadpoles appear to initiate a feeding response rather than an alarm response as has been previously proposed.

Kin-biased competitive interactions have been proposed as a possible functional explanation for kin discrimination in anuran larvae. Tadpoles may direct competitive interactions away from kin. I examined the role of kinship in growth and development of tadpoles of the Cascades frog (*Rana cascadae*) in both laboratory and field studies. In the laboratory, individuals reared in kin groups had a significantly smaller mass at metamorphosis than individuals reared in mixed groups. However, kinship effects in the field depended upon the treatment context. Depending upon tadpole density and access to flocculent substrate, tadpoles survived better (after adjusting for differences in mass) in kin groups than in mixed groups.

My results demonstrate that kinship factors can affect growth and development in tadpoles, depending upon the ecological conditions. Furthermore, my results provide a functional explanation for the kin discriminating behavior observed in *R. cascadae* and suggest why such behavior may be context dependent.
Ecological Aspects of Kin Discriminating Behavior With Implications of Functional Value

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Ecological Aspects of Kin Discriminating Behavior With Implications of Functional Value

General Introduction

Population and community level phenomena must ultimately be the result of interactions between individual organisms and/or between individuals and their resources. However, in the past, individual characteristics were seldom represented in most ecological models. More recently, individual variation has been recognized as an important component in population and community level models (Hassell and May 1985; Lomnicki 1988).

Variation in behavioral patterns may particularly influence interactions between individuals (e.g. Gilliam 1987; Lima and Dill 1990; Werner 1992). Social behavior, loosely defined as any behavioral interaction between conspecifics, can significantly influence population and community level phenomena (e.g. Wilson 1975; Clutton-Brock and Albon 1984). Such behavior can influence the degree of competition between conspecific or heterospecific individuals, the amount and nature of predation, and the distribution of individuals. Furthermore, social behavior affects the reproductive interactions between individuals and, therein, directly influences the fitness of individuals. Thus, social
behavior can have profound ecological and evolutionary consequences.

Perhaps the most significant contribution to the study of the evolution of social behavior has been Hamilton's (1964a,b) inclusive fitness concept. Hamilton proposed that an organism may increase its contribution to the future gene pool of a population either by producing its own progeny (direct fitness), or by helping relatives produce offspring (indirect fitness). These two fitness components combine to determine the inclusive fitness of the individual.

The concept of inclusive fitness has most often been used to explain apparent altruistic behaviors; behaviors in which the actor sacrifices some degree of direct fitness for the benefit of other individuals. Inclusive fitness theory predicts that such behaviors will only persist (be selected for) if the recipient of the benefit is, to an appropriate degree, a relative of the actor. The actor, although sacrificing direct fitness, gains indirect fitness through the enhanced survival of relatives. The degree to which an individual's indirect fitness increases by helping a relative is proportional to the coefficient of relatedness (r). Hamilton condensed his quantitative model into the simple rule; $B/C > 1/r$, where $B$ is the gain (in terms of fitness) the relative receives and $C$ is the sacrifice the actor makes. From this rule we would expect organisms to help other conspecifics only when there exists a
degree of relatedness such that the inequality of the model holds true.

However, Hamilton's rule and inclusive fitness concept are not restricted to explaining altruistic behaviors (Hamilton 1964a,b). The rule has been generalized to incorporate any type of behavioral interaction between individuals or groups of individuals (e.g. Maynard Smith 1964; West-Eberhard 1975; Michod 1982). Hamilton's rule may be generalized as follows:

$$\Delta W_p + \sum r \Delta W_a > 0$$

where; $\Delta W_p$ = the change in direct fitness (positive or negative) of the individual performing the behavior,

$\Delta W_a$ = the change in direct fitness (positive or negative) of an individual affected by the behavior, $r$ = the coefficient of relationship between the performer and the individual affected, and thus,

$\sum r \Delta W_a$ = the sum of the product of relatedness and change in fitness for all individuals affected by the behavior (i.e. more than one individual can be affected by an individual behavioral act).

Thus, Hamilton's rule and inclusive fitness concept may be used to predict the influence of relatedness on any social behavior, from altruistic to agonistic. To this end, Maynard Smith (1964) coined the term "kin selection" in reference to the evolutionary result of
individuals interacting in a non-random way with respect to kinship.

Kin selection does not require individuals to actually recognize relatives (Michod 1982, West Eberhard 1975). For example, demographic or geographic circumstances may be such that conspecific interactions are, by default, kin-biased. Relatives will remain in close contact in populations in which individuals show little dispersal (e.g. Sherman 1977). However, Hamilton (1964a,b) further predicted that an animal may possess the ability to recognize relatives to ensure that its behaviors are appropriately directed toward or away from kin.

Since Hamilton's prediction, numerous recognition studies have revealed a diverse array of taxa that exhibit kin-biased behavior; behavior that is preferentially directed toward or away from kin (reviewed in Fletcher and Michner 1987; Blaustein et al. 1988; Hepper 1991). The concepts of inclusive fitness and kin selection have been utilized to explain the kin-biased behavior in most kin recognition studies. However, such "kin recognition" research is significantly skewed toward mechanistic studies.

Recent criticisms of kin recognition research have noted that the adaptive value of kin-biased behavior has seldom been demonstrated or even tested (Blaustein 1988; Grafen 1990; Blaustein et al. 1991; Gamboa et al. 1991). Yet, alternative explanations exist. Rather than being adaptive, kin-biased behavior may be a by-product of some other behavioral
phenomenon. For example, species recognition mechanisms may result in kin-biased behavior (O'Hara and Blaustein 1982, Grafen 1990, Barnard 1991). An animal using phenotypic cues to discriminate among conspecifics may preferentially associate with kin simply because kin have more similar phenotypes. Alternatively, preferences toward environmental cues can lead to the observation of kin-biased behavior (Pfennig 1990; Barnard 1991). Kin groups may form simply because relatives have similar resource preferences.

Other predictions associated with Hamilton's rule have seldom been empirically examined. For example, the rule, as presented above, is a conditional statement. Whether individuals behave differently toward their kin depends upon whether the inequality of the model is satisfied, which in turn depends upon the prevailing ecological and social conditions. Ecological and social conditions are often variable and may lead to context-dependent kin-biased behavior (Reeve 1989). In some contexts, kin discrimination may not be expressed, even when individuals have the ability to "recognize" kin, because the behavior has no adaptive value (Reeve 1989; Beecher 1991). Thus, the context within which kin discrimination occurs may indicate the functional value of the kin-biased behavior. However, few empirical studies have examined kin discriminating behavior in alternative, experimentally manipulated contexts (Pfennig et al.
The vast majority of kin recognition studies manipulate no factor other than the relatedness between individuals. The goal of this thesis was to expand the perspective of kin recognition research and to assess further the ecological and evolutionary implications of kin-biased behavior. To this end, I established two objectives. First, I describe the ecological factors which affect kin-biased behavior. Second, I test alternative hypotheses concerning the adaptive value of kin-biased behavior.

Anuran amphibians are a model system often used for vertebrate kin recognition studies (Blaustein and Waldman 1992). In particular, anuran larvae (tadpoles) have been greatly utilized in mechanistic and ontogenetic kin recognition research (see reviews by Blaustein 1988; Waldman 1991; Blaustein and Waldman 1992; Blaustein and Walls in press). There are several advantages of using the anuran tadpole system for kin recognition studies (Blaustein and Waldman 1992). Larvae are contained in an easily definable environment (aquatic pond or lake) so population density and environmental factors are easily manipulated. Also, most anuran larvae are easily reared under laboratory conditions. Fertilization is external for most species and can result in definable egg masses representing sibling groups. Therefore, the genetic relatedness of interacting conspecifics is easily manipulated. Furthermore, several species have been shown to discriminate kin from nonkin (Blaustein 1988; Blaustein et al. 1991; Blaustein and Waldman 1992).
However, the adaptive value of kin discrimination in anuran larvae is poorly understood (Blaustein 1988; Blaustein et al. 1991; Blaustein and Waldman 1992).

Several hypotheses have been proposed as functional explanations for anuran kin discrimination (Blaustein et al. 1987; Blaustein 1988; Blaustein et al. 1991; Blaustein and Waldman 1992). For example, tadpoles may obtain inclusive fitness benefits by preferentially forming kin aggregations because the advantages of living in a group would be shared among relatives (Blaustein and Waldman 1992). In fact, the behavioral assay most often used to test for kin discrimination in anuran tadpoles is a spatial affinity test. Individuals are tested to see if they preferentially associate with relatives over non relatives (Blaustein 1988; Blaustein et al. 1991; Blaustein and Waldman 1992).

The tadpoles of many anurans are known to form aggregations (Beiswenger 1972, 1975; Wassersug 1973; O'Hara 1981; Blaustein 1988). Tadpole aggregations may offer many benefits of group living, including enhanced foraging opportunities and the ability to detect and avoid predators more effectively (Bertram 1978; Hamilton 1971; Pulliam and Caraco 1984). For example, groups of tadpoles may stir up organic detritus in the substrate, thereby gaining access to food unavailable to a solitary forager (Beiswenger 1975; Wilbur 1977). Due to the dark color of tadpoles, tadpole aggregations may elevate body temperature and
increase growth and development rates (Beiswenger 1972; O'Hara 1981). Tadpoles in groups may be able to detect predators more effectively than individuals (Wassersug 1973; O'Hara and Blaustein 1981). Furthermore, some tadpoles release a chemical cue when captured by a predator. Such cues may warn cohorts of a predator's presence (Hrbacek 1950; Waldman 1982; 1986; Hews and Blaustein 1985). Any of these group benefits may be further enhanced by associating with relatives because of the inclusive fitness benefits accrued by individuals within the group.

Kin discrimination may be important for reasons other than forming kin aggregations. Anuran kin discrimination may be important for mate choice decisions. Kin discrimination may allow individuals to balance the costs of inbreeding and outbreeding (Bateson 1983). Some anurans are known to discriminate kin from non-kin after metamorphosis (Blaustein et al. 1984) and molecular analysis suggests that matings between siblings may be rare (Waldman et al. 1992). Furthermore, kin discrimination may allow anurans with cannibalistic tendencies to direct their cannibalism away from relatives (Blaustein and O'Hara 1982; Blaustein et al. 1987; Pfennig et al. 1993).

The Cascades frog (*Rana cascadae*) has a number of attributes that make it ideally suited for this study. The natural history of the frog is well known (O'Hara 1981; Nussbaum et al. 1983; Blaustein 1988) and they are locally abundant in high altitude ponds and lakes. Furthermore, the tadpoles have a
Sensitive kin discriminating mechanism (O'Hara and Blaustein 1981; Blaustein and O'Hara 1982; O'Hara and Blaustein 1985; Blaustein and O'Hara 1987). Individual tadpoles spatially associate with kin in laboratory tanks and form kin-biased aggregations in the field. The specific questions addressed in this thesis were; 1) what are the socio-ecological factors that affect aggregation behavior in _R. cascadae_, 2) does aggregation behavior in _R. cascadae_ have a function, and, if so, 3) do kinship factors influence the adaptive value of aggregation behavior.

The investigations in chapter two were an analysis of context dependent aggregation behavior in _R. cascadae_. I first observed tadpole aggregation behavior in the field to determine which ecological and environmental factors may be important in the formation of tadpole aggregations. I then tested for interactions between kinship and ecological factors in their effects on aggregation behavior.

The remaining chapters consist of experiments designed to directly test specific hypotheses concerning the adaptive value of kin discriminating behavior in _R. cascadae_. In chapter three, I tested two predator avoidance hypotheses in a series of laboratory experiments. First, I tested whether _R. cascadae_ tadpoles may detect and avoid predators more effectively while in groups, and thus obtain additional inclusive fitness benefits by forming groups composed predominantly of kin. Second, I tested
whether *R. cascadae* release an alarm cue when captured by a predator and whether such an alarm cue is kin specific.

In the experiments contained in chapters four and five, I tested whether growth and development is different in tadpoles reared in cohorts of kin versus cohorts of non-kin. The study in chapter four was conducted under laboratory conditions while the one in chapter five was performed in field enclosures. Additionally, observations in chapter two suggested that tadpoles in aggregations may "dig" pits into suitable substrate and enhance their foraging opportunities. Manipulations in the field enclosure experiment in chapter five allowed direct testing of this hypothesis.

The function (or lack thereof) of kin-biased aggregation behavior in *R. cascadae* may be revealed by comparing the results of the experiments in chapters two, through five. In the final chapter, I summarize the key findings of the previous chapters and make general conclusions. Furthermore, I suggest a new perspective for kin recognition research; one which will empirically link ecological and evolutionary processes.
Kinship Effects on Aggregation Behavior in Tadpoles and the Influence of Different Ecological Conditions

D. Grant Hokit and Andrew R. Blaustein
ABSTRACT

Kin discrimination is a behavior whereby individuals differentially interact with conspecifics based upon relatedness. Despite its observance in many organisms, few studies have examined kin discrimination in different contexts. Yet, theory suggests that kin discrimination may interact with ecological factors and, therefore, may influence ecological processes. We examined kin-biased aggregation behavior in tadpoles of the Cascades frog (Rana cascadae) in different ecological conditions. Field observations revealed ecological factors that were influential on tadpole aggregation behavior. We manipulated food distribution, predator presence, thermal heterogeneity, and relatedness in a multifactorial mesocosm experiment. All four factors interacted to influence tadpole dispersion. We then manipulated predator abundance, thermal heterogeneity, and relatedness in a multifactorial field experiment. Kinship was the only factor that significantly influenced group size at artificially established food patches in natural ponds. Our results suggest that kinship is an important factor in aggregation behavior dependent upon ecological conditions. Kin-biased aggregation behavior in tadpoles may result in improved thermoregulation, predator avoidance, or foraging efficiency. Thus, kinship is potentially important to population and community level phenomena.
INTRODUCTION

Aggregate distributions of organisms are common in nature and considerable theoretical attention has been given to understanding their consequences (e.g. Huffaker 1958; Fretwell and Lucas 1970; Hassell and May 1974; Charnov 1976). Individuals may aggregate to avoid predators, to increase foraging efficiency, or in response to patchily distributed resources (Hamilton 1971; Alexander 1974; Wilson 1975; Bertram 1978; Pulliam and Caraco 1984). Thus, aggregation behavior may be due to, and may result in, spatial heterogeneity, consequently affecting the dynamics of species interactions (Chesson and Rosenzweig 1991), habitat choice (Rosenzweig 1991), or foraging behavior (Stephens and Krebs 1986).

Additionally, kinship factors may influence social structure and, thus, the aggregation behavior of organisms (Hamilton 1964a,b; Williams 1964; Alexander 1974; West-Eberhard 1975; Wilson 1975). Kinship factors should interact with ecological factors that affect aggregation behavior (Reeve 1989). However, although many organisms are known to discriminate between related and non-related conspecifics (Fletcher and Michener 1987; Hepper 1991), the persistence of kin discrimination in various ecological conditions has seldom been tested.

Tadpoles of many anurans (frogs and toads) are known to form aggregations. These aggregations are influenced by a variety
of factors. Distribution of food and cover resources, predator avoidance, substrate type, thermotaxy, and conspecific attraction have all been implicated as cues in aggregation formation in tadpoles (Wassersug 1973; Beiswenger 1975; O'Hara 1981; Caldwell 1989; Blaustein and Walls in press). Furthermore, tadpoles of certain anurans are known to discriminate between kin and non-kin (commonly known as kin recognition) and form aggregations composed mainly of related individuals (reviewed by Waldman 1982; O'Hara and Blaustein 1985; Blaustein and Waldman 1992).

We examined the persistence of kin discrimination in tadpoles of the Cascades frog (Rana cascadae) under different ecological conditions. Rana cascadae tadpoles discriminate between kin and nonkin by spatially associating more closely with kin in choice tests in the laboratory (O'Hara and Blaustein 1981; Blaustein and O'Hara 1982; Blaustein and O'Hara 1987; Blaustein 1988). Furthermore, tadpoles form kin-biased aggregations in field experiments (O'Hara and Blaustein 1985). Rana cascadae tadpole aggregations are often observed in nature and are sensitive to temperature and substrate changes (O'Hara 1981; Wollmuth et al. 1987). We initially observed natural populations of tadpoles to assess factors that were most likely to affect aggregation behavior. We then chose a subset of factors to manipulate in a mesocosm experiment and field experiment to assess further the contribution of each factor to aggregation
formation and to what extent they interact with kinship effects. These three approaches have not been utilized in conjunction with one another to test the interrelationships between kinship and ecological parameters.
FIELD OBSERVATIONS

Study Site and Methods

Our study site was a subalpine meadow (elevation = 2300 m) located 40 km west of Bend, Oregon, U.S.A.; 3 km north of Todd Lake in the Deschutes National Forest. Two hundred by 100 meters in area, this meadow contains 38 individual potholes or small pools, a permanent stream, and several ephemeral springs. Although most of the potholes are too small and ephemeral to sustain tadpoles (average volume = 5.22 m\(^3\)), *R. cascadae* adults have been observed breeding in 12 of the larger pools (average volume of pools containing *R. cascadae* tadpoles = 14 m\(^3\)).

With the exception of an occasional boreal toad (*Bufo boreas*), the Pacific treefrog (*Pseudacris regilla*) was the only other anuran found in the meadow. Other amphibians included salamanders that are potential predators of tadpoles (roughskinned newt, *Taricha granulosa*; long-toed salamander, *Ambystoma macrodactylum*, Peterson and Blaustein 1991). No other vertebrates were found in the potholes. However, there were aquatic invertebrates that may prey on tadpoles (dytiscid larvae and adults, notonectids, and odonate larvae, Peterson and Blaustein 1992).

Tadpole aggregation behavior was observed from 25 June to 23 July 1991, with all potholes being surveyed on the same day, 2-3 times a week. *Rana cascadae* adults are explosive breeders,
with breeding often completed within 2-3 days (Nussbaum et al. 1983). Female R. cascadae lay one clutch per year and appear to mate with one male (O'Hara and Blaustein 1981), reducing the chance of a half-sib relationship within clutches. Thus, the offspring from within a clutch are probably full siblings. During breeding, multiple egg masses were oviposited in the two largest pools (12 in one and four in the other). Oviposition in six other pools included only one clutch each. Therefore, after hatching, it was possible to observe tadpole aggregation behavior in potholes containing siblings and in potholes containing tadpoles of mixed relatedness.

We measured several pothole variables, including volume, substrate type, temperature, number of potential predators (salamanders and invertebrate predators), number of potential competitors (P. regilla tadpoles), and number of conspecifics. Volume (m$^3$) was estimated by averaging several diameter measures combined with depth measures taken every 0.5 m. We categorized substrate type at each depth measure as dominated by silt, sand, moss, gravel, or sedge. Potential predators, competitors, and conspecifics were counted after trapping in minnow traps and during visual surveys. We measured temperature (°C) in the middle of each pool at 10 cm depth.

We performed an exploratory analyses to assess which pothole variables were correlated with the frequency of aggregation and how these correlations may be influenced by
kinship factors. First, Spearman Rank correlation was used to assess the association between the number aggregations found within a pothole and the volume, proportion of silty substrate type, number of potential predators, number of potential competitors, and number of conspecifics within a pothole. To extend this analysis and assess kinship effects, we constructed two regression models (one including and one excluding data points from the mixed relatedness pools) relating aggregation frequency with each pothole factor found to be strongly correlated with aggregation frequency. Because of large differences between pools in the number of predators and the number of conspecifics, these two data sets were log transformed.

*Rana cascadae* aggregations may be described as being loose, nonpolar, stationary, and small (O'Hara 1981; Blaustein and O'Hara 1982). They usually consist of less than 40 individuals resting or feeding on the substrate. For our observational purposes, an aggregation was arbitrarily defined as five or more individuals within a 15 cm² area. Several aggregation variables were measured, including number of tadpoles, temperature at the aggregation site, substrate type at the aggregation, depth of the aggregation, and the location of the aggregation within each pool. Aggregation location was mapped for each pool by taking a compass bearing along a line that intersected a central stake and the location of the aggregation. The compass bearing was
combined with a measure of the distance from shore for each aggregation, measured along the same intersection line.

Having mapped the location of each aggregation within each pothole, we assessed whether or not there were preferred sites for forming aggregations within a pothole. We used nearest neighbor analysis (Krebs 1989) to test if aggregations were clumped in distribution or randomly distributed across a pothole. This analysis was not performed on potholes with fewer than five aggregations due to potential sample size bias (Krebs 1989).

Results

The majority of aggregations were observed in shallow, warm water on silty substrates. Seventy five percent of the aggregations occurred over silty, flocculent substrates. The difference between the mean depth of a pool and the depth of aggregations within the same pool was positively skewed (Fig. II.1a). The mean depth of a pool was, on average, 12 cm deeper than the mean depth of aggregations. Likewise, the mean temperature of aggregation sites was, on average, 30°C warmer than the temperature at the middle of the pool (Fig. II.1b). Strong thermal preferences have been shown to affect correlates of fitness in *R. cascadae* (Wollmuth et al. 1987).

Aggregations were repeatedly observed at or near the same location within a pool. Nearest neighbor analysis (Krebs 1989) of
mapped aggregations revealed that, for the majority of pools, the aggregations were non-randomly distributed (Table II.1). Silty substrates may have been the key to the site specific nature of aggregations. Tadpoles within aggregations were observed "digging" small pits (4-8 cm in diameter and 4-6 cm deep) through repeated swimming and feeding behavior. Much of the silty, flocculent substrate is composed of organic detritus, providing a potentially rich food resource for the tadpoles (O'Hara 1981). Aggregations were often observed over several days in the same pit.

Given that there were eight potholes with *R. cascadae* tadpoles, only two of which were potholes with multiple clutches, we regard the assessment of kinship effects on aggregation behavior with caution. However, there were more aggregations per pool in the pools containing only siblings (mean=9.0 aggregations per pool, SE=2.71) than in the pools containing mixed siblings and nonsiblings (mean=5.5 aggregations per pool SE=0.50). Also, the average number of tadpoles per aggregation was slightly greater in sibling only pools (mean=9.4 tadpoles per aggregation SE=0.53) than in the mixed pools (mean=8.4 tadpoles per aggregation SE=1.47).

Spearman Rank correlation revealed strong correlations (0.5 or greater) between aggregation frequency and the proportion of silty substrate, the number of predators, and the number of conspecifics in a pothole (Table II.2). However, the effects of the
proportion of silty substrate and the number of conspecifics may be confounded due to their strong correlation with each other. The number of competitors and volume had considerably weaker correlations with aggregation frequency.

Regression analysis indicated that both the proportion of silty substrate and the log of the number of conspecifics were positively associated with aggregation frequency but were only significantly associated after the elimination of the mixed data points (Fig. II.2a,2c; Table II.3). Such a relationship may be expected of silty substrates if such substrates are a common food resource. Additionally, a greater number of conspecifics may increase the probability of finding aggregations. The log of the number of predators was significantly negatively associated with aggregation frequency (Fig I1.2b; Table I1.3). Thus, predators appear to disrupt aggregation behavior in R. cascadae.

The elimination of the mixed pool data points in the regression analysis resulted in improvements of fit for all three pothole variables (Fig. II.2; Table II.3). The influence of each data point is generally greater in regressions with small sample sizes and, thus, the removal of any data point was likely to improve the fit of the regression model. However, for the regression models which included all data, mixed pool data points consistently influenced the model in the same direction and often to a considerable degree (Fig. II.2, e.g. a Cook's statistic greater than 1.0 suggests an outlier, Tabachnick and Fidell 1989). These
patterns at least suggest that pure sibling and mixed populations are potentially different with regard to aggregation behavior.
EXPERIMENTAL ANALYSIS

Methods: Mesocosm Experiment

Based upon the field observations, we hypothesized that the distribution of food resources (e.g. silty substrate), thermal gradients, and predator abundance were particularly influential in the formation of tadpole aggregations. These three factors and a kinship factor were manipulated in plastic wading pools in a completely orthogonal design to test for their effects on tadpole dispersion.

We collected 10 newly oviposited clutches of *R. cascadae* eggs from the field site on 26 May 1992. We kept the clutches separate while transporting them to our laboratory where they were placed into aerated 38 L aquaria filled with dechlorinated tap water. Egg masses were divided and equal portions were placed into sibling only aquaria and mixed sibling and nonsibling aquaria. After hatching (developmental stage 22, Gosner 1960), tadpoles were transported to an outdoor site for the duration of the experiment. We introduced tadpoles into wading pools (1.5 m in diameter, filled to a depth of 15 cm), 100 individuals to a pool. This density was comparable to densities observed in similar sized pools at the natural field site (personal observation). Water and food levels were equilibrated across all pools.

Manipulations consisted of tadpoles being reared in 1) sibling versus mixed pools (a mixture of all 10 egg masses); 2)
pools with homogeneously versus patchily distributed food (rabbit chow); 3) pools with versus without predators; 4) pools with versus without a thermal gradient, and every possible combination of these four factors. This resulted in 16 treatments which we replicated four times, requiring a total of 64 pools. The four replicates were spatially blocked.

Pools with predators each contained one predatory newt (T. granulosa) housed in a plastic mesh cage (9 cm in diameter). Thus, all potential predator effects were sublethal. We placed equivalent plastic mesh cages in all pools to control for potential thigmotactic responses from the tadpoles. Thermal gradients were established within pools by mounting submersible heating elements inside 1 L glass jars filled with cooking oil and water (equal ratio of oil to water), and placing these "heating units" inside the pools. The heaters created warm water pockets, up to 5° C warmer than the surrounding water mass. Although the amount of food was equivalent for all pools, it was deposited in haphazardly located patches in some pools, and broadcast evenly across others.

We sampled each pool five times, allowing ample time between trial sampling periods (2-4 days) maximizing the likelihood that each trial was independent. Sampling of tadpole dispersion began two weeks after introduction to the wading pools to allow tadpoles to equilibrate to their rearing environment and continued for 17 days. During this period, we photographed each
pool on five different, randomly assigned days. A camera was suspended over the pools on a sliding rail system which allowed the pools to be photographed remotely, minimizing disturbance to the tadpoles.

We projected each slide image onto a grid and recorded the number of tadpoles within each grid cell. Knowing the total number of tadpoles, the number of tadpoles per cell, and the total number of cells, we calculated Morisita's standardized index of dispersion (Krebs 1989) for each slide image. This index ranges from -1.0 to +1.0. Random dispersion patterns give an index of 0.0, aggregated patterns are positive, and uniform patterns are negative. Additionally, this index has 95 percent confidence limits at +0.5 and -0.5. Therefore, any population with a dispersion index greater than 0.5 is 95 percent certain to be significantly more aggregated than a randomly distributed population.

We combined the five trial samples from each pool to obtain a mean tadpole dispersion index for each pool. The index data were transformed by the Box-Cox method (Sokal and Rohlf 1981) to meet the parametric assumption of normality. Before testing for treatment effects, we used analysis of variance (ANOVA) to test for spatial block effects. We then used ANOVA to test for treatment effects on tadpole dispersion.
Methods: Field Experiment

We manipulated kinship factors, predator abundance, and temperature factors under natural conditions to test for their effects on size of aggregations. We collected nine newly oviposited egg masses from our field site on 9 July 1993 and redistributed them into 12 potholes chosen for their similarity in volume and substrate type. Portions of the egg masses were allotted to each pool such that all pools received an approximately equal number of eggs. Egg masses were distributed such that six pools contained only sibling tadpoles and six pools contained mixed sibling and nonsiblings. Sibling and mixed pools were assigned randomly.

We removed predators from three of the sibling pools and three of the mixed pools by seining with a dip net (two times a week). The remaining pools were also seined without removing the predators to control for the potentially disruptive effect of seining. During each seining event, we recorded the number of *R. cascadae* tadpoles and the number of predators in each pool. Two artificial food pits were established within each pool by sinking plastic drinking cups (volume=200 ml) into the substrate. To manipulate temperatures, we positioned one of the cups in shallow water (5 cm) and one in deep water (20-30 cm). To begin a sampling trial, we created a rich food patch by filling each cup with 15 rabbit chow pellets (3 g of food).
Our manipulations of kinship, predators, food pit depth (i.e. temperature) and all possible combinations resulted in a total of eight treatments, each replicated in three different potholes for a total of 24 different food pits (Fig. II.3). We established and sampled all 24 food pits on five different days from 27 July to 13 August 1993. Food pits were placed a minimum of 1 m apart to minimize activity at one pit influencing another, and each pit was placed in a different location within each pothole at each sampling period. After establishment, food pits were left undisturbed for one hour, at which time we measured temperature and counted the number of tadpoles at each pit.

We combined the five trial samples from each pool to obtain the mean number of tadpoles per pit per pool. Transformation of data was not necessary. Several uncontrolled factors in the natural potholes may have potentially affected the number of tadpoles per pothole. Because tadpole abundance may be positively associated with aggregation frequency (see field observations), we used analysis of covariance (ANCOVA) to test for treatment effects, using the number of tadpoles per pool as a covariate. We first tested the ANCOVA assumption of homogeneity of regressions to assess whether the treatments themselves may have been the cause for differences in tadpole abundance (Tabachnick and Fidell 1989). We then used ANCOVA to test for treatment effects on the number of tadpoles counted.
per food pit. ANCOVA adjusted the means to account for differences in the number of tadpoles per pool.

**Results: Mesocosm Experiment**

ANOVA revealed no significant spatial block effect or interaction between blocks and treatments. Therefore, we pooled the blocks for the remaining tests and treated them as replicates (Zar 1984). Kinship, thermal heterogeneity, presence of predators, and the distribution of food all interacted to significantly influence tadpole dispersion behavior (Table II.4, Fig. II.4). Therefore, kin-biased aggregation behavior in tadpoles of *R. cascadae* depends upon the ecological conditions.

Sibling groups responded to ecological factors as would be predicted from field observations if ecological effects are additive. Mean dispersion indices for sibling pools were lowest when heaters were absent, predators were present, and food was homogeneously distributed, and were highest when heaters were present, predators were absent, and food was patchily distributed (Fig. II.4). Mean dispersion indices for mixed pools were higher than would be expected for the heater absent, predator present, homogeneous food treatment. Three other treatments for mixed pools had lower dispersion indices than the expected low treatment. Therefore, we suggest that the source of the four way
interaction appears to be the unpredictable nature of the mixed groups.

All 16 treatments had a positive index of dispersion which indicates a tendency to aggregate. However, only six of the treatments had mean dispersion indices that were significantly aggregated (i.e. indices greater than 0.5). Of these six, half were sibling and half were mixed treatments, half were homogeneous food and half were patchy food treatments, four were heater present and two were heater absent treatments, and all six were in predator absent treatments. Thus, it appears that temperature and predators had disproportionate effects, at least in the significantly aggregated treatments.

Results: Field Experiment

Preliminary ANOVA revealed no significant interaction between the covariate (number of tadpoles per pool) and any of the treatments. Therefore, we assumed that the treatments were not the cause of differences in the number of tadpoles per pool and that the ANCOVA assumption of homogeneity of regressions was satisfied (Tabachnick and Fidell 1989). Kinship significantly influenced the number of tadpoles per food pit (ANCOVA, Table II.5). The number of tadpoles per food pit was significantly greater in sibling pools than in mixed pools (Fig. II.5). Predator abundance, depth (i.e. temperature) of food pit, and interactions
between the factors did not significantly influence the number of tadpoles per food pit.
DISCUSSION

Our results indicate that kinship factors influence tadpole aggregation behavior. In nature, the number of tadpoles per food pit (group size) was significantly greater in sibling pools than in mixed pools. Moreover, kinship factors may interact with ecological factors. The distribution of food resources, presence of predators, and thermal heterogeneity all interacted with kinship to affect tadpole dispersion in the wading pools. Therefore, kin-biased aggregation behavior in tadpoles of *R. cascadae* is context dependent.

Although theory predicts context dependent kin discrimination (Reeve 1989), few studies have examined kin discriminating behavior in different conditions. In laboratory tests, kin directed aggressive behaviors in the marbled salamander (*Ambystoma opacum*) depended upon food levels and initial body size differences (Hokit et al. submitted manuscript). Tadpoles of the spadefoot toad (*Scaphiopus bombifrons*) preferentially associated with siblings or with nonsiblings in laboratory tests, depending upon whether they were omnivores or cannibalistic carnivores respectively (Pfennig et al. 1993). Together with our mesocosm results, these studies strongly suggest that kin discriminating behavior depends upon social, morphological, and ecological conditions.
However, kinship factors influenced group size in the field experiment without interacting with other factors. There are at least three reasons for this lack of context dependence in the field. First, sibling aggregation behavior in tadpoles may be more prevalent in natural conditions than in laboratory or mesocosm contexts (Waldman 1982; O’Hara and Blaustein 1985). Second, each experiment measured a different response variable (tadpole dispersion versus group size). It is possible that group size at rich, but temporary, food resources is independent of general tadpole dispersion. Third, ecological factors may have been unsuccessfully manipulated in the field study. For example, experimental pools with predators had substantially fewer predators (mean=9.33 predators per pool SE=4.30 in 1993) than pools from the previous year (mean=28.50 predators per pool SE=13.50 in 1992). This was probably due to a very late spring thaw which resulted in low numbers of invertebrate predators in 1993. Thus, predators may not have been at a sufficient density to have significantly affected group size. Additionally, during the weeks of sampling for the field study, central Oregon experienced abnormally high temperatures which warmed even the deeper sections of the potholes. Temperatures taken at each food pit indicate that, on average, there was less than 1.56 °C (SE=0.525) difference between shallow and deep food pits. Thus, very little thermal heterogeneity was established during the experiment, possibly resulting in no temperature effect.
Tadpoles of *R. cascadae* may form kin-biased aggregations for several reasons. First, groups of tadpoles, by virtue of their dark color, may form thermal sinks, which elevate body temperatures more than a solitary individual can achieve and increase growth rates (e.g. Beiswenger 1972; O'Hara 1981). Individuals may accrue additional benefits through increased inclusive fitness if they preferentially associate with kin in such thermal aggregations. Second, as with *S. bombifrons* (Pfennig et al. 1993), tadpoles may preferentially cannibalize nonkin. However, we know of no reports indicating carnivorous morphologies in *R. cascadae* or anything other than opportunistic cannibalism (e.g. feeding on already dead individuals).

Third, as theory would predict (Hamilton 1971; Bertram 1978; Pulliam and Caraco 1984), groups of tadpoles may be able to detect and to avoid predators more efficiently than solitary individuals. Individuals that preferentially associate with kin may accrue additional inclusive fitness benefits through cooperative predator vigilance. Furthermore, when captured by a predator, many tadpoles release an alarm substance (Hrbacek 1950; Hews and Blaustein 1985; Waldman 1986; Hews 1988) which could warn relatives in kin-biased aggregations (Hews and Blaustein 1985; Blaustein and Waldman 1992). However, our field observations and mesocosm results indicate that predators increase tadpole dispersion (i.e. reduce aggregation) which would seem to limit the opportunity for such an alarm mechanism.
Finally, groups of tadpoles are known to stir up the substrate, which may increase foraging efficiency (Beiswenger 1972, 1975; Wilbur 1977). We similarly observed R. cascadae tadpoles stirring up the substrate in our field observations, resulting in pits in suitable locations. Furthermore, both interference and exploitative competition have been demonstrated in anuran larvae (Steinwascher 1978). Our field observations do not indicate a strong relationship between potential interspecific competitors (P. regilla) and aggregation frequency. However, P. regilla were rarely observed in food pits in the field experiments even though they were present in the pools.

It is also possible that kin-biased aggregations may dominate non-related conspecifics, leading to kinship effects on intraspecific competition. In fact, many studies suggest that kinship factors influence growth and development in tadpoles (Shvarts and Pyastolova 1970; Waldman 1986; Jasienski 1988; Smith 1990; Hokit and Blaustein in press), although not always to the advantage of kin groups (Shvarts and Pyastolova 1970; Waldman 1991; Hokit and Blaustein in press). Furthermore, larval body size in amphibians has been positively associated with reproductive output (Bervin and Gill 1983; Smith 1987; Semlitsch et al. 1988). In our study, tadpoles in sibling pools formed larger groups both during field observation and in the field experiment. Group size at such foraging patches may vary according to patch
characteristics (Pulliam and Caraco 1984) and the relatedness of the individuals (Giraldeau and Caraco 1993; Higashi and Yamamura 1993). Equilibrium group size may increase with relatedness if tadpoles in groups choose between repelling or accepting solitary individuals (Giraldeau and Caraco 1993). Thus, kin-biased aggregations may lead to improved foraging efficiency and increased inclusive fitness.

Predator avoidance and foraging behavior are often considered important factors in aggregation/social behavior (Hamilton 1971; Bertram 1978; Pulliam and Caraco 1984; Stephens and Krebs 1986). More recently, individual behavior mechanisms have been used to explain population and community level phenomena (e.g. Gilliam 1987). Predator avoidance and foraging behavior have been incorporated into population dynamics and species interactions models (e.g. Rosenzweig 1987; Abrams 1991, 1992; Chesson and Rosenzweig 1991; Gordon 1991; Werner 1992).

Kinship factors are also considered important to social behavior (Hamilton 1964a,b; Williams 1964; West-Eberhard 1975; Wilson 1975). Yet kinship factors have not been incorporated into general models describing population or community level phenomena. This oversight seems unwarranted based upon several sources of evidence. For example, the densities and distributions of many organisms, spanning several taxa, are dependent upon kinship effects (e.g. urochordates, Grosberg and
Quinn 1986; insects, Crozier et al. 1987; fish, Brown and Brown 1993; amphibians, Waldman et al. 1992; reptiles, Werner et al. 1987; birds, Reyer 1980; rodents, Lambin and Krebs 1991; carnivores, Rogers 1987; and primates, Makwana 1978). Additionally, intraspecific competition in some organisms may be influenced by kinship factors, leading to kin-biased asymmetric competition (e.g. Shvarts and Pyastolova 1970; Waldman 1991; Hokit and Blaustein in press). Asymmetric competition can lead to size structured populations and behavioral allometry which, in turn, affects population dynamics and species interactions (Wilbur and Collins 1973; Wilbur 1980; Werner and Gilliam 1984; Werner 1986).

There is a need to incorporate multiple testing conditions in kin recognition research. Manipulating context may not only illuminate the functional value of kin discrimination, but may also describe the conditions and limits of kinship effects. However, it is apparent that kinship factors affect individual behavior and interact with ecological conditions. These effects may influence predator avoidance behavior, foraging behavior, intraspecific competition, and the distribution of organisms. Thus, the incorporation of kinship factors into general ecological models seems warranted.
ACKNOWLEDGMENTS

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Table II.1. Results of nearest neighbor analysis of aggregations for each pool with five or more aggregations including the number of aggregations per pool (n), the observed mean distance (D) to nearest neighbor (i.e. nearest aggregation), the expected mean distance, and Z tests for whether distributions were aggregated.

<table>
<thead>
<tr>
<th>Pothole</th>
<th>n</th>
<th>Observed D</th>
<th>Expected D</th>
<th>z Statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>1.07</td>
<td>1.79</td>
<td>-2.48</td>
<td>0.013</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.89</td>
<td>1.41</td>
<td>-2.86</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>1.36</td>
<td>3.14</td>
<td>-2.16</td>
<td>0.031</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>1.80</td>
<td>2.81</td>
<td>-1.50</td>
<td>0.134</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>0.95</td>
<td>2.37</td>
<td>-2.89</td>
<td>0.004</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>0.60</td>
<td>3.14</td>
<td>-3.07</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Table II.2. Spearman rank correlation analysis between pothole variables and the frequency of observed aggregations. Pothole variables include the volume of potholes, the proportion of silt per pothole, the number of potential predators per pothole (salamanders and invertebrate predators), the number of potential competitors per pothole (*P. regilla*), and the number of conspecifics.

<table>
<thead>
<tr>
<th></th>
<th>Aggregations</th>
<th>Silt</th>
<th>Predators</th>
<th>Competitors</th>
<th>Conspecifics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregations</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silt</td>
<td>0.63</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predators</td>
<td>-0.86</td>
<td>-0.22</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competitors</td>
<td>-0.14</td>
<td>0.01</td>
<td>0.25</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Conspecifics</td>
<td>0.56</td>
<td>0.91</td>
<td>-0.16</td>
<td>0.14</td>
<td>1.00</td>
</tr>
<tr>
<td>Volume</td>
<td>-0.34</td>
<td>0.11</td>
<td>0.59</td>
<td>-0.14</td>
<td>0.24</td>
</tr>
</tbody>
</table>
Table II.3. Results of regression analysis to assess the influence of the proportion of silt, the (log) number of predators, and the (log) number of conspecifics per pool on aggregation frequency (y). Regressions were performed with and without data points from pools of mixed relatedness.

<table>
<thead>
<tr>
<th>x Factor</th>
<th>Equation</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prop of Silt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with mixed</td>
<td>y = 0.11 x + 1.29</td>
<td>0.659</td>
<td>0.076</td>
</tr>
<tr>
<td>without mixed</td>
<td>y = 0.20 x - 0.83</td>
<td>0.994</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log no. Predators</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with mixed</td>
<td>y = -4.61 x + 17.75</td>
<td>0.845</td>
<td>0.008</td>
</tr>
<tr>
<td>without mixed</td>
<td>y = -6.41 x + 20.07</td>
<td>0.930</td>
<td>0.007</td>
</tr>
<tr>
<td>Log no. Conspecifics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with mixed</td>
<td>y = 3.59 x - 6.74</td>
<td>0.618</td>
<td>0.102</td>
</tr>
<tr>
<td>without mixed</td>
<td>y = 6.01 x - 14.10</td>
<td>0.905</td>
<td>0.013</td>
</tr>
</tbody>
</table>
Table II.4. ANOVA: effects of food distribution (either homogeneous or patchy), presence or absence of predators, presence or absence of heaters, and kinship (either siblings or mixed siblings and nonsiblings) on tadpole dispersion in the mesocosm experiment.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>1</td>
<td>230.59</td>
<td>5.26</td>
<td>0.026</td>
</tr>
<tr>
<td>Predator</td>
<td>1</td>
<td>1011.21</td>
<td>23.09</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Heater</td>
<td>1</td>
<td>123.64</td>
<td>2.82</td>
<td>0.099</td>
</tr>
<tr>
<td>Kinship</td>
<td>1</td>
<td>1.72</td>
<td>0.04</td>
<td>0.844</td>
</tr>
<tr>
<td>Food x Predator</td>
<td>1</td>
<td>76.56</td>
<td>1.75</td>
<td>0.192</td>
</tr>
<tr>
<td>Food x Heater</td>
<td>1</td>
<td>24.79</td>
<td>0.57</td>
<td>0.455</td>
</tr>
<tr>
<td>Food x Kinship</td>
<td>1</td>
<td>133.56</td>
<td>3.05</td>
<td>0.087</td>
</tr>
<tr>
<td>Predator x Heater</td>
<td>1</td>
<td>127.68</td>
<td>2.97</td>
<td>0.094</td>
</tr>
<tr>
<td>Predator x Kinship</td>
<td>1</td>
<td>50.17</td>
<td>1.15</td>
<td>0.290</td>
</tr>
<tr>
<td>Heater x Kinship</td>
<td>1</td>
<td>170.04</td>
<td>3.88</td>
<td>0.055</td>
</tr>
</tbody>
</table>
(Table II.4, continued)

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food x Predator x Heater</td>
<td>1</td>
<td>86.58</td>
<td>1.98</td>
<td>0.166</td>
</tr>
<tr>
<td>Food x Predator x Kinship</td>
<td>1</td>
<td>0.74</td>
<td>0.02</td>
<td>0.897</td>
</tr>
<tr>
<td>Food x Heater x Kinship</td>
<td>1</td>
<td>44.34</td>
<td>1.01</td>
<td>0.319</td>
</tr>
<tr>
<td>Predator x Heater x Kinship</td>
<td>1</td>
<td>396.26</td>
<td>9.05</td>
<td>0.004</td>
</tr>
<tr>
<td>Food x Predator x Heater x Kinship</td>
<td>1</td>
<td>319.64</td>
<td>7.30</td>
<td>0.010</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>43.78</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table II.5. Results of ANCOVA testing for treatment effects on group size at food pits in the field experiment. Treatments include kinship (either siblings or mixed siblings and nonsiblings), presence or absence of predators, and depth (either shallow or deep), with the number of tadpoles per pool as the covariate.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinship</td>
<td>1</td>
<td>3.91</td>
<td>6.17</td>
<td>0.030</td>
</tr>
<tr>
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Figure II.1. The distribution of the difference between mean pool depth and aggregation depth (a), and the difference between temperature at the aggregation site and temperature at the middle of the pool (b), for all pool.
Figure II.2. Regression analysis of the number of aggregations and the proportion of silt per pool (a), the log number of predators per pool (b), and the log number of conspecifics per pool (c). Regression statistics are included in table 3. Solid lines represent the best fit model including both the mixed pool data (open points) and the sibling pool data (solid points). Dashed lines represent the best fit model including only the sibling pool data. The numbers in parentheses next to mixed pool data points represent Cook's statistic, which is a measure of the influence these data points have on the model.
Figure II.2

A. Proportion of Silt

B. Log Number of Predators

C. Log Number of Conspecifics

(0.98) (1.34) (0.16) (1.23) (0.32) (0.99)
Figure II.3. Design of the field experiment. Each oval represents one pool. Each solid circle represents one food pit.
Figure II.4. Interaction diagram including the mean standardized Morisita's index of dispersion for all sixteen treatments in the mesocosm experiment.
Figure 11.4

Mean Standardized Index of Dispersion
Figure II.5. Mean number of tadpoles per food pit (± 1 SE) adjusted for the covariate (number of tadpoles per pool) for sibling and mixed sibling and nonsibling pools.
Predator Avoidance and Alarm Response Behaviour in Kin Discriminating Tadpoles

D. Grant Hokit and Andrew R. Blaustein
ABSTRACT

When captured by predators, the tadpoles of some species of frogs and toads may release an alarm substance that alters the behaviour of conspecifics. Such "alarm response" behaviour has been proposed as a potential mechanism whereby related conspecifics may "warn" relatives of a predator's presence and, thus improve their inclusive fitness. We examined predator avoidance and alarm response behaviour in tadpoles of the Cascades frog (Rana cascadae) and tested whether such behaviour is influenced by kinship factors. Tadpoles reduced activity when in the presence of a predatory newt (Taricha granulosa). Individuals in groups of sibling tadpoles were more active than solitary tadpoles and individuals in groups of mixed relatedness. However, we found no evidence of alarm response behaviour in R. cascadae. Behaviour of tadpoles in groups exposed only to predators was not different from that of tadpoles in groups exposed to predators plus crushed conspecifics. Tadpoles in groups exposed to crushed tadpoles were as active as tadpoles in groups exposed to water controls, and some test individuals fed upon the dead tadpoles. Thus, while R. cascadae tadpoles reduce activity in response to newt predators, crushed tadpoles appear to initiate a feeding response rather than an alarm response as has been previously proposed. Additionally, tadpoles in sibling groups may be better than solitary individuals and individuals in
mixed groups at balancing trade-offs between predator response behaviour and other activities.
INTRODUCTION

Hamilton's (1964a,b) kin selection theory predicts that, under certain conditions, an organism may treat related conspecifics differently than nonrelatives (kin discrimination or kin recognition). Since Hamilton's prediction, kin discrimination has been demonstrated in numerous studies including species from many taxa (reviewed in Fletcher and Michener 1988; Hepper 1991). However, kin recognition studies have been criticized for failing to provide functional explanations for kin discriminating behaviour (e.g. Grafen 1990; Gamboa et al. 1991; Barnard 1991; Blaustein et al. 1991). For example, although the larvae of several anuran (frogs and toads) species discriminate kin from non-kin (e.g. Blaustein 1988; Waldman 1991; Blaustein and Waldman 1992), the adaptive value of such behaviour is poorly understood (Blaustein 1988; Blaustein et al. 1991; Blaustein and Waldman 1992).

Kin-biased predator defense mechanisms have been proposed as a possible functional explanation for kin discrimination in anuran larvae (Waldman 1986, 1991; Blaustein 1988; Blaustein and Waldman 1992). There are at least two distinct, but non-exclusive hypotheses. First, groups of tadpoles may be able to detect and avoid predators more efficiently than solitary tadpoles (e.g. Hamilton 1971; Bertram 1978; Pulliam and Caraco 1984). Individuals which preferentially form groups with
kin may accrue additional inclusive fitness benefits by cooperating in predator vigilance with relatives. Specific predictions associated with this hypothesis are that 1) tadpoles will be more aggregated when in predator dense habitats, and 2) groups of tadpoles will respond differently to a predator's presence than will solitary tadpoles (i.e. solitary tadpoles will be less effective at detecting predators).

Second, tadpoles may release a chemical alarm substance when injured by a predator that "warns" nearby tadpoles of a predator's presence (Hrbacek 1950; Hews and Blaustein 1985; Waldman 1986; Hews 1988). Anuran larvae also use chemical cues to distinguish kin from non-kin (Blaustein and O'Hara 1982; Waldman 1986). If the chemical alarm substance contains kin specific chemical cues, injured tadpoles could preferentially warn relatives of the predator's presence. Specific predictions associated with this hypothesis are that 1) nearby tadpoles will respond to the presence of an injured conspecific (i.e. they will possess an alarm response), and 2) tadpoles will respond more to an injured sibling than to an injured non-sibling.

We examined predator vigilance and alarm response behaviour in tadpoles of the Cascades frog (Rana cascadae). Rana cascadae is a model system for kin recognition studies. Tadpoles of R. cascadae can discriminate between kin and non-kin and preferentially associate with kin in laboratory and field
experiments (e.g. O’Hara and Blaustein 1981; Blaustein and O’Hara 1982; O’Hara and Blaustein 1985; Blaustein and O’Hara 1987).

Hews and Blaustein (1985) suggested that tadpoles of *R. cascadae* exhibit an alarm response to conspecific extracts. Tadpoles increased swimming activity when presented with extracts of conspecific tadpoles versus when presented with water controls. Similar increases in activity have been interpreted as an alarm response in other anuran species (Pfeiffer 1974; Hews and Blaustein 1985; Hews 1988).

In a series of three laboratory experiments, we tested whether or not *R. cascadae* tadpoles 1) behaviourally respond to the presence of predators, 2) exhibit an alarm response, 3) exhibit different predator avoidance behaviour when solitary versus when in groups, and/or 4) exhibit kin-biased avoidance or alarm response behaviour. Our study is the first to test for a kin specific alarm response and kin-biased predator avoidance behaviour.
METHODS

Rearing and General Procedures

For all experiments, we collected newly oviposited clutches of *R. cascadae* eggs from a natural oviposition site 100 km east of Corvallis, Oregon (Cache Lake in the Deschutes National Forest, Jefferson Co.). Females of *R. cascadae* lay one clutch per year and appear to mate with only one male per season (O'Hara and Blaustein 1981), reducing the chance of a half-sib relationship within clutches. Thus, offspring from within a clutch are probably full siblings.

We kept clutches separate, transported them to our laboratory in Corvallis, and placed them into aerated 38 L aquaria filled with dechlorinated tap water. We subdivided each clutch and distributed subclutches into separate aquaria, approximately 100 sibling individuals per aquarium. Thus, there were multiple replicates (5-8 depending on initial clutch size) of each sibling group reared in different aquaria. After hatching, tadpoles were fed rabbit chow ad libitum, and water was changed every 5-7 days. We began testing tadpoles 3-4 weeks after hatching (tadpoles with rear limb buds, developmental stages 27-34, Gosner 1960).

We tested tadpoles in a rectangular aquarium (100 x 60 cm) with opaque walls (Fig. III.1). A center line divided the aquarium into width-wise halves and cylindrical cages (9 cm diameter)
made from plastic mesh were placed at each end and in the middle. We thoroughly rinsed the tank and cages with tap water before each test and filled the test tank with dechlorinated tap water to a depth of 5 cm. Test animals were placed in the central cage and allowed to acclimate for 10 minutes. Each end cage had a lid made from an inverted funnel with a plug which could be removed via a drawstring. End cages contained either nothing or predators (roughskinned newt, *Taricha granulosa*) depending upon the ultimate treatment stimulus (see specific experiments below). The inverted funnel lids contained either water or a stimulus tadpole. To mimic a predator attack, stimulus tadpoles were first humanely dispatched and then lightly crushed with a mortar and pestle. An observer concealed behind an opaque blind could remove both end plugs (releasing the funnel contents into the end cages) and the central cage by pulling on the drawstring (Fig. III.1).

A test began after the 10 minute acclimation period by pulling the draw string, which introduced the stimulants and released the test animals. Each test included two five minute trials separated by a five minute pause. Both activity level and tadpole distribution may vary with the presence or absence of predators (Hews 1988; Lawler 1989; Skelly and Werner 1991). As a measure of activity level, we counted the number of times a test animal crossed the center line during each trial. To assess spatial avoidance of particular stimuli (experiment 1 only), we
counted the number of test individuals on each end of the tank at 30 second intervals. These 30 second counts were then averaged for each test and recorded in relationship to the position of the treatment stimulus. Test individuals were never used in more than one test.

Experiment 1

We tested whether groups of *R. cascadae* tadpoles 1) respond to the presence of newt predators, 2) possess an alarm response (in addition to a predator response), and 3) possess an alarm response that is influenced by kinship factors. We tested three different sibling groups under five different stimulus conditions (treatments). Stimulus conditions included 1) a control with nothing in the end cages and only water in the funnel lids; 2) a predator in one end cage and water in the funnel lids; 3) a predator in both end cages and water in the funnel lids; 4) a predator in both end cages, water in one lid and water with a crushed, unrelated tadpole in the other lid; and 5) a predator in both end cages, water in one lid, and water with a crushed sibling tadpole in the other lid. We altered the position of stimulants from end to end for each test within a treatment, and the treatments were presented in random order. We used eight sibling tadpoles from the same rearing tank for test animals in each test. Thus, test individuals were both related and familiar.
Each of the three sibling groups were tested 15 times (five times for each stimulus condition) for a total of 75 tests in experiment 1.

Experiment 2

To assess whether solitary individuals respond to the presence of predators differently than groups, we tested predator response behaviour of solitary tadpoles, groups of sibling tadpoles, and groups of mixed relatedness. We measured the activity level of tadpoles in these three social conditions with predators present (predators in both end cages) and with predators absent (nothing in the end cages). Solitary individuals were alternately chosen from each of three sibling groups. Mixed groups were formed by selecting three individuals from each sibling group (a total of nine test individuals), with the individuals within each sibling group coming from a different rearing tank. Sibling groups were formed by selecting nine individuals from the same sibling group (which alternated for each test), with each individual coming from a different rearing tank. Thus, both the mixed and sibling groups contained unfamiliar tadpoles. Solitary individuals were tested 18 times with predators present and 18 times with predators absent. Mixed and sibling groups were tested nine times for each predator condition. Thus, we performed a total of 72 tests in experiment 2.
Experiment 3

Hews and Blaustein (1985) suggested that tadpoles of *R. cascadae* exhibit an alarm response to damaged conspecifics. Tadpoles increased their activity levels in response to extracts of macerated tadpoles. To assess further the nature of this behavioural response, we measured activity levels in groups of sibling tadpoles when exposed to 1) nothing in the end cages, 2) predators in both end cages, and 3) a crushed conspecific in both end cages (because groups were composed of sibling tadpoles, crushed conspecifics were always siblings). Groups of tadpoles were composed of nine familiar siblings and each test alternately selected from three different sibling groups. We tested each stimulus condition 18 times for a total of 54 tests in experiment 3.

Statistical Analyses

For experiment 1, we summed the number of times tadpoles crossed the center line for each test and calculated the mean number of individuals in the stimulus end (arbitrarily determined for treatments one and three) for each 30 second reading. We then used multivariate analysis of variance (MANOVA) to test for stimulus effects, sibling group effects, and interactions between stimulus and sibling group effects. After MANOVA, we used univariate analysis of variance (ANOVA) on each response variable within each main effect to help assess which variables were responsible for significant main effects.
Likewise for experiment 3, we summed the number of times tadpoles crossed the center line for each test and used ANOVA to test for stimulus effects.

For experiment 2, we summed the number of times tadpoles crossed the center line for each test, but then divided this total by the number of test tadpoles to get the number of crossings per test tadpole. We then used ANOVA to test for social condition effects, predator effects, and interactions between social condition and predator effects. For all experiments, parametric assumptions were met and no data transformations were necessary.
RESULTS

Experiment 1

MANOVA revealed that the type of stimulus presented in the end cages significantly affected tadpole behaviour (Table III.1). Subsequent ANOVA showed that tadpole crossing frequency was significantly affected by the type of stimulus (Table III.1). Tadpoles were most active when nothing was in the end cages, less active when a predator was present at one end, and least active for the remaining three stimulus conditions (Fig. III.2a). There was no difference in activity between regimes containing predators alone at both ends and predators with a crushed tadpole, whether the crushed tadpole was a sibling or not.

The average number of tadpoles counted on the stimulus side was not significantly affected by the stimulus type (Table III.1). Approximately four (out of eight) individuals were counted on the stimulus side for all five stimulus conditions (Fig. III.2b), suggesting that tadpoles were distributed randomly. Additionally, MANOVA revealed no significant sibling group effect and no significant interaction between sibling group and stimulus effects (Table III.1).

Experiment 2

ANOVA revealed significant predator and social condition effects on tadpole activity but, there was no significant interaction
between predator and social condition effects (Table III.2). As in experiment one, the presence of a predator significantly reduced activity level in all three social conditions (Fig. III.3). Solitary individuals and tadpoles in mixed groups were less active than tadpoles in sibling groups, both when predators were present and when predators were absent (Fig. III.3).

**Experiment 3**

Stimulus type significantly affected tadpole activity (Table III.2). Tadpole activity was greater in both the control tests and in tests with a crushed sibling than in the tests with predators present (Fig. III.4). Additionally, tadpoles forced their way inside of the cages in six of the 18 crushed sibling tests and were observed feeding upon the dead tadpole.
DISCUSSION

Our results show that *R. cascadae* tadpoles become less active in the presence of newt predators. Tadpoles in sibling groups were more active than solitary tadpoles and tadpoles in groups of mixed relatedness. We found no evidence of an alarm response in addition to the observed predator response, and no evidence of a kin-biased alarm response. In fact, tadpoles were sometimes observed feeding on crushed conspecifics.

Tadpoles of many anuran species reduce activity in the presence of predators (Lawler 1989; Skelly and Werner 1990; Stauffer and Semlitsch 1993). Low activity in the presence of some kinds of predators can reduce predation on tadpoles (Lawler 1989). However, decreased activity in tadpoles may reduce foraging activity and prolong development (Wilbur and Collins 1973) or reduce size at metamorphosis (Werner 1986). When in the presence of predators, some tadpoles reduce activity levels, metamorphose at a smaller size, and grow slower (Skelly and Werner 1990; Skelly 1992). Larval body size in amphibians has been positively associated with reproductive output (Bervin and Gill 1983; Smith 1987; Semlitsch et al. 1988). Thus, there are costs to avoiding predators and tadpoles must balance predator avoidance behaviour with foraging activity.

Trade-offs between predator avoidance behaviour and other activities are observed in many organisms (Sih 1987; Lima and
Dill 1990). Such individual behavior has been increasingly utilized to explain population dynamics and species interactions (e.g. Rosenzweig 1987; Chesson and Rosenzweig 1991; Abrams 1992; Werner 1992). In particular, relative changes (due to predators) in activity levels may modify competitive interactions between species (Werner 1991). Activity related differences in competitive interactions may also occur intraspecifically between individuals in different size or age classes (Werner 1992) or perhaps, between individuals in different social conditions.

Our results show that sibling groups are more active than solitary tadpoles and tadpoles in mixed groups. Such relative activity levels may result in different competitive abilities between individuals in different social conditions and suggest that tadpoles in sibling groups may be better at balancing the trade-offs between foraging activity and predator avoidance. Growth and development variables (survivorship and mass at metamorphosis) are known to differ between R. cascadae tadpoles reared in sibling groups versus tadpoles reared in groups of mixed relatedness, but not always to the advantage of the kin groups (Hokit and Blaustein in press; Hokit and Blaustein submitted manuscript). Predator vigilance may be more cooperative in groups of related tadpoles, but the degree and frequency of aggregation formation in tadpoles of R. cascadae has been shown to be inversely related to predator density (Hokit and Blaustein
submitted manuscript). Thus, the significance of greater activity in sibling groups of *R. cascadae* is unclear.

Many organisms possess an alarm reaction in response to chemical cues released from injured conspecifics (e.g. cnidarians, Howe and Sheikh 1975; molluscs, Stenzler and Atema 1977; insects, Hölldobler 1977; fish, Pfeiffer 1977; anurans Pfeiffer 1974). In particular, anuran larvae often avoid injured conspecifics and increase activity when presented with conspecific extracts (Pfeiffer 1974; Hews and Blaustein 1985; Hews 1988). Because tadpoles also use chemical cues to discriminate kin from nonkin (Blaustein and O'Hara 1982; Waldman 1986; Blaustein et al. 1993), it has been suggested that an alarm response may be chemically based and kin-biased (Hews and Blaustein 1985; Waldman 1986; Waldman 1991; Blaustein and Waldman 1992). Tadpoles living in sibling groups would warn relatives if they released an alarm cue when injured by a predator.

Our results do not suggest such an alarm response in *R. cascadae*. In experiment 1, tadpoles reduced activity when predators were present. There was no significant difference in tadpole behaviour between treatments where predators were present without crushed conspecifics and where predators were with crushed conspecifics, regardless of whether the crushed conspecific was related to the test animals. The increased activity observed in Hews and Blaustein (1985) was not detected in our study where predator and injured conspecific cues were
simultaneously present. In experiment 3, we observed higher tadpole activity levels in treatments with crushed conspecifics than treatments with predators present. Additionally, tadpoles were observed mouthing the cages, and, in some cases, forced their way into cages and fed on the dead tadpoles. Tadpoles were never observed inside of cages in any of the other testing conditions, including those conducted in experiments one and two. Thus, the increased activity observed in Hews and Blaustein (1985) may actually be a feeding response in R. cascadae tadpoles rather than an alarm response.

Although R. cascadae tadpoles responded to the presence of a predator, we found no evidence in support of the original predictions associated with kin-biased predator vigilance or alarm response behavior. Previous studies (Hokit and Blaustein submitted manuscript) indicate that R. cascadae tadpoles are less aggregated rather than more aggregated in predator dense habitats. In this study, the significance of greater activity in sibling groups is unclear, and all social conditions (solitary or not) responded to the presence of predators in a similar manner. There was no evidence of a predator alarm response, kin-biased or otherwise, and previous observations of a behavioral response to conspecific extracts may be interpreted as a feeding response. Our results demonstrate the need for testing hypotheses concerning the functional value of kin recognition behaviour.
ACKNOWLEDGMENTS

We thank Ronald Altig, Bruce Menge, Peter Dawson, and Robert Anthony for reviewing an early draft of the manuscript. We thank Miles Bennel, Becky Driscoll and Dan Kaufman for all their help. This research was funded by Zoology Research Funds (ZORF) to DGH and National Science Foundation grant 91-07171 to ARB.
Table III.1. Results of MANOVA for overall effects of stimulus type, sibling group, and stimulus x sibling group interaction, and ANOVAs for each response variable within each of the main effects. Response variables are number of times a tadpole crossed the center line (activity) and the number of tadpoles counted on the stimulus side of the apparatus (side).

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* Significance level for univariate tests is 0.025 (Bonferroni-adjusted for two response variables).
Table III.2. Results of ANOVAs for experiment two testing for predator and social condition effects on tadpole activity, and experiment three testing for stimulus treatment effects on tadpole activity.

**ANOVA for Experiment 2**

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**ANOVA for Experiment 3**

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Figure III.1. Apparatus used for all three experiments.
Figure III.2. Tadpole activity (A) and mean number of individuals counted on the stimulus side of the apparatus (B) for all five stimulus types in experiment one. Stimulus types include nothing in the end cages (a), a predator in one end cage (b), a predator in both end cages (c), predators in both end cages and a crushed nonrelated conspecific in one end cage (d), and predators in both end cages and a crushed related conspecific in one end cage (e).
Figure III.2
Figure III.3. Tadpole activity (mean number of times tadpoles crossed the center line), for solitary individuals, sibling groups, and groups of mixed relatedness, when predators were present and when predators were absent (experiment two).
Figure III.4. Tadpole activity for groups exposed to nothing (control), predators, and a crushed sibling tadpole (experiment three).
The Effects of Kinship on Growth and Development
in Tadpoles of *Rana cascadae*

D. Grant Hokit and Andrew R. Blaustein

This paper is in press: *EVOLUTION*
ABSTRACT

We examined the role of kinship in growth and development in tadpoles of the Cascades frog (*Rana cascadae*). Four different sibling cohorts were reared in the laboratory until metamorphosis in either pure kin groups or in mixed groups of kin and nonkin and at two levels of density. At metamorphosis, we measured body length, mass, length of larval period, and the number of metamorphs produced per tank. High density regimes produced proportionally fewer metamorphs that were significantly smaller in body length and mass and with longer larval periods than did low density regimes. Individuals reared in kin groups had a significantly smaller mass at metamorphosis than individuals reared in mixed groups. There was no significant difference between kin groups and mixed groups in body length, proportion of metamorphs produced, and larval period. Moreover, there was no significant interaction between density and kinship. Thus, in the context of our study, growth is depressed among kin in *R. cascadae*. These results suggest that intraspecific competition may be a significant cost to kin-biased social behavior.
INTRODUCTION

Tadpoles of the frog, *Rana cascadae* can discriminate between kin and non-kin (kin recognition) and preferentially associate with kin in laboratory and field experiments (e.g. O'Hara and Blaustein 1981, Blaustein and O'Hara 1982, O'Hara and Blaustein 1985, Blaustein and O'Hara 1987). Such association, combined with rapid metamorphosis from small, ephemeral breeding sites and the tendency to form aggregations in nature may lead to intense intraspecific competition among *R. cascadae* tadpoles (O'Hara 1981, Blaustein 1988). Kin recognition may allow tadpoles to direct competitive interactions away from kin, perhaps by altering behavioral interference or by selectively releasing chemical growth inhibitors (Waldman 1986, Blaustein 1988, Waldman 1991, Blaustein and Waldman 1992).

In tadpoles, the effects of kin association on growth and development remain controversial (Shvarts and Pyastolova 1970, Travis 1980, Waldman 1986, Jasienski 1988, Smith 1990, Waldman 1991; reviewed by Blaustein and Waldman 1992). In particular, the direction of the kinship effect (i.e. whether individuals in kin groups grow faster/larger or slower/smaller than those in mixed groups) appears to depend upon the species examined and the experimental conditions. For example, kinship effects vary for American toads (*Bufo americanus*). Some family groups show increased larval growth rate when reared with
siblings, whereas the growth rate in other families is slower when reared with sibling competitors (Waldman 1986). Individual growth rate is greater in sibling groups of the chorus frog (*Pseudacris triseriata*) than in mixed groups, but only at high density (Smith 1990). However, with the exception of *B. americanus* (Waldman 1986, 1991), none of the species previously examined for kinship effects on growth and development are known to discriminate kin from nonkin.

Using *R. cascadae*, we examined whether individuals within groups of full sibling tadpoles and groups of tadpoles of mixed relatedness differ in several key components of reproductive success including the lengths of the larval period, proportions of metamorphs produced, and body lengths and masses at metamorphosis. These traits correlate with fitness in some amphibians (e.g. Berven and Gill 1983, Smith 1987, Semlitsch et al 1988).
METHODS

We collected four newly oviposited (less than 8 hours old) clutches of *R. cascadae* eggs on 28 March 1992 from an ephemeral pond adjacent to Cache Lake in the Deschutes National Forest of central Oregon, U.S.A., (100 km east of Corvallis, Jefferson County). *Rana cascadae* adults are explosive breeders, with breeding often completed within 2-3 days (Nussbaum et al. 1983). Female *R. cascadae* lay one clutch per year and appear to mate with one male (O'Hara and Blaustein 1981), reducing the chance of a half-sib relationship within clutches. Thus, the offspring from within a clutch were probably full siblings. We kept the clutches separate while transporting them to our laboratory where they were placed into aerated 38 L aquaria filled with dechlorinated tap water. Only tadpoles that hatched on 7 April (developmental stage 18, Gosner 1960), were used in this study.

We randomly assigned tadpoles to low density (48 animals per aquarium: 1.26 tadpoles per liter) and high density (200 animals per aquarium: 5.26 tadpoles per liter) treatments. The small size and ephemeral nature of *R. cascadae* breeding sites can produce density levels within this range (Hokit and Blaustein, unpublished data). Within each density treatment, we assigned tadpoles to kin groups (sibling tadpoles from the same clutch) and mixed groups (an equal number of tadpoles from each of the four clutches). Thus, a factorial design resulted with four treatments:
1) low density kin group, 2) low density mixed group, 3) high density kin group, and 4) high density mixed group, with four replicates of each treatment (a total of 16 aquaria). We considered each sibling group as a replicate for the kin treatments.

We fed tadpoles ground rabbit chow daily, with high density treatments receiving four times as much food as low density treatments. We augmented food throughout the experiment to adjust for tadpole growth, with amounts ranging from 0.5 to 1.0 g per day for low density treatments and 2.0 to 4.0 g per day for high density treatments. On any given day all treatments of the same density received the same amount of food.

We positioned all 16 tanks on the same bench centered in the middle of a windowless room, under a 14L:10D photoperiod. We randomly assigned tank positions and made no markings to indicate kinship (pure kin or mixed) composition. Thus, measurements were blind with respect to kinship. Tanks were cleaned and filled with dechlorinated tap water every three to five days with all tanks being cleaned on the same day.

When tadpoles metamorphosed (forelimb emergence, stage 42, Gosner 1960), we measured four variables: body length (snout to tail base), mass, number of days to metamorphosis, and the proportion of tadpoles metamorphosed per tank. Tanks were checked daily for metamorphs. We used metal dial calipers to measure body length to the nearest 0.1 mm and measured mass,
after blotting each individual dry, to the nearest 0.001 g on an electronic scale. We measured metamorphs up to 100 days after hatching (total number of metamorphs measured = 1037), at which time we terminated the experiment. One hundred days is a long larval period for *R. cascadae* (Nussbaum et al. 1983), with many natural sites drying up within this period (O'Hara 1981).

We tested for density and kinship effects by using multivariate analysis of variance (MANOVA). To meet parametric assumptions of normality, measurements of body length, mass, and days to metamorphosis were loge transformed, whereas the proportion of metamorphs produced per tank was arcsine transformed (Zar 1984). Because individuals in tank cohorts may not be independent of one another, these measures were analyzed as tank means. We performed univariate analysis of variance (ANOVA) on each response variable within each main effect to assess which variables were responsible for significant main effects.
RESULTS

Kinship and density both affected the growth and development of tadpoles (MANOVA, Table IV.1). Density levels significantly influenced larval period, body length and mass at metamorphosis, and the proportion of metamorphs (ANOVAs, Table IV.1). Tadpoles took longer to develop, were shorter and weighed less, and proportionally fewer metamorphs were produced in the high density treatments (Fig. IV.1). There were no significant differences between kin and mixed groups with respect to body length, larval period, or the proportion of tadpoles metamorphosed per tank (ANOVAs, Table IV.1). However, the individual mass of tadpoles raised in kin groups was smaller than for tadpoles raised in mixed groups (Fig. IV.1).

Travis (1980) found significant differences in larval growth rates between different sibships of *Hyla gratiosa*. To assess whether differences in mass among sibships might alone have caused the differences between kin and mixed groups, we performed a post hoc variance ratio test on mass. This test revealed that the range in variance among kin groups did not differ significantly from the range in variance among mixed groups ($F_{0.05(2),7,7}=1.78$, $p>>.5$, Zar 1984). Additionally, when the performance of the four sibling groups are compared, the standard deviation of metamorph mass is noticeably greater within the sibling groups than among the sibling groups in both
the low and high density treatments (Table 2). Thus, it is unlikely that the kinship effect on mass is the result of differences in mass among sibling groups. Although mortality may alter densities enough to affect the mass at metamorphosis, mortality was low in all our tanks (less than five percent), and there was no apparent relationship between mortality and the experimental treatments.

Although the MANOVA revealed no significant interaction between density and kinship, there was a trend toward an interaction in all four response variables (Fig. IV.1). In particular, the difference in mass between kin and mixed tanks was much greater in the high density treatments than in the low density treatments.
DISCUSSION

Our results revealed density-dependent effects on metamorphic characters of *R. cascadae* that are consistent with previous studies of other anuran species (e.g. Brockelman 1969, Wilbur 1977, Steinwascher 1978, Wilbur 1980, Dash and Hota 1980, Semlitsch and Caldwell 1982, Smith 1990). Additionally, our results revealed a negative kinship effect on mass at metamorphosis. Consequently, the effects of intraspecific competition on mass were greater in kin groups than in mixed groups of *R. cascadae*, and these effects may be greater at high density.

Several other studies report negative effects of kinship on growth and development. For example, Shvarts and Pyastolova (1970) found that solitary *Rana arvalis* tadpoles reared in water conditioned by siblings grew slower than tadpoles reared in water conditioned by nonsiblings. Waldman (1986) observed the same effect in some families of *Bufo americanus* but not in others. The negative kinship effect in our study is particularly intriguing given that *R. cascadae* tadpoles can discriminate between kin and non-kin (i.e. kin recognition) and, therefore, could potentially avoid competitive interference with kin. Moreover, *R. cascadae* kin recognition is relatively sensitive: Individuals prefer to associate with siblings over nonsiblings and even half siblings over nonsiblings (Blaustein and O'Hara 1982), and kin recognition
persists after metamorphosis (Blaustein et al. 1984). Why do _R. cascadae_ tadpoles form aggregations composed primarily of kin in the field (O'Hara and Blaustein 1985), given that the negative effects of intraspecific competition appear to be greater within kin groups?

Smith (1990) suggested that either type of kinship effect (positive or negative) could be favored by kin selection depending upon how body size and larval period affect fitness. For example, rapid growth and development through metamorphosis may lower the risk of predation (e.g. Calef 1973, Heyer et al. 1975, Werner 1986) and reduce the risk of desiccation where the aquatic habitat is ephemeral (e.g. Heyer et al. 1975, O'Hara 1981). However, there are costs associated with early metamorphosis. Early metamorphosis may not allow full exploitation of the aquatic habitat, resulting in lower mass at metamorphosis and potential reductions in subsequent reproductive output (e.g. Berven and Gill 1983, Smith 1987, Semlitsch et al. 1988). Presumably, smaller mass at metamorphosis in kin groups of _R. cascadae_ may be offset by enhanced performance in other metamorphic traits, such as increased per capita probability of metamorphosis or acceleration of the larval period. However, none of the other variables measured in our study showed an increased response for kin groups except for a slight, but nonsignificant, increase in the proportion of metamorphs produced in the high density treatment (Fig. 1).
It is possible that the results observed in our study are context dependent (i.e. depend upon the testing conditions). For example, Smith (1990) found that growth rate and the proportion of tadpoles that attain metamorphosis are greater in sibling groups of *P. triseriata* than in mixed groups, but only at high density and with a particular size of container. Our results also indicate that kinship effects may be greater at high density. Although MANOVA did not reveal a statistically significant interaction between kinship and density, this could be a result of the conservative nature of MANOVA analysis (a bias toward avoiding type I errors) and the relatively small sample sizes. In anycase, the data indicate a trend toward an interaction in all response variables, particularly in the case of mass.

The kinship effect may also be specific to the particular sibling groups involved and their relative ratios in the mixed groups. Classic studies on genotypic viabilities (e.g. Dobzhansky and Spassky 1944, Levene et al. 1954, Lewontin 1955, Bhalla and Sokal 1964, Bell 1991) indicate that genotypic interactions are often nonlinear and difficult to predict. For example, the relative survival of different strains of *Drosophila* depends upon their rearing densities, the particular combination of genotypes, and their relative ratios in mixed groups (Dobzhansky and Spassky 1944, Levene et al. 1954, Lewontin 1955). These complex interactions may result in certain combinations of genotypes (i.e. mixed groups) out-performing isolated genotypes via more
efficient or harmonious resource use (Lewontin 1955). However, working with genotypically distinct populations of Chlamydomonas, Bell (1991) found no evidence of genotypic interactions, yet mixed cultures had higher productivity than did pure cultures. Rather than complementary resource use, replacement of one genotype by another resulted in a performance which was higher for mixed cultures than for the average pure culture.

In our experiment, as with previous studies (Travis 1980, Jasienski 1988, Smith 1990), each sibship was equally represented in each mixed tank. This design reduced the chance that differences in growth and development between sibships (Travis 1980, Smith 1990) might alone have caused differences between kin and mixed groups. However, with this design, every mixed tank had the same genetic structure. The kinship effect observed in our study could indicate more intense competition in kin groups or, conversely, complementary resource use in mixed groups (e.g. different tadpoles may feed in different regions of the tank or may feed by coprophagy versus eating rabbit chow). Alternatively, certain sibships may outperform (in terms of growth rate) other sibships, resulting in a "replacement" response (i.e. competition is asymmetrical with respect to sibships). Replacement could be exacerbated if the initial condition (e.g. size) of individuals were biased toward a particular sibling group. This latter possibility was minimized in our study, given that all sibling
groups were collected at the same time, and all treatment replicates were maintained under identical laboratory conditions. However, other factors, such as maternal investment in egg size, may produce clutch-biased competitive advantages (Kaplan 1989).

Nevertheless, because individual tadpoles in our study were not identifiable by sibship in the mixed tanks, it is difficult to distinguish between complementary resource use and "replacement." Ideally, experiments utilizing tadpoles that are individually or genetically marked would allow examination of sibships in all treatments and allow examination of facilitation or replacement processes. Regardless, the kinship effect observed in our study may only be relevant to the particular sibships involved, their relative ratios, and the particular densities used in our study.
We thank Ross Alford, Peter S. Dawson, Bruce Menge, and David Parichy for reviewing an early draft of the manuscript. We thank Susan C. Walls for laboratory help and critical review of the manuscript. For technical assistance we thank Rusty Ryan, John Brickley, and Boots MulCahey. This research was supported by Oregon State University Zoology Research Funds to DGH and National Science Foundation grants BNS 9020957 and BNS 9107171 to ARB.
Table IV.1. Results of MANOVA for overall effects of density, kinship, and density x kinship interaction, and of ANOVAs for each response variable within each of the main effects. Response variables are number of days to metamorphosis (days), body length (length), mass at metamorphosis, and the proportion of metamorphs produced per tank (mets).

<table>
<thead>
<tr>
<th>Kinship</th>
<th>Density</th>
<th>Density x Kinship</th>
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</table>
| F       | d.f.    | P
| MANOVA  | 4.64    | 4,9              | .026 (Wilks Criterion) |
| ANOVAs  |         |                  |
| Days    | 1.34    | 1                | .269                  |
| Length  | 3.13    | 1                | .102                  |
| Mass    | 10.20   | 1                | .008*                 |
| Mets    | 1.90    | 1                | .193                  |
|         | 265.58  | 4,9              | <.001                 |
|         | 2.51    | 4,9              | .116                  |
|         | 140.67  | 1                | <.001*                |
|         | 0.33    | 1                | .574                  |
|         | 145.72  | 1                | <.001*                |
|         | 3.27    | 1                | .096                  |
|         | 302.63  | 1                | <.001*                |
|         | 7.08    | 1                | .021                  |
|         | 714.46  | 1                | <.001*                |
|         | 1.90    | 1                | .193                  |

* Significance level for univariate tests is 0.0125 (Bonferroni-adjusted for four response variables.)
Table IV.2. Means, standard deviations, and ranges for number of days to metamorphosis (days), body length (length), mass at metamorphosis, and the proportion of metamorphs produced per tank (mets) for each tank within each treatment group. Treatment groups are low density kin groups (LDKG), low density mixed groups (LDMG), high density kin groups (HDKG), and high density mixed groups (HDMG).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Days</th>
<th>Length</th>
<th>Mass</th>
<th>Mets</th>
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<tr>
<td>LDKG</td>
<td>47</td>
<td>69.77±11.29 (50-97)</td>
<td>16.63±1.51 (14.0-19.7)</td>
<td>0.601±0.204 (0.34-1.16)</td>
<td>0.98</td>
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<td>47</td>
<td>70.40±12.09 (52-94)</td>
<td>16.24±1.27 (13.6-19.2)</td>
<td>0.563±0.153 (0.33-1.03)</td>
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<td>45</td>
<td>76.51±7.93 (65-94)</td>
<td>16.56±1.22 (13.5-19.5)</td>
<td>0.607±0.130 (0.35-0.93)</td>
<td>0.94</td>
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<td></td>
<td>48</td>
<td>67.52±12.39 (45-95)</td>
<td>16.44±1.57 (12.9-19.6)</td>
<td>0.606±0.193 (0.33-1.11)</td>
<td>1.00</td>
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<tr>
<td>LDMG</td>
<td>48</td>
<td>68.79±13.09 (48-94)</td>
<td>16.39±1.69 (13.1-19.7)</td>
<td>0.618±0.225 (0.30-1.38)</td>
<td>1.00</td>
</tr>
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<td></td>
<td>48</td>
<td>70.91±12.55 (49-97)</td>
<td>16.64±1.43 (13.9-19.6)</td>
<td>0.612±0.168 (0.39-1.12)</td>
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<td>44</td>
<td>71.18±11.79 (50-95)</td>
<td>16.69±1.48 (13.4-19.7)</td>
<td>0.620±0.189 (0.32-1.08)</td>
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<td></td>
<td>47</td>
<td>71.21±11.36 (52-91)</td>
<td>16.16±1.39 (13.2-19.1)</td>
<td>0.578±0.163 (0.32-1.01)</td>
<td>0.98</td>
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<tr>
<td>HDKG</td>
<td>80</td>
<td>87.83±7.92 (71-99)</td>
<td>14.88±1.05 (11.8-17.0)</td>
<td>0.399±0.068 (0.22-0.54)</td>
<td>0.40</td>
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(Table IV.2, continued)

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<tr>
<td>78</td>
<td>86.19±9.42</td>
<td>13.85±0.98</td>
<td>(12.0-16.8)</td>
<td>0.348±0.076</td>
<td>(0.21-0.61)</td>
</tr>
<tr>
<td>101</td>
<td>84.47±10.42</td>
<td>14.60±0.96</td>
<td>(12.6-17.4)</td>
<td>0.382±0.090</td>
<td>(0.24-0.67)</td>
</tr>
<tr>
<td>88</td>
<td>86.17±9.09</td>
<td>14.13±1.14</td>
<td>(11.6-17.3)</td>
<td>0.371±0.092</td>
<td>(0.21-0.63)</td>
</tr>
<tr>
<td>HDMG</td>
<td>88</td>
<td>84.60±10.38</td>
<td>14.90±1.03</td>
<td>(12.9-18.2)</td>
<td>0.428±0.113</td>
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<tr>
<td>76</td>
<td>84.40±9.70</td>
<td>14.78±1.39</td>
<td>(12.0-18.6)</td>
<td>0.422±0.131</td>
<td>(0.23-0.82)</td>
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<td>66</td>
<td>82.77±10.85</td>
<td>14.73±1.29</td>
<td>(11.5-17.7)</td>
<td>0.424±0.123</td>
<td>(0.23-0.80)</td>
</tr>
<tr>
<td>70</td>
<td>84.01±10.16</td>
<td>15.14±1.36</td>
<td>(12.1-17.9)</td>
<td>0.464±0.139</td>
<td>(0.20-0.79)</td>
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Figure IV.1. Mean (±se) mass (a), body length (b), larval period (c), and proportion of metamorphs produced per tank (d) for kin groups (open bars) and mixed groups (solid bars) at both low and high density.
Interactions Between Ecological and Kinship Factors in Their Effects on Growth and Development of Tadpoles

D. Grant Hokit and Andrew R. Blaustein
ABSTRACT

We examined the effects of kinship on growth and development in tadpoles of the Cascades frog (Rana cascadae) in different ecological contexts. Replicates of eight different sibling cohorts were reared in field enclosures until near metamorphosis in a three way completely factorial experiment. Factors consisted of rearing tadpoles in 1) groups of all sibling tadpoles versus groups of a mixture of siblings and non-siblings; 2) in low versus high density; and 3) with versus without access to the substrate. Density, access to the substrate, and kinship all interacted to significantly influence growth and development of tadpoles, as measured by mean mass and the proportion projected to metamorphose. There was no difference between tadpoles reared in sibling versus mixed groups in the low density, open substrate treatment. Tadpoles reared in sibling groups had lower mass than tadpoles reared in mixed groups but more were projected to survive through metamorphosis in the low density, closed substrate treatment and in the high density, open substrate treatments. However, tadpoles in sibling groups had lower mass and fewer were projected to survive through metamorphosis in the high density, closed substrate treatment. Subsequent analysis suggested that this interaction effect was due to differences between kin versus mixed groups in the distribution of individual tadpole mass. The distribution of individual mass was more
skewed (i.e. more small and fewer large individuals) in mixed groups than in kin groups. Our results demonstrate that kinship factors affect growth and development in tadpoles under field conditions. Furthermore, our results provide a functional explanation for the kin discriminating behavior observed in *R. cascadae* and suggest why such behavior may be context dependent. Survivorship may be higher for tadpoles that form sibling groups, depending upon the ecological conditions.
INTRODUCTION

Many animals exhibit kin-biased behavior; behavior that is preferentially directed toward or away from kin (reviewed in Fletcher and Michener 1987; Blaustein et al. 1988; Hepper 1991). The concepts of inclusive fitness (Hamilton 1964a,b) and kin selection (Maynard-Smith 1964) have been utilized to explain kin-biased behavior in most kinship studies. However, recent criticisms of kin recognition research have noted that the adaptive value of kin-biased behavior has seldom been demonstrated or even tested (Blaustein 1988; Grafen 1990; Blaustein et al. 1991; Gamboa et al. 1991), yet, alternative explanations exist.

Rather than being adaptive, kin-biased behavior may be a by-product of some other behavioral phenomenon. For example, species recognition mechanisms may result in kin-biased behavior (O'Hara and Blaustein 1982, Grafen 1990, Barnard 1991). An animal using phenotypic cues to discriminate among conspecifics may preferentially associate with kin simply because kin have more similar phenotypes. Alternatively, preferences toward environmental cues can lead to the observation of kin-biased behavior (Pfennig 1990; Barnard 1991). Kin groups may form simply because relatives have similar resource preferences.

In particular, tadpoles of several anuran species can discriminate between kin and non-kin (reviewed by Blaustein and Waldman 1992; Blaustein and Walls in press), although the
adaptive value of kin recognition in tadpoles is unclear (Blaustein and Waldman 1992). One proposed explanation is that kinship factors may influence growth and development in tadpoles to the advantage of tadpoles living in kin groups. For example, groups of tadpoles may stir up organic detritus in the substrate, thereby gaining access to food unavailable to a solitary forager (Beiswenger 1975; Wilbur 1977). Tadpoles in kin groups may gain additional inclusive fitness benefits by cooperatively foraging with relatives. Also, kinship factors may influence competitive interactions. Inclusive fitness may be enhanced by competing more intensely with unrelated individuals. Kin recognition may allow tadpoles to direct competitive interactions toward non-relatives and away from kin, perhaps by altering behavioral interference or by selectively releasing chemical growth inhibitors (Waldman 1986, Blaustein 1988, Waldman 1991, Blaustein and Waldman 1992).

However, the effects of kinship on growth and development in tadpoles remains poorly understood (Waldman 1991; Blaustein and Waldman 1992). In particular, the direction of the kinship effect (i.e. whether individuals in kin groups grow larger or smaller, or have higher survivorship than those in groups of mixed relatedness) appears to depend upon the species examined and the particular experiment. For example, kinship effects vary for *B. americanus*. Some family groups show increased larval growth rate when reared with siblings, whereas the growth rate
in other families is slower when reared with sibling competitors (Waldman 1986, 1991). Individual growth rate is greater in sibling groups of the chorus frog (*Pseudacris triseriata*) than in mixed groups, but only at high density (Smith 1990).

The larvae of anuran amphibians are often used in studies of intraspecific competition (e.g. Wilbur 1980, 1984; Alford and Harris 1988). Intraspecific competition has been inferred from density dependent effects on such factors as survival, growth rate, larval period, and the size at metamorphosis, and these traits correlate with fitness in some amphibians (e.g. Berven and Gill 1983; Smith 1987; Semlitsch et al. 1988). However, density effects often do not explain large amounts of variation in competition studies. For example, in Wilbur's (1977) study of competition between tadpoles of *Bufo americanus*, 36% of the variation in tadpole mass was unexplained by experimental factors. Kinship effects may account for some of the unexplained variation in tadpole competition studies.

We examined kinship effects on growth and development in tadpoles of the Cascades frog (*Rana cascadae*) in a multifactorial field experiment. Tadpoles of *R. cascadae* can discriminate between kin and non-kin and preferentially associate (form groups) with kin in laboratory and field experiments (e.g. O'Hara and Blaustein 1981, Blaustein and O'Hara 1982, O'Hara and Blaustein 1985, Blaustein and O'Hara 1987). Such association, combined with rapid metamorphosis from small, ephemeral
breeding sites and the tendency to form aggregations in nature, may lead to intense intraspecific competition among *R. cascadae* tadpoles (O'Hara 1981, Blaustein 1988).

Growth in *R. cascadae* tadpoles was influenced by kinship factors in a previous laboratory study (Hokit and Blaustein in press), although not to the advantage of kin groups. Individuals reared in sibling groups in laboratory aquaria had a significantly smaller mass at metamorphosis than individuals reared in mixed groups. However, groups of *R. cascadae* tadpoles have been observed in the field foraging in pits that were dug into silty substrate by tadpole swimming activity (Hokit and Blaustein submitted manuscript). Silty, flocculent substrate contains organic detritus, providing a potentially rich food resource for the tadpoles (O'Hara 1981). Such opportunities were not available for tadpoles in the laboratory study. Therefore, in field enclosures, we examined whether individuals reared in groups of full siblings versus groups of mixed relatedness differed in mass and survivorship, and whether tadpole density and access to the substrate interacted with such kinship effects.
METHODS

Our study site was a subalpine meadow (elevation = 2300 m) located 40 km west of Bend Oregon U.S.A.; 3 km north of Todd Lake in the Deschutes National Forest, Deschutes county. Two hundred by 100 meters in area, this meadow contains 38 individual potholes or small pools, a permanent stream, and several ephemeral springs. Although most of the potholes are too small and ephemeral to sustain tadpoles (average volume = 5.22 m$^3$), R. cascadae adults were observed breeding in 12 of the larger pools (average volume of pools containing R. cascadae tadpoles = 14 m$^3$).

We chose one pothole (volume = 35 m$^3$) for this experiment that had a consistent history of breeding adults and was relatively homogeneous with respect to depth (average depth = 20.63 cm) and substrate. We placed eight newly oviposited (less than 8 hours old) clutches of R. cascadae eggs into separate incubation enclosures on 5 July 1993. Rana cascadae adults are explosive breeders, with breeding often completed within 2-3 days (Nussbaum et al. 1983). Female R. cascadae lay one clutch per year and appear to mate with one male (O'Hara and Blaustein 1981), reducing the chance of a half-sib relationship within clutches. Thus, the offspring from within a clutch were probably full siblings. Only tadpoles hatched on 15 July were used in this study.
We randomly assigned hatchlings to, 1) low versus high density enclosures; 2) enclosures with versus without access to the substrate; 3) kin (sibling tadpoles from the same clutch) versus mixed (an equal number of tadpoles from each of the eight clutches) enclosures; and every possible combination of these three factors. This resulted in eight treatments, which we replicated four times, producing a total of 32 enclosures. All enclosures used in this study consisted of fiberglass mesh (mesh size = 2 mm) supported by a 0.5 m³ wood frame. We spatially blocked each of the four replicates within the pothole and randomly determined the position of treatments within each block.

We attached fiberglass mesh across the bottoms of enclosures for the substrate closed treatments to prevent tadpoles from digging into the substrate. Eight cm wide vinyl molding was nailed around the bottom of the remaining enclosures (substrate open treatments) and sunk into the substrate to keep tadpoles from escaping. We shoveled a thin layer of silty, flocculent substrate from the same pothole into the closed-bottomed enclosures. Thus, tadpoles in both open and closed-bottomed enclosures were reared on the same substrate. However, tadpoles in the closed bottom enclosures were limited in their ability to "dig" pits. We stirred the substrate inside the open-bottomed enclosures so that both types of enclosures were equally disturbed.
We placed 48 animals per enclosure (6 tadpoles per clutch for mixed relatedness enclosures) in the low density treatments, resulting in approximately 0.91 tadpoles per L, and 104 animals per enclosure (13 tadpoles per clutch for mixed relatedness enclosures) in the high density treatments, resulting in approximately 1.98 tadpoles per L. The small size and ephemeral nature of *R. cascadae* breeding sites can produce density levels within this range (O'Hara 1981; Hokit and Blaustein submitted manuscript).

We initially planned to collect metamorphs as they emerged from the enclosures. However, an unusually warm and dry summer caused potholes at our study site to dry prematurely. With the pothole containing our enclosures in danger of drying, we collected all surviving tadpoles on 12 September. At this point only six individuals had reached metamorphosis (forelimb emergence, stage 42, Gosner 1960). Thus, we measured the mass of all surviving tadpoles to the nearest 0.001 g on an electronic scale. However, tadpole survivorship does not always correlate with survival through metamorphosis and is often not significantly affected by density-related factors (Wilbur 1977). Small, stunted tadpoles may persist for indefinite periods of time without ever metamorphosing. In an attempt to remove these stunted individuals with effectively zero fitness from our initial analysis, we utilized the mass of the smallest metamorph (0.200 g) in our previous laboratory study (Hokit and Blaustein in press)
as a cutoff for including individuals in our initial analysis. Thus, we assumed that all individuals collected on 12 September with a mass greater than or equal to 0.200 g would have reached metamorphosis had the pothole not prematurely dried.

We used multivariate analysis of variance (MANOVA) to test for density, substrate and kinship effects on the proportion (calculated from initial densities) and mean mass of individuals per enclosure projected to metamorphose. Mass measures were analyzed as enclosure means because individuals within enclosures may not be independent of one another. We performed univariate analysis of variance (ANOVA) on both response variables within each main effect to help assess which variables were responsible for significant main effects. However, because there was a strong pooled, within cell correlation (greater than 0.30) between mean mass and proportion projected to metamorphose, it was likely that the two response variables were correlated in their response to the treatments. Therefore, we performed a more conservative stepdown F test for the proportion projected to metamorphose to assess better the influence of the treatment effects on the response variables (Tabachnick and Fidell 1989). This stepdown F was essentially an analysis of covariance using mean mass as the covariate and thus, was an analysis of the combined influence of the treatment effects on both response variables (Tabachnick and Fidell 1989).
To meet parametric assumptions of normality, measurements of the proportion of individuals projected to metamorphose were arcsine transformed (Zar 1984). Four enclosures were dislodged during a wind storm on 27 July and were eliminated from the experiment. These four enclosures were evenly distributed across the replicates and no treatment lost more than one enclosure. However, to correct for unequal sample sizes within cells, we applied the regression method (method 1 of Overall and Spiegel 1969) to assess treatment effects. This method assesses each treatment effect after adjusting for all other effects and is a recommended correction for unequal sample sizes for experimental research (Tabachnick and Fidell 1989).

To assess further the source of treatment effects, we constructed paired histograms of the mass of all surviving individuals (i.e. including those less than 0.200 g) in kin versus mixed enclosures within each of the other four treatments (low density substrate open, low density substrate closed, high density substrate open, high density substrate closed). These four treatments were ranked from least to most harsh in terms of the magnitude of their effects upon the mean mass of individual tadpoles. Such graphical representation helped to visualize the effects of treatments on the distribution of individual tadpole mass. Additionally, we used ANOVA to test for the effects of kinship and environmental harshness on the coefficient of
variation of tadpole mass calculated for each enclosure. All analyses were conducted using Systat version 5.1 (Wilkinson 1986).
RESULTS

Preliminary MANOVA revealed no significant spatial block effect or interaction between blocks and treatments. Therefore, we pooled the blocks for the remaining tests and treated them as replicates (Zar 1984). Density, access to substrate, and kinship all interacted to significantly affect tadpole growth and development (MANOVA, Table V.1a). The source of the interaction was a combined effect on the two response variables (Stepdown F, Table V.1c). There was little difference in mean mass and the proportion projected to metamorphose for tadpoles reared in sibling versus mixed groups in the low density open substrate treatment (Fig. V.1). Tadpoles reared in sibling groups had lower mass than tadpoles reared in mixed groups, but more were projected to reach metamorphosis in the high density open substrate and low density closed substrate treatments (Fig. V.1). However, mean mass and proportion projected to metamorphose were both lower for tadpoles in sibling enclosures in the high density closed substrate treatment (Fig. V.1). Thus, the effect of each factor depended upon the other factors.

Paired histograms revealed increased skewness of tadpole mass for both kin and mixed treatments as environmental harshness increased (Fig. V.2). However, the degree of skewness was not symmetrical across the treatments, resulting in differences in the proportion projected to metamorphose. Because
of differences in the distributions, kin enclosures produced more individuals over 0.200 g than did mixed enclosures in the low density closed substrate and high density open substrate treatments, and fewer individuals over 0.200 g in the high density substrate closed treatment. There was little difference between kin and mixed enclosures in the low density open substrate treatment. These differences in distribution were reflected in calculations of the coefficient of variation for each enclosure. Both environmental harshness and kinship significantly influenced the coefficient of variation (Table V.2). The coefficient of variation increased with environmental harshness and mixed enclosures had higher coefficients than did kin enclosures (Fig. V.3).
DISCUSSION

Our results revealed a significant interaction between kinship, density, and access to substrate in their effects on R. cascadae tadpole growth and development. Density-dependent effects were consistent with previous studies of other anuran species (e.g. Wilbur and Collins 1973; Wilbur 1977; Steinwascher 1978; Wilbur 1980; Semlitsch and Caldwell 1982; Alford and Harris 1988; Smith 1990). We found both a decrease in mass and increasingly skewed distributions of mass at higher densities, resulting in fewer individuals projected to metamorphose. However, the magnitude of this density-dependent effect depended upon kinship and substrate access factors.

The distribution of individual tadpole mass was less skewed for tadpoles reared in sibling versus mixed enclosures. This difference in distribution resulted in differences in mean tadpole mass and the number projected to metamorphose depending upon the density and substrate factors. There was little difference between tadpoles reared in sibling versus mixed groups in the low density, open substrate treatment. Tadpoles reared in sibling groups had lower mass than tadpoles reared in mixed groups but more were projected to survive through metamorphosis in the low density, closed substrate treatment and in the high density, open substrate treatments. However, tadpoles in sibling groups had lower mass and fewer were projected to survive through
metamorphosis in the high density, closed substrate treatment. In general, preventing tadpoles from digging into the substrate produced a similar effect to increasing density. This suggests that pits observed in previous studies (Hokit and Blaustein submitted manuscript) may provide tadpoles with increased foraging opportunities.

The results of our previous laboratory study (Hokit and Blaustein in press) revealed that size at metamorphosis was smaller for *R. cascadae* tadpoles reared in sibling groups than tadpoles reared in groups of mixed relatedness. This most closely matches the results observed in the high density closed substrate treatment in this study, suggesting that the laboratory conditions most closely mimicked the most harsh environmental conditions in the field study.

Our results provide a possible explanation for the disparate findings of previous studies of kinship effects on tadpole growth and development (Shvarts and Pyastolova 1970; Waldman 1986; Jasienski 1988; Smith 1990; Waldman 1991; Hokit and Blaustein in press). We suggest that kinship effects are highly dependent upon ecological (and thus, experimental) conditions and may simultaneously affect multiple correlates of fitness in a manner not detected by independent analyses of each fitness correlate. *Rana cascadae* tadpoles reared in sibling groups had higher survivorship (at some cost to mass) depending upon the density and substrate factors. Similar contextual kinship effects have
been observed in other tadpoles. For example, Smith (1990) found that growth rates and the proportion of tadpoles that attained metamorphosis were greater in sibling groups of *Pseudacris triseriata* than in mixed groups, but only at higher densities.

Kinship effects on growth and development may result from genetic factors such as the particular sibling groups involved and their relative ratios in the mixed groups. Competitive interactions between different genetic strains are often nonlinear and difficult to predict (e.g. Dobzhansky and Spassky 1944; Levene et al. 1954; Lewontin 1955; Bell 1991). For example, the relative survival of different strains of *Drosophila* depends upon their rearing densities, the particular combination of genotypes, and their relative ratios in mixed groups (Dobzhansky and Spassky 1944; Levene et al. 1954; Lewontin 1955).

Kinship effects may be enhanced by kin-biased behavior. Small initial differences in competitive ability between individuals may result from such processes as differential maternal investment (Kaplan 1980, 1989), priority effects (Bryant 1978; Prado et al. 1993), and/or genetic factors (Lomnicki 1978, 1980; Travis 1980). Any of these initial sources of variation may be greatly enhanced by intraspecific competition, resulting in the asymmetric distribution of resources and the performance of individuals (Wilbur and Collins 1973; Rubenstein 1981; Begon 1984). Because tadpoles of *R. cascadae* can discriminate between
kin and non-kin (e.g. O'Hara and Blaustein 1981, Blaustein and O'Hara 1982, O'Hara and Blaustein 1985, Blaustein and O'Hara 1987), individual tadpoles may direct competitive interactions away from kin (Waldman 1986, Blaustein 1988, Waldman 1991, Blaustein and Waldman 1992). Both exploitative and interference mechanisms of competition have been observed in tadpoles (Steinwascher 1978). Larger tadpoles may exploit food resources better due to their larger feeding structures (Wassersug 1975) and/or agonistic behavior (Savage 1952; John and Fenster 1975). Also, larger tadpoles may release chemically-based growth inhibitors (Richards 1958; Steinwascher 1978; although this mechanism may be mostly a laboratory phenomenon, Biesterfeldt et al. 1993). Any of these mechanisms may enhance initial differences in competitive ability and result in increasingly skewed distributions of mass with increases in density (Wilbur and Collins 1973; Rubenstein 1981; Begon 1984). Furthermore, any of these mechanisms may be kin-biased.

Regardless of the specific mechanism, kinship effects have important implications for size-structured populations. Size structuring is common in the populations of many organisms (Werner and Gilliam 1984) and is most dramatic in organisms that undergo metamorphosis (Wilbur 1980; Blakley 1981; Policansky 1983; Werner and Gilliam 1984). Furthermore, size structuring can greatly affect population dynamics and species interactions (e.g. Wilbur 1980; Werner and Gilliam 1984; Begon 1984; Werner
1986, 1994; Mittelbach and Osenberg 1993; Wootton 1993). As our results demonstrate, kinship factors can influence the number and size of individuals recruited into higher size classes and thus, may influence the dynamics of size-structured populations. Additionally, kinship may influence population dynamics through other means. For example, the densities and distributions of many organisms are dependent upon kinship factors (e.g. Reyer 1980; Grosberg and Quinn 1986; Crozier et al. 1987; Rogers 1987; Lambin and Krebs 1991; Waldman et al. 1992).

Given that the influence of kinship in our study was dependent upon ecological conditions, kin-biased competitive behaviors may also be context dependent. Theory predicts context dependent kin discrimination (Reeve 1989), and we previously demonstrated context dependent aggregation behavior; tadpole aggregation behavior in *R. cascadae* was dependent upon interactions between kinship, food distribution, thermal heterogeneity, and the presence of predators (Hokit and Blaustein submitted manuscript). Thus, not only is kin-biased aggregation behavior context dependent, but the possible benefits of aggregating with kin is also context dependent.

Many animals can discriminate kin from non-kin (Fletcher and Michener 1987; Hepper 1991), although the adaptive value of such behavior is often not known (Grafen 1990). Our results revealed that *R. cascadae* tadpoles in kin groups respond to intraspecific competition differently than do tadpoles in mixed
groups. Additionally, *R. cascadae* tadpoles form kin aggregations in the field (O'Hara and Blaustein 1985) and these aggregations can "dig" foraging pits into suitable substrate (Hokit and Blaustein submitted manuscript). We suggest that one function of kin discrimination in *R. cascadae* is to form kin-biased aggregations that favor kin groups in competitive interactions. However, this kin advantage will depend upon ecological conditions and tadpoles may form kin-biased aggregations accordingly.
ACKNOWLEDGMENTS

We thank Karla Hokit, Russ Burchard, and Fran Beck for help in the field work. We thank Susan C. Walls, Bruce Menge, Peter Dawson, and Robert Anthony for critical comments on earlier drafts of the manuscript. This research was supported by American Museum of Natural History Theodore Roosevelt Memorial Funds to DGH.
Table V.1. Results of MANOVA for overall effects of density, access to substrate, kinship, and all possible interactions, and univariate analysis (ANOVAs and Stepdown Fs) of each response variable within each main effect. Response variables are the mean mass and proportion projected to metamorphose per enclosure.

**A. MANOVA for Overall Effects**

<table>
<thead>
<tr>
<th>Source</th>
<th>Wilks'Lambda</th>
<th>F</th>
<th>d.f.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>0.285</td>
<td>23.78</td>
<td>2,19</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Substrate</td>
<td>0.683</td>
<td>4.40</td>
<td>2,19</td>
<td>.027</td>
</tr>
<tr>
<td>Kinship</td>
<td>0.664</td>
<td>4.82</td>
<td>2,19</td>
<td>.020</td>
</tr>
<tr>
<td>Density x Substrate</td>
<td>0.834</td>
<td>1.89</td>
<td>2,19</td>
<td>.178</td>
</tr>
<tr>
<td>Density x Kinship</td>
<td>0.983</td>
<td>0.17</td>
<td>2,19</td>
<td>.846</td>
</tr>
<tr>
<td>Substrate x Kinship</td>
<td>0.974</td>
<td>0.25</td>
<td>2,19</td>
<td>.780</td>
</tr>
<tr>
<td>Density x Substrate x Kinship</td>
<td>0.648</td>
<td>5.17</td>
<td>2,19</td>
<td>.016</td>
</tr>
</tbody>
</table>

**B. Univariate Analysis (ANOVA) of Mean Mass**

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>1</td>
<td>31.61</td>
<td>17.16</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Substrate</td>
<td>1</td>
<td>5.94</td>
<td>3.35</td>
<td>.082</td>
</tr>
<tr>
<td>Kinship</td>
<td>1</td>
<td>5.49</td>
<td>3.10</td>
<td>.094</td>
</tr>
<tr>
<td>Density x Substrate</td>
<td>1</td>
<td>1.69</td>
<td>0.94</td>
<td>.344</td>
</tr>
<tr>
<td>Density x Kinship</td>
<td>1</td>
<td>0.39</td>
<td>0.22</td>
<td>.646</td>
</tr>
<tr>
<td>Substrate x Kinship</td>
<td>1</td>
<td>0.53</td>
<td>0.29</td>
<td>.594</td>
</tr>
</tbody>
</table>
Density x Substrate x Kinship  & 1  & 0.86  & 0.48  & .498  
Error  & 20  & 17.84  

C. Univariate Analysis (ANOVA and Stepdown F Test) of Mean Proportion Projected to Metamorphose

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>P*</th>
<th>StepF</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>1</td>
<td>665.71</td>
<td>49.46</td>
<td>&lt;.001</td>
<td>16.18</td>
<td>.001</td>
</tr>
<tr>
<td>Substrate</td>
<td>1</td>
<td>122.02</td>
<td>9.16</td>
<td>.007</td>
<td>4.78</td>
<td>.042</td>
</tr>
<tr>
<td>Kinship</td>
<td>1</td>
<td>81.38</td>
<td>0.58</td>
<td>.464</td>
<td>6.19</td>
<td>.025</td>
</tr>
<tr>
<td>Density x Substrate</td>
<td>1</td>
<td>524.41</td>
<td>3.75</td>
<td>.067</td>
<td>2.53</td>
<td>.128</td>
</tr>
<tr>
<td>Density x Kinship</td>
<td>1</td>
<td>51.74</td>
<td>0.35</td>
<td>.562</td>
<td>0.12</td>
<td>.728</td>
</tr>
<tr>
<td>Substrate x Kinship</td>
<td>1</td>
<td>0.21</td>
<td>0.01</td>
<td>.992</td>
<td>0.24</td>
<td>.631</td>
</tr>
<tr>
<td>Density x Substrate x</td>
<td>1</td>
<td>471.73</td>
<td>3.58</td>
<td>.073</td>
<td>9.43</td>
<td>.006</td>
</tr>
<tr>
<td>Error(anova)</td>
<td>20</td>
<td>135.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean mass (stepdown covariate)</td>
<td>19</td>
<td>76.75</td>
<td></td>
<td></td>
<td>16.71</td>
<td>.001</td>
</tr>
</tbody>
</table>

* Significant P=0.025, Bonferonni-adjusted for two response variables (Tabachnick and Fidell 1989)
Table V.2. Results of ANOVA for the effects of environmental harshness and kinship on the coefficient of variation of mass.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>3</td>
<td>0.037</td>
<td>6.60</td>
<td>.003</td>
</tr>
<tr>
<td>Kinship</td>
<td>1</td>
<td>0.184</td>
<td>32.46</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Environment x Kinship</td>
<td>3</td>
<td>0.010</td>
<td>1.74</td>
<td>.191</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure V.1. The proportion projected to metamorphose versus mean tadpole mass per enclosure coded for kinship (kin groups = filled circles, mixed groups = open circles) and subdivided into high density closed substrate, high density open substrate, low density closed substrate, and low density open substrate treatments.
Figure V.1
Figure V.2. Frequency histograms of individual tadpole mass for each treatment type pooled across replicates. Kin groups (filled) and mixed groups (open) are paired for each density/substrate treatment. Density/substrate treatments are ranked from least to most harsh by their effects on mean tadpole mass. Numbers in parentheses are the proportion (of the initial density) projected to metamorphose (i.e. all those above 0.200 g).
Figure V.2

Increasing Environmental Harshness

Density: Low  High
Figure V.3. Coefficient of variation of mean tadpole mass versus environmental harshness, coded for kin groups (filled) and mixed groups (open). Environments from least to most harsh include low density open substrate (a), low density closed substrate (b), high density open substrate (c), and high density closed substrate (d) treatments.
Summary and Conclusions

The objectives of this thesis were to: 1) describe the socio-ecological factors that affect kin-biased aggregation behavior in *R. cascadae*, and 2) test alternative hypotheses concerning the adaptive value of kin-biased aggregation behavior in *R. cascadae*. Such questions are not often addressed in kin recognition research. Behavioral assays are often used to assess the kin discriminating ability of different species, or to examine the mechanisms of kin discrimination, without considering kin discrimination in different contexts. Furthermore, it is often assumed that kin discriminating behavior is functionally explained by inclusive fitness and kin selection processes. This assumption is rarely tested.

The results from chapter two reveal several socio-ecological factors that affect aggregation behavior in *R. cascadae*. Food distribution, thermal heterogeneity, substrate type, the presence of predators, and kinship factors all influence aggregation behavior in *R. cascadae*. Furthermore, these factors interact in their influence on aggregation behavior.

Despite Hamilton's rule being a conditional statement, there are few examples of studies that experimentally manipulate the context of behavioral assays when testing for kin discriminating behavior. Hokit et al. (submitted manuscript) found that aggressive behavior between individual marbled salamander
(Ambystoma opacum) larvae depends upon the social (kinship and relative size of the individuals) and environmental (low or high food levels) context. Aggressive behavior directed toward siblings was significantly lower than aggression directed toward non-siblings only when individuals were of similar size and in a high food environment. Similarly, Pfennig (1993) found that cannibalism in spadefoot toad (Scaphiopus bombifrons) tadpoles was directed away from siblings in high food environments. However, such kin discrimination was less prevalent in low food environments.

Many studies have observed changes in kin discriminating behavior without experimentally manipulating the ecological and social context. For example, in some animals, including amphibians, kin discrimination appears to change with ontogeny (Pfennig et al. 1983; Blaustein et al. 1984; Breed et al. 1988; Waldman 1989; Walls 1991; Blaustein et al. 1993). Observations suggest that, sibling aggression in birds may be mediated by food levels or food type (O'Connor 1978; Mock 1985; Mock et al. 1987; Drummond and Garcia Chavelas 1989; Parker et al. 1989).

Maternal care in prairie dog (Cynomys ludovicianus) colonies shifts from infanticide (females killing each others young) to communal nursing once litters become mixed a few weeks after parturition (Hoogland 1985; Hoogland et al. 1989). Female acorn woodpeckers (Melanerpes formicivorus) will cannibalize a sister's eggs or incubate them in a communal nest, depending upon the
intensity of intraspecific competition (Stanback and Koenig 1992). Similarly, sibling cannibalism was found to be density dependent in catfish (*Clarias gariepinus*, Hecht and Appelbaum 1988).

Thus, it is apparent that kin discrimination depends upon factors other than the relatedness of individuals and that test conditions may influence the detection of kin recognition abilities. Animals that discriminate kin from non-kin may not do so in all contexts, whereas animals not known to discriminate kin from non-kin (e.g. O'Hara and Blaustein 1988; Fishwald et al. 1990) may do so if test conditions are altered. Manipulating test conditions in kin recognition studies may produce more examples of context dependent kin discrimination. Furthermore, testing kin discrimination in different contexts may illuminate the functional basis of kin-biased behavior.

As discussed in chapter one, although there are several proposed explanations for kin discriminating behavior in anuran larvae, these hypotheses have seldom been tested (Blaustein et al. 1987; Blaustein 1988; Blaustein et al. 1991; Blaustein and Waldman 1992). A better understanding of the possible functional value of kin discrimination in *R. cascadae* may be obtained by comparing the results found in chapters two through five with the predictions made in table I.1.

There was little evidence that kin discriminating behavior in *R. cascadae* is associated with predator avoidance or vigilance. First, in both the field observations and the mesocosm study
(chapter two), tadpole aggregation behavior was less frequent in the presence of a predator than when predators were absent. The frequency of observed tadpole aggregations was negatively correlated with the abundance of predators in unmanipulated natural potholes. Furthermore, tadpoles were more dispersed in artificial wading pools with predators than in pools without predators. Second, the results of the field experiment in chapter two did not demonstrate enhanced survivorship for tadpoles in kin groups over tadpoles in mixed groups when in the presence of a predator. However, this could be due to the overall low number of predators in the field study. Third, in the predator avoidance experiments (chapter three), tadpoles in groups did not respond to the presence of predators differently than did solitary individuals. Tadpoles in groups and as solitary individuals decreased activity in the presence of a predator in a similar manner. However, overall, tadpoles in sibling groups were more active than solitary tadpoles and tadpoles in mixed groups.

Although there was no interaction between the presence of predators and the social condition of the tadpoles (i.e. the relative decrease in tadpole activity due to predators was the same for each social condition), higher activity in kin groups in the presence of predators may be the result of kin-biased foraging/vigilance trade-offs. Tadpoles in kin groups may be more cooperative in balancing the trade-offs between time spent foraging (associated with high activity in anurans) and predator
vigilance. Thus, although the evidence supporting it is weak, the predator vigilance hypothesis cannot be eliminated.

There was no evidence in support of the alarm response hypothesis. First, as discussed above, tadpole aggregations were less prevalent in the presence of predators. Thus, there would appear to be limited opportunity for such an alarm mechanism. Second, and most critical, there was no evidence of an alarm response, kin-biased or otherwise (chapter three). Instead, the cues released from crushed conspecifics appeared to elicit a feeding response in test animals.

There was strong evidence that the growth and development of *R. cascadae* tadpoles is kin-biased. First, tadpoles form aggregations in response to resources normally associated with growth and development in anuran larvae (chapter two, e.g. food patches, substrate type, and thermal pockets). Second, and most critical, growth and development was different when tadpoles were reared in kin versus mixed groups (chapters four and five). However, the difference was not always in favor of tadpoles in kin groups. Tadpoles reared in kin groups had lower mass at metamorphosis under the conditions of the laboratory growth and development study (chapter three). In the field study (chapter five), tadpole growth and development was dependent upon the ecological conditions of the field enclosures. Depending upon tadpole density and access to the substrate, and after adjusting for differences in mass, survivorship was sometimes
higher in kin groups, sometimes lower, and sometimes the same as mixed groups.

Kinship effects on growth and development in the field enclosures were associated with differences in the distribution of individual tadpole mass. Generally, the distribution of mass was more normally distributed in kin groups and more skewed in mixed groups. This difference in the distribution of tadpole mass could be due to the genetic make up of kin versus mixed groups, differences in behavioral interactions within kin versus mixed groups (e.g. kin may be less agonistic towards one another), or a combination of both.

Such context dependent growth and development is particularly significant given the results of previous tadpole growth and development studies. For example, the direction of the kinship effect (i.e. whether individuals in kin groups grow faster/larger or slower/smaller than those in mixed groups) appears to depend upon the species examined and the experimental conditions (Shvarts and Pyastolova 1970, Travis 1980, Waldman 1986, Jasienski 1988, Smith 1990, Waldman 1991; reviewed by Blaustein and Waldman 1992). The results of chapter five demonstrate that testing conditions may determine the nature and direction of kin-biased growth and development and that such context dependence may be consistent with Hamilton's rule.
As discussed in chapter one, kin discrimination may be important for reasons other than forming kin aggregations. For example, kin discrimination may allow anurans with cannibalistic tendencies to direct their cannibalism away from relatives (Blaustein and O'Hara 1982; Blaustein et al. 1987; Pfennig et al. 1993). Although I did not directly test the cannibalism hypothesis, this is an unlikely explanation for kin discrimination in *R. cascadae*. There are no reports in the literature of cannibalistic morphologies or behavior in *R. cascadae* tadpoles except for opportunistic cannibalism after a tadpole has already been killed. Alternatively, kin discrimination may be important for mate choice. In fact, *R. cascadae* individuals are known to discriminate between kin and non-kin after metamorphosis (Blaustein et al. 1984). Kin discrimination may allow individuals to balance the costs of inbreeding and outbreeding (Bateson 1983). The kin-biased mate choice hypothesis remains untested in amphibians. However, it maybe possible to test such a hypothesis by utilizing molecular techniques (e.g. DNA fingerprinting) that can assess the relatedness between mating pairs of frogs.

The results of this thesis clearly indicate that kin discriminating behavior in *R. cascadae* may yield inclusive fitness benefits. Alternative explanations for kin discriminating behavior (e.g. species recognition, resource preferences) do not predict kin-biased effects on fitness components of metamorphs (size and
survivorship to metamorphosis). Depending upon tadpole density and access to flocculent substrate, tadpoles survived better (after adjusting for differences in mass) in kin groups than in mixed groups. Furthermore, the context dependent nature of kin-biased aggregation behavior is consistent with the conditionality of Hamilton's rule and may be directly related to the growth and development opportunities offered by certain ecological conditions. For example, tadpoles may preferentially associate with kin only when there exists an opportunity for enhanced survivorship.

There is a need to incorporate multiple testing conditions in kin recognition research. It is apparent that kinship factors can affect individual behavior and that such effects may interact with ecological conditions. The context dependent nature of kinship effects directly demonstrate the ecological conditions necessary for kin selection to operate, thus, linking ecological and evolutionary processes. Therefore, kin recognition research may expand beyond the mechanistic studies of the past, to functional explanations based upon testing the conditionality of kin-biased behavior.

Just as kin selection processes may be affected by ecological factors, ecological processes may be affected by kinship. Kinship may influence social behavior, predator avoidance behavior, foraging behavior, intraspecific competition, and the distribution of organisms. For example, the densities and distributions of
many organisms are dependent upon kinship effects (e.g. urochordates, Grosberg and Quinn 1986; insects, Crozier et al. 1987; fish, Brown and Brown 1993; amphibians, Waldman et al. 1992; reptiles, Werner et al. 1987; birds, Reyer 1980; rodents, Lambin and Krebs 1991; carnivores, Rogers 1987; and primates, Makwana 1978).

Furthermore, the results from chapters four and five demonstrate that kinship factors can influence the number and size of individuals recruited into higher size classes and, therefore, may influence the dynamics of size structured populations. Size structuring can greatly affect population dynamics and species interactions (Wilbur 1980; Werner and Gilliam 1984; Begon 1984; Werner 1986, 1994; Mittelbach and Osenberg 1993; Wootton 1993). Thus, kinship effects have important implications for population studies.

Recently, individual behavior mechanisms have been used to explain population and community level phenomena (e.g. Gilliam 1987; Rosenzweig 1987; Abrams 1991, 1992; Chesson and Rosenzweig 1991; Gordon 1991; Werner 1992). Furthermore, individual variation has been recognized as an important component in population and community level models (e.g. Hassell and May 1985; Lomnicki 1988). As mechanistic approaches are becoming more important in interpreting ecological phenomena (Schoener 1986), research into the effects of individual variation on population dynamics and species interactions is becoming
increasingly relevant. Kin recognition studies may greatly contribute to the mechanistic understanding of ecology because of the possible effects of kinship on behavioral interactions between individuals. Such effects may be particularly important for highly social species.

The evolutionary implications of kin-biased behavior extend beyond explanations for altruistic behavior. Likewise, the ecological implications of kin-biased behavior are just beginning to be realized. Thus, there is great opportunity to expand the perspective of kin recognition research.
BIBLIOGRAPHY


Kaplan, R.H. 1980. The implications of ovum size variability for offspring fitness and clutch size within several populations of salamanders (Ambystoma). Evolution 34:51-64.


