

**Conservation and ecology of the endangered fire  
salamander (*Salamandra infraimmaculata*)**

**Ori Segev**

**A THESIS SUBMITTED FOR THE DEGREE  
"DOCTOR OF PHILOSOPHY"**

**University of Haifa  
Faculty of Science and Science Education  
Department of Evolution and Environmental Biology**

**NOVEMBER, 2009**

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**Supervised by: Leon Blaustein**

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## Acknowledgments

## Table of contents

<b>Abstract</b> .....	vii
<b>List of Tables</b> .....	x
<b>List of Figures</b> .....	xii
<b>General Introduction</b> .....	1
<i>Amphibian decline</i> .....	1
<i>Salamandra distribution and conservation status</i> .....	2
<i>Population size and breeding phenology</i> .....	3
<i>Invasive species</i> .....	4
<i>Breeding strategies and habitat selection</i> .....	5
<i>Priority effects</i> .....	6
<b>Chapter 1. Population size, structure and phenology of an endangered salamander at temporary and permanent breeding sites</b> .....	8-29
Abstract.....	9
Introduction.....	10
Methods.....	13
Results.....	16
<i>Population size</i> .....	15
<i>Residence timing and duration, phenology, and synchronization with rain</i> .....	16
Discussion.....	17
<i>Conservation management implications</i> .....	19
<i>Figures 1-4</i> .....	22
<i>Tables 1-4</i> .....	26

**Chapter 2. Deleterious effects by mosquitofish (*Gambusia affinis*) on the endangered fire salamander (*Salamandra infraimmaculata*).....30-54**

Abstract.....31

Introduction.....32

Materials and Methods.....34

*Natural pool observations*.....34

*Mesocosm experiment*.....36

Results.....39

*Natural pool survey*.....39

*Mesocosm experiment*.....40

Discussion.....42

*Figures 1-5*.....47

*Tables 1-3*.....52

**Chapter 3. Spatio-temporal progeny allocation strategies in the fire salamander:**

**a model and empirical test.....55-82**

Abstract.....56

Introduction.....57

The model.....59

*The environment*.....60

*Larval load and survival*.....61

*Increment in Reproductive Success*.....61

*Accumulated reproductive success*.....63

*Accumulated reproductive success of fixed clutch strategy*.....64

<i>Model predictions</i> .....	65
Larviposition Experiment.....	66
<i>Statistical analysis</i> .....	67
<i>Experimental results</i> .....	67
Discussion.....	68
<i>Figures 1-8</i> .....	72
<i>Tables 1-3</i> .....	80

**Chapter 4. Priority effects of the early breeding fire salamander on the late**

<b>breeding banded newt (<i>Triturus vittatus</i>)</b> .....	83-101
Abstract.....	84
Introduction.....	85
Methods.....	87
<i>Urodele survey at Secher pool</i> .....	87
<i>Artificial pool experiment</i> .....	88
<i>Statistical analysis</i> .....	90
Results.....	91
<i>Field survey</i> .....	91
<i>Artificial pool experiment 1</i> .....	91
<i>Effects on invertebrates</i> .....	91
<i>Effects on <i>Triturus</i> during <i>Salamandra</i> period</i> .....	92
<i>Overall effects on <i>Triturus</i></i> .....	92
<i>Artificial pool experiment 2</i> .....	93
<i>Combined results of experiments 1 and 2</i> .....	93
Discussion.....	93

<i>Figures 1-4</i> .....	96
<i>Tables 1-2</i> .....	100
<b>General Discussion</b> .....	102
<b>References</b> .....	110

# Conservation and ecology of the endangered fire salamander

(*Salamandra infraimmaculata*)

By: Ori Segev

## Abstract

Amphibian faunas have been declining globally for the last three decades, including in protected, supposedly pristine as well as unprotected areas. This process, which seems to be non-random and for many species unidirectional, is apparently driven by multiple factors acting independently or in synergism driving amphibian populations' decline, range constriction and extinction. Although the fire salamander, *Salamandra infraimmaculata* has been classified globally and locally endangered, critical information on its phenology, interspecific interactions and habitat selection are lacking to critically assess its true status and to properly develop conservation and management plans. *Salamandra* breeding sites and surrounding terrestrial habitats are threatened by various factors including contamination, desiccation, introduced species, and habitat destruction, alteration and fragmentation. As a keystone amphibian species, the absence of larval *Salamandra* can also potentially impact the entire ecological community.

In this thesis, I addressed and found the following:

(1) Although *S. infraimmaculata* is considered an endangered species, little is known about its population size and structure. Mark-recapture estimates of population size in several breeding sites in northern Israel revealed populations larger than previously assumed and a strong effect of breeding site permanence - populations at sites having permanent aquatic breeding bodies were significantly larger than temporary site populations. These results



delineate the need to prioritize the species conservation and management in accord with breeding site permanence.

(2) A major threat for local aquatic faunas in permanent water bodies is invasion by non-native predatory fishes. In Israel, the mosquitofish, *Gambusia affinis*, is stocked into permanent water bodies, some of which are *S. infraimmaculata* breeding sites. In field observations comparing *Salamandra* breeding sites before and after mosquitofish (*Gambusia affinis*) invasion and at different fish densities, I found circumstantial evidence for strong negative effects of *Gambusia* on larval performance and number of emerging metamorphs. In a follow-up mesocosm experiment, I found that *Gambusia* had very strong negative effects on *S. infraimmaculata* larvae regardless of whether artificial habitat heterogeneity had been added. In order to protect *S. infraimmaculata*, these studies call for the cessation of *Gambusia* stockings into *Salamandra* breeding ponds.

(3) Understanding habitat selection is essential for wise management of endangered species. Breeding site selection has significant implications on parental fitness through consequences on offspring survival and performance. A state-dependent dynamic model suggests, and a free-choice outdoor enclosure experiment corroborates, that *Salamandra* distribute larvae temporally, between rain events, and spatially over a number of small-size pools, adjusting batch size according to pool volume by placing more larvae in deeper pools. These results have theoretical and practical implications. By explaining the adaptive significance of batch size variation it contributes to the theory of breeding site selection. Furthermore, understanding *Salamandra* larviposition behavior may prove essential for predicting the consequences of deterioration in habitat quality.

(4) Interspecific interactions and species composition in temporary pool communities may reflect species differences in breeding phenology and within-season variation in breeding timing. Priority effects may partially explain negative spatial association between species

and may have particular conservation applications when these species are considered endangered. I conducted a mesocosm experiment and a field survey to assess the potential effects of *S. infraimmaculata* larvae on another endangered urodele species, banded newt (*Triturus vittatus*). These studies demonstrated that early breeding *Salamandra* strongly and negatively affect the late breeding *Triturus* and that increased habitat heterogeneity may enhance the likelihood of coexistence between the two urodeles.

A better understanding of *Salamandra* status and the extent of current and future threats is vital for developing protection measures and management programs, for this and other amphibian species. The information presented in this work also contributes to better understanding of conceptual aspects in spatial ecology, habitat selection, and metapopulation dynamics.

## LIST OF TABLES

<b>Table</b>	<b>Page</b>
Table 1.1: Site ephemerality, location and description of the five breeding sites studied.....	27
Table 1.2: Yearly population size estimates, standard errors and number of samples at the two permanent and three temporary breeding sites. The year listed provides the beginning of the breeding season. For example, 2002 represents fall 2002 through spring 2003.....	28
Table 1.3: Correlation analysis (Spearman Rank) of individual breeders' first arrival date across consecutive seasons.....	29
Table 1.4: Analysis of variance assessing the influence of site, gender, year and gender by site interaction on breeders' residence time at the breeding site.....	30
Table 2.1: Repeated measures analysis of variance assessing structural complexity and time effects on adult (9 dates) and juvenile (5 dates) mosquitofish abundance. Greenhouse-Geisser adjustments of degrees of freedom were used because of violations of the sphericity assumptions.....	54
Table 2.2: Repeated measures analysis of variance assessing effects of <i>Gambusia affinis</i> and structural complexity on larval <i>Salamandra</i> (a) survival (proportion, arcsin-square root transformed); (b) mass (natural log-transformed); (c) tail:body ratio (arcsin-square root transformed) during three, six and nine weeks after introducing <i>Salamandra</i> larvae. Greenhouse-Geisser adjustments of degrees of freedom were used in	

analyzing mass due to violations of the sphericity assumptions.....	55
Table 2.3: Structural complexity (presence or absence of radiating cords) effects in the absence of <i>Gambusia</i> on <i>Salamandra</i> number surviving, time to (days), size (wet mass) and tail:body ratio at metamorphosis assessed by t-tests.....	56
Table 3.1: The model's physical characteristics and frequency of pools.....	84
Table 3.2: The model's weather patterns. Rain probabilities were estimated from data in Warburg (1985). Time begins on 1 December, each rain event adds 1cm to the depths of all the pools.....	85
Table 3.3: Model parameter values and units.....	86
Table 4.1: Analyses assessing the influences of larval <i>Salamandra salamandra</i> and artificial vegetation on survival and size (total length ) of <i>Triturus</i> larvae measured one week after the removal of <i>Salamandra</i> (df=10, 1). Survival is assessed using Mann-Whitney U due to nonhomogenous variances. Length is assessed by analysis of variance. N equals number of pools. There are fewer pools for length because of zero survival in some pools. P-values are based on two-tailed tests.....	106
Table 4.2: Mann-Whitney U for measures of <i>Triturus vitattus</i> at metamorphosis: survival, size and development time. N gives number of pools. There are fewer pools for size and time to metamorphosis because of zero survival in some pools. P-values are two tailed.....	107

## LIST OF FIGURES

Figure	Page
Figure 1.1: Geographic maps of the study regions. On the aerial photos, <i>Salamandra infraimmaculata</i> breeding sites appear in capital letters and marked with dots while small letters signify nearby villages. The upper right hand (a) shows the Lower Galilee sites and the lower right hand map (b) shows the Mt. Carmel sites.....	23
Figure 1.2: <i>Salamandra infraimmaculata</i> average yearly population size estimates related to breeding pool maximum volume. Error bars are $\pm 1$ standard error.....	24
Figure 1.3: Male and female breeding sites residence time. Sites are paired per year and with geographic region; (a) Carmel and (b) Lower Galilee. Error bars are $\pm 1$ standard error. N=number of individual.....	25
Figure 1.4: Temporal distribution of the numbers of <i>S. infraimmaculata</i> individuals censused across sampling dates and daily amount of rain at: (a) permanent site (Ein-El-Balad) and (b) temporary site (Secher) in the Carmel during 2002.....	26
Figure 2.1: (a) Mosquitofish caught per sweep (mean of 15 one-meter sweeps) at “Kaukab 2” Pool during 20 May 1999 (prior to mosquitofish introduction) and “Alon”, “Kaukab 1” and “Kaukab 2” pools on 28 May 2003. Error bars are one standard error; (b) tail:body (snout-vent) ratio of <i>Salamandra</i> larvae from the same pools and dates. Error bars are one standard error. Numbers in parentheses indicate number of larvae	

measured. Photo insert: typical specimens of <i>Salamandra</i> larva with intact tail from “Kaukab 1”, the low fish density pool (Left), versus <i>Salamandra</i> with a truncated tail larva from “Kaukab 2”, the high fish density pool (Right).....	49
Figure 2.2: Number of <i>Salamandra</i> larvae caught per 15 minute sweep in “Kaukab 2” Pool in 1999, prior to mosquitofish introduction and during 2003, after mosquitofish introduction.....	50
Figure 2.3: Mosquitofish adults and juveniles (Mean $\pm$ SE) caught per pool in pools with and without increased structural complexity.....	51
Figure 2.4: Mosquitofish and structural complexity effects on <i>Salamandra</i> larvae (Mean $\pm$ SE): (a) survival; (b) mass; (c) tail to body (snout-vent) ratio; (d) percent of damaged gills; and (e) percent of damaged limbs, three, six and nine weeks following the introduction of <i>Salamandra</i> larvae.....	52
Figure 2.5: Mosquitofish and structural complexity effects on <i>Salamandra</i> survival to metamorphosis (Mean $\pm$ SE).....	53
Figure 3.1: Fitness increment from a single larviposition, $\Phi(c,i,d,t)$ , as a function of time and clutch size, $c$ , and current water depth, $d$ , combinations in three pool types, $i$ .....	76
Figure 3.2: Female seasonal fitness expectation $W(l_0,h,1,1 c_f)$ , as a function of fixed clutch size, $c_f$ .....	76
Figure 3.3: A comparison of maximal maternal fitness over the entire season for females employing a fixed clutch size strategy, $W(l_0,h,1,1 c_f^*)$ , versus a plastic clutch size strategy $F(l_0,h,1,1)$ , for four initial larval loads, $l_0=40, 80, 115, 150$ .....	77

Figure 3.4: Distribution of optimal clutch size, $c^*(150,i,d,t)$ , over time for different combinations of pool types, $i$ , and current depths, $d$ , at a constant load of $l=150$ larvae.....	78
Figure 3.5: Number of tubs used for larviposition per female.....	79
Figure 3.6: Number of larviposition nights per female (a); interval between individual female larviposition events (b).....	80
Figure 3.7: Distribution of larviposition events during the first night in the deep (14cm) versus the shallow (4.5cm) tubs. O=complete female avoidance; 1=deposition in only one tub of each depth pair; 2=deposition in both tubs of each depth pair.....	81
Figure 3.8: Mean number of larvae deposited per female in the deep (14cm) versus shallow (4.5cm) tubs during the entire stay within the enclosure and only the first night.....	82
Figure 4.1: Temporal distributions of <i>Salamandra salamandra</i> larvae and <i>Triturus vittatus</i> adults and larvae at the temporary pool, Secher Pool: (a) abundance (number caught per ten sweeps); (b) size (total length). Error bars are $\pm$ one standard error.....	102
Figure 4.2: Effects of larval <i>Salamandra salamandra</i> and artificial vegetation on the abundance of: (a) <i>Arctodiaptomus similis</i> (number per sweep); (b) <i>Culiseta longiareolata</i> egg rafts; (c) <i>Culiseta longiareolata</i> larvae (number per sweep). The left hand side of the graph is during the period in which <i>Salamandra</i> was present in <i>Salamandra</i> pools. The right hand side of the graph is the period after <i>Salamandra</i> was	

removed. Error bars are  $\pm$  one standard error.....103

Figure 4.3: Effects of larval *Salamandra salamandra* and artificial  
vegetation on larval *Triturus vittatus* survival (a), and size  
(total length) (b) five days after the removal of *Salamandra*  
*salamandra*. Error bars are  $\pm$  one standard error.....104

Figure 4.4: Effects of larval *Salamandra salamandra* and artificial  
vegetation on *Triturus vittatus* survival to (a), size at (b), and  
time to (c) metamorphosis. Error bars are  $\pm$  one standard error.....105



## **General Introduction**

### *Amphibian Decline*

Many amphibian faunas have been globally collapsing over the last three decades in both unprotected and protected, supposedly pristine areas (Halliday 2005). Scientists agree that amphibian decline is a unidirectional process and suspect there is more than a single cause for the phenomena. Moreover, multiple factors may act in synergism and result in amphibian mortality and/or sublethal effects (Kiesecker and Blaustein 1995, 1999; Kiesecker et al. 2001; Kiesecker and Skelly 2001). The list of anthropogenic factors underlying amphibian populations' declines, range constrictions and extinctions includes: habitat destruction (Blaustein et al. 1994a), alteration and fragmentation (Dodd and Smith 2003; Cushman 2006); invasive species (Kats and Ferrer 2003); climate change (Pounds et al. 1999; Blaustein et al. 2001); UV-B radiation (Blaustein and Kiesecker 1997; Kats et al. 2000); chemical contaminants (Berrill et al. 1997; Hayes et al. 2002); commercial exploitation (Jensen and Camp 2003); disease and pathogens (Morell 1999; Blaustein et al. 1994b; Daszak et al. 1999).

The challenges facing conservation biologists in determining the nature, extent and causes for amphibian decline are great as historical data on species presence and abundance is mostly scarce or completely absent for most species, and many amphibian populations tend to highly fluctuate with environmental conditions (Pechmann et al. 1991). Moreover, populations may be in decline in some regions yet maintain stability in others. Amphibian declines highlight the need for a better understanding of species-specific habitat associations for populations monitoring, management and conservation.

## *Salamandra Distribution and Conservation Status*

The fire salamander (*Salamandra infraimmaculata*) is listed globally as “near threatened with decreasing population trend” (Papenfuss et al. 2008). The species range includes Israel, Lebanon, Syria, and Turkey (Steinfartz et al. 2000). In Israel, at the genus’ southern-most edge of distribution, it is classified as endangered (Dolev and Perevelotzky 2004). Populations in Israel are found in several localities - on the Carmel Mountain and upper and lower Galilee mountain ranges (Degani 1996) - and some but not all of these populations are found within protected areas (national reserves). *Salamandra*, like other complex life cycle organisms and pond-breeding amphibians, requires aquatic habitats for reproduction and for larval development and terrestrial habitats for juvenile and adult stages and dispersal. The two habitats are biologically linked and must be managed mutually. As habitat requirements differ among life stages, there is a need to recognize and quantify the type and degree of threats facing *Salamandra* in both terrestrial and aquatic habitats.

I used a combination of field surveys, experiments and modelling to study *Salamandra*. In particular, I addressed the following:

1. Population size, structure and phenology at temporary and permanent breeding sites.
2. Deleterious effects of the invasive mosquitofish (*Gambusia affinis*).
3. Spatio-temporal larval deposition strategies.
4. Priority effects on the late-breeding banded newt (*Triturus vittatus*).

### *Population Size and Breeding Phenology*

Estimation of a species population size has significant applications for most conservation activities. Small populations are particularly vulnerable to extinction through random variation in birth and death rates, variation in resource or habitat availability, predation, competitive interactions, single-event catastrophes, and inbreeding. *Salamandra infraimmaculata* is recognized as endangered species in Israel yet population size estimates are scarce. *Salamandra* utilizes both permanent and temporary water bodies for reproduction thus making it an appropriate model for evaluating different population level dynamics and characteristics in the context of breeding site permanence and size. Short hydroperiods may result in catastrophic death of all pre-metamorphic larval stages (Smith 1983; Semlitsch 1987; Wilbur 1987; Banks and Beebee 1988; Newman 1988; Murphy 2003) while negative density-dependent effects are known to strongly influence larval stages of pond-breeding amphibians (Wilbur 1976). Breeding phenology i.e., the timing and duration of breeders' residency, may also differ between temporary and permanent sites populations. Initial breeding may be constrained by the timing of pool inundation at temporary sites while rain pattern may dictate the early season above-ground dispersal activity to and between permanent breeding ponds. In the first chapter, I used capture-recapture of adult breeders during up to three years at five sites (two permanent and three temporary) to estimate population size and address the hypothesis that permanent breeding sites support larger populations. In addition, I used these mark recapture data to address several hypotheses regarding population, size and structure: (1) permanent breeding sites support larger populations because there is no loss of recruitment due to pool desiccation; (2) breeding activity would be largely at the beginning of the season in permanent sites because later in the season risk of cannibalism and the intensity of intraspecific

competition increases; (3) permanent sites breeders would show reduced plasticity on first arrival date between seasons because there is no uncertainty regarding pool filling date; (4) a gender-related variation in site occupancy duration - males were predicted to stay longer at the vicinity of the breeding site to increase female encounter rate since male *Salamandra* are capable of multiple copulations with different females per season.

### *Invasive Species*

Alien or invasive species have been generally recognized as detrimental for amphibians and as one of the causes for amphibian population declines and local extinctions (Bradford et al. 1993; Morgan and Buttemer 1996; Skelly 2001; Kats and Ferrer 2003). These are species that did not occur naturally in a system and have been transported either purposefully or accidentally by human activities (Kiesecker 2003). Freshwater ecosystems are particularly susceptible to impacts by introduced species. Fishes are stocked in natural water bodies mainly for fishing but also as biological control agents. The mosquitofish *Gambusia affinis* has been introduced extensively around the world in an attempt to control mosquito populations (Courtenay and Meffe 1989). Accumulated evidence suggests *Gambusia* not only may fail to reduce mosquito populations efficiently (Hoy et al. 1972; Arthington and Lloyd 1989; Blaustein 1992), but may negatively impact non-target organisms (Courtenay and Meffe 1989; Alcaraz et al. 2008). Mosquitofish effects on amphibians are not consistent across species and systems but may be species and system specific (Baber and Babbitt 2004; Ling 2004; Gregoire and Gunzburger 2008). The potential magnitude of the effect of *Gambusia* on endangered species may be influenced by habitat structure such as aquatic vegetation type and amount (Heck et al. 1991). Habitat structure may affect predator-prey interactions through the amount of prey refuge it provides and has been often suggested as the cause for reduced

fish predation in various aquatic systems (Werner et al. 1983; Baber and Babbitt 2004; Lehtiniemi 2005; Sass et al. 2006). *Gambusia* was first stocked in Israel at the beginning of the century to control mosquitoes (Kligler 1930). The fish are currently widespread and have recently occupied several permanent water bodies traditionally used as breeding sites by the fire salamander. In the second chapter, I present field observations to compare *Salamandra* larval abundance and size at the same site before and after *Gambusia* introduction and compared simultaneously three different sites that varied in mosquitofish densities. Next, I present the results of a follow-up mesocosm experiment designed to quantify the effects of *Gambusia* and habitat complexity on *Salamandra* larval survival, growth and development.

#### *Breeding Strategies and Habitat Selection*

Spatial and temporal progeny allocation entail direct and indirect fitness consequences for the mother as selection favours the adjustment of reproductive effort with environmental context (Resitarits 1996). Active discrimination by the female among potential oviposition sites based on their perceived suitability was empirically shown for numerous organisms (e.g., Mayhew 1997; Wilson 1998; Blaustein et al. 2004; Blouin-Demers et al. 2004; Rieger et al. 2004). In complex life cycle organisms such discrimination requires available information at the time of decision-making that would be reliable for at least most of the larval period. Amphibian oviposition site selection has been studied in the context of predators and competitors (Resetarits and Wilbur 1989; Crump 1991; Sexton et al 1994; Petranka et al. 1994; Marsh and Borrel 2001; Glos et al. 2008), parasites (Kiesecker and Skelly 2000), timing of deposition (Matsushima and Kawata 2005) and pool volume (Crump 1991; Apsbury and Juliano 1998, Spieler and Linsenmair 1997; Rudolf and Rodel 2005). Despite this body of literature, the mechanisms underlying

plastic oviposition strategies remained mostly unexplored at the individual level. In the third chapter, I present a state-dependent-variable model that incorporates data on the individual female physiological state (offspring complement) and the hydrologic environment properties (rainfall probabilities, pool size, pool permanence, and pool frequency). The model predicts that to optimize lifetime reproductive success, gravid females will deposit their larvae across multiple pools and adjust the number of larvae deposited according to pool size. To test the model predictions I conducted an outdoor enclosure experiment and allowed individual gravid salamander to freely choose between four artificial pools, two deep and two shallow.

#### *Priority Effects*

The composition of a natural community at any point in time is determined by the historical sequence of past colonizations and the outcomes of interactions between colonists and inhabitants. One important community interaction that can potentially affect community assembly is priority effects. A priority effect occurs when the first colonizer to a patch gains an advantage over later colonists by monopolizing shared resources. Priority effects have been demonstrated for species over a wide range of taxa (Insects: Cole 1983; Hodge et al. 1996; Palmer et al. 2002; Amphibians: Wilbur and Alford 1985; Lawler and Morin 1993; Fish: Almany 2003; and Birds, Lockwood et al. 1999). In Mediterranean climates, late breeding amphibians may entail costs related to higher risk of pool desiccation though the level of food resources in temporary pools may be higher as the season progresses depending on early colonization history of potential competitors. Late breeders may experience negative priority effects by early breeding competitors and intraguild predators. By reducing the level of food resources, early breeders may continue

to have negative residual effects on late breeders, even after the early breeders have metamorphosed and left the breeding pool.

Habitat heterogeneity may interact with order of entrance of amphibian species to affect larval survival, growth and development by providing more substrate for food resources consumed by the larvae, and it may also reduce predation rates by concealing individuals from predators (Sih 1987; Semlitsch and Reyer 1992; Jackson and Semlitsch 1993; Kats et al. 1994; Walls 1995; Niecieza 2000, Baber and Babbitt 2004). In the fourth chapter, I present results of a field survey conducted to examine intra-season temporal distributions of *Salamandra* and *Triturus vitattus* in a temporary breeding site and an outdoor artificial mesocosm experiment designed to assess priority effects of large *Salamandra* larvae at the end of their larval development period, on recently hatched *Triturus* larvae. In this experiment I also assessed how artificial vegetation, in the form of submerged plastic strips, may influence *Triturus* larval performance in the presence or absence of *Salamandra*.

**Chapter 1. Population size, structure and phenology of an endangered  
salamander at temporary and permanent breeding sites**

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## **Abstract**

The fire salamander, *Salamandra infraimmaculata*, is listed as an endangered species in Israel and Israeli populations represent the genus' worldwide southern-most limit. This endangered classification was based largely on limited mark-recapture data and on using the Lincoln index whose assumptions of equal catchability, time homogeneity and a closed population are unlikely kept for salamander populations. We estimated population size at five breeding sites in northern Israel – two permanent and three temporary breeding sites - for up to three years using a non-parametric procedure that allows the probabilities of recapture to vary both with time and with individual animal. We also compared breeding phenology and population size structure at these same sites. Population estimates at some breeding sites were larger than previously thought. Adult population size was not correlated with the size of the water body, but instead, sites with permanent water bodies had significantly larger populations. First arrival date to the breeding site of individuals on consecutive years was positively correlated at all sites suggesting that some individuals breed consistently early while others breed consistently late. Activity abundance was correlated with daily rainfall at a permanent site but not at an adjacent temporary site. Instead, activity abundance at the temporary breeding site was synchronized with pool inundation, which did not occur during the first rains. Males remained longer than females at all breeding sites. This study provides clear management implications both in terms of determining the vulnerability of specific populations, and in suggesting that permanent breeding sites are much more likely to support larger populations.

## Introduction

A key but elusive parameter for evaluating species' conservation status or extinction threat level is population size (IUCN 2001). Populations sizes are often estimated using the Lincoln index even in cases where the assumptions of this index cannot be met. Violations of the assumptions of a closed population and of equal catchability over individuals and time may cause over- and under-estimates of population size, respectively (Bohlin and Sundström 1977; Lindeman 1990; Arntzen 2002).

Populations of the fire salamander, *Salamandra infraimmaculata*, in Israel represent the southern-most edge of the genus' range (Warburg 2007). Although *S. infraimmaculata* is considered endangered in Israel (Dolev and Prevolotzky 2004) and near endangered worldwide (Papenfuss et al. 2008), population size estimates using mark-recapture are scarce (Degani 1996). Mark-recapture studies are needed on this species to better evaluate its endangered status. Moreover, mark-recapture studies can be an important tool in elucidating the factors that affect carrying capacity, which is essential information to protect an endangered species. Although there is increasing recognition of the importance of quality and quantity of terrestrial habitat for conservation of amphibians (Loredo et al. 1996; Semlitsch 1998; Skelly et al. 1999; Porej et al. 2004; Schmidt et al. 2005), quality and quantity of aquatic habitat can certainly be the limiting factor for amphibian populations.

One factor that might affect adult population size is breeding pond size. Small ponds may produce fewer recruits than could otherwise be supported by the terrestrial environment. Such recruitment limitation could occur if small ponds are of poorer quality and less preferred than larger ponds. Small ponds could be of poorer quality as the result of stronger and negative density-independent processes (e.g., per capita immigration rates are lower in small ponds) or density-dependent processes (e.g., larval survival may be lower in small

ponds if small ponds have greater larval densities) affecting the number of recruits. Furthermore, we have observed that *Salamandra* is more likely to choose to larviposit in a larger pond and deposit more larvae in larger ponds (unpublished data). A second factor is the ephemerality of the aquatic breeding habitat. Desiccation is one of the primary factors that prevent amphibian larvae from reaching metamorphosis (Newman 1989; Ryan 2007). Habitat ephemerality is also an important selective force on life history traits of many temporary pool breeders (Blaustein and Schwartz 2001; Williams 2006) and on amphibians in particular (Semlitsch 1985; Wilbur and Alford 1985; Skelly 2001). Ecological and evolutionary responses of biota to ephemerality have been brought to the forefront of ecological science due to the threat of global climate change (Kareiva et al. 1993; Alford and Richards 1999; Walther et al. 2002). Changes in temperature and rainfall pattern may result in faster habitat desiccation and can thus have particularly large impacts on species that use ephemeral habitats (Blaustein et al. 2001; Araújo et al. 2006).

Additional constraints and factors are relevant for aggregate breeders like terrestrial salamanders. Their movement to, from and among breeding sites may be determined by the distance and habitat structure between aestivation sites and the breeding ponds, thus affecting the time of arrival to the breeding site. Different factors may constrain breeders at permanent versus temporary sites. Early breeders at permanent sites may be limited by the frequency and distribution of rainy nights suitable for terrestrial activity. However, temporary-site breeders also may be constrained by the timing of pond inundation (Griffith 1997; Semlitsch et al. 1993) – i.e. due to percolation into dry soils, pools may not fill during the first rains after an extended dry season. Temporary Mediterranean winter ponds are characterized by high variation in hydroperiod and the unpredictability of annual pond filling (Jakob et al. 2003). Strategies to cope with this unpredictability mostly involve plasticity in the onset of breeding (Diaz-Paniagua 1992). We expected to find differences

in breeding phenology between populations utilizing permanent breeding sites and those utilizing temporary sites. *Salamandra* species make for an excellent model organism to study intraspecific variation in breeding phenology in the context of site permanence because they breed in both permanent and temporary sites, because of their rather isolated populations and because of their remarkable plasticity in terms of life history, behavior and morphology (Alcobendas and Castanet 2000; Weitere et al. 2004).

*S. infraimmaculata* is found in Turkey, Syria, Lebanon, and Israel (Steinfartz et al. 2000). They are found in three distinct geographic regions in northern Israel. Breeding adults show considerable pond fidelity with part of the breeding population returning to the same breeding site every year (Warburg 2006); however, there is some dispersal among breeding sites (Bar-David et al. 2007). Activity of adult *S. infraimmaculata* is largely restricted to rainy nights (which occurs mostly between late fall and late winter) when gravid females deposit larvae in a variety of aquatic habitats including springs, rock pools, quarry cisterns and wells (Degani 1996). The larvae are predaceous and cannibalistic, and exhibit strong density-dependent effects (Degani et al. 1980; Blaustein et al. 1996; Eitam et al. 2005; Segev and Blaustein 2007). Larvae of temporary breeding sites must metamorphose by early spring, while a fraction of the larvae in permanent sites continue to grow and metamorphose the following fall when the rains begin (Blaustein pers. obs.).

In this study, we use mark-recapture data to assess the following adult population characteristics at three temporary and two permanent breeding sites: (1) Adult population size. We predicted that permanent sites would support larger adult populations (no loss of recruitment to desiccation) but that adult population size would also increase with pool size as we expect larger pools to be capable of supporting more larvae; (2) Within-season residence time at breeding site. If females leave the site after larvipositing and mating, then we would expect breeding activity to be largely at the beginning of the breeding season for

permanent sites, because there appears to be no advantage to depositing larvae late in a permanent pond (no risk of desiccation at the beginning, and delaying larviposition will increase the risk of cannibalism and intraspecific competition). However, for temporary sites, there are tradeoffs between desiccation risk occurring during the early part of the hydroperiod where rains are sporadic with risks of cannibalism and competition later (Crump 1983; Warburg 1994; Lawler and Morin 1993; Griffiths 1997; Lehtinen 2004), so we would thus expect greater temporal bet-hedging and thus a longer residence time at temporary breeding sites; (3) Among-season individual-specific timing in first appearance at breeding site. We predicted that permanent-site breeders will show reduced plasticity on first arrival date since there is no uncertainty regarding date at which the pond holds standing water; (4) Gender-related variation in site occupancy duration. We predicted that males will stay longer than females near the breeding site to increase female encounter rate as male *Salamandra* are capable of multiple copulations within a season (Steinfartz et al. 2006) while females are capable of long-term sperm storage and repeated usage (Sever 2002).

## **Methods**

We studied five *S. inframaculata* populations (two permanent and three temporary breeding sites) for 2-3 seasons per site (Figure 1; Tables 1, 2). We use the term "population" for the breeding aggregation around the sampled water body. Breeding sites were located at two geographic regions ~25km apart in northern Israel: Mt. Carmel and the Lower Galilee. Adult *Salamandra* were monitored on rainy nights throughout the breeding season for a total of 43 nights (generally 2 sites were sampled on a given night) in which 670 captures were made over the entire study. We used visual search using head torches and kept duration (2 person hours) and area searched (~1000 m<sup>2</sup>) constant among sites and

censuses. As *Salamandra* are slow moving, virtually every salamander that was detected was caught. For each individual, we recorded a digital dorsal photo and gender. Individuals were then released at their capture site. Later in the laboratory, a hard-copy picture of every individual captured was compared with a photograph database of all previously caught individuals. The unique dorsal yellow spot pattern was used to make individual-specific identifications (Doody 1995; Warburg 2006).

We used the mark-recapture data to estimate adult population size using a non-parametric procedure developed by Chao et al. (1992), allowing probabilities of recapture to vary both with time (different probabilities of capture at the  $t$  different sampling times) and with individual animal (different individuals have different probabilities of capture). Given this general framework, Chao et al. (1992) provide three different bias corrections for dealing with these heterogeneities; we chose the model that gave the lowest variance. This procedure is especially appropriate for species like terrestrial salamanders that show high heterogeneity between individual capture probabilities (Grover 2000; Petranka and Murray 2001).

To examine the evidence for our hypothesis that permanent pools carry larger populations, we compared the natural log-transformed population values averaged over years between permanent and temporary breeding sites by a t-test. We also examined evidence that pool size may influence population size and considered the evidence that population size might be correlated with urban characteristics (Spearman rank correlation). Using Google Earth Pro, we estimated proportion of urbanized area, distance to nearest road and settlement, and total length of dirt and paved roads over a radius of 1.28 km radius (maximum dispersal distance detected to date by a *Salamandra* individual: Bar-David et al. 2007).

To determine if time of first appearance relative to conspecifics was consistent for specific individuals between years, we ranked all individuals that were caught on consecutive years for each year based on their first occurrence, and conducted Spearman-rank correlations across years. We also compared residence time between sites and years within the Carmel (Ein-El-Balad and Secher) and within the Galilee (Kaukab and Manof). When an individual was observed once, it was signed a value of 1, while for individuals with multiple captures, we used the interval between the first and last capture to estimate the minimal annual duration of stay at the breeding site.

We assessed whether breeding site activity (number of individuals observed on a given sampling night) at a permanent (Ein El-Balad) and a temporary (Secher) breeding site during 2002 was correlated (Spearman Rank). These two sites on Mt. Carmel are only ~5 km apart and were sampled on the same nights. We assess the data for only these two sites on this one year due to insufficient sampling nights for the other sites and years.

## **Results**

### *Population size*

Estimated adult population sizes ranged considerably among breeding sites from >500 to ~20 (Table 2). Based on 95 percent confidence intervals, there were no significant differences between population sizes across years within a site although there were many significant contrasts between sites within a year. Comparing sites averaged over years, population estimates could not be explained by the size of the breeding pools – either maximum pool volume (Pearson Correlation:  $r=-0.38$ ;  $p=0.532$ ; Figure 2) or maximum surface area (Pearson Correlation:  $r=-0.37$ ;  $p=0.542$ ). Instead, the two permanent sites had considerably larger populations than the three temporary sites ( $t=4.33$ ;  $df=3$ ;  $p=0.027$ ). Despite three orders of magnitude difference in pool volume, the three temporary sites

show relatively small variation in population size (Figure 2, Pearson Correlation:  $r=-0.074$ ;  $p=0.953$ ). A normalized test of mean differences on paired temporary sites sampled during the same year revealed no significant differences ( $p \gg 0.05$  for all cases).

Average yearly population size was positively correlated with proportion of urbanized cover area (Spearman's  $Rho=0.9$ ;  $p=0.037$ ), negatively correlated with the distance to the nearest settlement (Spearman's  $Rho=-0.9$ ;  $p=0.037$ ) and tended to be, but not significantly, negatively correlated to road proximity (Spearman's  $Rho=-0.6$ ;  $p=0.285$ ) and to road length (Spearman's  $Rho=-0.7$ ;  $p=0.188$ ).

#### *Residence timing and duration, phenology and synchronization with rains*

Specific individuals tend to consistently arrive either early or late to the breeding site. Individuals' first arrival date across seasons was positively correlated in all the breeding sites tested (we did not assess data from Damun site due to small sample size) (Table 3). Males stayed at the breeding site significantly longer than females with no site or site by sex interaction (Figure 3; Table 4).

The number of active *Salamandra* at Ein-El-Balad was positively correlated with the daily amount of rain occurring on the census day throughout the season (Spearman  $Rho=0.708$ ;  $p=0.002$ ) (Figure 4a). However, at the temporary Secher site, *Salamandra* numbers peaked on Nov. 12th, the first time during the rainy season that the pool had standing water. Aside from this peak, numbers at Secher were low throughout the rainy season showing no correlation with the daily rainfall (Spearman's  $Rho=-0.142$ ;  $p=0.586$ ) (Figure 4b).



## Discussion

Good population estimates for assessing the endangered status are uncommon for amphibians in general and rare for *Salamandra* species in particular (Miaud et al. 2001; Rebelo and Leclaire 2003; Schmidt et al. 2005; Schmidt et al. 2007). For *S. infraimmaculata*, prior to our study, a long-term study of the Damun breeding site by M. R. Warburg (Warburg 1994, 2007; Bar-David et al. 2007), based on activity data, indicated a fluctuating, very small population: only 131 different individuals were detected over an 18 year period. This long-term study was influential in the local assessment that *S. infraimmaculata* was highly endangered in the Mt Carmel Region. Our own study also indicated a small population at this site. However, we have since found that populations at various breeding sites are linked by dispersal (Bar-David et al. 2007). Moreover, we have demonstrated that population size varies greatly among sites, ranging from hundreds (Kaukab, Ein-El-Balad) to tens (e.g., Damun).

We were also interested in explaining among-site variation in population size. We are limited in inferring what factors are important because of a small number of sites censused; careful censusing of numerous sites is problematic because rains at night are infrequent and unpredictable, long processing times, and the number of breeding sites, particularly permanent sites, are very limited. Nevertheless, our data strongly support our *a priori* hypothesis that permanent breeding sites support larger adult populations than temporary ones. Breeding site permanence may drive population dynamics at both pre-metamorphic larval stages and post metamorphic terrestrial stages and can have strong effects on both survival and reproductive fitness. Breeders at permanent sites can start the breeding season earlier since they do not depend on pool inundation as temporary sites breeders. Additionally, larvae developing at permanent sites are free from desiccation risks and may prolong their larval period and consequently metamorphose at a larger size, a trait

that can have high selective value in amphibian species (See review by Altwegg and Reyer 2003). Pond volume differed among sites by several orders of magnitude, yet it did not show any importance in explaining the high variance in adult population size among these five breeding sites. Although greatly increasing the number of breeding sites checked is likely to reveal that breeding pond size can also be important, the overriding effects of pool permanence in this study suggest it to be most important. Quality and quantity of terrestrial habitat surrounding the breeding site should be important for the supporting populations of amphibians (Semlitsch 1998; Porej et al. 2004; Regosin et al. 2005). Urban cover, generally expected to be negatively correlated with amphibian population size, was *positively* correlated with *Salamandra* population size. This undoubtedly is confounded with pond permanence; historically, due to the paucity of water sources that are available all year, human settlements were established close to permanent springs; large villages of Isifya and Kaukab exist close to Ein-El-Balad and Kaukab springs respectively, while no villages exist close to the temporary breeding sites. We suggest then that relatively larger *Salamandra* populations exist at the permanent sites not because of, but despite, the proximity to human settlements. We think it likely that the populations would be larger at the permanent sites were there no large urban settlements.

We found that males remain longer than females near the breeding site. This may reflect the species mating system and inter-sex conflicts as different selection pressures shape each gender's reproductive behavior. *Salamandra* demonstrate multiple paternity (Steinfartz et al. 2006) - i.e., males are capable of multiple mating with different females during a single season (Adams et al. 2005). Females however are capable of long-term sperm storage and thus may not be limited by copulation every year to reproduce each year (Sever 2002).

We found that individuals, in comparison to their conspecifics, were consistently early arrivers or late arrivers. The consistency in breeders' first arrival may be related to individual differences in orientation abilities or use of the same aestivation refuge year after year, resulting in individuals that use refuges closer to the breeding site consistently arriving earlier. This "refuge fidelity" may be prominent in habitats where high quality refuges are limited. Consistent ordered arrival to breeding site was shown for *Ambystoma maculatum* (Stenhouse 1985) but not for *Ambystoma talpoideum* (Semlitsch et al. 1993).

The temporal distribution of *Salamandra* across a breeding season may also be tied to breeding site permanence. At the temporary site, in contrast to the permanent site, salamanders seem to synchronize breeding-site activity with temporary pool filling (Jakob et al. 2003; Semlitsch et al. 1993) and the numbers observed was not correlated with daily amount of rain. This breeding strategy may maximize the duration of larval period and reduces the probability for catastrophic mortality of entire larval cohorts due to early pool desiccation (Semlitsch 1987; Pechmann et al. 1991; Skelly 1996; Blaustein et al. 2001). Additionally, breeding shortly after pond-filling reduces inter-guild competition with other aquatic predators.

#### *Conservation Management Implications*

Small isolated populations are generally associated with higher probability of local extinction. The picture created by the long-term data of Warburg (1994, 2007) at the Damun site was alarming for two reasons: (1) it indicated a very low population size and (2) it was assumed that due to strong pond fidelity (Warburg 2006), populations were isolated and thus more susceptible to local extinctions. Our information provides a somewhat more optimistic picture. First, although our estimated population size at the Damun site in recent years is within the range of that found by Warburg (1974 - 1999),

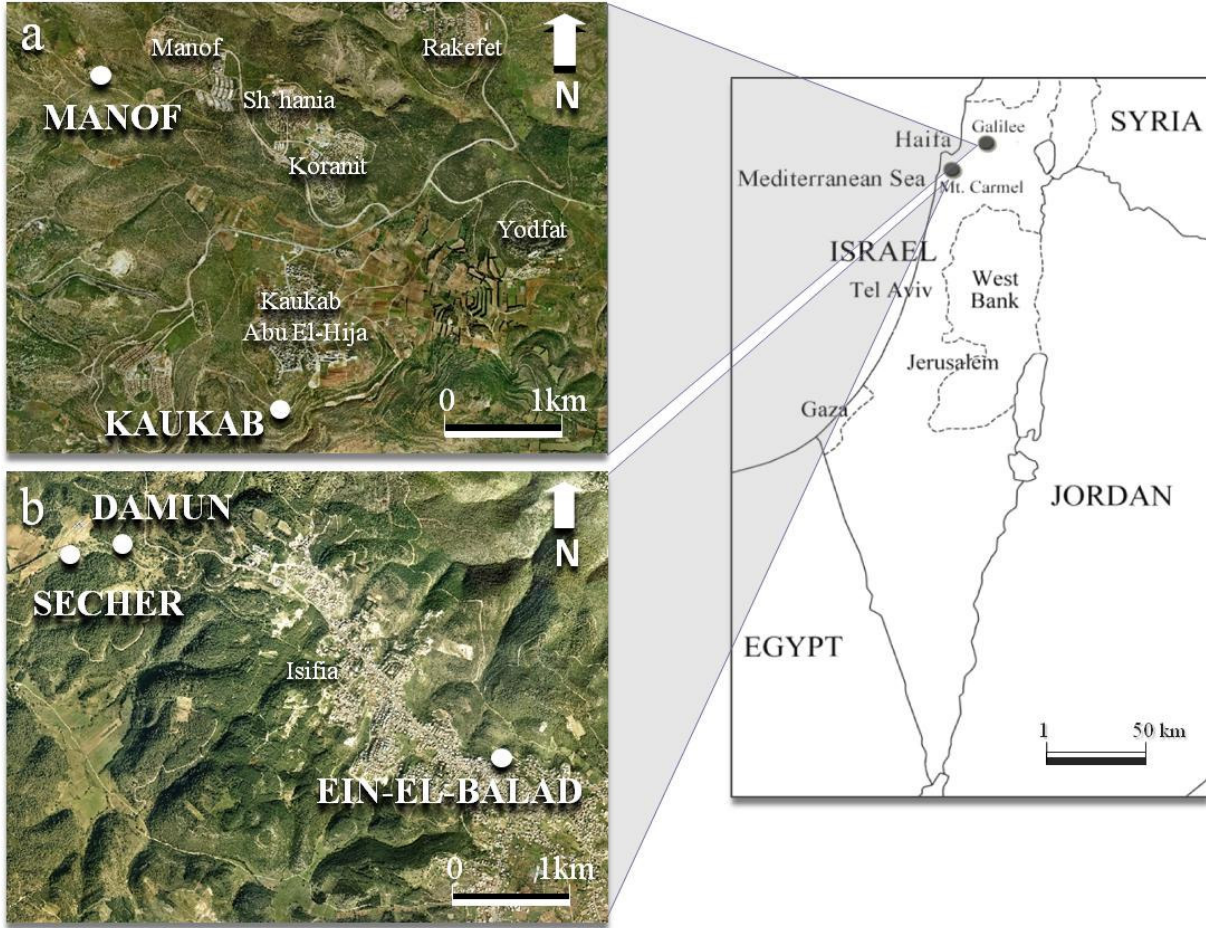
other populations are several orders of magnitude greater. Degani (1996) has also demonstrated higher populations in Israel in the Galilee region. Second, we now know that breeding site populations on Mt. Carmel are linked as metapopulations by some among-site dispersal (Bar-David et al. 2007); which is also consistent with dispersal studies of another *Salamandra* species in Europe (Schmidt et al. 2005, Schulte et al. 2007).

Although some of the population sizes that we found are encouraging regarding conservation of this species, current activities in and around these sites raise considerable concern. The Kaukab site was stocked with *Gambusia* in 2002 which have since been decimating the larval cohorts (Segev et al. 2009). As it takes 4-5 years for *Salamandra* juveniles to become reproductively mature and join the adult population at the breeding site, we are likely to begin to see population declines if *Gambusia* is not removed. The Ein-El-Balad site, according to development plans for the nearby village of Isifya, will soon be encroached further. A wide dirt road built less than 5 meters from the Manof site endangers the population as we have observed a number of roadkills (Segev and Blaustein, unpublished). Secher Pond is privately owned. It has been used for irrigation in the past and can be used for such purposes again.

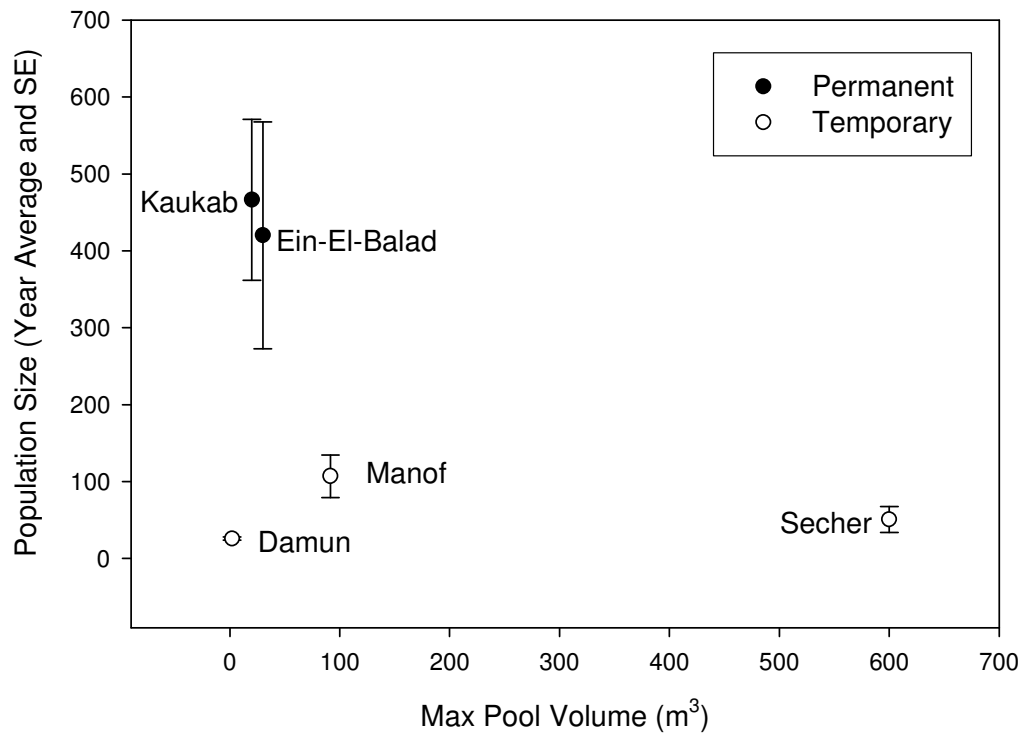
The most significant information coming from our study regarding future management is the evidence that permanent ponds of similar size to temporary ponds will support a much larger adult population size of *Salamandra*. Over the past decade, numerous temporary ponds have been constructed to provide breeding sites for *Salamandra* within nature reserves. Our findings suggest that larger, more stable populations can be established if the ponds can be made to hold water year round.

## **Acknowledgments**

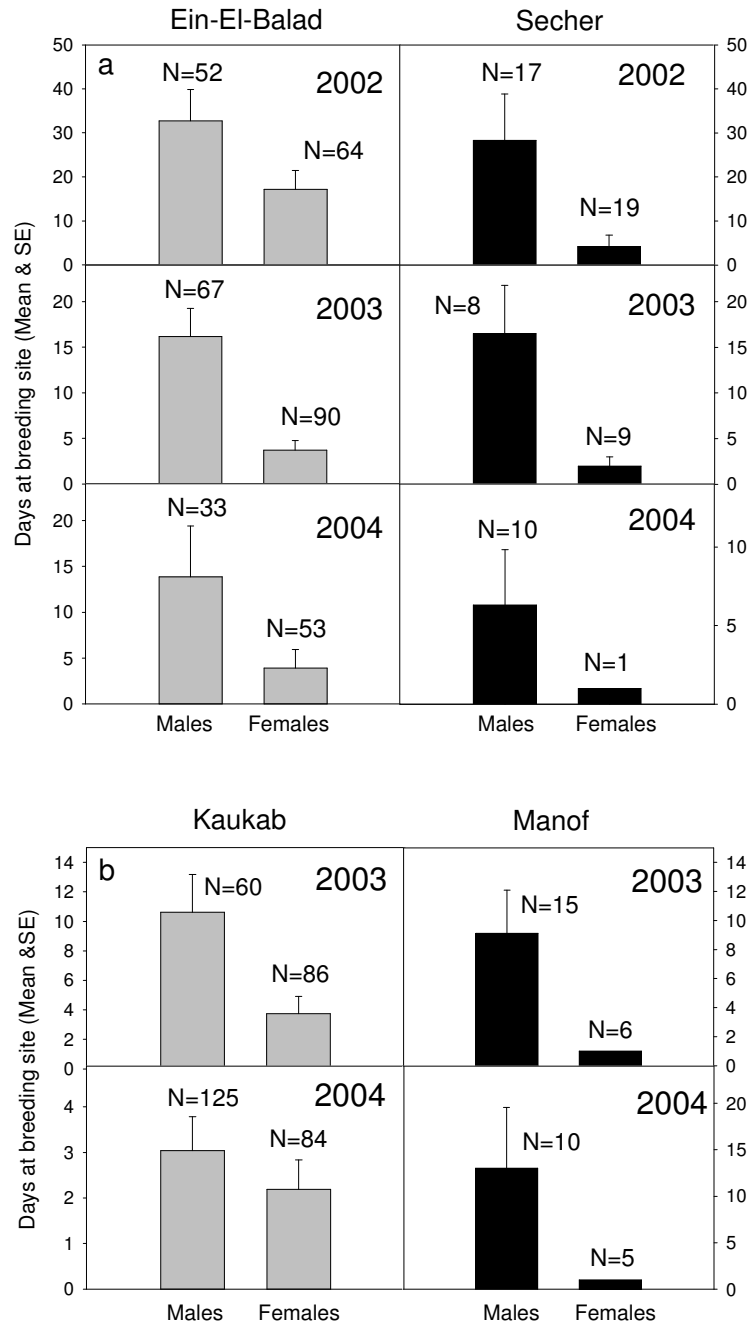
We thank Alon Silberbush, Asaf Sadeh, Nir Peleg, Oren Pearlson, Shirli Bar-David, and Shai Markman for field help and fruitful discussions, Haim Kutiel for rain data and two anonymous reviewers for excellent comments. We also thank the Israel Parks and Nature Reserves Authority for providing aerial photos of the study areas and for permission to conduct this study. This project was partially funded by U.S-Israel Binational Science Foundation grant 2002-365 awarded to LB and Marc Mangel.



**Fig. 1:** Geographic maps of the study regions. On the aerial photos, *Salamandra inframaculata* breeding sites appear in capital letters and marked with dots while small letters signify nearby villages. The upper right hand (a) shows the Lower Galilee sites and the lower right hand map (b) shows the Mt. Carmel sites.

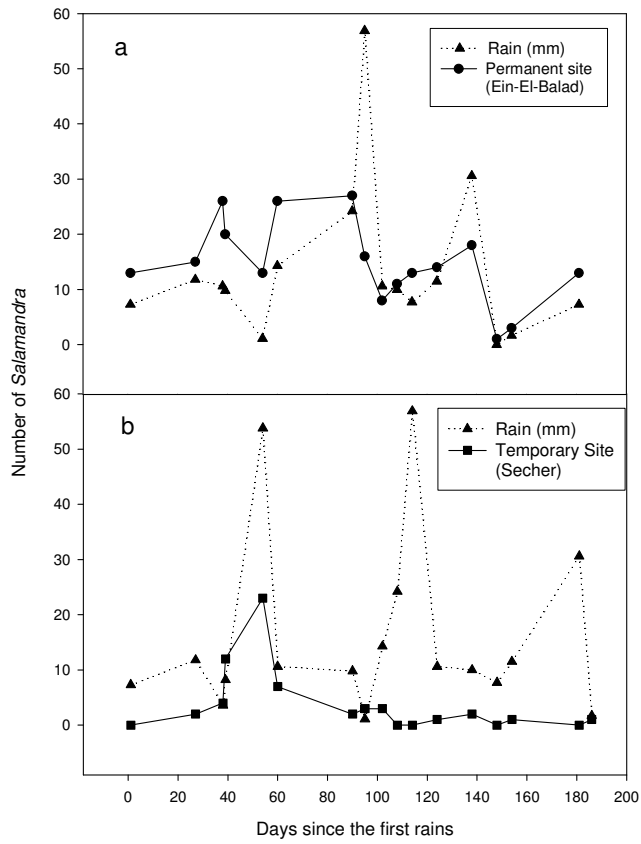


**Fig. 2:** *Salamandra infraimmaculata* average yearly population size estimates related to breeding pool maximum volume. Error bars are  $\pm 1$  standard error.



**Fig. 3ab:** Male and female breeding sites residence time. Sites are paired per year and with geographic region; (a) Carmel and (b) Lower Galilee. Error bars are  $\pm 1$  standard error. N=number of individuals.





**Fig. 4ab:** Temporal distribution of the numbers of *S. infraimaculata* individuals censused across sampling dates and daily amount of rain at: (a) permanent site (Ein-El-Balad) and (b) temporary site (Secher) in the Carmel during 2002.

**Table 1.** Site ephemerality, location and description of the five breeding sites studied.

<b>Ephemerality</b>	<b>Site</b>	<b>Description</b>
<b>Permanent</b>	<b>Ein-El-Balad</b>	Spring-fed man-made pool (32°43'13"N; 35°04'17"E), ~20m <sup>2</sup> surface area and ~1.2m maximum depth.
	<b>Kaukab</b>	Spring (32°49'23"N; 35°14'51"E), two permanent rock pools, ~40m apart; each ~15m <sup>2</sup> surface area and ~0.8m maximum depth).
<b>Temporary</b>	<b>Secher</b>	Large pool (32°44'04"N; 35°01'52"E), generally, but not always, dry in summer, ~400 m <sup>2</sup> and ~2m max depth (See detailed site description at Segev and Blaustein 2007).
	<b>Damun</b>	Collection of temporary small volume and shallow rock pools (32°44'06"N; 35°02'00"E), (See detailed site description at Spencer, Blaustein, Schwartz, and Cohen 1999).
	<b>Manof</b>	Collection of temporary rock pools (32°50'58"N; 35°13'52"E). <i>Salamandra</i> deposit larvae in two large (~40m <sup>2</sup> ), one medium (~15 m <sup>2</sup> ) surrounded by few small volume natural rock pools.

**Table 2.** Yearly population size estimates, standard errors and number of samples at the two permanent and three temporary breeding sites. The year listed provides the beginning of the breeding season. For example, 2002 represents fall 2002 through spring 2003.

<b>Site</b>		<b>2002</b>	<b>2003</b>	<b>2004</b>	<b>2005</b>
<b>Ein-El-Balad (permanent)</b>	Population	257.5	581.9	559.8	-
	SE	39.5	117.9	184.9	-
	Sampling	15	8	6	
<b>Kaukab (permanent)</b>	Population	-	567.9	272.5	-
	SE	-	136.7	95.4	-
	Sampling	-	6	4	-
<b>Secher (temporary)</b>	Population	84.1	31.3	36.8	-
	SE	20.3	11	15.4	-
	Sampling	15	8	5	-
<b>Damun (temporary)</b>	Population	-	23.8	-	28
	SE	-	24.2	-	21.3
	Sampling	-	3	-	3
<b>Manof (temporary)</b>	Population	-	134.8	79.5	-
	SE	-	70.2	53.5	-
	Sampling	-	6	4	-

**Table 3.** Correlation analysis (Spearman Rank) of individual breeders' first arrival date across consecutive seasons

<b>Site</b>	<b>Seasons Compared</b>	<b>N</b>	<b>Spearman Rho</b>	<b>p</b>
<b>Ein-El-Balad</b>	2002- 2003	38	0.437	0.006
	2003-2004	30	0.51	0.004
<b>Kaukab</b>	2003-2004	34	0.527	0.001
<b>Secher</b>	2002-2003	10	0.806	0.005
<b>Manof</b>	2003-2004	6	0.871	0.024

**Table 4.** Analysis of variance assessing the influence of site, gender, year and gender by site interaction on breeders' residence time at the breeding site

<b>Geographic region</b>		<b>df</b>	<b>F</b>	<b>p</b>
<b>Carmel</b> (Ein-El-Balad vs. Secher)	Site	1	2.456	0.118
	Gender	1	11.638	<0.001
	Year	2	11.42	<0.001
	Gender*site	1	0.012	0.911
<b>Galilee</b> (Kaukab vs. Manof)	Site	1	0.143	0.705
	Gender	1	8.907	0.003
	Year	2	7.981	0.005
	Gender*site	1	2.062	0.152

**Chapter 2. Deleterious effects by mosquitofish (*Gambusia affinis*) on the  
endangered fire salamander (*Salamandra infraimmaculata*)**

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## Abstract

Invasive fishes, via competition and predation, may be an important factor in endangering populations of amphibians and other aquatic fauna. The mosquitofish, *Gambusia affinis*, commonly stocked into amphibian breeding sites for mosquito control worldwide, has recently been found in some breeding sites of the endangered fire salamander, *Salamandra infraimmaculata*, in northern Israel.

A comparison of *Salamandra* larvae in natural pools ranging in *Gambusia* densities suggested that *Gambusia* negatively affects *Salamandra* by causing damage to its appendages, in particular, its tailfin (as manifested in a smaller tail:body ratio). Comparison of the same pool before and after mosquitofish introduction suggested strong negative effects on larval performance and number of emerging metamorphs. To explicitly test the hypothesis that *Gambusia* negatively affects *Salamandra* larval development, growth and survival, we conducted a replicated outdoor mesocosm experiment. In this experiment, we also tested how habitat structural complexity, in the form of radiating cords simulating artificial vegetation, might mediate damage caused by *Gambusia*. We used a two by two-factorial design: presence or absence of increased structural complexity crossed with the presence or absence of mosquitofish. After a short period, mosquitofish strongly reduced larval wet weight, survival, and increased body damage in the form of a reduced larval tail:body ratio and partially missing gills and limbs. No *Salamandra* larvae exposed to mosquitofish survived to metamorphosis in either habitat type.

We conclude that *Gambusia* strongly and negatively affects *Salamandra* larvae and that the introduction of *Gambusia* into sites containing *Salamandra* is inconsistent with the goal of preserving this endangered urodele.

## Introduction

Invasive predators may play an important role in population declines and perhaps extinctions of native species in general, including amphibian species (Skelly 2001; Kats and Ferrer 2003). Fish introductions are a global-scale phenomenon: Kiesecker (2003) reported the introduction of more than 160 species of predatory fishes in 120 different countries. Fishes are stocked in natural water bodies, not only for fishing, but also for attempts to biologically control mosquito populations, as is the case of stocking the mosquitofish *Gambusia affinis* (Courtenay and Meffe 1989). Introduced biological control agents may have deleterious ecological impacts (Diamond 1996; Simberloff and Stiling 1996). In the case of *Gambusia* for mosquito control, accumulating evidence suggests that this small poeciliid fish is far from a panacea; due presumably to their generalist feeding habits (Garcia-Berthou 1999), they are not always effective at reducing mosquitoes (Hoy et al. 1972; Arthington and Lloyd 1989; Blaustein 1992) and they may negatively impact non-target organisms including endangered amphibian species, fishes and invertebrates (Courtenay and Meffe 1989; Alcaraz et al. 2008). In the case of amphibians, negative effects of *Gambusia* species (Gamradt and Kats 1996; Lawler et al. 1999; Goodsell and Kats 1999; Hamer et al. 2002) and other fishes (Tyler et al. 1998; Funk and Dunlap 1999; Hoffman et al. 2004; Denoel et al. 2005) have been documented on populations of a number of amphibian species. Most of these studies have considered anurans, and not urodeles, and even fewer studies have considered how mosquitofish in particular affect urodeles. Moreover, the evidence accumulated thus far indicates that the effects of mosquitofish species on amphibians are not consistent across all amphibian species and systems, but may vary greatly among amphibian species from highly deleterious to minimal (e.g. Baber and Babbitt 2004; Ling 2004; Gregoire and Gunzburger 2008). The potential magnitude of the effect of *Gambusia* on endangered species may be influenced



by habitat structure such as aquatic vegetation type and amount. Habitat structure may affect predator-prey interactions through the amount of prey refuge it provides. Submerged vegetation in aquatic systems has been suggested as the cause for reduced fish predation (Werner et al. 1983; Baber and Babbitt 2004; Lehtiniemi 2005; Sass et al. 2006). However, the overall effect of submerged vegetation in influencing the effect of *Gambusia* on amphibian larvae may be complex because not only might it provide a refuge for the amphibian larvae, it may also provide a refuge for *Gambusia* juveniles from cannibalistic adults leading to larger *Gambusia* population size (Blaustein 1988; Benoit et al. 2000).

In Israel, *G. affinis* has been stocked into water bodies for mosquito control since the turn of the twentieth century (Kligler 1930). As a consequence of these introductions and their invasion abilities (Moyle and Marchetti 2006), they are widespread in Israel. Their recent occupancy includes several breeding sites of the fire salamander, *Salamandra infraimmaculata* (Blaustein pers. obs.) an endangered species in Israel (Dolev and Perevolotzky 2004). Most permanent water bodies that serve as breeding sites for fire salamanders are isolated thus limiting “natural” invasions, but the threat of introductions into additional salamander breeding sites for mosquito control exists. Like mosquitofish (Blaustein 1992; Courtenay and Meffe 1989), *Salamandra* larvae are generalist intraguild predators (Blaustein et al. 1996; Eitam et al. 2005; Segev and Blaustein 2007). Consequently, mosquitofish may thus negatively affect *Salamandra* larvae via predation and competition.

To assess the potential effects of *G. affinis* on *S. infraimmaculata* larvae, we present some field observations that compare *Salamandra* abundance and size at the same site before and during the presence of *Gambusia*, and compare three *Salamandra* breeding sites simultaneously having different mosquitofish densities. Additionally, we present a mesocosm experiment designed to study the effects of *Gambusia* and habitat complexity

on *Salamandra* larvae in a two-factorial design. The results of both field observations and experiment indicate that *Gambusia* has strong negative effects on larval *Salamandra* and that the introduction of *Gambusia* in permanent water bodies containing *Salamandra* is inconsistent with the goal of preserving this amphibian species.

## **Methods and materials**

### *Natural pool observations*

To collect preliminary field evidence that *Gambusia* influences *Salamandra*, we compared three breeding sites that differed in *Gambusia* densities ranging from none to high densities. We also compared some *Salamandra* data at one site before and after *Gambusia* was introduced. One pool, Alon Spring, located at Mount Carmel National Park (32°43'30"N; 35°1'26"E; ~ 4 m<sup>2</sup> surface area and 0.4 m maximum depth), did not contain mosquitofish based on visual inspection and the sampling described below. The two other pools, ~40 m apart from each other, were located in the Lower Galilee Mountains at Kaukab Springs (32°49'23"N; 35°14'51"E; each ~15 m<sup>2</sup> surface area and ~0.8 m maximum depth). The two Kaukab Pools, hereafter Kaukab 1 and Kaukab 2, are both independent end points of underground springs and they are not connected. Mosquitofish were stocked into the two Kaukab pools by an unknown source sometime in 2002, about a year prior to our post-introduction field observations.

On 28 May 2003, we sampled each pool for fish abundance by taking 15 one-meter sweeps using a D-net (0.3 cm mesh; 1600 cm<sup>2</sup>) along the bottoms and sides of the pools. *Salamandra* larvae were sampled during late afternoon by entering the water, dislodging rocks which exposed *Salamandra* larvae under rocks, and by sweeping for 15 minutes on 28 May, 10 July and 24 July 2003. This dislodging minimized any possibility of differential sampling efficiency of salamander larvae in the presence or absence of

*Gambusia* due to antipredator behavior in response to *Gambusia* (Holomuzki 1996; Blaustein and Spencer 2005; Schmidt and Van Buskirk 2005). On the first date, 15 larvae captured in each of the Kaukab Pools and 10 larvae captured at Alon Pool were also measured for wet weight, total length and snout-vent length. Larval lengths were measured using an electronic calliper and wet weights were taken with an electronic field scale. From the length data, we calculated tail:body ratio (i.e., the tail fin length [from the tail fin distal end to the vent] divided by the snout-vent length). Larvae were returned to the pools after measurements. From observations in previous years, we knew that *Salamandra* metamorphs would, during early fall, emerge and concentrate in a collection of ~15 rocks just behind a cave-like area of Kaukab 2 pool prior to dispersing during the rainy season. These collection of rocks, from just above the water surface to just about 10 cm above the highly consistent water level, were carefully lifted to count *Salamandra* metamorphs on 19 September and 9 October 2003. We then carefully returned the rocks.

We were able to compare the 2003 *Salamandra* data with some data collected from the Kaukab 2 Pool in 1999, before the introduction of *Gambusia*. We sampled for larval abundance in the same way as described for 2003 on three dates: 20 May, 10 July and 24 July 1999. This allowed us to compare larval abundances on the three similar Julian dates for this pool before and after *Gambusia* introduction. We also compared the 28 May 2003 tail:body ratios and the wet weight from this Kaukab pool to measurements taken from nine *Salamandra* larvae collected from the same pool on 20 May 1999. A count of *Salamandra* metamorphs in the rock pile behind Kaukab Pool 2 on 9, 16 and 30 October 1998, and 20 November 1998 (before mosquitofish introduction) allowed for comparisons of the 2003 counts made after mosquitofish introduction.

The data were analyzed with t-tests for any pair-wise comparison and by ANOVA accompanied by Tukey's HSD test for multiple comparisons. The tail:body ratios were arcsine-square root transformed prior to analysis.

### *Mesocosm Experiment*

We used a two by two factorial design - presence or absence of mosquitofish crossed with the presence or absence of increased structural complexity - to study the effects of these two factors on larval *Salamandra* survival and development. The experiment was conducted on the periphery of the University of Haifa Campus using 16 artificial pools (180 L rectangular-plastic containers; height, length, width: 35 x 91 x 57 cm) in a 2 x 8 array under a 90% shade net suspended at a height of 1.5 m. Distance between adjacent containers was 0.5 m and treatments were randomly assigned among containers. We filled the containers with tap water on 12 Feb 2005 and maintained maximum or near maximum water levels throughout the experiment using some rainwater, and mostly aged tap water. Containers were open at the top to allow insect colonization and allochthonous input. For structural complexity, we used a black plastic cord (3.5 mm diameter). In half of the containers, we added 30-one m sections of cord, each tied to a brick. The strings, which were positively buoyant, radiated toward the surface, simulating submergent vegetation. In order to control for any possible chemical effects of the cord material, we also added the same amount of cord (one 30 m cord) to the eight low structural complexity pools by tightly rolling the cord around the brick. On 14 February, to provide food resources for the mosquitofish and *Salamandra* larvae, we inoculated equal aliquots of invertebrates (containing primarily cladocerans, ostracods and copepods) collected from a nearby winter pool, to each experimental pool. Insect colonization/oviposition (mainly chironomids) also contributed to a prey base. Sweep

samples with a small net (16x11 cm; 250 micron mesh) taken once per four weeks for 16 weeks did not reveal any treatment or temporal differences in invertebrate abundance (repeated measures Anova) and is not discussed further.

On 17 Feb 2005, we added 8 male and 8 female *Gambusia* collected from the Kaukab pools to each of four pools of each habitat structure type. This density was well below the densities reported elsewhere (Blotsford et al. 1987; Blaustein 1992). Female mosquitofish averaged 48.1 mm in total length (SE: 1.18; n=64) and 1.21 gr. in wet weight (SE: 0.11; n=64). Males averaged 24.3 mm total length (SE: 0.78; n=64) and 0.12 gr. wet weight (SE: 0.01; n=64).

We used *Salamandra* newborn larvae all deposited in the lab by four mothers collected from a breeding site on Mt. Carmel. On 21 February, we added 16 *Salamandra* larvae (mean total length  $\pm$  SE: 31.44 $\pm$ 1.47 mm; mean wet weight  $\pm$  SE: 0.18 $\pm$ 0.02 gr.; size estimates based on a subset of 40 newborns, 10 each from each mother) to each container. This is a moderate density (0.09 per liter) compared to what we have found in natural pools where densities of newborns can be more than an order of magnitude higher ( $\gg$  1 per liter; L. Blaustein, unpublished data). Particularly because this species is listed as endangered, we used the minimum number of individuals and replicates that would lead to detection of moderate effects. Given that this particular population is  $>500$  adults with an approximately 1:1 sex ratio as determined by mark-recapture (Segev et al., unpublished data), and that females deposit on average  $>100$  larvae, the 96 individuals placed in *Gambusia* pools represented  $<0.3$  percent of the total deposited larvae for that one season. Each container included larvae from all four mothers (an experiment design that would also assess for maternal effects would have required many more pools and larvae).

Containers were sampled for mosquitofish monthly for nine months. *Salamandra* were sampled every three weeks until metamorphosis began, and then additional

observations were made more frequently. Fish and larval *Salamandra* abundances were assessed by first searching for and removing individuals with a net. We estimate that this resulted in the capture of most individuals, and for *Salamandra*, all of the larvae, because the water was clear and larvae were relatively large and visible. We systematically swept for 5 additional minutes after the last individual had been captured. Captured *Salamandra* larvae were counted and measured (wet weight, snout-vent length and tail fin length as described in the survey). Mosquitofish were classified as adults or juveniles and according to gender. After measuring, both mosquitofish and *Salamandra* larvae were returned to their respective pools. As in the field survey, these data allowed us to calculate a tail:body ratio. Additionally, we recorded the number of the *Salamandra* larvae containing missing or partially missing limbs and gills. Once metamorphosis began, in addition to the regular sampling, we made observations several times per week for new metamorphs. Completely metamorphosed individuals were measured and weighed and returned to their natural breeding site. These data provided us with survival to, time to, and size at metamorphosis. Based on circumstantial evidence, some metamorphosing individuals apparently escaped prior to capturing and measuring. Therefore, survival to metamorphosis is probably not an exact estimate but a still a good comparative estimate among treatments.

For *Gambusia* abundance data, we conducted repeated measures analyses of variance on adults for nine monthly samples and on juveniles for five monthly samples (because there were no juveniles for the first four months). We also conducted repeated measures analyses of variance on larval *Salamandra* survival (arcsin-square root transformed proportions), wet weight (log-transformed) and tail:body ratios (arcsin-square root transformed) over three sampling dates – 3, 6 and 9 weeks after introducing the newborn *Salamandra*. After that, survival in all *Gambusia* pools reached zero presenting homogeneity of variance problem for the survival variable and there was an absence of any

data in *Gambusia* pools for the other variables. We made Greenhouse-Geisser adjustments to degrees of freedom for within-subjects sources of variation when data failed to meet sphericity assumptions.

In the cases of percent limb damage, percent gill damage and survival to metamorphosis, Fisher exact tests were used instead of analyses of variance for *Gambusia* effects, because of the absences of homogeneity of variance, and t-tests were used to assess structural complexity effects.

## **Results**

### *Natural Pools survey*

Neither Alon Pool sampled in 2003 nor Kaukab 2 Pool sampled in 1999 had mosquitofish. In May 2003, Kaukab 1 Pool had relatively low densities of *Gambusia* (hereafter “low-fish-density pool), while Kaukab 2 Pool had relatively high densities (hereafter “high-fish-density pool”), a statistically significant difference ( $t=3.16$ ,  $DF=28$ ,  $p=0.004$ ; Fig. 1a).

Several pieces of circumstantial evidence are in line with the hypothesis that mosquitofish negatively affect *Salamandra* larvae. First, damage consistent with biting was evident on the bodies of the salamander larvae when mosquitofish densities were high. Tail:body ratios of *Salamandra* larvae were highest (longest tails) when mosquitofish were absent (i.e. in Alon Pool and the Kaukab 2 Pool prior to mosquitofish introduction), lower but not statistically so in the low-fish-density pool, and significantly lower in the high-fish-density pool ( $F_{3,46}=53.77$ ,  $p<0.0001$ ; Tukey HSD; Fig. 1b). Second, larval densities were similar from late May through July 1999 in Kaukab 2 Pool when *Gambusia* was absent, but in 2003, when *Gambusia* was present in high densities in this pool, there were no *Salamandra* detected in the two July sampling dates (Fig. 2). Third, the larvae sampled 20

May 1999 – i.e., before the introduction of mosquitofish - were more than twice as heavy (Mean= 3.30 gr; SE=0.30) as the larvae sampled in the same pool later during the same month (28 May 03) after mosquitofish introduction (Mean= 1.31; SE=0.24) ( $t=5.17$ ;  $DF=22$ ;  $p<0.0001$ ). Fourth, we found many more metamorphosed *Salamandra* in the rock pile just above the water line in the high fish density Kaukab pool before the mosquitofish introduction (126, 106, 27, and 1 individuals on 9 October 1998, 16 October 1998, 30 October 1998, and 20 November 1998, respectively) than after introduction (2 and 1 individuals on 19 September 2003 and 9 October 2003, respectively). The drastic drop in through time in 1998 almost certainly represents dispersal away from the breeding site during the rainy season.

#### *Mesocosm Experiment*

A repeated measures analysis on all nine monthly samples (with Greenhouse-Geisser adjustments of degrees of freedom) showed no statistically significant effects of structural complexity, time or the structural complexity x time interaction on mosquitofish adult abundance (Table 1; Fig. 3). The first newborn mosquitofish appeared on 6 June. Juvenile mosquitofish abundance was higher in the increased structural complexity treatment over the final five months of the experiment (Table 1; Fig. 3).

A repeated measures analysis on three sample dates over the first nine weeks, show mosquitofish had a large negative effect on *Salamandra* survival with the effect increasing with time as evidenced by the highly significant *Gambusia* x time interaction (Table 2; Fig. 4a). By the next sample date, no salamander remained in the *Gambusia* pools. During these first three sample dates, structural complexity did not significantly influence the negative effect of mosquitofish on *Salamandra* survival (Table 1). *Salamandra* wet mass was strongly reduced by *Gambusia* but unaffected by structural complexity (Table 2; Fig. 4b).



The magnitude of the mosquitofish effect on wet mass tended to increase with time, but the *Gambusia* x time interaction was not statistically significant ( $p=0.169$ ). Tail:body ratio (Fig. 4c) was strongly reduced by *Gambusia* but not by structural complexity (Table 2; Fig. 4c). The magnitude of the effect did not appear to increase with time (*Gambusia* x time interaction:  $p \gg 0.05$ ).

The body damage caused by *Gambusia* was not limited to tail fins. To assess gill and limb damage, we used Fisher exact tests on the last date, where effects appeared greatest, instead of repeated measures ANOVA because of homogeneity of variance violations. The occurrence of larvae with partially missing limbs (Fisher exact test:  $p=0.0014$ ) and partially missing gills (Fisher exact test:  $p=0.0014$ ) were drastically higher in the mosquitofish treatments (Fig. 4d, e). In the pools containing mosquitofish, the proportion with damaged limbs and gills did not appear to be affected by structural complexity (Fisher exact tests:  $p=0.143$ ; Fig. 4d, e).

Observations and sampling indicated that it is likely that not a single *Salamandra* larva survived to metamorphosis in any of the *Gambusia* pools regardless of structural complexity while many metamorphosed in the absence of mosquitofish (Fisher Exact test:  $p=0.0014$ ; Fig. 5). Because some metamorphosing individuals may have escaped the pools in between sampling events after 9 weeks, the number surviving to metamorphosis in *Gambusia*-free treatments shown in Figure 5 was likely higher. Given that the size of the remaining larvae in *Gambusia* pools were considerably below the minimum size for metamorphosis (Eitam et al. 2005), it is unlikely that any larvae survived to metamorphosis in mosquitofish pools. In *Gambusia*-free pools, habitat type did not affect survival to, time to, or tail:body ratio at metamorphosis, but *Salamandra* metamorphosed at a larger size in the lower structural complexity pools (Table 3).

## Discussion

Invasive fishes in general, and mosquitofish in particular, by acting as predators and competitors, pose concern regarding how they might negatively influence the native community including endangered fish and amphibian species (Kats and Ferrer 2003; Rogowski and Stockwell 2006). However, although there are clear demonstrations of *Gambusia* negatively affecting specific amphibian species (Courtenay and Meffe 1989; Gamradt and Kats 1996; Goodsell and Kats 1999; Hamer et al. 2002), the effect of mosquitofish, if any, varies greatly and depends on the specific amphibian of interest (Lawler et al. 1999; Komak and Crossland 2000; Walls et al. 2002; Ling 2004). We present here convincing evidence for strong detrimental effects of the mosquitofish on fire salamander larvae, an endangered species in Israel. Our observations in natural *Salamandra* breeding pools provide circumstantial evidence of strong negative effects as follows. First, comparative temporal observations within the same pool during the same time of year before and after mosquitofish introduction showed that larvae were in higher densities, were larger in size, and had larger tail:body ratios prior to mosquitofish introduction. Moreover, high metamorph abundance in the rock pile prior to mosquitofish introduction followed by a very low abundance after mosquitofish introduction suggested a drastic reduction of successful metamorphosis after mosquitofish introduction. Second, a spatial comparison of three different pools containing no, low and high fish densities showed a negative correlation between fish density and tail:body ratio.

The mesocosm experiment results are congruent with our field survey findings and show a strong negative effect of *Gambusia* on *Salamandra* in the form of larval survival, size and body damage (larval tail:body ratio and the number of damaged appendages). A significant fraction of the reduced tail:body ratio is unlikely attributed to some phenotypic plastic response to *Gambusia* – i.e. the tails were not shorter whole tails, but shorter because they

were partial tails with raw wounds and absence of fin tissue at the distal end. *Gambusia*, being a gape-limited predator, cannot consume an entire larva and instead inflicts multiple injuries through tail, limb and gill bites (Richards and Bull 1990; Komak and Crossland 2000). This damage likely eventually caused mortality in all or almost all larvae in all mosquitofish pools before any could complete metamorphosis.

Repeated nonlethal injuries can accumulate to cause mortality (Harris 1989). Even under conditions where there is some survival to metamorphosis in the presence of *Gambusia*, the tissue damage inflicted by the fish likely causes reduced fitness for the surviving larvae. Predator-inflicted tail damage has been studied primarily in anuran tadpoles (Wilbur and Semlitsch 1990; Blair and Wassersug 2000) and has been shown to affect tadpole development and swimming performance (Parichy and Kaplan 1992; Figiel and Semlitsch 1991; Van Buskirk and McCollum 2000). The first fish-inflicted damage in the mesocosm experiment was seen on the larval tail while gill and limb damage followed only later. One possible explanation is that when a mobile salamander larva flees, its most vulnerable tissue to the pursuing predator is its tail fin. Then, significant tail damage caused a reduction in larval swimming performance which increased larval vulnerability to bites in other, possibly more, vital body parts. The reduction in gill surface-area due to gill biting may affect larval gas exchange performance and consequently fitness as urodele gills are responsible for 60-70% of O<sub>2</sub> uptake and CO<sub>2</sub> elimination (Burggren and Just 1992).

Lower body weight in the presence of *Gambusia* in the mesocosm experiment and the field observations may be in part due to exploitative and interference competition. The two species are considered generalist predators showing a high overlap in their diets (Mansfield and Mcardle 1998; Gophen et al. 1998; Blaustein 1992).

In the natural pool survey, we initially surmised that the absence of any detectable effect of mosquitofish on *Salamandra* larvae in the low-fish-density pool was due in large part to the lower *Gambusia* densities (~4 X lower) but it might also have been due to increased refuge for *Salamandra* larvae; this pool had considerably denser algal filamentous mats (comprising >50% of the water volume) in contrast to the high-fish-density pool which had low algal abundance (<5% of the water volume). Comprising part of mosquitofish diet (Harrington and Harrington 1982; Meffe and Snelson 1989; Gophen et al. 1998), the abundance of algae in the low fish density pool might even reduce exploitative competition between *Salamandra* and *Gambusia*. In the mesocosm experiment, increased structural complexity did not reduce the negative effect of *Gambusia* on *Salamandra* larvae. One possible reason for the lack of a refuge effect is that structural complexity in the form of artificial vegetation does not provide much of a refuge for amphibian larvae; *Gambusia* have been shown to be effective predators in vegetation on invertebrates (Linden and Cech 1990) and on larger prey such as amphibian larvae (Baber and Babbitt 2004). A second possibility might be that other types or amounts of structural complexity not simulated by our radiating cords provide a better refuge for *Salamandra* larvae. Stones, which *Salamandra* larvae are often found beneath, may provide a better refuge than the plastic cords we used. These cords, bundled around the brick in the low-structural complexity treatment, might have also provided some additional refuge. Lastly, the artificial cords, unlike the filamentous algae, could not have provided an alternative food source for the *Gambusia*.

The radiating cords caused an increase in juvenile recruitment in *Gambusia*. Other studies have shown that vegetation in the water column causes increased survival and increased population growth rates of *Gambusia* (Blaustein 1988; Winkelman and Aho 1993; Benoit et al. 2000). However this increased indirect positive effect on mosquitofish

abundance, could not have had an effect in the mesocosm experiment; by the time there was mosquitofish reproduction, all *Salamandra* larvae had been killed.

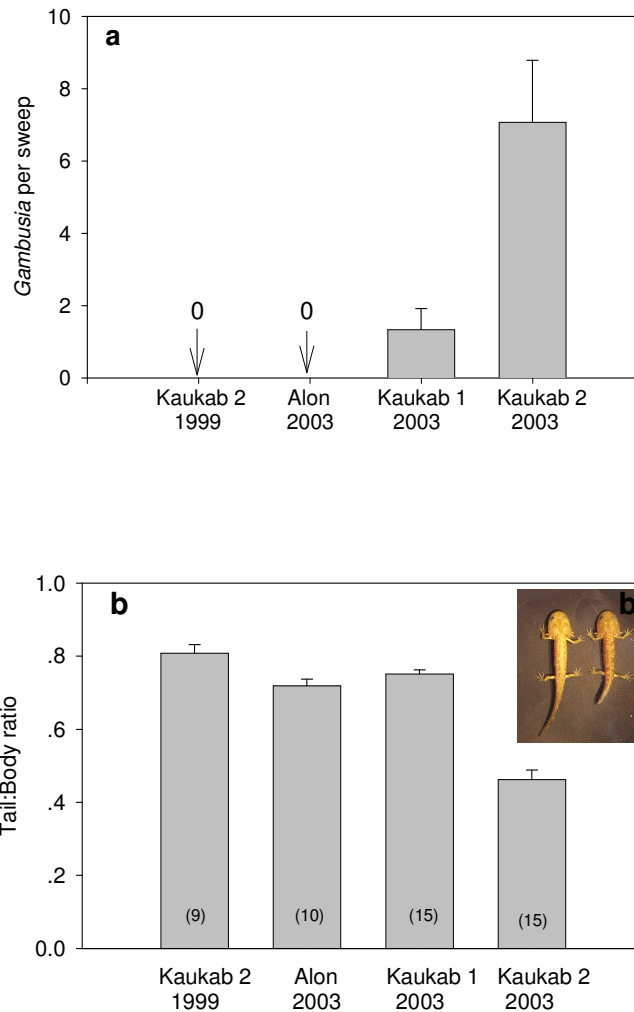
The introduction of *Gambusia* to a specific permanent *Salamandra*-breeding site may not just threaten the salamander population at that breeding site; *S. infraimmaculata* is capable of long-term dispersal (Bar-David et al. 2007). The absence of recruits from *Gambusia*-stocked pools to other breeding sites may endanger the long-term persistence of other small subpopulations.

With regards to mosquito control in permanent ponds, mosquitofish and *Salamandra* larvae may be ecologically redundant; *Salamandra* larvae themselves can be very effective predators of mosquitoes (Blaustein unpublished data). As a consequence of the results from this paper, the Israel Nature Reserve Authority has decided to remove *Gambusia* from *Salamandra* breeding sites and not allow further introductions. *Gambusia* is widely distributed globally (e.g., Meffe and Snelson 1989; Caiola and Sostoa 2005; Alcaraz et al. 2008) where various species and subspecies of salamandrids are found. Many urodele species that lay eggs instead of larvipositing may be even more vulnerable to mosquitofish since they undergo longer and probably more vulnerable stages. In summary, the evidence shown here clearly demonstrates that the goal of *Salamandra* preservation is inconsistent with the goal of using mosquitofish for mosquito control.

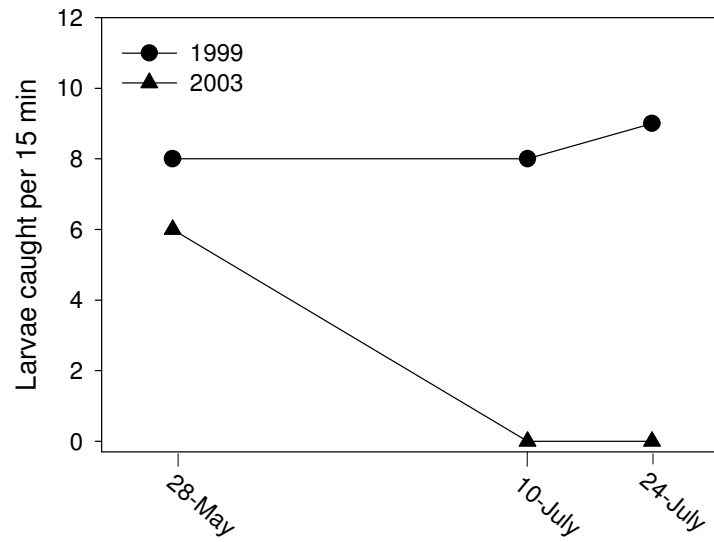
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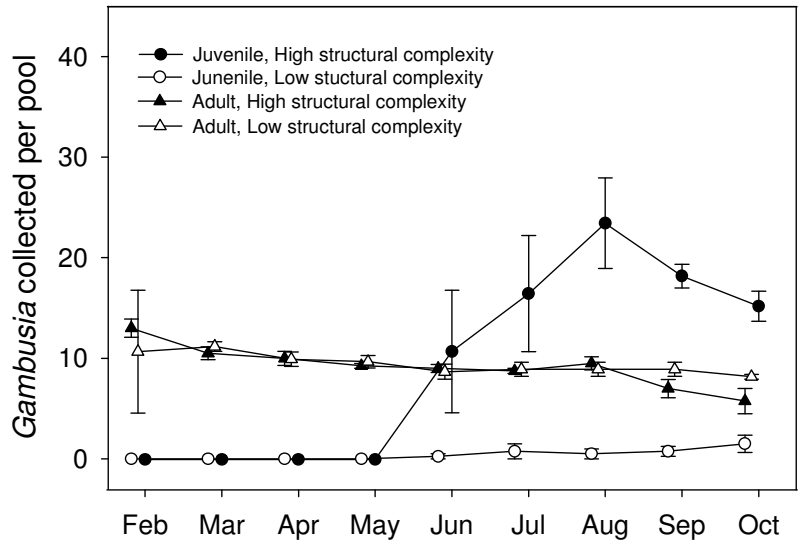


**Fig. 1:** (a) Mosquitofish caught per sweep (mean of 15 one-meter sweeps) at “Kaukab 2” Pool during 20 May 1999 (prior to mosquitofish introduction) and “Alon”, “Kaukab 1” and “Kaukab 2” pools on 28 May 2003. Error bars are one standard error; (b) tail:body (snout-vent) ratio of *Salamandra* larvae from the same pools and dates. Error bars are one standard error. Numbers in parentheses indicate number of larvae measured. Photo insert: typical specimens of *Salamandra* larva with intact tail from “Kaukab 1”, the low fish density pool (Left), versus *Salamandra* with a truncated tail larva from “Kaukab 2”, the high fish density pool (Right).

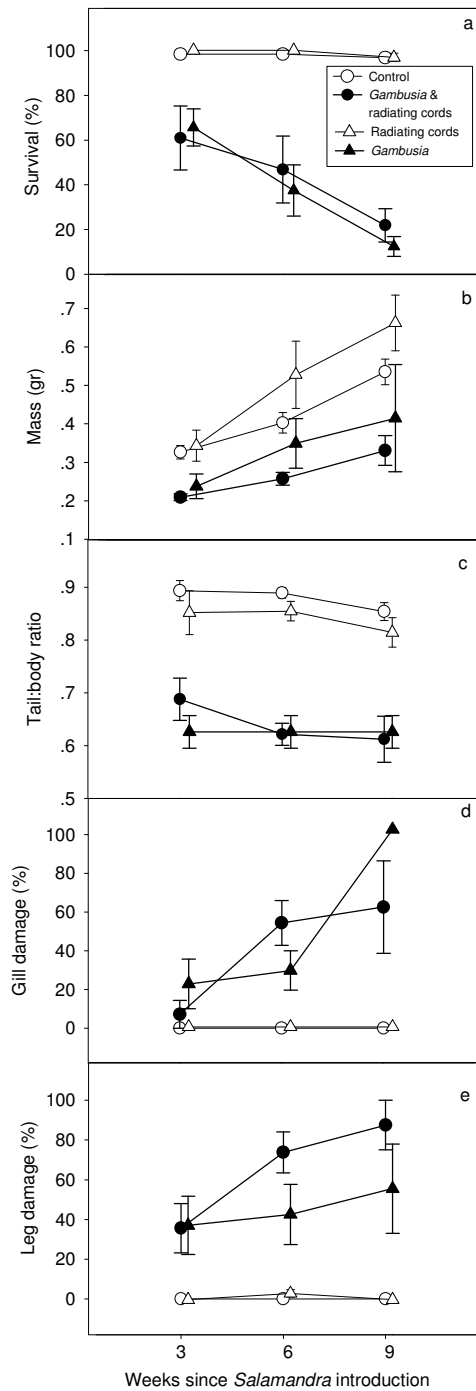


**Fig. 2:** Number of *Salamandra* larvae caught per 15 minute sweep in “Kaukab 2” Pool in 1999, prior to mosquitofish introduction and during 2003, after mosquitofish introduction.



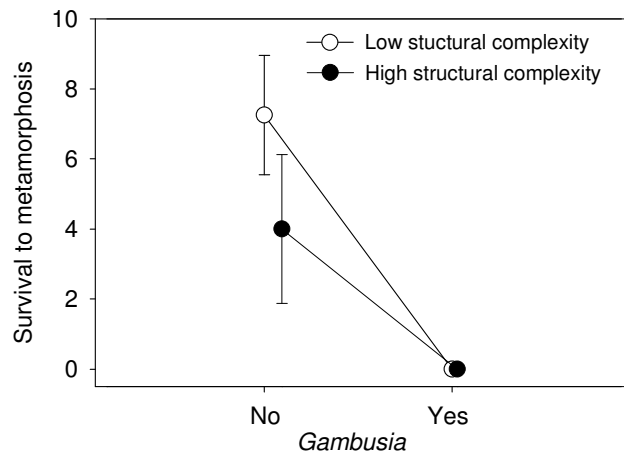


**Fig. 3:** Mosquitofish adults and juveniles (Mean  $\pm$  SE) caught per pool in pools with and without increased structural complexity.



**Fig. 4:** Mosquitofish and structural complexity effects on *Salamandra* larvae

(Mean  $\pm$  SE): (a) survival; (b) mass; (c) tail to body (snout-vent) ratio; (d) percent of damaged gills; and (e) percent of damaged limbs, three, six and nine weeks following the introduction of *Salamandra* larvae.



**Fig. 5:** Mosquitofish and structural complexity effects on *Salamandra* survival to metamorphosis (Mean  $\pm$  SE).

**Table 1.** Repeated measures analysis of variance assessing structural complexity and time effects on adult (9 dates) and juvenile (5 dates) mosquitofish abundance. Greenhouse-Geisser adjustments of degrees of freedom were used because of violations of the sphericity assumptions.

	Adult Mosquitofish			Juvenile Mosquitofish		
	DF	F	<i>p</i>	DF	F	<i>p</i>
Structural Complexity	1, 6	0.12	0.745	1, 6	29.28	0.0016
Time	1.27, 7.64	1.85	0.218	1.81, 10.85	1.68	0.232
Interaction	1.27, 7.64	0.42	0.583	1.81, 10.85	1.66	0.234

**Table 2.** Repeated measures analysis of variance assessing effects of *Gambusia affinis* and structural complexity on larval *Salamandra* (a) survival (proportion, arcsin-square root transformed); (b) mass (natural log-transformed); (c) tail:body ratio (arcsin-square root transformed) during three, six and nine weeks after introducing *Salamandra* larvae. Greenhouse-Geisser adjustments of degrees of freedom were used in analyzing mass due to violations of the sphericity assumptions.

	Survival		Mass		Tail:Body	
	F <sub>1, 12</sub>	p	F <sub>1, 12</sub>	p	F <sub>1, 12</sub>	p
<i>Gambusia</i>	134.12	<0.001	18.33	0.001	97.27	<0.001
Structural Complexity	0.42	0.527	2.49	0.143	0.98	0.343
<i>Gambusia</i> x Structural Complex.	0.004	0.949	0.13	0.720	1.04	0.329
	F <sub>2, 24</sub>	p	F <sub>1.31, 14.36</sub>	p	F <sub>2, 24</sub>	p
Time	15.43	<0.001	30.62	<0.001	1.44	0.257
Time x <i>Gambusia</i>	7.73	0.003	2.08	0.169	0.28	0.758
Time x Structural Complex.	0.23	0.794	0.82	0.413	0.63	0.541
Time x <i>Gam</i> x Structural Complex.	0.60	0.560	0.51	0.532	0.50	0.614

**Table 3.** Structural complexity (presence or absence of radiating cords) effects in the absence of *Gambusia* on *Salamandra* number surviving, time to (days), size (wet mass) and tail:body ratio at metamorphosis assessed by t-tests.

	High Structural Complexity		Low Structural Complexity		Test	
	Mean	SE	Mean	SE	t	p
Survival	5.33	2.33	7.25	1.70	0.68	0.524
Time	137.71	50.75	240.10	64.47	1.17	0.294
Mass	0.73	0.05	0.98	0.04	3.78	0.013
Tail:body	0.77	0.06	0.80	0.03	0.49	0.645

**Chapter 3. Spatio-temporal progeny allocation strategies in the fire salamander:  
a model and empirical test**

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## Abstract

Spatial and temporal progeny allocation entails fitness consequences for the mother as selection favours the adjustment of reproductive strategies according to environmental conditions. We used modelling and experimental approaches to predict and assess how the endangered fire salamander, *Salamandra atra*, distributes offspring in a set of temporary pools. We developed a state variable model that incorporates the female's physiological state (larval load) and stochastic variation in breeding pool conditions (pool volumes and hydroperiods). The model predicts that gravid females will split their larval load during the rainy season and across multiple pools and that female will adjust the number of larvae deposited into a pool according to pool quality i.e., current and maximum depth. To explicitly test these predictions, we conducted an outdoor enclosure experiment where individual gravid salamanders could choose to deposit their larvae among four artificial pools of different depths (two shallow and two deep pools). *Salamandra* females distributed larvae temporally (60% of the females larviposited during more than one night) and spatially (87% of the females deposited into more than one pool), and they deposited more larvae in deeper pools. Larviposition habitat selection has implications for population dynamics and species distributions, especially in disturbed, fragmented ecosystems through processes of colonization and recruitment. Our results can be used to set conservation priorities for specific breeding sites, identify new sites for relocations or introduction programs and to delineate breeding-pool properties in cases where artificial pools are constructed.



## **Introduction**

Locally optimized life history phenotypes, such as propagule size and number (Lack 1947; Wilbur 1977), cannot evolve without a consistent environmental context (Resetarits 1996). This consistency can be achieved by philopatry or site fidelity (McPeck 1989) when little spatio-temporal variation exists in the relative qualities of available habitats, as is the case in many anadromous fishes and some amphibians. Alternatively, when such variation is considerable, consistency may be achieved by active discrimination of breeding females among potential oviposition sites based on their perceived suitability (Resetarits 1996), as was empirically shown for numerous organisms (e.g., Mayhew 1997; Wilson 1998; Blaustein et al 2004; Blouin-Demers et al 2004; Rieger et al 2004).

Oviposition site selection has direct implications on the reproductive success of amphibians, particularly when larvae are spatially confined in small, temporary water bodies (Resetarits 1996; Blaustein 1999). In order to respond to pool variables that determine its quality as potential larval habitat, females must be capable of assessing them (Clark and Mangel 1984; Boulinier and Danchin 1997; Doligez et al. 2003). Although temporary pools are highly variable and unpredictable in many aspects, some physical and biological properties may provide breeding females with reliable information regarding the pool's current and future quality. Pool depth, for instance, may provide some indication on its hydroperiod (Dennis and Golet 2005).

Amphibian oviposition habitat selection was empirically studied in the context of risks of predation and competition (Resetarits and Wilbur 1989; Crump 1991; Kats and Sih 1992; Sexton et al 1994; Petranka et al 1994; Aragon et al 2000; Dilon 2000; Marsh and Borrel 2001; Rieger et al. 2004; Orizaola and Brana 2003; Brown et al. 2008; Glos et al. 2008; Sadeh et al 2009), risk of parasitism (Kiesecker and Skelly 2000), timing of deposition (Matsushima and Kawata 2005) pool volume (Crump 1991; Spieler and

Linsenmair 1997; Rudolf and Rodel 2005; Brown et al. 2008; May et al. 2009) and pool hydroperiod (Egan and Paton 2004). Few studies however have attempted to explore the spatial and temporal distribution of offspring in an individual-based experimental setting (Spieler and Linsenmair 1997). Breeding habitat selection has implications for population dynamics (Pearman and Wilbur 1990) and species distributions, and its understanding is essential for conservation and restoration (Jonzen 2008).

The fire salamander, *Salamandra infraimmaculata*, uses breeding pools characterized by high spatial and temporal variability. *Salamandra* in Israel utilizes permanent water bodies but mostly temporary ones. The latter are much more abundant but vary in volume, duration and onset of hydroperiod. Small size temporary rock pools in regions containing *Salamandra* are often found in wadis or exposed bedrock areas where many pools can be found clustered in an area of less than one hundred square meters (e.g., Spencer et al. 2002). Three major causes for larval *Salamandra* mortality in temporary pools are cannibalism (Reques and Tejedo 1996; Degani 1993; Degani et al. 1980), competition (Eitam et al. 2005; Cohen-Koren et al. 2006), and time-dependent, stochastic pool desiccation followed by death of all the larvae that could not metamorphose by that time. It was previously reported that *Salamandra* demonstrate a high degree of philopatry or site fidelity (*S. salamandra*: Rebelo and Leclair 2003; *S. infraimmaculata*: Warburg 2007). Amphibian species demonstrate high plasticity in breeding modes; the same species may deposit multiple clutches per season in the tropics but a single clutch per season or even once in two years in temperate regions (Duellman and Treub 1994). However, if we consider intraseason single clutch distribution, many amphibian species cannot or do not spread their progeny and practice one deposition bout; once they begin to deposit, all progeny are placed at the same pool and at the same time. Very few studies have demonstrated intraclutch temporal and spatial spread (Spieler and Linsenmair 1997). While

gravid *Salamandra* carry complements of ~100-150 developed larvae at the start of the rainy season, field surveys in breeding sites revealed high variability in the number of newborns per pool, including numerous pools containing <20 newborn larvae (Segev unpublished).

In this study, we developed stochastic dynamic state variable model (Clark and Mangel 2000) incorporating larval density dependence as a partially predictable stress factor combined with stochastic pool depth and risk of desiccation. The model was used to generate predictions regarding temporal and spatial larval spread of larvipositing salamanders.

We specifically address the following questions:

- 1) Given the larval threats mentioned above, are *Salamandra* expected to spread their offspring spatially and/or temporally?
- 2) Are *Salamandra* expected to fine-tune larval distribution by plastically adjusting clutch sizes according to the depths of encountered pools?

Qualitative model predictions were then tested in an outdoor pool-choice experiment.

### **The model**

State dependent life history theory (Mangel and Clark 1988, Mangel and Ludwig 1992, Houston and McNamara 1999, Clark and Mangel 2000) requires characterizing the environment, the physiological state and survival of the larvipositing female, the increment in reproductive success obtained from deposition of a known number of larvae, and the overall accumulated reproductive success during the season. In the course of computing the latter, we will be able to predict larviposition as a function of the female's larval load and the environment and compare plastic and fixed clutch size strategies.

### *The Environment*

We assume that there are three kinds of pools indexed by  $i=1, 2, 3$ . The frequency of pool type  $i$  is denoted by  $p_i$ , and its maximal depth by  $D_{max(i)}$  (see Table 1 for pool characteristics). All pools are assumed to be full at  $t=1$  and dry by  $t=T$ , where the time units are nights. We assume that pool depth is linearly proportional to pool volume and floor area (a determinant of density-dependent interactions among larvae, see below). For computational simplicity, we assume that all pools that have not yet dried experience parallel changes in depth due to rainfall and evaporation, and that each pool type has only one useable hydroperiod (i.e., once a pool dries out, it remains dry for the rest of the breeding season). These assumptions are added to simplify the model, but are also conservative since they restrict the possibility of larval spreading. Thus, at time  $t$ , the environment is characterized by the vector  $H(t) = \{D_1(t), D_2(t), D_3(t)\}$ .

The probability of rain on night  $t$ ,  $\Psi(t)$ , as in Mediterranean climate simulated here, varies across the season (see Table 2). On a rainy night, the female has  $U$  time units to search for pools, with the probability  $\rho$  of finding a pool in each time unit, allowing her to find up to  $u$  pools per rainy night. On dry nights, she does not search for pools.

### *Larval Load and Survival*

We characterize the larvipositing female with a single state variable

$$L(t) = \text{Female larval load at the beginning of night } t \quad (1)$$

At the start of the breeding season, females have a full complement of larvae so that

$$L(1)=l_0.$$

The female experiences a constant rate of mortality,  $\mu$ , so that the probability of surviving from  $t$  to  $t+1$  is  $e^{-\mu}$ .

*Increment in Reproductive Success*

When a pool of type  $i$  is encountered and the female larviposits, her lifetime reproductive success is incremented. We characterize this increment in reproductive success by:

$$\Phi(c, i, d, t) = \text{Expected number of grand-offspring produced by successfully metamorphosed larvae from a clutch of } c \text{ larvae deposited on night } t \text{ into a pool of type } i \text{ with a current depth of } d \quad (2)$$

In order to compute this increment, we must consider the growth, survival, and fecundity of the larvae in the pool.

Imagine larvae deposited on night  $t=\tau$  and let  $S(t)$  denote the mean size of the larvae on the nights following deposition. If  $A$  is the floor area of the focal pool and  $n(t)$  is the number of other larvae in the focal pool, we assume that

$$S(t+1) = S(t) + \min \left[ S(t) \cdot k, \frac{P \cdot A}{n(t)} \right] \quad (3)$$

where  $k$  is the maximum specific growth rate of the larvae and  $P$  is the productivity of the focal pool (see Table 3 for parameterization). We assume a value of 0.2g for the mass of a newly deposited larva,  $S_0$  (Degani 1994). Maximum specific growth rate,  $k$ , can be estimated as follows: an individual larva grows from 0.2g to 2.0g in 90 days (for one larva growing alone in a 0.24m<sup>2</sup> pool; Blaustein, unpublished data). Solving Eqn. (3) for  $k$  when  $n(t)=0$  produces a value of 0.026g day<sup>-1</sup>.

To estimate  $P$  from empirical data (Blaustein, unpublished data) we note that at a density of 4 larvae in a pool with an area of  $0.24\text{m}^2$ , a larva grows from 0.2g to 1.2g in 100 days. Solving Eqn. (3) for  $P$  produces a value of  $0.167\text{g m}^{-2} \text{day}^{-1}$ . In natural temporary pools there may be high heterogeneity in food resources due to, for example, differences in pool substrate, organic input, or illumination conditions. However we assume a constant productivity among pools since we are interested in pool hydroperiod in this study.

We assume that larvae experience both density-independent and density-dependent survival, characterized by  $\mu_d$  and  $\mu_t$ , respectively, so that the probability of survival from night  $t$  to  $t+1$  under the same conditions as above is:  $\exp\left(-\frac{\mu_d n(t)}{A} - \mu_t\right)$ .

Larvae that survive and successfully metamorphose will produce offspring themselves if they exceed a critical size  $S_c$  and we assume that the lifetime reproduction of offspring is given by  $aS_m^b$ , where  $S_m$  is the size at metamorphosis.

In order to compute the fitness increment, we used a backwards iteration algorithm, starting at each possible date upon which a pool might dry for each possible size of larvae and number of larvae in the pool. For each combination of previous time  $t'$  and values of  $n(t')$ ,  $S(t')$ , and  $d(t')$ , the expected increment in fitness is calculated as the sum of all possible future fitness possibilities multiplied by their associated prior probabilities. The process repeats for  $t = t-2, t-3$ , etc., until the initial larval mass  $S_0$  is reached. Such a backward algorithm accounts for the mother not knowing at the time of larviposition when the pool will dry. Figure 1 shows how the female's fitness increment from a single larviposition ( $\Phi$ ) changes with respect to clutch size, time, pool type and current depth. Fitness increment is highest in the deepest ( $i=1$ ) pool even when its current depth is equal to that of the shallower pools. Within any pool type, fitness increment is positively related to both current pool depth ( $D_i$ ) and time remaining for larviposition ( $T-t$ ).

### *Accumulated Reproductive Success*

Once  $\Phi(c, i, d, t)$  is known, we are able to compute the accumulated reproductive success of the focal female, and through that the optimal larviposition strategy throughout the season as a function of state. We define accumulated reproductive success by:

$$F(l, h, u, t) = \text{Maximum expected number of grand-offspring resulting from} \\ \text{larviposition from period } u \text{ on night } t \text{ to night } T, \text{ given that } L(t)=l \text{ and} \\ H(t)=h \tag{4}$$

Since there is no evidence for maternal re-absorption of remaining fetuses towards the end of the breeding season in this iteroparous species, we set the end condition:

$$F(l, h_T, U, T) = 0 \tag{5}$$

The female's state at the end of search on night  $t$  and at the start of night  $t+1$  must be the same, so that we have the sequential coupling condition  $F(l, h, U, t) = F(l, h, 1, t+1)$  (*sensu* Mangel and Clark 1988, Clark and Mangel 2000).

If it does not rain on night  $t$ , then the female must survive to the next night, and her larval load does not change. Similarly, if it does rain but she does not encounter a pool, then she must survive and her larval load does not change. If it does rain and she encounters a pool, she must survive and then we assume that she optimizes the number of larvae deposited, accumulating fitness increment due to this deposition, and reducing her larval load. Taking

these three possibilities into account allows us to link fitness from one search period to another on each night:

$$\begin{aligned}
F(l, h, u, t) = & (1 - \Psi(t))e^{-\mu U} F(l, h', 1, t+1) + \Psi(t)(1 - \rho)e^{-\mu} F(l, h, u+1, t) \\
& + \Psi(t)\rho e^{-\mu} \sum_{i=1}^3 p_i \max_c \{ \Phi(c, i, d, t) + F(l-c, h, u+1, t) \}
\end{aligned}
\tag{6}$$

Where  $h'$  denotes the value of  $H(t+1)$ , given that  $H(t)=h$ . In the course of solving Eqn. 6 backwards in time, we generate the optimal clutch sizes  $c^*(l, i, d, t)$ .

#### *Accumulated Reproductive Success of a Fixed Clutch Strategy*

The logic leading to Eqn. 6 can also be used to compute the accumulated reproductive success of a fixed clutch strategy. To do this, we let:

$$\begin{aligned}
W(l, h, u, t | c_f) = & \text{Expected number of grand-offspring resulting from} \\
& \text{larviposition from period } u \text{ on night } t \text{ to night } T, \text{ given that } L(t)=l \text{ and} \\
& H(t)=h, \text{ and that the female always deposits } c_f \text{ larvae}
\end{aligned}
\tag{7}$$

Reasoning analogous to that leading to Eqn. 6 leads to:

$$\begin{aligned}
W(l, h, u, t | c_f) = & (1 - \Psi(t))e^{-\mu U} W(l, h', 1, t+1 | c_f) + \Psi(t)(1 - \rho)e^{-\mu} W(l, h, u+1, t | c_f) \\
& + \Psi(t)\rho e^{-\mu} \sum_{i=1}^3 p_i \{ \Phi(c_f, i, d, t) + W(l-c_f, h, u+1, t | c_f) \}
\end{aligned}
\tag{8}$$

with the understanding that if  $l < c_f$  then only  $l$  larvae are deposited. The optimal fixed clutch strategy  $c_f^*$  is then the value of the fixed clutch that maximizes  $W(l_0, h, 1, 1 | c_f)$ .



### *Model Predictions*

We use the fixed clutch model in order to answer our first question, whether females are predicted to deposit all their larvae in a single pool or to spread their young across pools. An optimal fixed clutch of equal size to the maternal full complement at the beginning of the season ( $c_f^* = l_0$ ) would indicate that females are predicted to deposit all of their larvae in the first encountered pool, thus supporting the possibility of the salamanders practicing pond fidelity, in accordance with previous reports (Rebelo and Leclair 2003; Warburg 2007). However, our model predicts that  $c_f^* < l_0$ , i.e. that a *Salamandra* female will spread larvae in time and across pools (Fig. 2).

In order to answer our second question, whether a plastic clutch size strategy is predicted to evolve, by which females adjust the number of deposited larvae according to pool quality, we compare the expected fitness of the optimal fixed clutch size strategy (Eqn. 8), with that of the optimal plastic clutch size strategy (Eqn. 6). The results of this comparison for different initial larval loads are shown in Figure 3, predicting that the plastic clutch size strategy will be favoured by natural selection. A 3-5% difference in fitness may seem insignificant but on evolutionary time scale in a constant environment, it may accumulate to a considerable difference and for the prevalence of a plastic deposition strategy. The model also predicts changes in optimal clutch size with time, pool type and current depth. Optimal clutch size shows a general hump shape curve throughout the season when all three pool types are completely full (Fig. 4a), and a general decline with time when water capacity in the pools is less than maximal (Fig. 4b,c).

## Larviposition Experiment

To test some of the model's predictions, we conducted a larviposition experiment to determine whether given a choice of several pools, a gravid *Salamandra* will:

- 1) Spread her complement of larvae across time and/or into multiple pools
- 2) Adjust clutch sizes according to pool depths/volumes.

We collected gravid females from two breeding sites located within Mt. Carmel National Park. One, Secher Pool (32°44'04"N; 35°01'52"E) is a large, rainwater-fed winter pool, approximately 400m<sup>2</sup> surface area and 2m deep at its maximum capacity in mid-winter. This pool generally completely dries up by mid-summer. The second, Ein al-Balad (32°43'13"N; 35°04'17"E) is a spring-fed, concrete permanent pool of 15m<sup>2</sup> surface area and 0.8m deep. We used 6 enclosures (each 4x4m in area), each containing four identical plastic tubs (surface area: 60x40 cm). In each enclosure, two tubs were 13.5cm deep, and the other two were 4.5cm deep. The tubs were dug into the ground and were equally spaced while the depth treatment locations were randomized within each enclosure. Each trial began with the placement of a single gravid female into an enclosure on the same night it was collected from the field. Trials were conducted at the onset of the wet season to assure that gravid females were still carrying nearly full larval complement. We made daily counts of the number of larvae in each pool, leaving all larvae in the tubs where they were deposited. Each female was kept inside her enclosure for two weeks to allow the female to experience several rain events, since they mostly only give birth at rainy nights. After each two-week trial, all the tubs in the enclosure were cleaned, dried and refilled with aged tap water and rain water for another trial with a different female. This was done to eliminate possible chemical traces of the previous trial's larvae. The experiment was conducted using 15 gravid females and these 15 trials were completed over 10 weeks (Oct

18<sup>th</sup>-Dec 29<sup>th</sup> 2002). Each female and her deposited larvae were returned to their capture site once the trial ended.

### *Statistical analysis*

To analyze the females' responses to pool depths, we compared the number of deposition events within each pool depth class (regardless of number of larvae deposited in each event). We also compared the mean numbers of larvae deposited into each pool depth, both during the first night of the trials and mean numbers accumulated throughout the experiment. The first night's data would more closely approximate conditions of all else being equal in the tubs except for pool depth/volume. The accumulated data would also incorporate any ideal free distribution effects – i.e. the mothers might first choose deeper pools but then later begin to deposit into the shallower pools once larvae were found in the deeper pools.

As the number of larvae in each depth class did not distribute normally, we performed a non-parametric rank test (Mann-Witney U) to assess the effect of pool depth on the number of larvae deposited on the first trial night and throughout the females stay within the enclosures. One of the 15 females that participated in the experiment escaped from her enclosure following the first night, and was excluded from our temporal larval spread analysis.

### **Experimental Results**

The 15 females combined for a total of 39 larval deposition events, yielding a total of 1008 larvae. Six of 15 females (40%) larviposited during the first night, and all six larviposited into more than one tub. Eventually, 13 of 15 females (86%) larviposited in more than one tub, and eight (53%) larviposited into at least three tubs (Fig. 5). These

results provide evidence that *Salamandra* are capable of spreading their larvae spatially, across different pools. Nine of 14 females (64%) larviposited during more than a single night (Fig. 6a), and the interval between larviposition events varied between 1-5 days (Fig. 6b). These results imply that *Salamandra* capable of spreading their larvae temporally. Female larviposited mostly during rainy nights; 20 of 24 (86%) larviposition events occurred during rainy nights or not more than 48 hours after a major ( $\geq 7.3\text{mm}$ ) rain event. During the first night in the enclosure, when all four tubs still contained no larvae, 7 of 15 females (47%) completely avoided larviposition in either of the shallow tubs. Instead, at least one of each pair of the deep tubs was chosen for larviposition (Fig. 7). Overall during the first night, more larviposition events occurred in the deep tubs (Paired t test:  $df=14$ ,  $t=2.17$ ,  $p=0.024$ )

During their first night, females larviposited significantly more larvae into the deep pools ( $N=15$ ; Mann-Whitney  $U=168.5$ ;  $p=0.019$ ; Fig. 8). Overall, during their entire stay within the enclosures females deposited significantly more larvae into deep pools ( $N=15$ ; Mann-Whitney  $U=168.5$ ;  $p=0.025$ ; Fig. 8).

## **Discussion**

Our model predicted that gravid salamanders will spread their larval complement spatially and temporally throughout the breeding season, and that their distribution of larvae will incorporate plastic responses in clutch size to perceived pool quality factors, specifically pool volume. The predictions of both temporal and spatial larval spread, as well as the prediction of a plastic response to pool volume were supported by the results of our outdoor pool choice experiment.

Marsh (2001) highlights the need to distinguish spatial patterns resulting from individual behavior i.e., habitat selection and dispersal, from changes in population

demographics. In a set of natural temporary pools that vary in depth, pond fidelity may be responsible for higher larval abundances in deeper pools if *Salamandra* return yearly to sites where they were born (Rebelo and Leclair 2003; Warburg 2007), since females that larviposit into shallow, short-lived pools will have fewer emerging descendants. However, field observations of small pools often containing less than 20 individuals (Segev unpublished), as well as observations of long-range dispersing adults (Bar-David et al. 2007) that likely do not return to their natal pond, do not support such a mechanism. Our model, in agreement with theory for parasitic insects (Mangel 1987), predicts that larval density-dependent effects are responsible for the spreading of larvae in time and space as an optimal resolution of the trade-off between current and future reproduction. In our experiment, females showed the ability of assessing pool depths, but still used more than one pool, including inferior (shallow) ones, and spread their larvae among them.

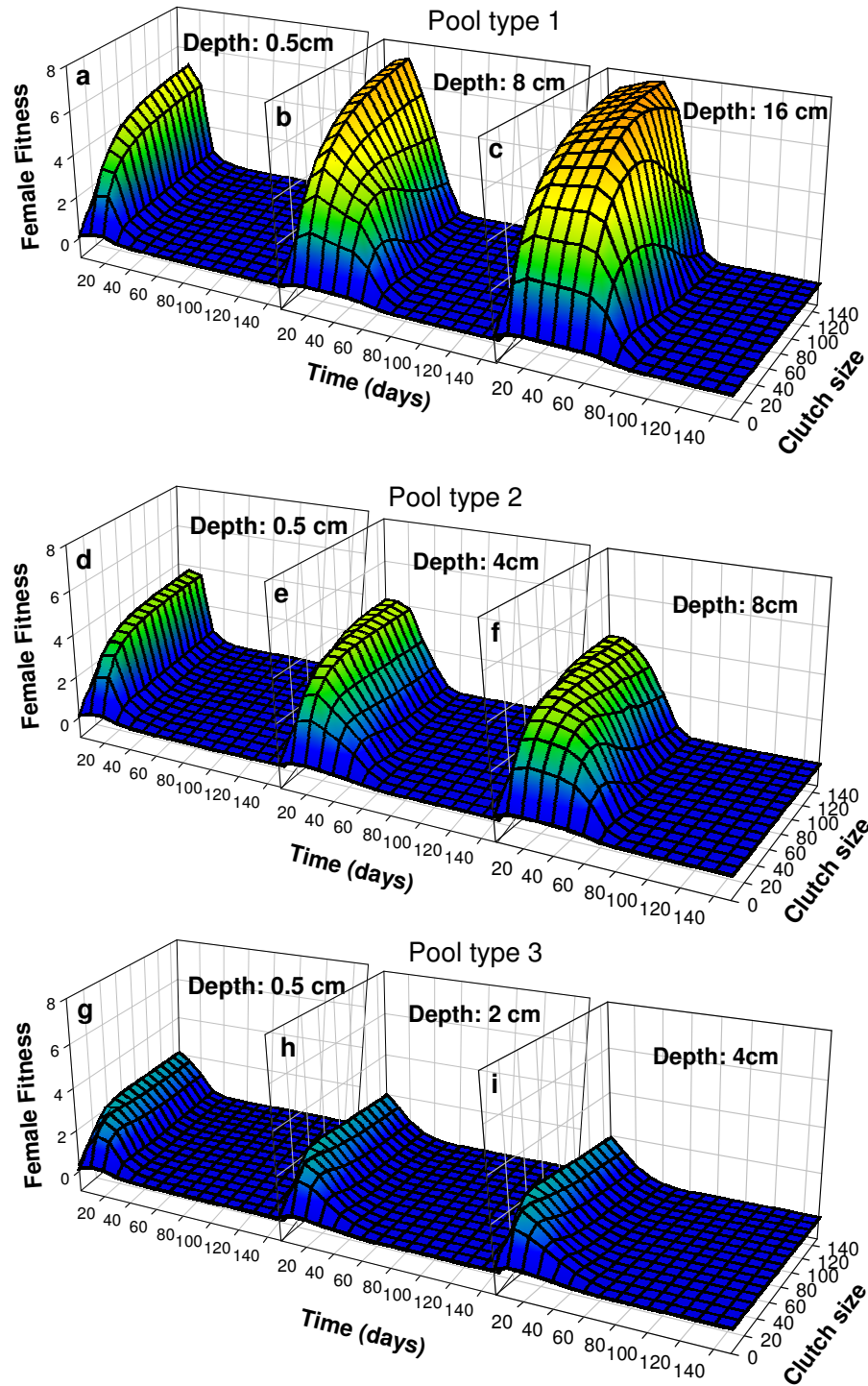
The temporal association of rain combined with breeding pools' spatial distribution may dictate the interval between breeding bouts within a single breeding season. In our experiment, the temporal distribution of rain was primarily responsible for timing of larviposition. Our experiment confirms that gravid *Salamandra* are physiologically capable of delaying deposition long enough to utilize temporally distinct rain events i.e., temporally spread their larvae.

Greater accuracy in assessment of breeding patch qualities is favoured by natural selection when costly penalties exist for its over- and under-estimation (Godfray 1994). Patch quality assessment has been treated extensively in the context of foraging theory (Valone and Brown 1989; Gotceitas and Colgan 1991; Schmidt and Brown 1996; Vásquez et al. 2006). In breeding habitat selection, a large body of literature was devoted to the effects of biotic factors (mainly the presence of predators and competitors (see reviews in Edgerly et al. 1998; Blaustein 1999; and Skelly 2001), while few studies examined

whether species breeding in temporary waters can assess water-holding capacities (Crump 1991; Spieler and Linsenmair 1997; Rudolf and Rodel 2005; Arav and Blaustein 2006; Brown et al. 2008; May et al. 2009). Aquatic insects like mosquitoes and chironomids that do not submerge during egg deposition may lack the ability to assess water depth (Arav and Blaustein 2006; Mokany and Mokany 2006). Our study indicates that *Salamandra* can evaluate pool depth or volume, probably while swimming prior to larviposition initiation. The mechanism may involve sight, tactile cues received either by contact of the gravid female appendages with the pool floor or by sensing minute changes in water pressure while submerging. The evaluation of pools' water loss rates by changes in their water levels over repeated visits was previously suggested for two anurans, *Hoplobatrachus occipitalis* (Spieler and Linsenmair 1997) and *Phrynobatrachus guineensis* (Rudolf and Rodel 2005). Although not addressed in the current study, such dynamic pool quality assessment may be practiced by *Salamandra* females to gauge water-holding capacity, rate of water loss and hydroperiod. A mark-recapture study of *Salamandra* breeding populations revealed high within-season recapture rate in breeding pools vicinity (Segev et al. 2009) that may be attributed to multiple pool sampling. Moreover, observations of a breeding site containing numerous pools from the time of their first inundation revealed that females repeatedly visited the pools over a week before the first female deposited its larvae (Sadeh, personal observations).

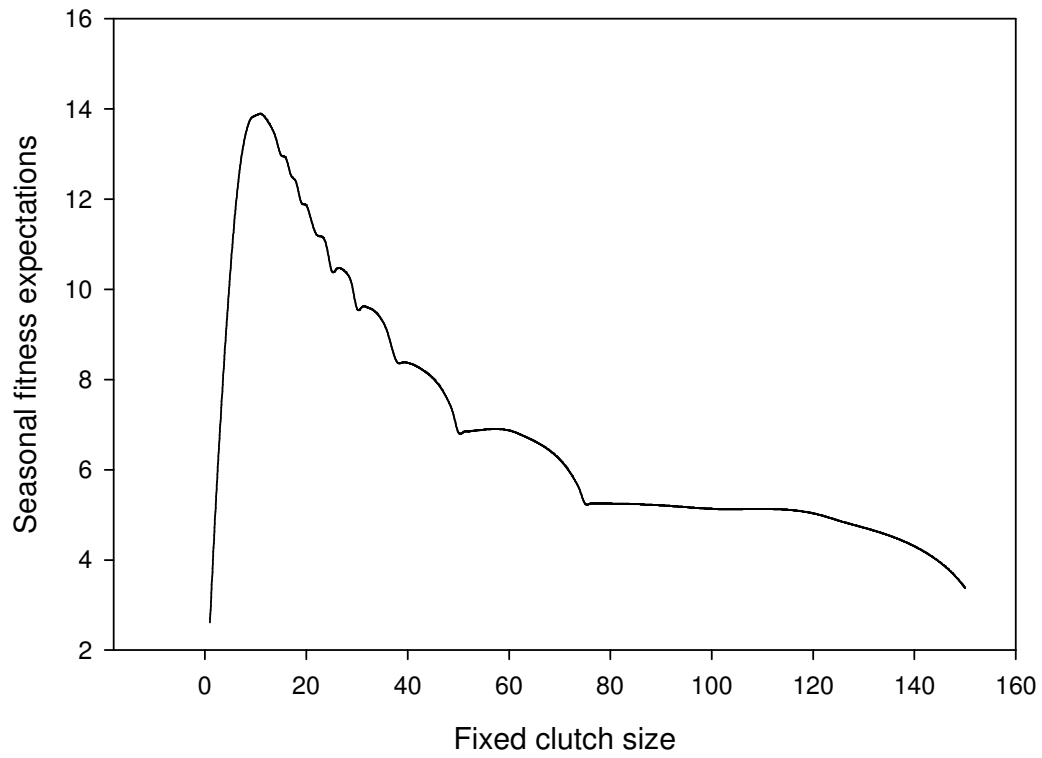
Beyond its contribution to life history theory and habitat selection theory this study through the investigation of mechanisms that underline the spatio-temporal distribution of threatened organisms, can facilitate the relation of ecological concepts to applied real-world problems facing natural resource managers. For example breeding habitat selection has consequences for the probability of colonization success in captive release, relocations

and translocation programs (Stamps and Swaisgood 2007), and on dispersal between sites as breeders movement is an integral part of active breeding site choice.

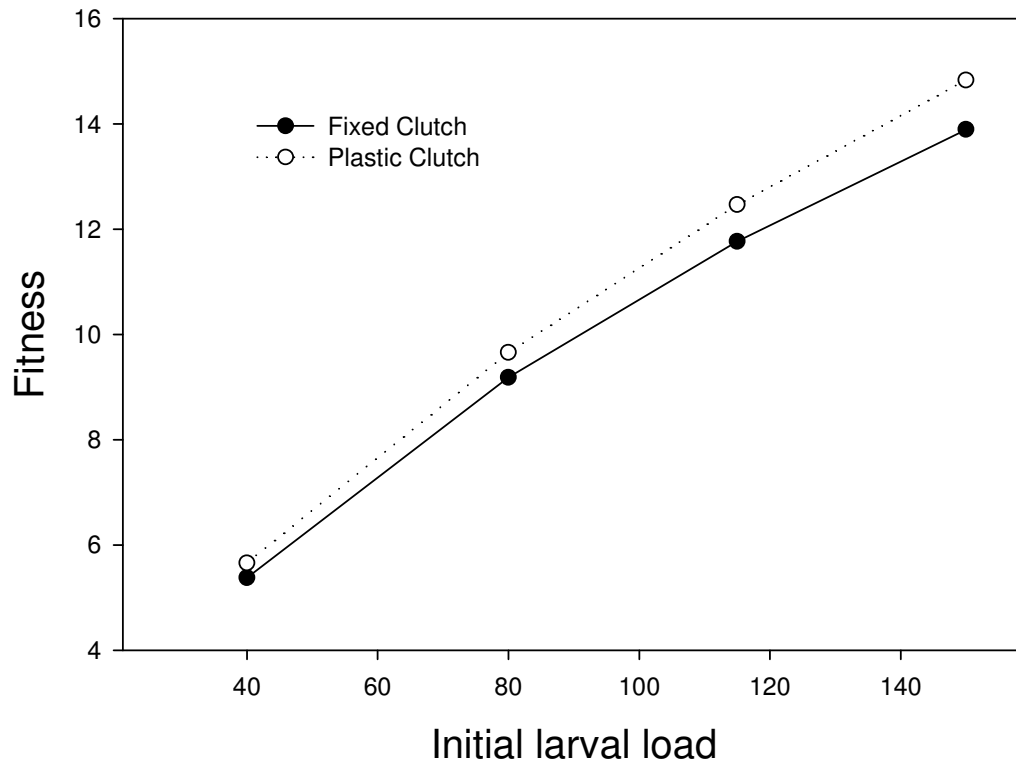


**Fig. 1:** Fitness increment from a single larviposition,  $\Phi(c,i,d,t)$ , as a function of time and clutch size,  $c$ , and current water depth,  $d$ , combinations in three pool types,  $i$ .

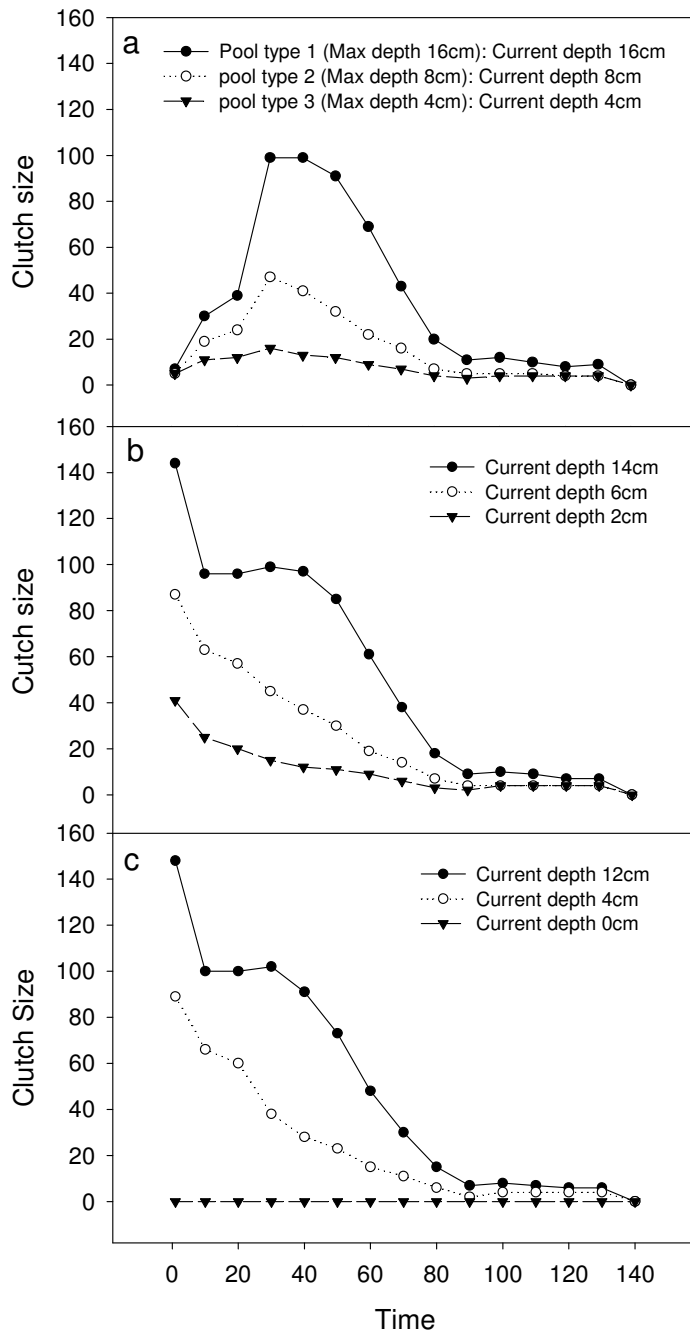




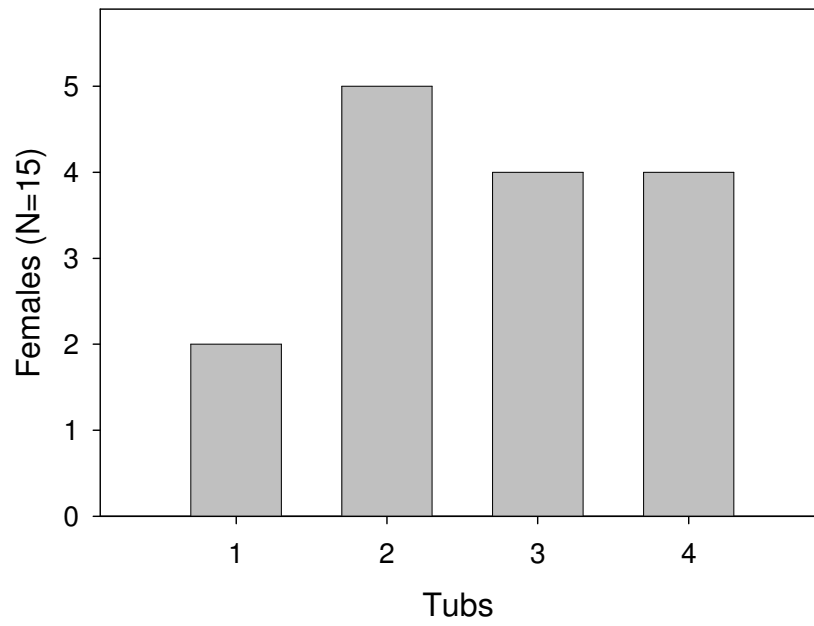
**Fig. 2:** Female seasonal fitness expectation  $W(l_0, h, 1, 1|c_f)$ , as a function of fixed clutch size,  $c_f$ .



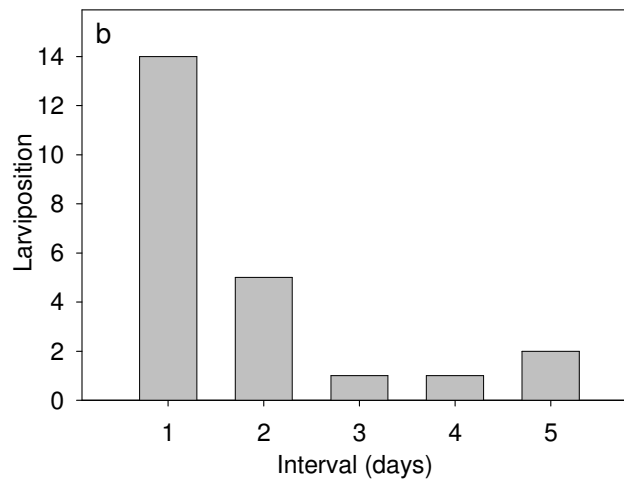
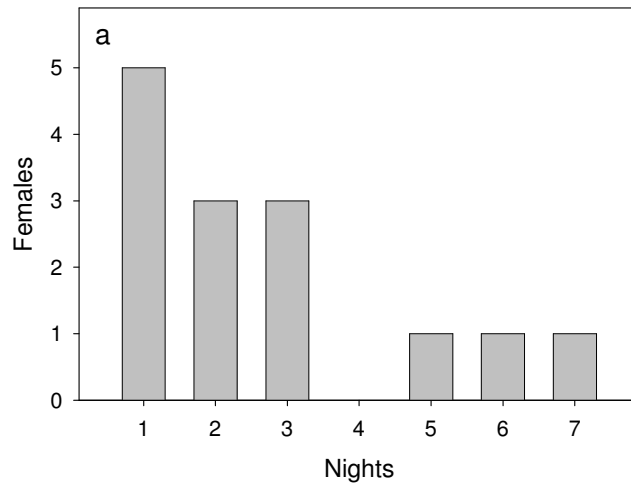
**Fig 3:** A comparison of maximal maternal fitness over the entire season for females employing a fixed clutch size strategy,  $W(l_0, h, 1, 1|c_f^*)$ , versus a plastic clutch size strategy  $F(l_0, h, 1, 1)$ , for four initial larval loads,  $l_0=40, 80, 115, 150$ .



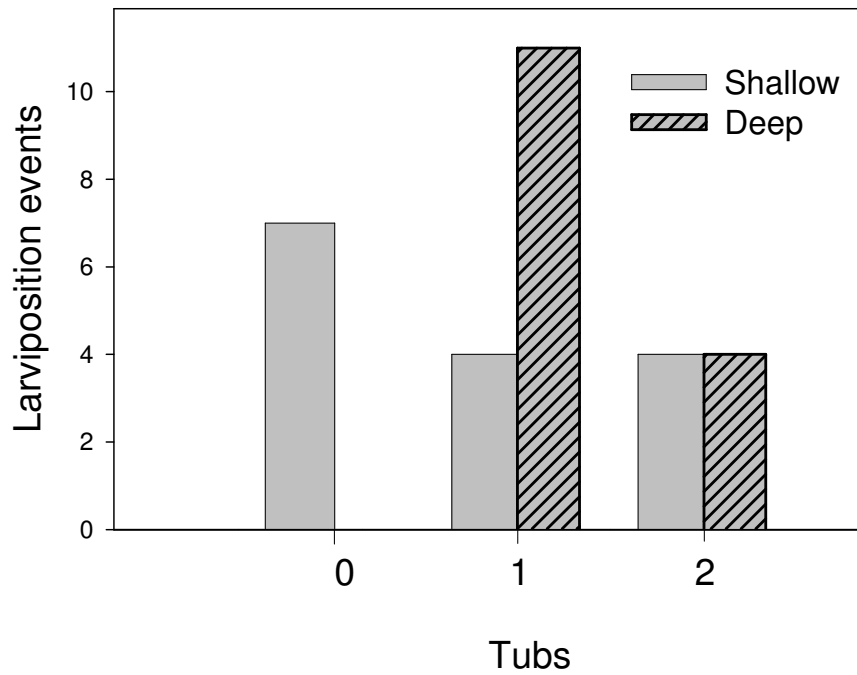
**Fig. 4:** Distribution of optimal clutch size,  $c^*(150, i, d, t)$ , over time for different combinations of pool types,  $i$ , and current depths,  $d$ , at a constant load of  $l=150$  larvae.



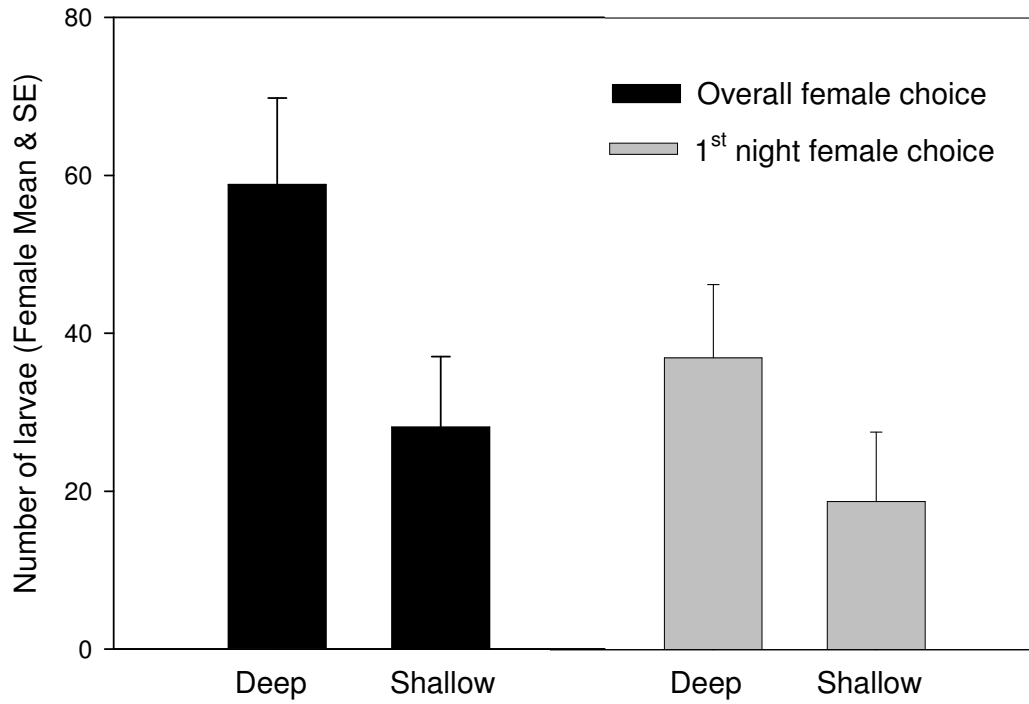
**Fig. 5:** Number of tubs used for larviposition per female.



**Fig. 6:** Number of larviposition nights per female (a); interval between individual female larviposition events (b).



**Fig. 7:** Distribution of larviposition events during the first night in the deep (14cm) versus the shallow (4.5cm) tubs. 0=complete female avoidance; 1=deposition in only one tub of each depth pair; 2=deposition in both tubs of each depth pair.



**Fig. 8:** Mean number of larvae deposited per female in the deep (14cm) versus shallow (4.5cm) tubs during the entire stay within the enclosure and only the first night.

**Table 1:** The model's physical characteristics and frequency of pools.

<b>Pool type (<math>i</math>)</b>	<b>Pool maximal depth (<math>D_{max(i)}</math>)</b>	<b>Pool frequency in landscape (<math>p_i</math>)</b>	<b>Pool slope</b>	<b>Pool productivity (<math>P_i</math>)</b>
1	16 cm	0.25	0.2m <sup>2</sup> cm <sup>-1</sup>	0.167 g m <sup>-2</sup> day <sup>-1</sup>
2	8 cm	0.25		(Blaustein unpublished)
3	4 cm	0.5		



**Table 2.** The model's weather patterns. Rain probabilities were estimated from data in Warburg (1985). Time begins on 1 December, each rain event adds 1cm to the depths of all the pools.

<i>t</i> =	Daily rain probability ( $\Psi$ )	Daily evaporation rate
1-30	.65	0.0 cm
31-60	.52	0.5 cm
61-90	.36	0.5 cm
91-120	.25	1.0 cm
121-150	.11	1.0 cm

**Table 3.** Model parameter values and units.

<b>Symbol</b>	<b>Meaning</b>	<b>Value and units</b>	<b>Reference</b>
$T$	Number of nights in the breeding season	150 nights	Goldreich 1995
$l_0$	Full larval load in uterus at $t=1$	150 larvae	Degani 1996
$i, A, p_i$ $D_{max(i)}$ $P_i$	Pool characteristics	(see Table 1)	
$\mu$	Nightly rate of female mortality		
$\Psi(t)$	Time-dependent nightly rain probability	(see Table 2)	
$u$	Number of time units per night available for searching for pools	5	
$\rho$	Probability of finding a pool per time unit	0.2	
$S_0$	Initial larval size at birth	0.2g	Degani 1994
$S_c$	Minimal larval size allowing metamorphosis	0.45g	Segev unpublished
$k$	Maximum specific larval growth rate	0.026g day <sup>-1</sup>	Blaustein unpublished
$\mu_d$	Density-dependent larval mortality factor	1·10 <sup>-4</sup>	
$\mu_t$	Time-dependent larval mortality factor	8.5·10 <sup>-4</sup>	
$a$	Allometric relationship constant for egg production	0.5	Peters 1986
$b$	Allometric relationship constant for egg production	0.26	Peters 1986

**Chapter 4. Priority effects of the early breeding fire salamander on the late  
breeding banded newt**

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## Abstract

Early breeding intraguild predators may have advantages over late breeding predators via priority effects; early breeding predators may reduce shared prey resources before late breeders appear and may also prey upon the late breeders. Here we show that predatory larvae of the late-breeding predatory banded newt, *Triturus vittatus vittatus*, occupy the same temporary pond toward the end of the developmental period of the early-breeding predatory fire salamander, *Salamandra salamandra*<sup>1</sup>, resulting in a large size disparity between larvae of these two species while they co-occur. We conducted outdoor artificial pool experiments to assess priority effects of large larval *Salamandra* at the end of their larval development period, on recently hatched larval *Triturus*. We also assessed how artificial vegetation may influence larval *Triturus* performance in the presence or absence of *Salamandra*. *Salamandra*, introduced into the experimental pools two weeks prior to the newt larvae, strongly reduced invertebrate prey abundance shared by these two predatory urodeles and with only a one week period of overlap, strongly reduced abundance of *Triturus* larvae. The artificial vegetation had only a small ameliorating effect on *Triturus* survival when *Salamandra* was present. *Triturus* size at metamorphosis (snout-tail length) was significantly larger in the *Salamandra* pools, presumably due to a combination of a strong “thinning effect” and greater vulnerability of smaller *Triturus* individuals to predation by *Salamandra*. Time to metamorphosis was not significantly affected by *Salamandra*. These results have conservation implications as *T. v. vittatus* is listed as highly endangered and may also explain the largely negative spatial association of the two species.

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<sup>1</sup> At the time of publication, we accepted the taxonomic assignment of this species in Israel as S.s. (Degani 1996). Since then, there has been a consensus among taxonomists that the populations in Israel should be assigned to the species S.i. (Steinfartz et al. 2000).

## **Introduction**

Breeding late by amphibians in temporary aquatic habitats has several potential advantages. For example, in Mediterranean climates where there are distinct rainy and dry seasons, breeding early may come at the cost of higher risk of desiccation since rains are less predictable and less frequent at the start of the rainy season (Warburg 1994), and food resources for amphibians have had less time to become abundant (Steiner and Roy 2003). However, breeding late also carries the risk of negative priority effects – i.e. the presence of early breeding competitors and intraguild predators may negatively affect larval performance of late breeders (Morin et al. 1990; Blaustein and Margalit 1996; Brodman 1999; Walls and Williams 2001; Eitam et al. 2005). Intraguild predators may not only affect larval success by competition with, and consumption of, these larvae, but there are costs of various responses to risk of predation (Gustafson 1994; Resetarits 1995). By reducing the level of food resources, the early breeders may continue to have negative residual effects on late breeders, even after the early breeders have metamorphosed and left the pool.

Vegetation, or other types of habitat heterogeneity, may affect amphibian larval performance in at least two ways: it may provide more substrate for food resources used by the larvae, and it may reduce predation rates by concealing individuals from predators (Sih 1987; Semlitsch and Reyer 1992; Jackson and Semlitsch 1993; Kats et al. 1994; Walls 1995; Niecieza 2000, Baber and Babbitt 2004).

Facultative shifts in larval life history (Werner 1986; Rowe and Ludwig 1991), and behavioral changes (Werner 1991; Semlitsch and Reyer 1992; Niecieza 2000), have both been suggested as anti-predator responses in amphibians. Predator presence might have a negative, non-lethal effect on prey developmental rate by affecting prey behavior. Prey behavioral responses might involve a reduction in activity level or a change in microhabitat

selection such as an increase in refuge use (Walls 1995; Turner and Montgomery 2003). Reduced foraging activity can consequently decrease growth rate, size at metamorphosis, and increase age at metamorphosis (Fauth 1990; Babbitt 2001; Barnett and Richardson 2002). Alternatively, a facultative shift in prey life history might increase the rate of development and thus might reduce time to metamorphosis (Peacor and Werner 2004).

Predation could also indirectly benefit surviving prey individuals by thinning the number of consumers, thus increasing per capita food density (Abrams et al. 1996; Van Buskirk and Yurewicz 1998; Brodin and Johansson 2002). Hence thinning may ultimately lead to increased growth rate, development and reduced time to metamorphosis. The beneficial effect of thinning on larval life history may be obscured or outweighed by the negative effect of behavioral avoidance from predators.

In the Middle East region, temporary pools generally first fill in autumn with the onset of the rainy season. The banded newt, *Triturus vittatus vittatus*, an endangered species in Israel due at least partly to a reduction in quantity and quality of suitable breeding habitats (e.g. Blaustein et al. 1996a; Gafny 2004), is a late season, temporary pool breeder. Late breeding by *Triturus* may be related to increased temperature or the presence of vegetation, which can be used by the female to wrap her eggs singly (Degani and Kaplan 1999). In the absence of an early season dominant predator, late breeding often provides a higher abundance of prey (primarily aquatic insects and crustaceans) and abundant submerged vegetation for oviposition. However, some pools inhabited by *Triturus* are also breeding sites of an early season breeder, the fire salamander *Salamandra salamandra* (Degani and Kaplan 1999). *Salamandra* larvae have strong negative impacts on the biomass of aquatic insects and crustaceans (Blaustein et al. 1996b, Blaustein 1997; Spencer and Blaustein 2001; Eitam et al. 2005). Consequently, *Salamandra* larvae may have strong competitive effects on *Triturus* larvae even after *Salamandra* metamorphose

and leave the pool. Moreover, since *Triturus* begins its larval life at a time when *Salamandra* larvae are quite large, larval *Salamandra* may potentially be an important predator of *Triturus* where the two species co-occur. In such pools, vegetation may be crucial in reducing predation rates of *Triturus* larvae by *Salamandra*. *Salamandra* and *Triturus* tend to be negatively associated spatially across pools (Degani and Kaplan 1999). This may be partly due to negative priority effects of *Salamandra* on *Triturus*. This, to date, has not been experimentally examined. In pools where they do coexist, ample submerged vegetation may prevent local extinction of *Triturus* (e.g., Sassa Pond, Degani and Kaplan 1999).

On Mt Carmel, Israel, we found populations of *Salamandra* and *Triturus* together in a large temporary pool, Secher Pool. *Salamandra* occurs there naturally while H. Mendelssohn introduced the *Triturus* population in 1985 (H. Lahav, personal communication). Here, we examine the temporal distributions of these two urodeles in this pool. We also report on experiments designed to isolate the effects of *Salamandra* larvae and artificial vegetation on survival and development of *Triturus* larvae.

## **Methods**

### *Urodele survey at Secher Pool*

Secher Pool (624N 690W) is a large pool (~400 m<sup>2</sup> surface area and ~2m depth at maximum size in early to mid winter), which generally dries completely by summer but during some years, holds water all year. We periodically sampled the pool for urodeles on 15 dates by sweeping with a D-net (0.3 cm mesh; 0.35 m<sup>2</sup>) between 16 November 1994 and 19 June 1995 (urodeles were no longer found by this last date). The total number of *Salamandra* and *Triturus* collected in 10 net sweeps of one-meter length was recorded. On

9 of those dates, individuals were also measured (total length), and then immediately returned to the pool.

#### *Artificial pool experiments*

In the first experiment, we used a two factorial design - presence or absence of larval *Salamandra* crossed with presence or absence of artificial vegetation - to study the effects of these two factors on larval *Triturus* survival and development. The experiment was conducted on the periphery of the University of Haifa Campus in 20 plastic tubs (length, width, depth: 60 x 40 x 14 cm). *Salamandra* larvae are often found in pools of this size and smaller (Spencer et al. 2002; L. Blaustein, pers. obs.). *Triturus* larvae can also be found in pools of this size. The tubs were arranged in 2 x 10 array under a 90% shaded net suspended at a height of 1.5m. Distance between neighbouring tubs was 0.5 m. On 20 April 2002, we filled pools to a depth of 12 cm with tap water. Water level was then maintained at 12-13 cm (~30 L) to some extent with rainwater, but mostly with aged tap water and distilled water. To prevent the escape of metamorphosing *Triturus*, an 8 cm wide plastic frame overhang was horizontally attached to the tub edges using Velcro<sup>®</sup> straps. The pools were otherwise open at the top to allow insect colonization and allochthonous input. To supply metamorphosing *Triturus* with a terrestrial platform for exiting the water, two 10 x10 x 6.5 cm bricks were placed one on top of the other into the center of each tub (total height: 13cm).

Treatments were randomly assigned to the tubs. On 21 April, three medium sized *Salamandra* larvae (mean total length and standard error: 48.5 g, 3.4 mm; mean weight and standard error: 0.73 g, 0.12) collected from a nearby spring were added to 10 pools. We placed a bundle of 100 black plastic strips (80 cm long, 3 cm width) tied together at their midpoints, (to simulate a vegetation cover) on one side of five *Salamandra* and five non-*Salamandra* tubs. On 22 April, to provide food resources for the urodeles, we inoculated



equal aliquots of invertebrates from other artificial pools containing primarily the crustacean *Arctodiaptomus similis*. Two weeks after introducing the *Salamandra* larvae (2 May), we introduced into each tub, four *Triturus* larvae collected from Secher Pool. Due to limited numbers and high variability in size (mean and standard error of total length: 18.9 cm, 5.9 mm) of *Triturus* found in the natural pool, larvae were sorted by size (4 per tub) in order to get approximately the same mean and range in sizes for all tubs. The groups of four were then randomly assigned to all 20 tubs. At the time of introduction, there were no statistically significant differences in *Triturus* snout-tail length between treatments (Anova:  $F_{3, 16}=0.246$ ,  $P=0.863$ ). Adding *Salamandra* larvae two weeks prior to the introduction of *Triturus* was expected to deplete invertebrate resources substantially (Blaustein et al. 1996b; Eitam et al. 2005) prior to the appearance of the *Triturus* larvae. One week after introducing the *Triturus*, several *Salamandra* were observed to start metamorphosis. We then removed all *Salamandra* larvae (8 May). Any *Triturus* larvae surviving in the *Salamandra* tubs should then still be potentially subjected to residual effects of the *Salamandra* – i.e. the depleted invertebrate prey caused by *Salamandra*. Thus, to reflect the natural phenology of urodeles, our experiment began with a *Salamandra*-only period (2 weeks), a second period of co-occurrence of both urodeles (one week) and a third period of *Triturus* only.

Invertebrate fauna in the tubs were sampled on April 29<sup>th</sup> (8 days after the introduction of the *Salamandra* larvae) and on June 10<sup>th</sup> by sweeping a small rectangular net (size: 6.4x7.85 cm, mesh size: 250  $\mu\text{m}$ ) in an S-shape (sample volume: 0.6  $\text{m}^3$ ) through the water while lifting the artificial vegetation. Mosquito egg rafts on the water surface were counted twice during the *Salamandra*-present period (7 and 8 May) and summed for analysis. Likewise, egg rafts were counted every 2-3 days during the entire post-*Salamandra* period and summed for analysis. Maximum and minimum temperatures, recorded every 3-5 days

from max-min thermometers placed at the bottom of three randomly chosen pools, ranged between 13-30 °C and 11-26 °C, respectively.

*Triturus* larvae were counted on the day that *Salamandra* was removed and then counted and measured (total length) every two weeks beginning 5 days after removing the *Salamandra* larvae. Once the first *Triturus* had emerged from the water (June 3<sup>rd</sup>), tubs were checked daily for metamorphosing individuals (without gills and generally out of the water), we recorded the metamorphs' length and weight which yielded time to, and size at metamorphosis.

Because the first experiment yielded unclear results of effects of the artificial vegetation on *Triturus* survival, we further assessed *Salamandra* predation on *Triturus* larvae in the presence or absence of artificial vegetation in a second, short-term experiment. The experiment was conducted at the same site and with identical tubs and water depth as the first experiment. The tubs were arranged in a 3 x 4 array placed in the shade of a pine tree (*Pinus halepensis*). The artificial vegetation, randomly assigned to half the tubs, consisted of 50 1-m length black plastic twine sections tied together at their midpoints. On 28 May 2006, we filled pools with tap water. On 30 May, we introduced 5 *Triturus* larvae (snout-tail length, mean and standard error: 23.23 mm, 3.72) and 2 *Salamandra* larvae (snout-tail length, mean and standard error: 61.9 mm, 4.56) into each tub. *Triturus* larvae were sorted by size to get very similar means and ranges for all tubs. The groups of five were then randomly assigned to each of the 12 tubs. We counted the number of *Triturus* larvae surviving for 5 consecutive days.

### **Statistical Analysis**

For all *Triturus* variables, analyses were conducted on mean values for each tub. Data were analyzed by analysis of variance when variables met the parametric test

assumptions. In all other cases, we used Mann-Whitney U tests. Bonferroni-adjusted alpha levels were made when necessary based on numbers of comparisons. In order to further assess a potential effect of vegetation reducing predation rates by *Salamandra* on *Triturus*, we combined data from *Salamandra* treatments of both experiments and tested for the effect of year (block) and artificial vegetation on the proportion of *Triturus* surviving (arcsine-square root transformed) using analysis of variance. Since our a-priori predictions were that *Salamandra* would reduce *Triturus* survival and that survival in the presence of *Salamandra* would be enhanced by vegetation, we used one-tailed tests for survival for these factors. For all other variables and factors, we used two-tailed tests, as there were no specific unidirectional predictions.

## **Results**

### *Field survey*

*Salamandra* and *Triturus* demonstrated strong temporal separation at Secher Pool (Fig. 1a). *Salamandra* larvae were abundant early in the season and disappeared by late April leaving several weeks overlap with *Triturus* larvae. Adult *Triturus* were most abundant in April. During April, when both species co-occurred in the pool, there were large size differences between the larvae of the two species (Fig. 1b).

### *Artificial Pool Experiment 1*

#### Effects on Invertebrates

Two prey species were sufficiently common to assess for treatment effects: the calanoid copepod, *Arctodiaptomus similis*, and the mosquito, *Culiseta longiareolata*. *Salamandra* larvae, while present, virtually eliminated *Arctodiaptomus* ( $U=100$ ;  $p<0.0001$ ; Fig. 2a), *Culiseta* egg rafts ( $U=85$ ,  $p=0.002$ , Fig. 2b), and *Culiseta* larvae ( $U=97.5$ ,

$p=0.0001$ ; Fig. 2c). During the post-*Salamandra* period, numbers of these prey types were considerably lower overall and there were no detectable treatment differences (Mann-Whitney tests:  $p>0.05$  in all cases). During both the *Salamandra* period and post-*Salamandra* period, there were no statistically significant effects of vegetation on any of the prey categories (Mann-Whitney tests:  $p>0.05$  in all cases).

#### Effects on *Triturus* during *Salamandra* period

*Salamandra* strongly and negatively affected *Triturus* survival. While present, *Salamandra* reduced *Triturus* larvae by 90% in the non-vegetation pools and by 70% in the pools containing artificial vegetation (Table 1; Fig. 3a). Because a Mann-Whitney U test cannot test for an interaction term, we also compared for effects of vegetation only in pools with *Salamandra* in a separate Mann-Whitney U test. The lower observed predation rate in the presence of artificial vegetation was not statistically significant (Mann-Whitney  $U=7.5$ ;  $n=10$ ;  $p=0.119$ , one-tailed test).

*Triturus* individuals that survived the *Salamandra* period were significantly larger in the tubs containing *Salamandra* (Table 1;  $p=0.001$ ; Fig 3b), indicating that *Salamandra* predation was greater on smaller individuals. However, the significant vegetation by *Salamandra* interaction indicates that smaller individuals were able to escape predation at a higher rate in the presence of vegetation but not in its absence (Table 1,  $p=0.006$ ; Fig 3b).

#### Overall Effects on *Triturus*

*Salamandra* strongly reduced survival to and time to metamorphosis, and increased size at metamorphosis of *Triturus* (Table 2; Fig 4). Neither vegetation nor vegetation by *Salamandra* interaction had significant effects on any metamorphosis variables (Table 2; Fig 4). The presence of a *Salamandra* by vegetation interaction on *Triturus* size for the early period ending when *Salamandra* was removed, compared with the absence of the

interaction for *Triturus* size at metamorphosis, indicates that the smaller surviving larvae in the vegetated pools caught up in size (Fig. 4b). This result should be treated with caution because the high predation rate by *Salamandra* resulted in few remaining *Triturus* larvae to make this comparison.

#### *Artificial Pool Experiment 2*

As in the first experiment, in the presence of *Salamandra* larvae, the measured increase in *Triturus* survival in the presence of vegetation (from 23.3% to 43.3%) was not statistically significant (Mann-Whitney  $U=10.5$ ;  $n=12$ ;  $p=0.107$ , one-tailed test).

#### *Combined results of Experiments 1 and 2*

To further increase statistical power, we conducted a statistical analysis combining the same two treatments from the 2002 and 2006 experiments into a single analysis. Neither year nor year x *Salamandra* interaction was statistically significant ( $p >> 0.05$  in both cases) but the overall increase in survival in the presence of vegetation was statistically significant ( $F_{1,18}=3.02$ ;  $p=0.049$ , one-tailed test).

### **Discussion**

Our observations in a natural pool reported here and in other observations of other pools (Degani and Kaplan 1999) indicate that where *Salamandra* and *Triturus* co-occur, strong interspecific priority effects should be expected. By the time *Triturus* larvae are in the pool, *Salamandra* larvae are quite large and *Triturus* larvae are vulnerable to being preyed upon by the larger *Salamandra* larvae. Moreover, *Salamandra* will deplete resources that *Triturus* utilize (Blaustein et al. 1996b; Eitam et al. 2005) both before and during *Triturus* is in the pool. The results of the experiment presented here support these

predictions. The strong decline in invertebrate numbers observed during the post-*Salamandra* period in the absence of *Salamandra* is likely due to predation by *Triturus* larvae. In the non-*Salamandra* tubs, *Triturus* larvae had nearly 100% survival and are predators of *Arctodiaptomus similis* and *Culiseta*.

In the outdoor artificial pool experiment, *Salamandra* larvae had a strong negative effect on *Triturus* survival. *Triturus* larvae were almost completely eliminated in the *Salamandra* pools without artificial vegetation but the plastic strips caused a marginally significant increase in *Triturus* larval survival in the presence of *Salamandra*. The increased survival is probably due to a partial refuge from predation afforded by the vegetation as has been shown with other predators and prey in aquatic systems (e.g., Morin 1986; Babbitt and Jordan 1996; Kupferberg 1998).

Although *Triturus* survival was drastically reduced in the presence of *Salamandra*, those that did survive metamorphosed at a larger size. We suggest two reasons for this. One is that the few remaining *Triturus* larvae in the presence of *Salamandra* enjoyed reduced intraspecific competition. Several anurans have previously been shown to be larger in the presence of *Salamandra*, again presumably because of reduced competition (Blaustein et al. 1996a). A second explanation that likely contributes to this is that as a gape-limited predator, *Salamandra* larvae appeared to demonstrate size-specific predation, resulting in higher predation on the smaller *Triturus* and greater survival of the larger *Triturus* larvae.

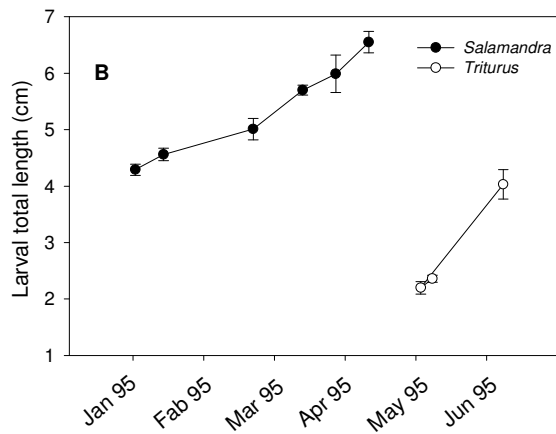
Although *Salamandra* and *Triturus* were in the same pools for only one week, given the quick and strong negative impact *Salamandra* has on larger invertebrates (Blaustein et al. 1996b, Eitam et al. 2005), we predicted that *Salamandra* presence should influence *Triturus* larvae well past the date of the *Salamandra* removal via priority effects. We would expect that *Salamandra*'s reduction of invertebrate prey shared by the two

predators should have contributed to slower growth and perhaps a smaller size at metamorphosis. We found the opposite. Given that *Triturus* metamorphosed at a much larger size in the *Salamandra* pools, we suggest that the reduced intraspecific competition caused by thinning was considerably more important than the negative effect of *Salamandra* in reducing invertebrate prey. We interpret reduced time to metamorphosis in the *Salamandra* pools to both survivors of the *Salamandra* period being bigger plus reduced intraspecific competition due to the thinning.

This study has several conservation implications. First it may supply at least a partial explanation for the apparent negative spatial association observed between the two endangered species, *Triturus vittatus* and *Salamandra salamandra*, in Israel (Degani and Kaplan 1999). Second, it suggests that increased habitat heterogeneity may increase the likelihood of coexistence between the two urodeles. Although the increased survival of *Triturus* in the presence of *Salamandra* when vegetation was present was statistically insignificant, this may be due simply to insufficient statistical power. Further studies may indicate that a higher density of vegetation or different kind of vegetation, e.g., the filamentous algae, may provide a higher degree of refuge for the *Triturus* larvae and support richer and more abundant communities of invertebrate prey resources.

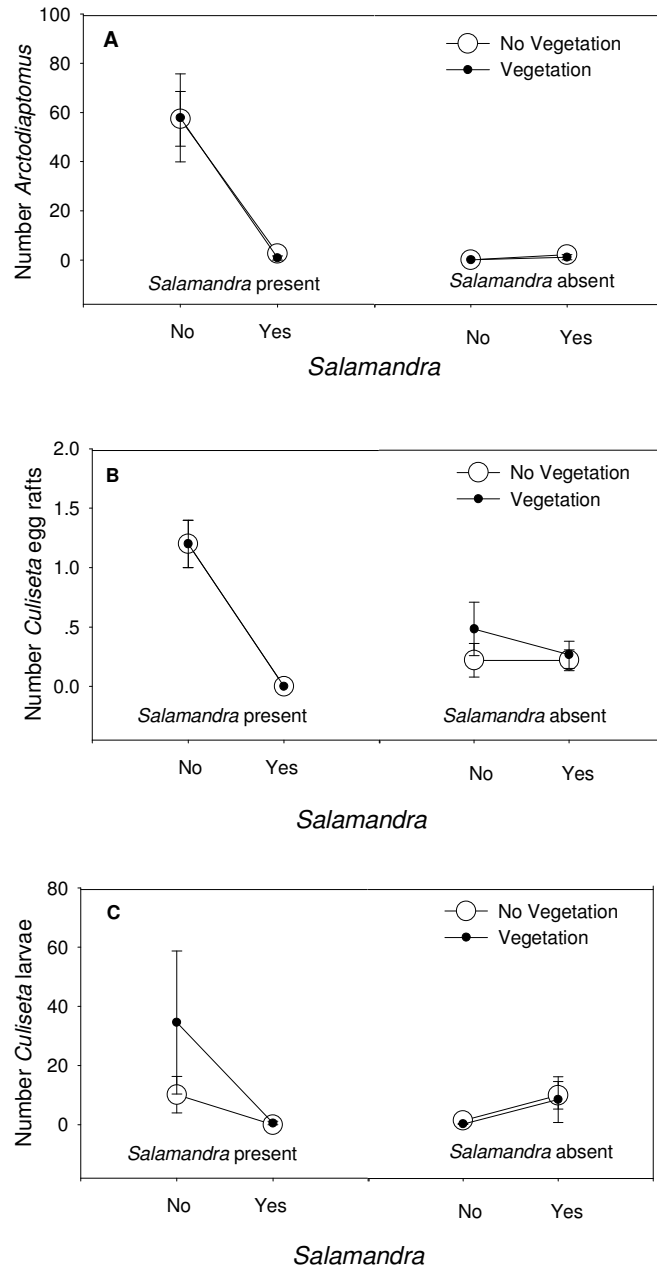
### **Acknowledgements**

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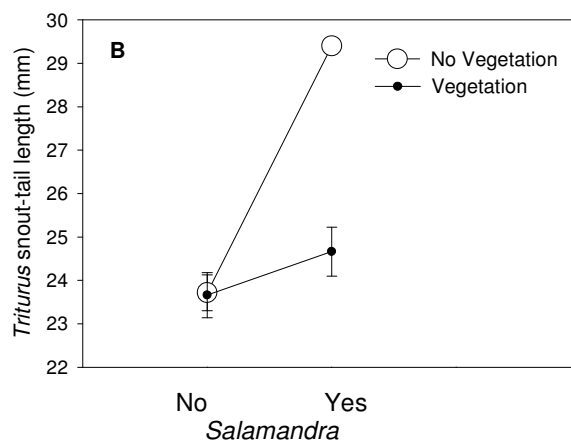
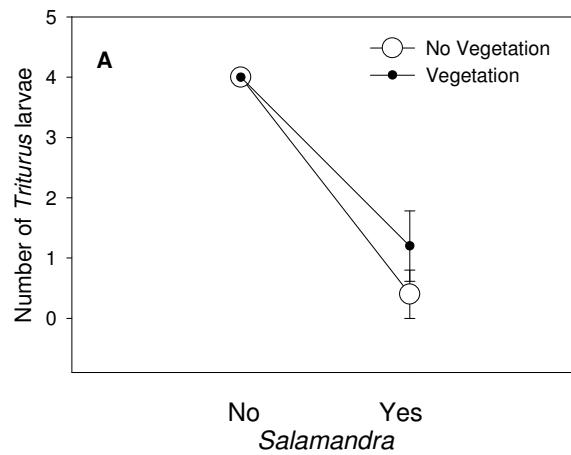


**Fig. 1:** Temporal distributions of *Salamandra salamandra* larvae and *Triturus vittatus* adults and larvae at the temporary pool, Secher Pool: (a) abundance (number caught per ten sweeps); (b) size (total length). Error bars are  $\pm$  one standard error.

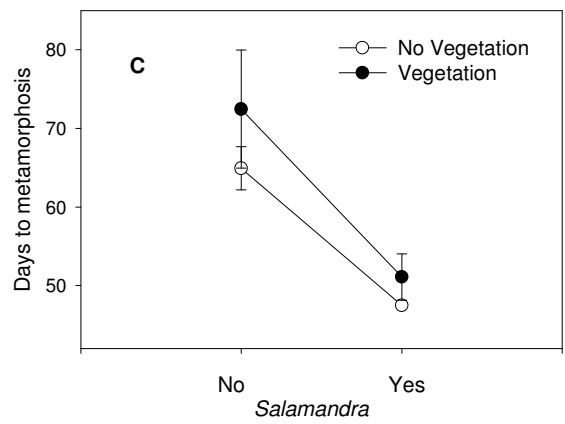
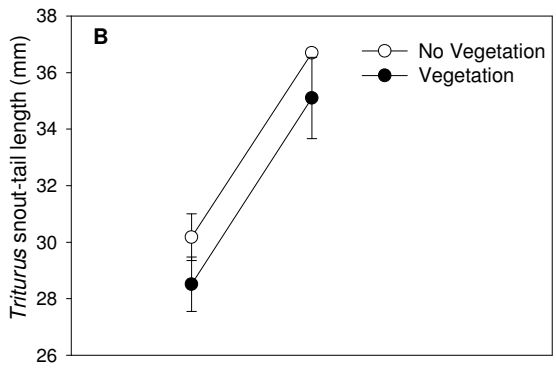
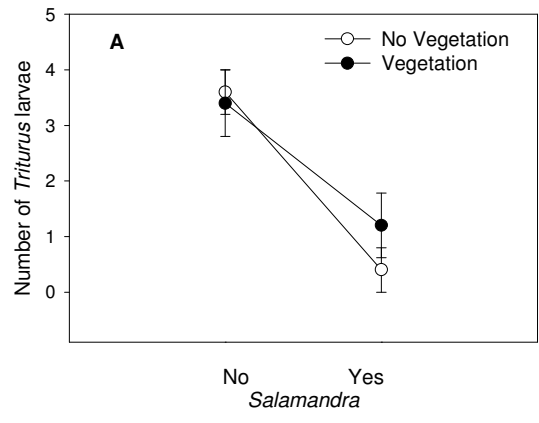




**Fig. 2:** Effects of larval *Salamandra salamandra* and artificial vegetation on the abundance of: (a) *Arctodiaptomus similis* (number per sweep); (b) *Culiseta longiareolata* egg rafts; (c) *Culiseta longiareolata* larvae (number per sweep). The left hand side of the graph is during the period in which *Salamandra* was present in *Salamandra* pools. The right hand side of the graph is the period after *Salamandra* was removed. Error bars are  $\pm$  one standard error.



**Fig. 3:** Effects of larval *Salamandra salamandra* and artificial vegetation on larval *Triturus vittatus* survival (a), and size (total length) (b) five days after the removal of *Salamandra salamandra*. Error bars are  $\pm$  one standard error.



**Fig. 4:** Effects of larval *Salamandra salamandra* and artificial vegetation on *Triturus vittatus* survival to (a), size at (b), and time to (c) metamorphosis. Error bars are  $\pm$  one standard error.

**Table 1.** Analyses assessing the influences of larval *Salamandra salamandra* and artificial vegetation on survival and size (total length ) of *Triturus* larvae measured one week after the removal of *Salamandra* (df=10, 1). Survival is assessed using Mann-Whitney U due to nonhomogenous variances. Length is assessed by analysis of variance. N equals number of pools. There are fewer pools for length because of zero survival in some pools. P-values are based on two-tailed tests.

<b>Source of variation</b>	<b>Survival (N=20)</b>		<b>Length (N=14)</b>	
	U	P	F	P
<i>Salamandra</i>	100	<0.0001	23.97	0.001
Vegetation	45	0.682	12.33	0.006
<i>Salamandra</i> *Vegetation	-	-	11.77	0.006

**Table 2.** Mann-Whitney U for measures of *Triturus vitattus* at metamorphosis: survival, size and development time. N gives number of pools. There are fewer pools for size and time to metamorphosis because of zero survival in some pools. P-values are two tailed.

Source of variation	Survival to (N=20)		Size at (N=14)		Time to (N=14)	
	U	P	U	P	U	P
<i>Salamandra</i>	97.5	<0.0001	0.0	0.005	39	0.007
Vegetation	43	0.578	25	0.897	23	0.897

## General Discussion

The four chapters in this work are particularly important in the backdrop of the suspected human-caused global environmental effects of climate change on global amphibian decline (McMenamin et al. 2008). The study of species at the edge of their global distribution may be particularly constructive for understanding species-environment interactions (Caughley et al. 1988; Lawton 1993; Gaston 2009) and in the case of amphibians, can provide insights regarding the effects of hydrologic changes on species abundance and distribution. As *Salamandra* populations in Israel are at the genus' southern-most, and most xeric habitats, changes in temperatures and precipitation can thus have particularly large impacts on spatial and temporal activity patterns, distribution and abundance.

Documenting population declines, which may lead to local or complete species extinctions, are central for conservation research and require knowledge of population size. Estimates of population size are imperative part of numerous conservation programs. However, reliable estimates are rare for *Salamandra* species (Miaud et al. 2001; Rebelo and Leclaire 2003; Schmidt et al. 2005; Schmidt et al. 2007) and particularly for *Salamandra infraimmaculata*, defined globally as “near threatened” (Papenfuss et al. 2008) and locally as “endangered” (Dolev and Perevolotzky 2004). Chapter 1 demonstrates that *S. infraimmaculata* population size varies greatly among sites, ranging from hundreds to tens. Moreover, despite relatively small number of sites censused, I provide strong evidence for a positive relationship between breeding site permanence and population size, as permanent breeding sites support larger adult populations than the temporary ones. Permanence of breeding water bodies may drive population dynamics at both pre-metamorphic larval stages and post metamorphic terrestrial stages and can thus strongly affect both survival and reproductive fitness. Breeders at permanent sites can start the

breeding season earlier since they do not depend on pool inundation as temporary sites breeders. Additionally, larvae developing at permanent sites are free from desiccation risks and may prolong their development (larval period) and consequently metamorphose at a larger size. Size at metamorphosis has been shown to be positively correlated to juvenile survival (Smith 1987; Semlitsch et al. 1988).

Quality and quantity of terrestrial habitat neighbouring the breeding site should also be important for supporting amphibian populations (Semlitsch 1998; Porej et al. 2004; Regosin et al. 2005). Counter-intuitively, I found that urban cover, generally expected to be negatively correlated with amphibian population size, was *positively* associated with *Salamandra* populations. This undoubtedly is confounded with pond permanence; historically, due to the paucity of year-round water sources, human settlements were established close to permanent springs while no settlements exist close to any of the temporary breeding sites. I suggest then that relatively larger *Salamandra* populations exist at the permanent sites not because of, but despite, the proximity to human settlements.

The relative contribution of different life cycle stages (i.e., aquatic egg and larval stages versus terrestrial sub-adult and adult stages) to amphibian population dynamics and demography is controversial (Vonesh and De la Cruz 2002; Biek et al. 2002). When weighing recruitment against adult survival, populations breeding in permanent sites may experience lower variation in larval survival and stable recruitment. However, populations from permanent breeding sites are prone to threats that do not exist in temporary sites such as introduced invasive fishes. Invasive fishes in general, and mosquitofish in particular, by acting as predators and competitors, may negatively affect the native aquatic communities in permanent water bodies including endangered amphibian species (Kats and Ferrer 2003; Rogowski and Stockwell 2006). Although studies of *Gambusia* frequently demonstrate its negative effect on local native fauna, the effects also prove to be species-specific and may

vary considerably between species (Lawler et al. 1999; Komak and Crossland 2000; Walls et al. 2002; Ling 2004). In chapter 2, I present evidence for strong detrimental effects by *Gambusia affinis* on fire salamander larval performance and number of emerging metamorphs by comparing the same natural *Salamandra* breeding pool before and after mosquitofish introduction. A comparison of *Salamandra* larvae in natural pools simultaneously ranging in *Gambusia* densities also suggested that *Gambusia* negatively affects *Salamandra* by causing damage to its appendages. In a replicated outdoor mesocosm experiment designed to test the effects of *Gambusia* on *Salamandra* larval stages, I found that mosquitofish strongly reduced larval wet weight and survival, and increased body damage in the form of a reduced larval tail:body ratio and partially missing gills and limbs. *Gambusia*, being a gape-limited predator, inflicts multiple injuries through tail, limb and gill bites (Richards and Bull 1990; Komak and Crossland 2000). This damage likely eventually caused mortality in all or almost all larvae in all mosquitofish pools before any could complete metamorphosis. Repeated nonlethal injuries can reduce the surviving larvae fitness or accumulate to cause mortality (Harris 1989). Predator-inflicted tail damage has been studied primarily in anuran tadpoles (Wilbur and Semlitsch 1990; Blair and Wassersug 2000) and has been shown to affect tadpole development and swimming performance (Parichy and Kaplan 1992; Figiel and Semlitsch 1991; Van Buskirk and McCollum 2000).

The introduction of *Gambusia* to specific *Salamandra*-breeding sites may not just threaten the salamander population locally. A recent study found that *S. inframaculata* is capable of long-term dispersal between breeding pools (Bar-David et al. 2007). The absence or reduction in number of recruits from *Gambusia*-stocked pools to other breeding sites may endanger the long-term persistence of other small subpopulations that are structured as metapopulation (Marsh and Trenham 2001). One of the consequences of this



study was a management policy change by the Israeli Nature and Parks Authority to forbid further introductions of *Gambusia* into *Salamandra* breeding sites and actively remove fish from these sites.

Habitat selection incorporates evolutionary implications as well as consequences for population and community ecology (Holt and Barfield 2008). Behavioral studies are an imperative tool for understanding the complex relationships between proximate mechanisms involved in habitat selection and the selective pressures that may drive their evolution (Stamps 2001). The dynamic state-dependent model presented in Chapter 3, suggest that spreading of larvae in time and space by mothers as an optimal resolution of the trade-off between current and future reproduction. In the experiment designed specifically to test the model predictions, females showed an ability to assess pool depths/volumes by depositing more larvae in the deeper, higher volume pools, but still used multiple pools, including inferior quality (shallow) ones, and spread their larvae among them. Moreover, the experiment confirms that gravid *Salamandra* are physiologically capable of delaying deposition long enough to utilize temporally distinct rain events i.e., for temporal intraclutch spread. Identifying the mechanism involved in pool assessment was not tested here, however it may include tactile cues received by the female prior to or during larviposition. The evaluation of pools' water loss rates by changes in their water levels over repeated visits was previously suggested for two anuran species,(Spieler and Linsenmair 1997; Rudolf and Rodel 2005). Although not addressed in the current study, such dynamic pool quality assessment may be practiced by *Salamandra* females to gauge water-holding capacity, rate of water loss and hydroperiod. Evidence of mark-recapture study (see chapter 1) revealed high within-season recapture rate in breeding pools vicinity that may be attributed to multiple pool sampling.

Greater accuracy in assessment of breeding patch qualities is favoured by natural selection when costly penalties exist for its over- and under-estimation (Godfray 1994). In breeding habitat selection, a large body of literature was devoted to the effects of biotic factors, mainly the presence of predators and competitors (see reviews in Edgerly et al. 1998; Blaustein 1999; and Skelly 2001), while relatively few studies examined whether species breeding in temporary waters can assess water-holding capacities (Crump 1991; Spieler and Linsenmair 1997; Rudolf and Rodel 2005; Arav and Blaustein 2006; Brown et al. 2008; May et al. 2009). Temporary pools are highly unpredictable in hydroperiod making risk spreading a major force promoting resource allocation in such environments (Roitberg et al. 1999). Spreading of offspring across time or space is frequently interpreted as a "bet-hedging" strategy, sometimes when its cause is density dependence avoidance (Hopper 1999). The suggested model presented in chapter 3 does not involve the multi-generational scale of environmental variability that is required to make predictions regarding bet hedging strategies. However, bet-hedging may be an important factor influencing *Salamandra* larviposition distribution in time and space, since many pools in a temporary pool system are prone to unpredictably early desiccation followed by catastrophic death of their entire larval populations (Smith 1983; Semlitsch 1987; Wilbur 1987; Banks and Beebee 1988; Newman 1988; Murphy 2003). In such circumstances, it might be beneficial for female *Salamandra* to only partially exploit an encountered pool to reduce variance in fitness should that pool be subject to an unpredictable, disastrous event. Dynamic life history theory may be suitable to distinguish between the contributions of risk spreading and of density dependence avoidance on offspring distribution behaviors, but a complete analysis of bet hedging in a dynamic life history framework is still lacking (Roitberg et al 1999).

Interspecific interactions and species composition in temporary pond communities may reflect species differences in breeding phenology and within-season variation in breeding timing. Priority effects may partially explain negative spatial association between species and may have particular conservation applications when these species are considered endangered. My field observations suggest that in breeding ponds where *Salamandra* and *Triturus* co-occur, strong interspecific priority effects should be expected. *Salamandra* ovoviviparous reproduction mode and early breeding ponds colonization at the start of the rainy season result in size discrepancy between the aquatic stages of the two urodeles and consequently strong predation pressure on *Triturus* larvae. Early pond breeding species can affect late breeding species indirectly even when there is no temporal overlap in pool occupancy between them. In the case of *Salamandra* and *Triturus*, two generalist predators, the two species diet overlap enabling *Salamandra* to deplete food resources in the pond even before *Triturus* lay their eggs (Blaustein et al. 1996b; Eitam et al. 2005). In my artificial pool experiment *Salamandra* significantly reduced *Triturus* survival while the artificial vegetation caused marginally significant increase in *Triturus* survival probably by providing only partial refuge. Contrary to my prediction that after the removal of *Salamandra* the remaining *Triturus* will metamorphose at slower rate and smaller size, the surviving *Triturus* metamorphosed faster and at larger size. This is probably the result of reduced intraspecific competition caused by strong thinning effect and size-dependent predation by the gape-limited *Salamandra*. These combined effects were considerably more important than the negative effect of *Salamandra* in reducing shared invertebrate prey items.

This work was intended to provide novel information regarding the ecology, biology and life history of a locally endangered amphibian species at several ecological scales and to relate this information to applied real-world problems in conservation and

management. The nature and intensity of threats and stressors for pond breeding amphibians can change considerably with breeding site permanence. Capture-recapture population size estimates confirmed that permanent breeding sites support larger adult populations than temporary ones. However, unlike populations breeding in temporary sites, these may be negatively affected by invasive fishes, as this study demonstrated in the case of the highly invasive mosquitofish *Gambusia affinis*. An additional risk factor for permanent breeding sites identified in this study entails the developmental pressures and expansion of human settlements historically built in the vicinity of permanent springs. Although permanent sites can apparently support larger populations, temporary waters are much more abundant in this work geographic scale that encompass *Salamandra* southern and xeric-most range of distribution. In disturbed fragmented ecosystems larviposition habitat selection can potentially affect *Salamandra* population dynamics and distribution through processes of colonization and recruitment. To cope with the unpredictability inherent within temporary pool system female *Salamandra* larviposition strategy consist of spreading larval load through time-between rain events, and space-across pools, and adjusting reproduction output, in the form of clutch size, according to pool volume. The conservation implication of this breeding strategy highlights the need for reevaluating ecologically relevant spatial scale of *Salamandra* breeding habitat, suggesting reserving or creating multiple adjacent temporary pools within the breeding female dispersal range. Temporal aspects of breeding phenology can potentially affect focal species' distribution and abundance but may also impact entire community structure through larval interactions with other pond breeding species. Priority effects suggested as the mechanism driving temporal separation in ponds were *Salamandra* and *Triturus*, co-occurs and may explain negative spatial association in their overall distribution. These interspecies interactions may be particularly important to the design and application of management measures that

incorporate relocations and introductions, and in defining spatial scales to protect these two endangered urodeles.

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# הסלמנדרה המצויה (*Salamandra inframaculata*) אקולוגיה

## ושמירת טבע

### אורי שגב

#### תקציר

קריסת פאונת הדו-חיים המתרחשת בשלושת העשורים האחרונים הינה תופעה כלל עולמית הכוללת היכחדות מינים, ירידה בגודל אוכלוסיות וצמצום אזורי תפוצה. נראה כי מספר גורמים הפועלים לעיתים בסנירגיזם אחראים לתהליכים המתרחשים באופן מדאיג הן באזורים שמורים והן באזורים מופרעים. למרות היותה מוגדרת גלובלית ומקומית כמין בסיכון, היעדר מידע או קיומו של מידע חלקי בלבד לגבי גודל ופנולוגיה אוכלוסיות הסלמנדרה המצויה (*Salamandra inframaculata*) בישראל כמו גם לגבי יחסי גומלין עם מינים אחרים וגורמים המשפיעים על בחירת בית גידול ואתרי רבייה, מהווים גורם מגביל לפיתוח ויישום של פעולות ממשק שמירה למין. גופי המים המשמשים כאתרי רבייה לסלמנדרה כמו גם בתי הגידול היבשתיים הסובבים אותם מצויים תחת מגוון איומים אינטנסיביים ההולכים ומתרבים וכוללים בין היתר זיהום, התייבשות, התפשטות מינים פולשים והרס וקיטוע בתי גידול. כמין מפתח פגיעה בסלמנדרה יכולה להשפיע לא רק על מין בודד אלא ברמת החברה האקולוגית כולה. מעבודה זו עולות המסקנות הבאות:

- 1) גודל אוכלוסיה הינו מדד חיוני להערכת מצבם של מינים בסיכון ולהשוואת כושר נשיאה של בתי גידול שונים. לכידות חוזרות באתרי רבייה של הסלמנדרה בכרמל ובגליל התחתון חשפו אוכלוסיות בוגרים גדולות מהערכות קודמות, וכן השפעה של הידרולוגיה אתר הרבייה. אוכלוסיות שנדגמו סביב מקווי מים קבועים המחזיקים מים גם במהלך הקיץ, היו גדולות מאלו שנדגמו סביב מקווי מים זמניים, ללא קשר לנפח מקווי המים. תוצאות אלו מעידות כי לפגיעה

במקווי מים קבועים עלולות להיות השלכות חמורות על מצב האוכלוסיות המתרבות בהם וכי יש לתת עדיפות לריכוז מאמצי שימור ו/או שחזור של בתי גידול אלו.

(2) אחד הגורמים המאיימים על הפאונה האקוטית דווקא במערכות מקווי מים קבועים הוא חדירה והתפשטות של מיני דגים פולשים. בישראל פוזרו דגי גמבוזיה (*Gambusia affinis*) במכוון לצמצום אוכלוסיות יתושים במגוון גופי מים שחלקם משמשים כאתרי רבייה גם לסלמנדרה המצויה. סקרי שדה באתרי רביית סלמנדרה לפני ואחרי התבססות אוכלוסיית דגי גמבוזיה ובצפיפויות דגים שונות חשפו עדויות נסיבתיות להשפעה שלילית של דגי הגמבוזיה. בניסוי המשך שנערך בבריכות מלאכותיות נמצאה עליה בתמותת ראשני סלמנדרה וירידה דרסטית במספר הראשנים שהצליחו לעבור מטמורפוזה ולעזוב את בריכת הרבייה גם בנוכחות צמחיה מלאכותית. מנתונים אלו עולה צורך בהגבלת התפשטותם של מינים פולשים במערכות אקוטיות ובמקרה של דגי הגמבוזיה בהוצאתם הפעילה ממקווי מים המשמשים אתרי רבייה לסלמנדרה.

(3) הבנת תהליכי בחירת בית גידול בסקאלות אקולוגיות שונות מהווה שלב הכרחי בשמירה, שיחזור וממשק של משאבי טבע. לבחירת אתר הרבייה יכולות להיות השלכות על שרידות וביצועי הצאצאים ומכאן גם על כשירות ההורים. מודל דינאמי תלוי-מצב וניסויי בחירת אתר השרצה במכלאות חשפו כי נקבות סלמנדרה מפזרות ראשנים במהלך עונת הרבייה בזמן - בין אירועי גשם, במרחב - בין בריכות, ומתאימות את מספר הראשנים המושרצים לעומק המים בבריכה בכדי להביא לאופטימום את ההצלחה הרבייתית בסביבת מקווי מים זמניים. לתוצאות אלו יכולות להיות השלכות תיאורטיות ומעשיות כאחד; תרומה תיאורטית לתיאוריית בחירת אתר רבייה דרך הסברת המשמעות האדפטיבית של שונות במספר הראשנים שמשריצה הנקבה לבריכה, ומעשית מאחר והבנת התנהגות השרצה של הסלמנדרה יכולה להתברר כחיונית לחיזוי ההשלכות של ההתדרדרות באיכות בתי גידול אקוטיים.

(4) לפנולוגיה ולתזמון הרבייה בסקלה העונתית חלק משמעותי ביחסי גומלין בין-מיניים ובעיצוב מבנה החברה בייחוד בבריכות זמניות בהן פרק הזמן העומד בפני השלבים האקוטיים במחזור החיים עד למטמורפוזה הינו מוגבל. אפקטים של קדימות (priority effects) יכולים להסביר באופן חלקי דגמי פיזור מרחביים שליליים בין מינים וכאשר מינים אלו מוגדרים כמינים בסיכון

גם להיות בעלי השלכות יישומיות לשמירת טבע. מערך ניסוי בבריכות מלאכותיות וסקרי שדה תוכננו להעריך את ההשפעה של ראשני הסלמנדרה המצויה על מין דו-חי אחר המוגדר כמין בסיכון, טריטון הפסים (*Triturus vittatus*). מחקרים אלו חשפו כי לסלמנדרה המתרבה מוקדם בעונה השפעה שלילית על הטריטון המתרבה מאוחר וכי עליה בהטרוגניות בית הגידול יכולה להגדיל את ההסתברות להימצאותם של שני המינים יחד.

ניטור מצבן הנוכחי של אוכלוסיות הסלמנדרה, הערכת איומים קיימים ועתידיים על בתי הגידול והבנת תהליכים ומנגנונים העומדים בבסיס אסטרטגיות בחירת בית גידול מהווים שלב חיוני בפיתוח ויישום תוכניות ממשק ושמירה למין זה ולמיני דו-חיים אחרים.

**הסלמנדרה המצויה (*Salamandra infraimmaculata*)**

**אקולוגיה ושמירת טבע**

**מאת: אורי שגב**

**בהדרכת: פרופסור לאון בלאושטיין**

**חיבור לשם קבלת התואר "דוקטור לפילוסופיה"**

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