Detection of non-consumptive effects of predation and intraspecific aggression in fire salamander larvae: environmental issues

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Abstract. The identification of injury causes may reveal important insights on the factors that influence intra and interspecific predation pressures. In this study we evaluated the proportion of injuries occurring in fire salamander larvae (*Salamandra salamandra*) in caves and epigean springs to understand the factors determining non-consumptive effects that may affect larval survival. We surveyed 25 sites (13 cave pools, 12 spring pools) during the day. We applied two consecutive removal samplings to collect fire salamander larvae and examined every removed larva to detect if it had injuries and we evaluated the proportion of the injured larvae on the total number of collected larvae. For each site we recorded different environmental variables including predator occurrence and prey density. The proportion of injured larvae was significantly linked only to predators occurrence. Our results show that interspecific predation pressure is a major source of non-consumptive effects that may affect larval survival Overall, our study underlines the role that environmental features may play on the non-consumptive effects of selective pressures that affect larval survival. Predator occurrence determines the levels of non-consumptive effects and larval survival across their developmental cycle. These results deriving from a field survey could furnish useful insights for further experimental studies.

Key words: predation, non-consumptive effects, cannibalism, cave, spring, subterranean, trophic, invertebrates.

Introduction

Amphibian population decline is a world-wide problem (Beebee & Griffiths 2005) and amphibians are among the most vulnerable vertebrates of the world with several threats to them acting at a global level (Ficetola et al. 2015). There are two main factors affecting amphibian population density and survival that, although obvious, are important to be underlined: death (or removal) of the individuals and recruitment failure.

As far as natural mortality factors, there are several cases that can affect the health of local amphibian populations. First, a major role may be played by an increase in the rate of disease spread with a higher number of individuals dying before breeding (Rollins-Smith & Conlon 2005, Rudolf & Antonovics 2007, Gonzalez-Hernandez et al. 2010). Another factor is the availability of food; climate change or other elements determining a decrease in the density of prey available may increase the deaths of juveniles and adults (Kusano 1981, Vaissi & Sharifi 2016). Moreover, some populations can exploit food deprived habitats like deserts or caves in which starvation may be a common limiting factor (Manenti et al. 2009a). Breeding sites may also be subject to high disturbance phenomena like desiccation during dry seasons or flooding during wet periods, determining mortality of larvae and mating adults (Blaustein et al. 2001, Tockner et al. 2006). Finally the most common pressure affecting amphibian survival in natural environments is predation (Wizen & Gasith 2011, Drake et al. 2014, Wesner et al. 2015).

Predators may affect prey through consumption, but also through non-consumptive effects (NCEs) altering the morphology, life histories and behaviour of prey (Preisser & Bolnick 2008, Winandy & Denoël 2013), with major impacts on prey population dynamics (Davenport & Chalcraft 2013). For instance, semi-aquatic organisms with complex life cycles like amphibians are able to assess the predation risk in bodies of water and modulate breeding activity, by selecting habitats with fewer predators (Winandy et al. 2015). The ef-

fects of these factors on the mortality rate may be slightly different depending on life stage. Eggs and larvae are generally considered the stages in which mortality is higher (Anderson et al. 1971).

In amphibian systems it is well documented that larval survivor is density-dependent: high conspecific densities may enhance competition and aggressive interactions reducing the larvae survival rates (Kishida et al. 2011, Manenti et al. 2015). At the same time, reduced larval densities, linked for example to higher egg mortality or reduced female fecundity, may yield the same number of metamorphosed individuals if competition is lower and larvae survival higher (Vonesh & De la Cruz 2002). Widespread and common amphibian species would be particularly suitable for assessing non consumptive effects of predation and intraspecifc aggressiveness and may reveal processes that may also affect endangered species. One example is the fire salamander *S. salamandra,* a widespread species in Europe that is extensively studied in its use of different breeding sites. *S. salamandra* generally breeds in small lotic environments (Manenti et al. 2009b), but can use also a wide variety of habitats like ponds (Steinfartz et al. 2007, Denoël & Winandy 2014) and hypogean (i.e. subterranean) springs or pools (Manenti et al. 2009a, Manenti et al. 2011) and artificial tunnels (Covaciu-Marcov et al. 2017). Subterranean environments can provide advantages such as stable environmental conditions and limited predation risk. On the other hand, in these habitats salamanders face major challenges such as lack of light and food scarcity. Subterranean populations of salamanders have therefore developed local adaptations including a higher behavioural plasticity and a better ability to capture prey in complete darkness, compared to conspecific populations from epigeous streams (Manenti & Ficetola 2013).

With this study we recorded the proportion of injured larvae within food and predator deprived cave pools and predator and prey rich epigean pools to assess the pressure played by predation and cannibalism.

Several studies have shown the behavioural responses linked to non-consumptive effects of predation risk (Amo et al. 2003), while few have used the non-consumptive effect to assess the ecological determinants of them. Recently (Munshaw et al. 2014) underlined how recording data on the frequencies of injuries (such as bitten tails or missing limbs) may be a useful non-manipulative approach to identify the levels of conspecific aggression in stream-dwelling salamanders. With this study we applied the same methodology to identify correlates of natural injuries in predator rich and interspecific predator deprived environments such as spring and cave pools. In caves, where larval development is generally low (Manenti et al. 2011, Limongi et al. 2015), it is likely that old larvae from former cohorts prey upon newly laid larvae; we hypothesise that injuries reflect intraspecific aggressiveness levels.

Materials and Methods

Field surveys

We studied fire salamander populations from Italian Prealps in Lombardy (NW Italy; around 45°48'N, 9°02'E). In this area, *S. salamandra* usually deposit larvae in streams, but also uses for breeding a number of caves with small pools or streams close to the cave entrance (Manenti et al. 2016). All the fire salamander larvae are fully aquatic and have external gills. In the studied subterranean sites, adult females actively enter caves and select them as breeding sites; no larvae could have drifted into these environments from surface waters, because all these selected sites are emitting caves that receive water only from the subterranean aquifer (Manenti et al. 2009a, Manenti & Ficetola 2013). In these underground habitats, salamander larvae are able to successfully develop and reach metamorphosis (Manenti et al. 2011).

To assess the proportion of injured larvae we surveyed 25 sites (13 cave pools, 12 spring pools). Every site was independent from each other (average distance between the sampling sites: 9.25 Km). Every site was surveyed once or in April 2013 or in April 2014. We chose pools relatively isolated, fed only by the subterranean aquifer with very limited risk of overflow and drift for the larvae and with relatively limited surface area in which the detectability of fire salamander larvae was likely enhanced. Surveys were performed using two successive removal samplings with a fine mesh. The removal method is particularly useful and effective for salamander larvae collection (Schmidt et al. 2015). During each removal sampling all possible shelters, constituted by stones, mud substrate, leafs and rock crevices, were attentively investigated.

We stored every removed larva in a tank for at maximum 30 minutes and we attentively examined it to detect if it had injuries and recorded the proportion of injured larvae on the total number of larvae sampled. We considered as injured the larvae that showed tail bites and limbs missing. We considered larvae with multiple injuries only once.

For environmental variables we estimated: a) occurrence of predators by deep-netting the entire pool surface after having estimated b) prey density through pipe sampling (diameter: 25 cm) (Dodd 2010); see (Limongi et al. 2015) for additional details on invertebrate samplings. Furthermore, we distinguished between c) cave and epigean springs, and we considered the d) fire salamander larvae density. The latter was obtained by applying the removal method analysis proposed by (Chao & Chang 1999) to estimate the total number of larvae occurring in the pool on the basis of the two successive removal samplings performed (Schmidt et al. 2015).The estimated abundance for each site was the divided for the pool area in order to obtain fire salamander density per square meter.

Data analysis

We used linear models (LMs) to assess the factors determining the proportion of injured larvae. For the analysis, the site typology (hypogean / epigean), fire salamander larvae abundance, predator occurrence and prey density were considered as independent variables. If needed, independent variables were transformed using logarithm (larvae abundance, prey and predator biomass) to reduce skewness and improve normality. Prior to the analysis, we used variance inflation factors (VIF) to identify collinearity among explanatory variables. We calculated the VIF values for all explanatory variables; as the maximum value was 2.6 for the site typology variable, we could be confident that collinearity levels were weak and we kept all the variables (Zuur et al. 2010). We assessed significance of variables using a likelihood ratio test (Bolker et al. 2008). We performed all the analyses in R 3.31 environment using the, nlme, HH and car packages.

Results

In caves, predators were generally absent except for one site in which the native freshwater crayfish *Austropotamobius pallipes* occurred and one site in which we detected dragonfly larvae of the species *Cordulegaster bidentata* at the entrance. In epigean sites we detected predators in 36 % of the weekly surveyed pools. Predators were comprised of dragonfly larvae of the genera *Cordulegaster* and *Aeshna* (Table 1). On average the number of detected prey was slightly higher in epigean sites (average ± standard error = 44.2 n/m^2 ± 19.8 n/m^2) than in caves (average ± standard error = 5.8 n/m^2 ± 2.1 n/m^2); also the density of larvae varied between the two site typologies (Table 1). In caves we detected an average ± standard error of 12 n/m^2± 2.6 n/m^2 larvae, while in epigean sites an average ± standard error of 37.2 n/m^2± 11. n/m^2 larvae. Considering the number of injured larvae, the differences were not evident as we detected in epigean sites on average ± standard error = 6.8 ± 2. 3 injured larvae and in caves an average ± standard error of 4 ± 1 larvae. Considering the proportion of injured larvae, however, on average ± standard error we detected 0.24 ± 0.06 larvae in caves and 0.36 ± 0.09 larvae in epigean sites.

The LMs analysis revealed that the only factor that significantly affected the number of inured larvae was the predator occurrence (Table 2). 2

Discussion

In this study we used the rate of injuries occurring in fire salamander larvae, also to understand the factors that may determine non-consumptive damages and reflect the long term pressures acting on larvae survival. Our results can help to provide further insights on the roles played by habitat features on salamander larvae survival. Considering the rate of injuries occurring in aquatic salamander populations may be a helpful way to understand pressures acting on a populations survival (Munshaw et al. 2014).

The rates of injured larvae were significantly higher where predators occur, demonstrating that predation pressure is detectable and at least partially quantifiable by considering its non-consumptive effects. Non-consumptive effects may be as important as consumptive effects in deter-

Table 1. Features of the spring and cave pools surveyed. The number and the proportion of fire salamander larvae collected in the study sites are reported together with the predators detected and the density of prey recorded.

Site	Typology	N injured	Proportion injured	Density of larvae (n/m²)	Prey density (n/m²)	Predators
G11	Hypogean	12	0.55	27.55	10	*Austropotamobius pallipes*
G16	Hypogean	3	0.19	18.37	3	
G19	Hypogean	2	0.17	30.00	0	
G3	Hypogean	2	0.20	3.37	0	
G171	Hypogean	4	0.14	5.69	20	
G172	Hypogean	1	0.17	1.03	0	
G173	Hypogean	0	0.00	3.75	0	
G9	Hypogean	9	0.75	8.89	8	
G7	Hypogean	0	0.00	17.50	0	
G24	Hypogean	8	0.44	6.94	1	.
G1	Hypogean	0	0.00	10.99	25	
G22	Hypogean	2	0.11	2.05	6	
G2	Hypogean	9	0.47	21.11	3.5	
Campsirago pool	Epigean	2	0.40	31.25	2	*Cordulegaster* sp. *Aeshna* sp.
Colle brianza spring	Epigean	16	0.36	42.42	8	*Aeshna* sp.
Monte Barro high	Epigean	30	0.34	63.91	4	*Cordulegaster* sp. *Aeshna* sp.
Civate	Epigean	1	0.06	31.06	1.5	*Aeshna* sp.
Lecco 1	Epigean	2	0.15	8.66	10	
Lecco 2	Epigean	1	0.09	10.89	8	
P01	Epigean	4	1.00	1.43	26	*Cordulegaster* sp.
P03	Epigean	2	1.00	0.77	11	*Cordulegaster* sp
Valzurio A	Epigean	7	0.54	32.65	102	*Cordulegaster* sp.
Valzurio B	Epigean	3	0.06	5.15	65	
Valzurio C	Epigean	9	0.20	81.25	43	
Valzurio D	Epigean	5	0.13	137	250	

Table 2. Results of the Linear Models analysis of the relationship between the proportion of injured fire salamander larvae and the environmental features of spring and cave pools.

Variable	NumDf	DenDf	*B*	F	P
Cave	1	20	0.06	1.67	0.2
Conspecifics density	1	20	-0.04	1.95	0.1
Prey density	1	20	0.02	0.92	0.3
Predators occurrence	1	20	0.42	16.03	**< 0.001**

mining prey populations dynamics (Matassa & Trussell 2011). For instance, when prey detect a predation risk, in most cases they shift their foraging activities, both in spatial and temporal terms, from risky conditions to safer ones (Matassa & Trussell 2011). In this case, non-consumptive effects may be strong determinants affecting the distribution and abundance of trophic resources, constituted by prey over time and space (Matassa & Trussell 2011). Our results indicate that predation pressure strongly acts also at the level of fire salamander larvae and it is likely to affect their behaviour and habitat choice. Recent studies showed that in predator free habitats the rate of active fire salamander larvae is higher than in habitats where predators occur (Manenti et al. 2016). Moreover, predator occurrence may drive breeding site choice by females (Manenti et al. 2016). Several cases of the role played by predators on salamander larvae survival are reported, but field data on no-consumptive effects generally lack.

No other effects were recorded among the variables considered. In particular, the abundance of invertebrate prey, was not related to a lower level of injured fire salamander

larvae as it could have been hypothesised. This lack of effect underlines that the occurrence of other potential targets for the predators, like dragonfly larvae, does not dilute their pressure on fire salamander larvae.

Intraspecific predation may also play a relevant role in affecting non-consumptive effects. High levels of cannibalism have been reported both in Hynobidae and in Ambystomatidae; when conspecific density is high, cannibalistic interactions are likely to occur between different cohorts born or laid in different periods affecting the survival rate of the smaller larvae (Kishida et al. 2009). In our case no effect of conspecific density was observed, however no significant differences were also recorded among the different habitat (cave and spring pools) considered. Cave environments have very different ecological features from nearby epigean habitats because of the lack of light and of the scarcity of trophic resources (Romero 2011), thus subterranean food webs are often simple, with very few predators. Our surveys confirm that fire salamander larvae were in most of the cases the top aquatic predator occurring in cave pools. This fact suggests that most of the proportion of injured larvae recorded in caves should reflect intraspecific aggressiveness.

Overall, our study underlines the role that environmental features may play on the non-consumptive effects of selective pressures that affect larval survival. Predator occurrence mediates the levels of non-consumptive effects and larval survival across their developmental cycle. These results could furnish useful insights for experimental studies dealing with disentangling of the factors allowing the colonisation of different/novel habitats.

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