1 **Safe as a cave? Intraspecific aggressiveness rises in predator-devoid and resource-depleted**

2 **environments**

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Abstract

Intraspecific aggressiveness can be affected by multiple environmental pressures. In several cases aggressiveness can grade into full-scale cannibalism, particularly when resources are scarce. However, limited information exists on how intraspecific aggressiveness varies among populations experiencing different environmental pressures, and on the role intraspecific predation plays for the exploitation of harsh habitats. The fire salamander, *Salamandra salamandra*, is an excellent model species to study factors affecting intraspecific aggressiveness, because of its ability to breed in habitats with contrasting food resources and predation pressure. Here, we evaluated the influence of predation risk and habitat of origin on aggressive interactions. To this extent, we reared larvae from cave (scarce resources; nearly-absent predators) and surface (abundant resources and predators) populations under different risk conditions and measured aggressive behavior towards conspecifics. During behavioral trials, larvae were exposed to different combinations of predator and wounded conspecific chemical cues. Intraspecific aggressiveness increased in large and late-development larvae. Larvae from all the populations significantly reduced aggressiveness under both typologies of risk experienced during rearing (constant presence of predator; pulses of high predation risk), and also when stimulated by chemical cues. However, larvae from cave populations exhibited a more pronounced aggressiveness, especially when exposed to wounded conspecific cues. Intraspecific aggressiveness can be modulated by the complex interaction between multiple variables, and both behavioral plasticity and local adaptations can determine its variation across populations. Our findings reveal that aggressive interactions are favored in cave environment, suggesting intraspecific predation can play a key role in the exploitation of resource-depleted habitats.

Keywords: Intraspecific aggressiveness, cannibalism, chemical signaling, cave environment, predation risk, *Salamandra*

Significance statement

In this study we investigated how intraspecific aggressiveness of salamander larvae is shaped under predation risk in populations originating from contrasting environments, such as cave and surface habitats. Larvae experiencing predator presence during their development or exposed to predator cues significantly reduced their aggressive interactions, both in cave and surface populations. Interestingly, cave-originating individuals reacted to wounded conspecific cues by increasing the frequency of their aggressive displays, suggesting cannibalistic behavior is locally enhanced in populations from resource-depleted habitats. The present study offers new insights on the importance of intraspecific aggressiveness for the adaptation to harsh environments.

Aknowledgements

Introduction

Intraspecific aggressiveness is a plastic behavioral trait that can deeply modulate interactions with conspecifics. Intraspecific aggressiveness can have profound impact on populations, by modulating

competition and determining access to resources, and often has cascading effects on fitness and

population dynamics (Reques and Tejedo 1996; Whitehouse 1997; Arnott and Elwood 2008). This

is particularly relevant in contexts where conspecifics become a potential trophic resource, and

aggressive interactions often result into full-scale cannibalism (Wise 2006; Manenti et al. 2015).

Cannibalistic behavior can, at the same time, provide additional foraging resources to the cannibals

and release them from intraspecific competition pressure, with potential benefits such as a faster

development rate and better survival (Polis 1981; Crump 1983). These benefits are particularly

relevant in environmental contexts where resources are limited, conditions are harsh or risk is

elevated (Fox 1975; Crump 1983). Therefore, aggressive interactions can be more frequent in

ephemeral habitats, when food availability is scarce or conspecific densities are elevated (Reques

and Tejedo 1996; Wildy et al. 2001; Amat et al. 2008; Cooper et al. 2015; Manenti et al. 2015).

Intraspecific aggressiveness can be higher in age- or size-structured populations (e.g. where cohorts

from different breeding seasons coexist). In these conditions, predation on conspecifics by large,

late-stage individuals is facilitated by size asymmetry (Ziemba and Collins 1999; Eitam et al. 2005;

Wissinger et al. 2010).

The benefits of intraspecific aggressiveness are often context-dependent. Aggressiveness is

frequently a plastic or conditional strategy which is subjected to multiple ecological trade-offs (Fox

1975; Polis 1981; Pizzatto and Shine 2008). The occurrence of predators is often a major

determinant of intraspecific aggressiveness, nonetheless its effects on cannibalism occurrence may

be complex and difficult to predict. First, predators directly affect the availability of conspecifics

through consumption, thereby reducing intraspecific encounter rate (Polis 1981). Second, predation

pressure often determines non-consumptive effects in prey populations, such as behavioral

responses that allow minimizing predation risk (Peckarsky et al. 2008; Davenport and Chalcraft

2013). Non-consumptive effects can affect the incidence of aggressiveness in diverse ways. On the

one hand, predation risk is known to determine the decrease of activity level across multiple taxa

(Lima and Dill 1990; Anholt et al. 2000; Barbosa and Castellanos 2005), which in many cases can

93 limit or even suppress the occurrence of cannibalistic behavior (Wissinger et al. 2010; Kishida

94 2011). For instance, dragonfly larvae are predators that can cause a dramatic reduction of activity

95 and aggressive interactions in the Ezo salamander (*Hynobius retardatus*) larvae, thus inhibiting the

96 occurrence of cannibalistic individuals (Kishida 2011).

97   On the other hand, when the risk of being predated is constantly elevated, a prolonged

98 reduction of activity can be too costly, as it would hamper foraging (Lima and Bednekoff 1999;

99 Ferrari et al. 2009). Under persistent risky conditions, an increase in foraging may even result more

100 advantageous, favoring faster growth and rapid development, which can limit exposure to predators

101 of most vulnerable stages or size-classes (Ferrari et al. 2009; Kishida et al. 2015; Manenti et al.

102 2016). An increase in cannibalistic behavior under heavy predation risk was observed in spadefoot

103 toads, which showed a higher frequency of cannibalistic tadpoles in sites where their main predators

104 (salamanders) were present (Ghioca-Robrecht et al. 2009).

105   Non-consumptive effects and the degree of anti-predatory responses strongly depend on the

106 ability of prey to perceive predator presence and to assess predation risk (Lima and Dill 1990;

107 Palmer et al. 2017). In aquatic environments, risk is typically perceived through chemical cues

108 released by the predator (kairomones) (Chivers and Smith 1998; Kats and Dill 1998). Chemical

109 signals can be also released by conspecifics, for instance when they are stressed, wounded or eaten

110 by a predator (stress, alarm and diet cues) (Mirza and Chivers 2001; Wisenden 2003; Ferrari et al.

111 2010b). The simultaneous perception of predator kairomones and conspecific cues often produces

112 synergic effects on prey behavior and can induce or enhance antipredator responses (Bryer et al.

113 2001; Keppel and Scrosati 2004; Dalesman et al. 2007). Moreover, anti-predator response can be

114 modulated on the basis of experienced conditions (Wisenden and Millard 2001; Gonzalo et al.

115 2007; Epp and Gabor 2008) thus previous encounters with predators or risk cues can determine

116 refined responses (McCollum and VanBuskirk 1996; Martin and Lopez 2003; Ferrari et al. 2007;

117 Ferrari et al. 2008). However, when exposure to predators is continuous, predator-associated stimuli

118 can lose their effectiveness and the intensity of anti-predator behavior can decrease (Turner 1997;

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Ferrari and Chivers 2011; Gonzalo et al. 2013). Overall, mechanisms regulating cannibalism under predation risk are difficult to disentangle, and complex trade-offs likely determine its occurrence and intensity (Nilsson et al. 2011; Kishida et al. 2015). Despite environmental conditions being expected to affect the benefits of cannibalism, only a few studies have analyzed the variation of aggressive interactions between populations subjected to considerably diverging predatory and environmental pressures (Griffiths 1994; Nilsson et al. 2011).

In our study, we evaluated how predation risk affects intraspecific aggressiveness of salamander larvae in populations originating from extremely diverging habitats, such as surface and cave environments. The fire salamander (*Salamandra salamandra*) typically breeds in surface streams, where predators are abundant (Lanza et al. 2009; Manenti et al. 2009b; Manenti et al. 2016). Nevertheless, several populations breed in underground streams or pools (Manenti et al. 2009a; Manenti et al. 2011). These environments are virtually devoid of interspecific predators, are characterized by limited trophic resources and can host high salamander densities (Manenti et al. 2015). Theory predicts that cannibalistic behavior is favored under these conditions (Polis 1981; Crump 1983).

Cannibalism is frequent in fire salamander larvae (Joly 1968) and is known to occur both in surface and cave populations (Manenti et al. 2015). In this species the frequency of aggressive interactions is associated with cannibalism intensity, thus intraspecific aggressiveness represents a good proxy for the occurrence of this behavior (Markman et al. 2009; Limongi et al. 2015; Manenti et al. 2015). To evaluate the complex interplay among factors determining cannibalism, we investigated the plasticity and variability of aggressive displays linked to cannibalistic behavior in salamander larvae from cave and surface populations, after the exposure to risk conditions and risk-associated cues. We predict that i) long-term exposure to predators during development decreases the occurrence of aggressive interactions among larvae; ii) the degree of anti-predator behavior is affected by the temporal pattern of experienced conditions (e.g. less pronounced response under constant risk regime compared to periodical exposure). Moreover, iii) acute exposure to chemical

145 cues associated to predation risk should affect the perceived level of risk, decreasing intraspecific

146 aggressiveness. Finally, iv) we expect higher aggressiveness in larvae facing environmental

147 conditions that favor intraspecific predation (e.g. cave populations). Understanding mechanisms

148 regulating cannibalism occurrence and intensity in natural environments can offer important

149 insights on the relative role played by cannibalism in favoring ecological plasticity and the

150 exploitation of harsh, resource-deprived habitats.

151

152 **Methods**

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154 <u>Collection of larvae</u>

155 The study individuals were collected in the districts of Como and Lecco (Lombardy, NW Italy.

156 Approximately 45.8° N, 9.2° E). This area is characterized by hilly and mountainous reliefs with

157 scattered woodlands, predominantly composed of deciduous trees and a dense hydrographic

158 network. In this region, karstic areas are frequent, with numerous cavities constituting a suitable

159 habitat for multiple terrestrial and aquatic invertebrates. In this area, the fire salamander is frequent

160 and gives birth to fully-aquatic larvae both in surface sites (e.g. pools and small streams (Manenti et

161 al. 2009b)) and in underground springs (Manenti et al. 2009a). In underground sites, larvae prey

162 upon cave-dwelling invertebrates, but food availability is scarce (Romero 2009). Therefore,

163 development rate is generally slower than in surface sites (Manenti et al. 2011; Limongi et al. 2015)

164 still salamanders are able to successfully complete metamorphosis.

165 In spring 2014, 142 newborn fire salamander larvae (developmental stage 1, (Zakrzewski

166 1987)) were collected from six underground pools (N = 56 individuals, average 9.3 individuals per

167 site; range: 5-12) and from eight surface sites (N = 86, average 10.7 individuals per site; range: 7-

168 12; Figure 1), located between 250 and 970 m a.s.l. The distance between sites ranged between 350

169 m and 29 km. Nearby sites were separated by natural barriers like deep valleys with steep slopes,

170 thus larvae from different sites belong to separate populations. All underground sites were emitting

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171 springs inside caves therefore no accidental drift of larvae from outdoor sites was possible (Manenti

172 et al. 2009a; Manenti and Ficetola 2013). For the surface sites, only habitats with permanent water

173 were selected, because temporary wetlands have very different selective pressures (Reinhardt et al.

174 2013). Fire salamanders are ovoviviparous, thus larvae could only be collected after deposition. To

175 limit age differences and minimize the effect of possible prior experiences, we daily monitored

176 breeding sites and collected newborn larvae immediately after deposition.

177

178 Rearing conditions

179 Immediately after collection, each larva was photographed to allow individual identification

180 through the unique pattern of their tail (Eitam and Blaustein 2002). Larvae were then individually

181 hosted in transparent plastic containers (10 cm diameter, 15 cm height), arranged in large plastic

182 tanks (40 x 50 cm) filled with 5 cm of aged tap water (blocks). All the individual containers were

183 perforated (2 mm perforations), therefore water freely flowed between the tank and the individual

184 containers. Water temperature (15°C) and oxygenation were kept constant and larvae were exposed

185 to natural photoperiod. Five days after collection, cave and surface larvae were equally divided into

186 three rearing conditions differing in risk exposure: constant predator exposure with no direct

187 predation risk (*No-contact*), short periodical predator encounters (*Contact*) and a control condition

188 with no exposure to predators (Figure 1). The predator used for the experiment was a large (35 mm)

189 dragonfly (*Cordulegaster bidentata*) larva. Dragonfly larvae were collected from surface sites,

190 where they represent a common predator of salamander larvae. Dragonfly larvae can also occurr in

191 cave environments, but their frequency is extremely low (Manenti et al. 2013b). In the No-contact

192 conditions (N = 43 larvae, subdivided in two blocks), two dragonfly larvae were added to the tanks

193 hosting the containers with salamanders and allowed to freely move inside the tank for the whole

194 rearing period. In this condition, predation was impossible but larvae were constantly exposed both

195 to predator's visual and chemical cues. In the Contact conditions (N=49 larvae, two blocks),

196 individuals were subjected to brief pulses of risk with an abrupt exposure to predator presence (with

the simultaneous perception of visual, chemical and tactile stimuli), larvae were exposed every four

days to direct encounters with predators, by inserting one dragonfly larva in the plastic container for

30 seconds (total: 11 encounters throughout the rearing period). This brief exposure prevented

predation attempts but was sufficient to be perceived as a threat by salamander larvae, which

showed startled escape responses when the dragonfly larva was inserted into their container. The

escape response was observed even at the last exposure, suggesting no habituation to this treatment.

Predator exposure in Contact and No-contact conditions differed both in time and modality: while

Contact-reared animals experienced risk during limited but acute stress events, individuals reared in

No-contact conditions were exposed to persistent and but less pronounced risk conditions. Contact

and No-contact conditions aimed at comparing differential risky conditions, which could

differentially affect behavior (Turner 1997; Lima and Bednekoff 1999; Sih and McCarthy 2002).

Finally, larvae under Control conditions never experienced predator presence (N = 50 larvae, three

blocks). Rearing lasted 45 days and during this period both fire salamander and dragonfly larvae

were fed *ad libitum* every second day with fresh *Chironomus* spp. larvae.

Chemical cues

To test the influence of predation-related cue exposure, two chemical treatments were

prepared: predator cues and wounded conspecific cues. Cue extraction was performed before

rearing and behavioral tests as in Manenti et al. (2016). Previous studies have demonstrated that

salamander larvae perceive dragonfly larvae as a threat, as they heavily reduce activity (Manenti et

al. 2016). Therefore, predator chemical cues were obtained leaving 6 *C. bidentata* specimens in 1.5

litres of decanted tap water for 24 hours. Conversely, in order to obtain cues from a wounded

conspecific, the tail tip (< 30%) of a fire salamander larva was cut off with a sterilized scalpel, and

the individual was left in 1.5 litres of decanted tap water for 24 hours. Tail loss is very frequent in

natural populations (up to 40% of salamander larvae in populations with abundant predators)

(Manenti et al. 2013b). Moreover, salamander larvae are able to quickly regenerate tail`, and this

operation does not impact larval survival or subsequent performance (Segev et al. 2015). The
wounded individual was separately reared, its conditions were monitored for 40 days, and it was
released in its site of origin. The two cue solutions were collected, divided into 10 ml aliquots and
stored at -20°C until the behavioral tests. Tap water was collected using the same procedure and
used as control treatment. Salmander and dragonfly larvae used during this procedure were not used
for any rearing treatment or behavioral trial, and were maintained separated from the individuals
used for the experiment.

Behavioral tests

The experimental design consisted of two behavioral sessions: before and after the 45-days rearing
period. During these sessions, the aggressive behavior of individuals was measured by different cue
exposures. At each behavioral session, each individual was tested in two replicates for all the
combinations of cue exposures (predator; wounded conspecific; predator + wounded; control; see
Figure 1). In total, individuals were subjected to 16 behavioral trials (N = 8 trials per individual per
session); in each session, the order of cues was randomized. This procedure made it possible to
perform all the test combinations in a reasonable and comparable time; the randomization of test
order allows the consideration of potential biases related to the sequence of cue exposure (Altmann
1974; Ferrari et al. 2010a). Aggressiveness trials were performed after a three-day starvation period
to promote the occurrence of aggressive interactions and standardize individual motivation to
attack. Before each experimental session, all larvae were photographed on graph paper to measure
total length. Behavioral tests were conducted under daylight conditions in a 13.5 x 18.3 cm plastic
arena filled with 250 ml of decanted tap water. To test for intraspecific aggressiveness, the focal
individual was exposed to a so-called "prey larva" in the experimental arena. The prey larva was a
newborn fire salamander larva originating from a different site of collection, and not subjected to
any rearing treatment. Both the focal and the prey larvae were gently introduced at the opposite side
of the same arena and let acclimatize for a 5-minute period. During acclimation, larvae were kept

separated by means of a removable plastic barrier which divided the arena into two equal-size

sectors. After the acclimation phase, 1 ml of the selected cue treatment was injected by the same

experimenter in the prey larva sector, then the plastic barrier separating the two larvae was

cautiously removed and the test started.

Aggressiveness tests lasted 7 minutes. During tests, three behavioral displays were recorded for

focal larvae: total number of bites or biting attempts towards the prey larva (Attack), the latency of

the first movement towards the prey (Latency) and the time spent by the focal larvae approaching

the prey larva (Following). An Attack was any effective bite, a snap trying to catch the conspecific,

or a sudden rush towards the prey larva. Latency was the time occurring until the first approach,

such as when the larva performed an attack or at least two consecutive movements towards the prey

larva. Following was the total duration of approaching events towards the prey larva (approaching

events were three or more consecutive movements towards the prey larva). Data were not blindly

recorded to avoid the risk of mismatching errors.

Between trials, both focal and prey larvae were placed in their plastic boxes for a 15-minute

recovery time, while arenas were carefully washed twice to remove cues; previous studies showed

that this procedure is sufficient to detect the effect of exposure to different cues (Manenti et al.

2016). In total, we used 47 prey larvae and randomly assigned them to the focal larva during each

trial to minimize repeated encounters. For each trial, we also calculated size difference between

larvae (i.e. the total length difference between focal and prey larva). During the trials, two clear

cannibalism attempts occurred. In both cases the consumption was promptly interrupted, and the

trial stopped. The prey larvae were replaced, separately allowed to recover and never used in any

subsequent trials.

Statistical analysis

Data were analyzed using linear mixed effects models (LMMs) and generalized linear mixed effects

models (GLMMs), which takes into account random factors determining non-independence of

observations (Pinheiro and Bates 2000). Gaussian LMMs were used to analyze the factors

determining Following and Latency displays, while Poisson GLMMs were used for the number of

attacks. As fixed effects, we considered size difference between focal and prey larva, period (before

or after rearing), origin (cave or surface population), rearing condition (No-contact, Contact,

Control) and chemical treatment. The two sessions of behavioral trials (before and after rearing)

were analyzed simultaneously; we included period in the mixed models to take into account

differences occurring through time. We also included length difference between larvae as a

covariate. Random factors were larva identity, rearing block and population of origin. Preliminary

analyses including the identity of prey larva as a further random factor showed higher AIC values

and were qualitatively identical.

We used orthogonal contrasts (or "planned comparisons") to perform the comparison

between rearing conditions. Orthogonal contrasts allow pairwise comparisons without increasing

Type I and Type II errors, as it would occur when using post-hoc tests (Field et al. 2012). We first

used contrasts to compare predator-rearing conditions (Contact and No-contact) against control-

larvae. Subsequently, we tested the significance of differences between Contact and No-contact

conditions. For each behavioral display, two-way interactions between period, origin, rearing

condition and chemical treatment were tested; non-significant interactions were not included in the

final models. Testing statistical interactions between between chemical stimuli (predator and

wounded conspecific cues) was used to assess the possibility of joint effects between them. The

analysis of latency and following behaviors was performed on the subset of trials in which larvae

approached or followed conspecifics (i.e. 196 and 160 trials, respectively; see Appendix S1).To

visualize the effects of GLMMs, we built conditional partial regression plots using visreg 2.4

(Breheny and Burchett 2017). All analyses were performed under the R 3.4.1 environment using the

packages lmerTest and lme4 (Pinheiro and Bates 2000), while visreg package was used to generate

figures presented hereafter.

300 The datasets during and/or analyzed during the current study available from the corresponding

301 author on reasonable request.

302

303 **Results**

304

305 Overall, we performed 1520 behavioral trials. During trials, differences between focal larva and

306 prey larvae for the total length ranged between -5 mm and 21 mm (mean=3.44, SD=5.37mm). None

307 of the correlations between behavioral displays was strong. We detected a significant correlation

308 between the duration of Following and the number of Attacks and ($r = 0.472$, $P < 0.001$), while

309 correlations between Attacks and Latency ($r = 0.002$, $P = 0.979$) and between Following and

310 Latency ($r = -0.111$, $P = 0.120$) were weak and non-significant. Survival rate after rearing was

311 70.4%. The mortality of salamander larvae in nature is often >90% even in environments with

312 limited predators (Limongi et al. 2015), therefore the observed values are not unexpected. Mortality

313 was not significantly different between rearing conditions or origin (binomial generalized linear

314 mixed model; all $P > 0.05$).

315

316 **<u>Attack</u>**

317 During behavioral tests, mean ($\pm$ SD) Attack rate was 0.34 ± 0.88, and the total number of attacks

318 ranged from 0 to 6 per trial. The number of attacks significantly increased when size differences

319 among larvae were largest ($P = 0.041$), and after 45 days ($P = 0.040$; Table 1; Figure 2A-B).

320 Furthermore, rearing under risky conditions significantly decreased the Attack rate ($P < 0.001$;

321 Figure 2D). Orthogonal contrasts showed that both rearing conditions significantly reduced attack

322 rate compared to controls ($\chi^2_1 = 11.37$; $P < 0.001$), while we did not detect differences between the

323 Contact and the No-contact conditions ($\chi^2_1 = 0.08$; $P = 0.781$). We found no significant effect of

324 origin, predator cues or wounded conspecific cues on this display (all $P > 0.05$; Table 1, Figure 2C-

325 F).

326   We also detected multiple significant interactions between independent factors (Figure 3,

327   Table 1). First, larvae reduced their attack rate in presence of predator cues, but only after 45 days

328   of rearing (P = 0.037; Figure 3A). Second, cave larvae reared under No-contact conditions reduced

329   attack rate more than surface larvae (P < 0.001; Figure 3B). Finally, larvae reared under Contact

330   conditions reduced their aggressiveness less when exposed to predator (P = 0.013; Figure 3C) and

331   to conspecific cues (P = 0.012; Figure 3D), compared to the other conditions. We found no

332   significant interaction of the paired exposure to the two chemical cues nor between any other fixed

333   factor.

334

335   **Latency**

336   Approaches towards prey larvae were detected in 12.9% of trials, with Latency time ranging

337   between 0 and 417 seconds (mean = 189.22 ± 127.85 s). The time before approaching prey was

338   significantly shorter when size differences between the two larvae were largest (P = 0.019; Table 1,

339   Figure 4A). By contrast, we did not detect a significant effect of period, origin, rearing, predator

340   cues or conspecific cues (all P > 0.05; Table 1, Figure 4B-4F). Besides, when exposed to wounded

341   conspecific cues, cave larvae showed a shorter time to first approach the prey larvae, compared to

342   surface larvae (P = 0.008; Figure 5A). No other interactions between factors showed significant

343   effects on display.

344

345   **Following**

346   The following behavior occurred in 10.5% of trials. The total time pursuing prey larvae ranged from

347   10 to 267 seconds (mean = 43.53 ± 46.48 s). The Following time was significantly longer when

348   length differences between larvae were largest (P = 0.013; Table 1; Figure 6A). Furthermore, less

349   prolonged following was observed in larvae from surface populations (P = 0.021; Figure 6C), in

350   presence of predator cues (P = 0.029; Figure 6E), and in larvae reared with predators (P < 0.001;

351   Figure 6C). Larvae reared under both risk conditions showed significantly shorter following

behavior, compared to controls (orthogonal contrasts: $F_{1, 118.4} = 13.9$; $P < 0.001$; Figure 6D), while differences between the Contact and No-contact conditions were not significant ($F_{1, 150.7} = 0.74$; $P = 0.390$). We did not detect significant effects of period or wounded conspecific cues on following (all $P > 0.05$; Figures 6B, 6F). Finally, a significant interaction between origin and wounded cues indicated that only cave larvae increased their following activity when exposed to wounded cues ($P = 0.028$; Figure 5B). No other interaction between fixed factors significantly affected following duration.

Discussion

Environmental pressure can determine strong variation of aggressive behavior, and we observed increased agonistic interactions in larvae from cave populations, where the fitness advantages of aggressive individuals can be particularly effective. Our study confirms that the expression of aggressive behavior can be affected by habitat pressures, and that populations can show differences in their responses to environmental conditions. Different typologies of risk exposure (constant presence of predator; pulses of high predation risk; presence of chemical cues) yielded a consistent reduction of aggressiveness through all the populations, still larvae from cave populations exhibited a more pronounced aggressiveness, especially when exposed to wounded conspecific cues. Individuals from cave populations also tended to follow more actively the perspective prey larvae, suggesting a stronger propensity to start agonistic interactions. This supports the prediction that aggressive interactions are favored in cave environment, and suggests a key role of intraspecific predation on the exploitation of resource-depleted habitats.

The higher aggressiveness of cave larvae is probably related to their peculiar environment. Theory predicts that food scarcity, high conspecific density and absence of predators shall enhance cannibalistic tendency (Fox 1975; Polis 1981), and these conditions are generally experienced by salamander larvae from cave populations (Limongi et al. 2015; Manenti et al. 2015). Moreover,

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when exposed to wounded conspecific cues, cave larvae considerably decreased their Latency to

approach prey larvae, and increased their Following duration. Examples of conspecific cues

eliciting an active foraging strategy are described for some cannibal species (Harvey and Brown

2004; Tran 2014; Carlson et al. 2015) and resource-poor environments, such as caves, may further

favor this response. Cannibalism is often favored in size- or age-structured populations (Fox 1975;

Reques and Tejedo 1996). As expected, size differences promoted aggressive displays by increasing

the number of attacks and the following intensity, and by decreasing the latency of aggressive

behaviors. Moreover, an increased aggressiveness was detected after 45 days of rearing, in

agreement with studies suggesting that aggressive behaviors and cannibalism are more frequent in

older larvae (Sadeh et al. 2009; Wissinger et al. 2010). In cave environments, prey are scarce and

new-born conspecifics likely constitute an important resource for older, larger and more aggressive

larvae to survive and successfully achieve metamorphosis. Our results suggest that larvae from cave

populations can be particularly prone to adopt risky behaviors and actively search for prey (Blecha

et al. 2018), and perceive the cues of wounded conspecifics as a signal of trophic opportunity rather

than an alarm signal.

The different behavioral strategies between cave and surface larvae (Figures 5 and 6C)

support the hypothesis of local adaptation in populations facing different environmental pressures

(Baur 1994; Caspers et al. 2015). High foraging plasticity and cannibalism are traits that can

facilitate the exploitation of resource-depleted environments (Polis 1981), and favor the

colonization of novel habitats such as caves (Romero 2009; Manenti et al. 2013a). Moreover, in

these environments strategies offering access to alternative trophic resources, such intraspecific

predation and active search for the prey, should be positively selected and can become established

as local adaptations (Hüppop 1987; Manenti and Ficetola 2013). Cave and surface populations often

live nearby, therefore some gene flow between populations is possible. Nevertheless, local

401   adaptations might be maintained by multiple processes, such as the strong difference in selective

402   pressure between the two environments and assortative mating (Caspers et al. 2009).

403   Predation pressure can determine complex anti-predatory responses. A reduction of activity

404   and higher refuge exploitation are frequent non-consumptive effects allowing to limit encounter rate

405   with predators (Lima and Dill 1990; Davenport and Chalcraft 2013; Manenti et al. 2016), thus we

406   expected a reduction of intra-specific aggression in salamander larvae exposed to predators. Our

407   findings were in agreement with this prediction, as a strong decrease of aggressiveness was evident

408   in salamanders from both cave and surface populations experiencing predator presence during

409   rearing (i.e. Contact and No-Contact conditions), or exposed to predator cues during behavioral

410   tests.

411   Attack rate and Following duration were significantly reduced in larvae reared under

412   both Contact and No-contact conditions, confirming the hypothesis that risk exposure can lead to

413   important non-consumptive effects in fire salamander (Manenti et al. 2016). On the one hand, the

414   strong risk pulses of Contact were expected to determine a pronounced increase of anti-predator

415   responses and thus limit the occurrence of aggressive interactions. On the other hand, a continuous

416   exposure to predator, without direct experience of negative consequences as in the No-contact

417   condition, was supposed to cause a lower anti-predator response (Turner 1997; Lima and Bednekoff

418   1999; Sih and McCarthy 2002). Nevertheless, we did not detect significant differences between the

419   Contact and No-contact conditions, as both caused a similar reduction in aggressiveness. While,

420   such lack of difference might also be favored by the good nutritional regime of larvae, which can

421   limit the need for an active search of resources, the reduction of aggressiveness in No-contact larvae

422   strongly suggests that any occurrence of habituation to predator presence was unlikely. In contrast,

423   these results support the idea that perceived predation risk, even if extremely differing both in time

424   and modality, can elicit a strong anti-predator response causing deep consequences on behavioral

425   interactions between conspecifics. Moreover, we detected complex interactions between chemical

cues and rearing conditions, as larvae reared under Contact condition showed a lower reduction in

their aggressiveness when exposed to cues of predators and wounded conspecifics (Figure 3).

During the Contact condition, animals experienced pulses of risk with the direct presence of the

predator (with co-occurring visual, tactile, and chemical stimuli). In contrast, during behavioral

trials, animals were exposed to just the chemical cues released by predators. The lower response to

predator cues in Contact-reared animals seems to suggest that animals experiencing the

contemporary exposure to multiple stimuli could have improved their capability to discriminate

between stimuli associated to predators, compared to individuals that never experienced direct

contact with predators. However, the assessment of this hypothesis would require focused tests.

Chemical signals are assumed to be the most common cues used for risk perception by

amphibian larvae (Chivers and Smith 1998). Our study showed that chemical signals are important

also for fire salamanders, as a small amount of cues from predators and/or conspecifics was enough

to entail significant behavioral responses. While predator cue exposure determined a general anti-

predator response, with a significant reduction of some aggressive displays, cues of wounded

conspecifics did not determine anti-predator behaviors, suggesting that in fire salamander they are

not perceived as alarm cues potentially inhibiting intraspecific aggressiveness. Several studies

showed that other amphibian larvae reduce activity rate when exposed to the cues of wounded

conspecifics (Gonzalo et al. 2007; Ferrari et al. 2010b). However, most of these studies have been

performed on anuran tadpoles, which rarely are predators (Wells 2007), and more studies are

necessary to unravel the complex effects of wounded conspecifics in aquatic predators.

Both exposure to predators during rearing, and the presence of predator cues during

behavioral trials, led to pronounced decreases of intraspecific aggressive interactions.

Aggressiveness decreased in both cave and stream larvae, and in both newborn and older larvae.

Such a reduction of aggressiveness led by predator occurrence may result in a waning or even a

complete suppression of cannibalism in risky habitats (Kishida et al. 2011; Líznarová et al. 2018).

As cannibalism plays a key role in many aquatic ecosystems (Rudolf 2008; Wissinger et al. 2010;

Takatsu and Kishida 2015; Takatsu et al. 2017), its suppression may impact the survival and life-history of individuals (Kishida et al. 2011), with potential broad consequences on population dynamics (Claessen et al. 2004; Rudolf 2008).

Behavioral decisions under predation risk are driven by a complex interplay between multiple conditions (e.g. background level of risk, experience, prey energetic state). Their expression can be fine-tuned by the multifaceted nature of the environmental pressures experienced, but is also affected by adaptations to local conditions. Integrated analyses, considering multiple behavioral displays, are essential to better understand how prey responses are modulated under the complex environmental variation that can occur in nature.

Ethics approval

The study design was approved by the ethical committee of the Lombardy Region Authority and was authorized as complying with the regional law 10/2008, p.n.: F1.2013.0002091. Larvae were subjected to the same rearing conditions as in Manenti et al. (2016). The aim of the present study was to evaluate the non-consumptive effects of dragonfly larvae on salamander larvae aggressive behaviour. Consequently, care was taken to plan the experiment accordingly and, thus, to avoid any larvae being exposed to any actual predation. To this end, during rearing, direct contact with predator was restricted to 30-second encounters which were short enough to prevent predation events. Whatever the case, we were always ready with tweezers to block snaps or stop them from extending their mouthparts. Similarly, full cannibalistic interactions were not allowed. During behavioral trials, when an aggressive interaction lasted more than a single bite event conspecifics were promptly separated, thus preventing injuries and intraspecific predation events. It should be also noted that in natural conditions fire salamander larvae show very often aggressive interactions (Joly 1968), facing much more severe outcomes (e.g. being cannibalized) than those experienced in the study design that we adopted during behavioral trials and that was approved by the ethical

477   committee. Conspecific cues were extracted from a single larva by removing the tail tip (far less

478   than 30% of the tail) with sterilized scissors, a procedure that has been assessed to have no impact

479   on individual survival or subsequent performance (Segev et al. 2015). After tail removal, the

480   conditions of this individual were monitored and it perfectly recovered during a 40-day rearing

481   period before its releasing in the site of origin. Overall, the survival rate of larvae was 70.4%, being

482   much higher than that observed in natural populations (Limongi et al. 2015). All individuals were

483   checked daily and fed every 2 days (see also (Winandy and Denoel 2013; Winandy and Denoel

484   2015)). All the larvae were released at their site of origin at the end of the study, following the

485   recommendations of the permit.

486

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661 **Table 1** Influence of independent variables on Attack, Latency and Following displays performed

662 by fire salamander larvae. For Attack, we used Poisson GLMMs (Test statistics: χ2); for Latency

663 and Following, we used Gaussian LMMs (Test statistics: F value). Significant effects are in bold

Display	Fixed effects	Df	Test statistic	P
Attack	Size difference	1	4.160	**0.041**
	Period	1	4.212	**0.040**
	Origin	1	3.099	0.078
	Rearing	2	14.962	**<0.001**
	Predator cues	1	0.003	0.960
	Conspecific cues	1	2.016	0.156
	Period × Predator cues	1	4.351	**0.037**
	Rearing × Origin	2	16.372	**<0.001**
	Rearing × Predator cues	2	8.735	**0.013**
	Rearing × Conspecific cues	2	8.875	**0.012**
Latency	Size difference	1, 126.9	5.68	**0.019**
	Period	1, 91.64	1.33	0.253
	Origin	1, 81.6	0.53	0.467
	Rearing	2, 100.4	1.64	0.199
	Predator cues	1, 168.0	0.001	0.972
	Conspecific cues	1, 176.3	0.02	0.893
	Origin × Conspecific cues	1, 172.1	7.32	**0.008**
Following	Size difference	1, 73.8	6.47	**0.013**
	Period	1, 48.8	3.52	0.067
	Origin	1, 151.99	5.43	**0.021**
	Rearing	2, 120.31	8.79	**<0.001**
	Predator cues	1, 148	4.81	**0.029**
	Conspecific cues	1, 151.99	0.19	0.666
	Origin × Conspecific cues	1, 150.7	4.93	**0.028**

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Figure 1 Experimental design and sample sizes. We collected 142 fire salamander larvae (*Salamandra salamandra*) from 6 cave (N = 56) and 8 surface (N = 86) populations. Larvae were exposed for 45 days to three rearing conditions: Control, absence of predator (N = 18 cave larvae; N = 32 surface larvae); Contact, periodical exposure to 30-second encounter with predator (N = 20 cave larvae; N = 31 surface larvae); No-contact, constant to non-lethal exposure to predator (N = 18 cave larvae; N = 23 surface larvae). We conducted two sessions of behavioral tests: before rearing period, and after rearing period. During each behavioral test, focal larva was exposed to each chemical treatment in two replicates (N = 8 test per larva per session)

Figure 2 Conditional partial residual plots, showing the relative influence of size difference (A), period (B), origin (C), rearing (D), predator cues (E) and conspecific cues (F) on Attack (number of attacks performed by fire salamander larvae toward conspecifics). Shaded areas are 95% confidence bands

Figure 3 Conditional partial residual plots, showing the significant interaction effects on fire salamander Attack: period and predator cues exposure (A), rearing conditions and origin (B), rearing conditions and predator cues exposure (C), rearing conditions and conspecific cues exposure (D). Shaded areas are 95% confidence bands

Figure 4 Conditional partial residual plots, showing the relative influence of size difference (A), period (B), origin (C), rearing (D), predator cues (E) and conspecific cues (F) on Latency (time

693   needed by fire salamander larvae to approach conspecifics). Shaded areas are 95% confidence

694   bands

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697   **Figure 5** Conditional partial residual plots, showing the significant interaction effects of origin and

698   conspecific cues exposure on fire salamander Latency (A) and on Following (time spent by focal

699   fire salamander approaching a conspecific; B). Shaded areas are 95% confidence bands

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702   **Figure 6** Conditional partial residual plots, showing the relative influence of size difference (A),

703   period (B), origin (C), rearing (D), predator cues (E) and conspecific cues (F) on fire salamander

704   Following time. Shaded areas are 95% confidence bands