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ORIGINAL ARTICLE

Influence of interspecific competitors on behavioral thermoregulation: developmental or acute plasticity?

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Abstract

Many ectotherms reduce their exposure to changing thermal conditions using behavioral thermoregulation. The effectiveness of behavioral thermoregulation in maintaining ectotherm body temperatures within the target range is influenced not only by environmental (operative) temperatures but also by the presence of other con- and heterospecific individuals. How species' interactions affect behavioral thermoregulation is largely unknown. Theory predicts that species' interactions could affect the plasticity of behavioral thermoregulation in two ways, i.e., by developmental plasticity of a preferred temperature range or by an acute shift in body temperatures. Empirical tests of these predictions are scarce. We examined the developmental and acute effects of heterospecific social interactions on the accuracy and effectiveness of thermoregulation in the larvae of two competing species, *Ichthyosaura alpestris* and *Lissotriton vulgaris*. The presence of heterospecifics during larval development had no effect on preferred body temperatures but it modified later acute thermoregulatory responses to heterospecifics. *Ichthyosaura alpestris* larvae from heterospecific tanks increased their thermoregulatory accuracy and effectiveness, while *L. vulgaris* larvae from conspecific tanks relaxed their thermoregulatory efforts. The thermal dependence of somatic growth suggests that modified behavioral thermoregulation has the potential to accelerate growth in competitively dominant *I. alpestris*. Acute thermoregulatory responses are affected by heterospecific social interactions in newt larvae, but not conspecific. A developmental plastic response modified body temperatures not the target thermoregulatory range, which shows that the influence of heterospecific social interactions is more complex than predicted by theory. Species interactions complicate estimating an ectotherm's vulnerability to ongoing climate change.

Significance statement

Many ectothermic animals control their body temperature through behavioral thermoregulation. Their thermoregulatory decisions are influenced not only by environmental temperatures, but also by the presence of other species. We show that the current thermoregulatory effort in interacting newt larvae is affected by previous experience with competing species. The influence of heterospecific social interactions is more complex than predicted by theory, which complicates estimating an ectotherm's vulnerability to ongoing climate change.

Keywords Climate change · Species interactions · Thermal niche · Preferred temperatures · Behavioral plasticity · Newt

Introduction

Many ectotherms respond to changing environmental temperatures primarily through behavioral thermoregulatory adjustments. In some species, these adjustments are so effective that it allows them to maintain similar body temperatures in populations distributed across various thermal conditions (Blouin-Demers and Nadeau 2005). The buffering potential of behavioral thermoregulation is therefore considered an important mechanism for reducing the selection pressure of environmental temperatures on morphological, physiological, and life

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history traits (Huey et al. 2003). Behavioral thermoregulation is also an important mechanism to cope with human-induced threats such as climate change (Kearney et al. 2009; Sunday et al. 2014), habitat modification (Tuff et al. 2016), or pathogen spread (Richards-Zawacki 2010). Contrary to its stabilizing role in ectotherm ecology and evolution, behavioral thermoregulation is a plastic trait affected not only by environmental temperature but also by other biotic and abiotic factors such as substrate humidity, food availability, predation, and competition (Huey and Slatkin 1976; Angilletta 2009; Mitchell and Angilletta 2009). How these factors influence behavioral thermoregulation still remains poorly understood.

Among biotic factors, con- and heterospecific social interactions, i.e., behavioral interactions among individuals of its own or another species, affect body temperatures across ectotherms, from crayfish to lizards (Magnuson et al. 1979; Downes and Bauwens 2002; Tattersall et al. 2012; Rusch and Angilletta 2017). In the presence of dominant individuals, either con- or heterospecific, subordinates usually decrease their body temperatures. How social interactions affect behavioral thermoregulation, i.e., an ectotherm's ability to maintain its body temperature within a target range, however, has received limited attention from both a theoretical and empirical view. Theory predicts that if individuals of two separate lizard species exploit the same resource, they should lower their thermoregulatory accuracy, which is the departure of actual body temperatures from the preferred body temperature range (Huey and Slatkin 1976). On the other hand, in territorial species, more accurate thermoregulatory behavior should provide an advantage in agonistic encounters, because it allows maintaining body temperatures around thermal optima for fighting performance. Unfortunately, to our knowledge, only one empirical study examined the intraspecific effect of social interactions on behavioral thermoregulation in a territorial lizard (Rusch and Angilletta 2017), which showed that social interactions reduce thermoregulatory efforts in both dominant and subordinate individuals. A few other studies have shown differences in body temperature due to presence of competitors (e.g., basking behavior—Regal 1971; Seebacher and Grigg 1997, and competitive exclusion from shelters—Downes and Shine 1998; Downes and Bauwens 2002). Hence, the available theory and empirical results about this topic are restricted to limited types of social interactions in only one taxonomic group. Given the enormous diversity of ectotherms and various forms of social interactions, biotic influences on behavioral thermoregulation remain largely unstudied.

Heterospecific social interactions may affect behavioral thermoregulation by both coevolution (Thompson 2005; Brockhurst and Koskella 2013; Northfield and Ives 2013) and phenotypic plasticity (see references above). In taxa with long generation times, such as lizards or newts, the evolution of thermoregulatory traits is rather slow (Muñoz et al. 2014; Gvoždík 2015), and so they should respond to this biotic factor primarily with plastic modifications of their phenotype. One unresolved issue is whether social interactions affect behavioral thermoregulation by inducing acute (activational), seasonal, or developmental plastic responses (Gvoždík 2012; Little and Seebacher 2016). This distinction is important, because the various types of behavioral plasticity vary in their response time, costs, and adaptive significance (Snell-Rood 2013). Behavioral thermoregulation has been defined as maintaining body temperatures within a target (preferred body temperature) range in an environment with varying sources of heat (IUPS Thermal Commission 2003). Accordingly, thermoregulating ectotherms may cope with changing environmental conditions either through an immediate shift in body temperature, i.e., acute plasticity, or through seasonal or developmental plasticity of a preferred body temperature range (Fig. 1). For example, the presence of a predator, dragonfly larva, induces an immediate thermoregulatory response in prey, newt larva, whereas predators respond to the presence of prey with a developmental shift of its preferred body temperature range (Smolinský and Gvoždík 2012; Gvoždík et al. 2013). Hence, interspecific variation of thermoregulatory plasticity may also occur in social interactions.

Newt larvae are a suitable system to examine the influence of social interactions on behavioral thermoregulation, among individuals of competing species. Larvae of the alpine newt *Ichthyosaura alpestris* and smooth newt *Lissotriton vulgaris* frequently develop in the same bodies of water (Szymura 1974; Kuzmin 1991; Braz and Joly 1994). The level of con- or heterospecific aggression varies considerably between species (Babik 1998; Hloušková et al. 2018), and so social interactions may affect their thermoregulatory behavior in disparate ways. Theory predicts that the adaptive significance of developmental plasticity depends on the predictability of future conditions (Moran 1992; Padilla and Adolph 1996; Ghalambor et al. 2007). The presence of other species in a small water body, such as temporary pools, provides a stable cue, and thus, it meets this condition. Accordingly, larvae of the subordinate species, *L. vulgaris*, should modify their thermal requirements through developmental plasticity to reduce encounter rates with the competitively dominant species, *I. alpestris* (Downes and Bauwens 2002). Specifically, we examined whether (i) the presence of heterospecific interactions induce a plastic shift in behavioral thermoregulation and (ii) larvae adjust their thermoregulation through developmental plasticity in the preferred body temperature range or an acute change in body temperature distribution (Fig. 1). Finally, we quantified the thermal dependence of growth rates to predict the impact of larval thermoregulatory modifications on life history in both species. These results provide understanding

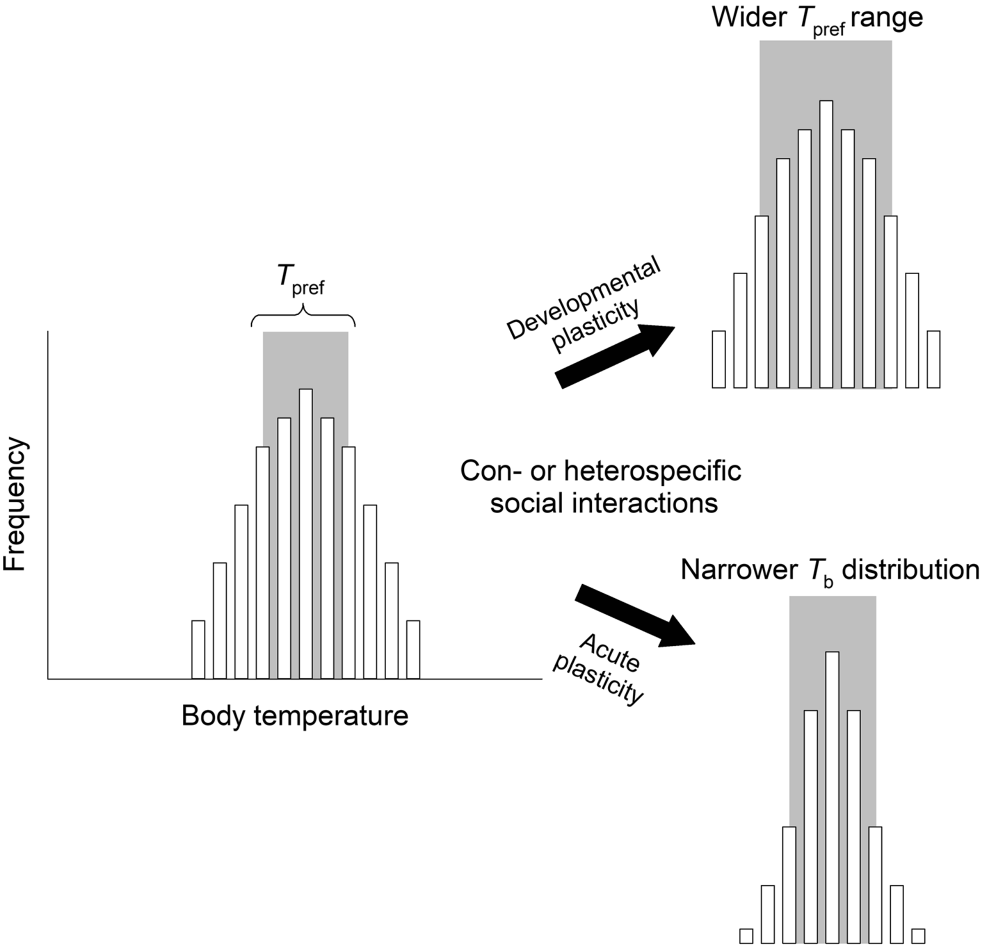

Fig. 1 The influence of social interactions on plasticity of behavioral thermoregulation. Behavioral thermoregulation is illustrated as the distribution of field body temperatures (T_b) relative to target temperatures (preferred body temperatures, T_{pref}) that an ectotherm aims to maintain in environments where conditions allow thermoregulation (left). Under the presence of con- or heterospecifics, an ectotherm may adjust behavioral thermoregulation through developmental or seasonal plasticity of the T_{pref} range (upper right) and (or) by an acute shift of T_b distribution (lower right). Note that the plastic shift may involve not only the breadth of body temperature distribution or T_{pref} range but also their horizontal position

of the complex influence of interspecific social interactions on the plasticity of behavioral thermoregulation and, accordingly, its potential to reduce an ectotherm's exposure to local climate change.

Material and methods

Study species and maintenance

Both *L. vulgaris* and *I. alpestris* newts are 11- and 12-cm-long tailed amphibians, respectively. They are distributed across most of Europe. Their oviposition period lasts from April to June. Larvae usually undergo metamorphosis during July–September or over winter under adverse thermal conditions. Newt larvae regulate their body temperatures with horizontal or vertical movements across water of different temperatures (Smolinský and Gvoždík 2012; Gvoždík et al. 2013). If both species develop together, larvae exploit the same food resource, zooplankton. The *I. alpestris* larvae are aggressive towards the second species. Both species compete using both food exploitation and physical interference, which results in slower growth and developmental rates in *L. vulgaris* (Hloušková et al. 2018).

For the purpose of this study, we reared larvae obtained from eggs of wild-caught females ($n = 20$ per species) under seminatural conditions in 2015 and 2016. Eggs were incubated in aquaria under outdoor conditions (mean hourly water temperature = 13.8 ± 4.2[SD] °C). For larval rearing, we used 30 tanks (90 × 63 × 47 cm) filled with non-chlorinated well water (90 L; see Online Resource 1 for water parameters). Each tank was inoculated with pond water (1 L) 2 weeks prior to larval introduction. To promote plankton growth and nutrient cycling, we added 12 g of dry beech soil, 3 g of fine hay, and four snails (*Lymnea stagnalis*) to each tank. Several stems of the aquatic plants (*Egeria densa*) and dry beech leaves were provided to act as hiding substrate for the larvae. Tanks were covered with a fine mesh to prevent egg laying of insect predators in the water.

Tanks were distributed into ten blocks. Within each block, one tank contained only *I. alpestris* larvae ($n = 30$), only *L. vulgaris* larvae ($n = 30$), or a mixture of both species ($n = 15$ per species). Larvae were haphazardly selected from female clutches, and so we assume that larvae from each female were distributed across all rearing tanks. Hereafter, we will refer to larvae without experience of interspecific interactions as naïve larvae, and larvae from heterospecific tanks as experienced larvae. Larval density was chosen intentionally to be in the upper ranges of a natural density range (Van Buskirk and Schmidt 2000) to provide sufficient cues for presumed plastic responses. For logistic reasons, we were unable to perform acute and developmental plasticity tests during only one season; however, thermal conditions during larval development were similar in both seasons (mean hourly water surface temperature in 2015, 16.8 ± 5.6 °C; 2016: 17.6 ± 5.3 °C), which suggests that this uncontrolled factor had little influence on the obtained results.

Developmental plasticity of preferred body temperatures

To find out whether the presence of interspecific competitors during larval development affects their thermal requirements, we measured the preferred body temperatures (T_{pref}, see also Table 1 for definition) in larvae of both species from all rearing groups. Since the method of measurement has been published in detail elsewhere (Smolinský and Gvoždík 2012), we provide only a brief description here. A horizontal thermal gradient (8–30 °C) was maintained in the stainless steel tank (240 × 60 × 60 cm), which was longitudinally divided into nine lanes.

Experimental larvae ($n = 9$ per trial) were haphazardly chosen from rearing tanks, photographed from the dorsal view (see below), and individually placed in each lane of the gradient ca. 15 h before the start of each trial (9:00). We carefully measured (so as not to disturb larvae) water temperatures (to 0.1 °C) close to each individual using a digital thermometer (HH 22, Omega Engineering, Stamford, USA) at hourly intervals from 10:00 to 16:00. All temperature-measuring devices used were calibrated using a certified mercury thermometer (0.2 °C resolution). Water has 24 times higher thermal conductivity and four times higher specific heat capacity than air (Denny 1993), which prevents small-to-medium-sized ectotherms from maintaining different body temperatures than the surrounding water (Lutterschmidt and Hutchison 1997). Accordingly, larval body temperatures were obtained indirectly by extrapolation from surrounding water temperatures (see also Smolinský and Gvoždík 2009, 2012). For further analyses, we used the mean, minimum, and maximum body temperatures from each individual. Minimum and maximum values were used as estimates of the lower (LBT_{pref}) and upper (UBT_{pref}) boundary of the T_{pref} range, respectively. Our previous study showed that minimum and maximum values obtained using this method correspond closely to the percentile method calculated from video recordings of larval movement of estimating T_{pref} range (Smolinský and Gvoždík 2009). It was not possible to record data blind because T_{pref} measurements were performed by one person.

Table 1 Definitions of acronyms used in this paper

Acronym	Definition
d_b	Index of accuracy of thermoregulation; the mean absolute deviation of body temperatures from the T_{pref} range
d_e	Index of thermal quality of habitat; the mean absolute deviation of operative temperatures from the T_{pref} range
E	Index of effectiveness of thermoregulation; $E = d_e - d_b$
LBT_{pref}	Lower boundary of T_{pref} range
T_b	Body temperature
T_{pref}	Preferred body temperatures; body temperatures that an ectotherm maintains in the laboratory thermal gradient under the absence of abiotic and biotic limitations
UBT_{pref}	Upper boundary of T_{pref} range

Acute plasticity of behavioral thermoregulation

We used seven experimental aquaria (60 × 10 × 40 cm high) with inclined bottoms (Marek and Gvoždík 2012; Gvoždík et al. 2013) to test the effect of intra- and interspecific social interactions on acute thermoregulatory responses. Aquaria were filled with non-chlorinated well water exactly to 30 cm. In each aquarium, a heater (50 W, Eheim/Jäger, Wüstenrot, Germany) located 5 cm below the water surface was used to create a vertical temperature gradient. The heater was separated from the rest of the aquarium by a mesh-bound recess, so as not to affect the behavior of the test animals. The aquaria were placed in an environmental room kept at 12 °C, resulting in aquaria temperatures ranging from 25 °C at the water surface to 13–14 °C at the bottom, closely matching the maximum vertical thermal gradient in natural newt habitats (Dvořák and Gvoždík 2010). The profiled bottoms (10 cm flat, 40 cm inclined, and 10 cm flat) allowed bottom-walking for newt larvae in water of different temperatures. In an extra aquarium not used for behavioral observations, a series of thermistor probes connected to dataloggers (H08-002-02, Onset Computer, Bourne, MA, USA) recorded hourly water temperatures at 5-cm intervals in water columns. In the other aquaria, the temperature gradient was checked regularly using a thermal-imaging camera (C2, Flir Systems, Wilsonville, OR, USA) to ensure thermal conditions were consistent throughout the experiments.

Prior to each trial, randomly chosen larvae from each rearing group were photographed from their dorsal side using a digital microscope (magnification × 8; DinoLite Pro, AnMo Electronics, New Taipei City, Taiwan). Total length of larvae were obtained from the digital images (to 0.001 mm;

DinoCapture 2.0, AnMo Electronics). All larvae (*I. alpestris*: total length = 28.1 ± 5.9[SD] mm; *L. vulgaris*: total length = 29.7 ± 5.8 mm) were at the same developmental stage, i.e., the fifth toe visible on the hindlimb, because newt larval thermal requirements vary during their development (Smolinský and Gvoždík 2009). We distributed larvae among aquaria about 16 h prior to the beginning of each trial (9:00) according to four experimental setups: naïve single individuals (i.e., no experience with interspecific interactions), naïve conspecific pairs, naïve heterospecific pairs, and experienced heterospecific pairs. The environmental room was regularly visited by the same person (BW), and larval vertical positions were recorded (2.5-cm resolution) at hourly intervals from 10:00 to 17:00. This period corresponds with a time window allowing larval thermoregulation in the wild (Hadamová and Gvoždík 2011). Individual larvae were recognized according to their species identity and total length differences. Larval body temperatures were extrapolated from known water temperatures at a given depth. As for T_{pref} measurements (see above), we assumed their body temperatures were identical with surrounding water temperatures (Lutterschmidt and Hutchison 1997). Accordingly, the distribution of available operating temperatures was calculated from water temperatures. Group identity was unknown to investigator during recording larval positions.

To quantify behavioral thermoregulation, we applied an index of accuracy and effectiveness of thermoregulation (Hertz et al. 1993). The index of accuracy of thermoregulation (d_{b}) is the mean deviation of body temperatures from the preferred body temperature range. The higher index values indicate less accurate thermoregulation and vice versa. The index of effectiveness of thermoregulation was calculated as $E = d_{\mathrm{b}} - d_{\mathrm{e}}$ (Blouin-Demers and Weatherhead 2001), where d_{e} is the mean deviation of operating temperatures from the preferred body temperature range. Higher values indicate active thermoregulation, whereas values close to zero suggest thermoconformity. We chose this calculation over the original formula (Hertz et al. 1993), which calculates E as division instead of subtraction of d_{e} and d_{b} indices, because it has better performance in ectotherms living under thermoregulatory-challenging conditions (Piasečná et al. 2015).

Thermal dependence of growth rates

In their native habitat, developing newt larvae are subjected to various diel temperature fluctuations (Dvořák and Gvoždík 2010), which affect their growth, development, and locomotor performance (Měráková and Gvoždík 2009). Accordingly, the thermal dependence of their growth rates was measured under three fluctuating thermal regimes with various day peaks (15, 20, and 25 °C) and a common night drop (12 °C). The magnitude of diel thermal fluctuations represents their variation in the wild (Dvořák and Gvoždík 2010). We used six custom-made water baths (two per regime) equipped with programmable heaters to induce desired temperature fluctuations. Each water bath contained 1 L plastic tubes filled with water (0.5 L), and these tubes were mildly aerated, preventing the creation of hypoxic conditions. For growth trials, we used 5–7-day-old larvae. Each larva was photographed from the dorsal view (see above) and randomly assigned to a tube. Equal amounts of live cyclops nauplii were added to each tube. At the end of the trial (7 days), larvae were repeatedly photographed using a digital microscope (magnification × 8; DinoLite Pro). Larval total length, i.e., distance between the tip of snout and tip of tail, was measured from digital images using DinoCapture 2.0 (AnMo Electronics; resolution 0.001 mm). The growth rate was calculated as (end total length − initial total length)/trial duration. Growth trials were performed during two consecutive weeks.

Statistical analyses

Given the sample size used, the non-normal distribution of some data (d_{b}), and the presence of outliers, we applied a semiparametric (randomization) approach for their statistical analysis. We applied a permutation general linear model (pGLM) for the analysis of T_{pref} with the rearing group (i.e., naïve or experienced), species identity, and their interactions as fixed factors. Mean T_{pref} is often negatively correlated with T_{pref} range (Hadamová and Gvoždík 2011), and thus, the mean T_{pref} was added as a covariate to correct for this effect during testing T_{pref} boundaries. The influence of con- and heterospecific social interactions on body temperatures and thermoregulatory indices was tested using pGLM with the experimental treatment (i.e., single, conspecifics, naïve heterospecifics, and experienced heterospecifics) as a fixed factor, and the experimental group as a random factor. Larval total length was added as a covariate. The model was applied separately to each species because the degrees of freedom would be artificially inflated within species groups in heterospecific pairing. To examine the variation between species in thermal dependence of growth rates, we applied a model with species, thermal regime and their interactions as fixed factors, and larval initial length as a covariate. In all models, we used a model simplification approach to find a model explaining the most trait variance with the minimum number of parameters, and thus, we dropped factors with zero or negative variance components. Results are reported as means [95% confidence intervals] unless otherwise stated. Intervals were calculated using the non-parametric bootstrap (“bca”) method in the “boot” package (Canty and Ripley 2017) available in R. All models were performed using the “PERMANOVA” package in Primer (version 6.1.16, PRIMER-E Ltd., Lutton, UK).

Data availability All data used in our paper are available from the Dryad Digital Repository at https://doi.org/10.5061/dryad.hr2t3m3.

Results

Developmental plasticity of preferred body temperatures

In the horizontal thermal gradient, we measured T_{pref} in 70 newt larvae representing naïve and experienced individuals of both species (*I. alpestris*, $n_{conspecific} = 18$; $n_{heterospecific} = 18$; *L. vulgaris*, $n_{conspecific} = 18$; $n_{heterospecific} = 16$). On average, *I. alpestris* (22.7[22.1, 23.4] °C) maintained lower body temperatures than *L. vulgaris* (24.3[23.6, 24.9] °C; $F_{1,65} = 18.61$, $P = 0.001$). Experience with heterospecifics had no significant effect on mean T_{pref} ($F_{1,65} = 1.95$, $P = 0.17$). Mean T_{pref} was negatively associated with T_{pref} range in both species (*I. alpestris*, $r = -0.44$, $P = 0.008$; *L. vulgaris*, $r = -0.49$; $P = 0.003$). When correcting for the effect of mean T_{pref}, range boundaries varied between species (LBT_{pref}, $F_{1,65} = 7.71$, $P = 0.009$; UBT_{pref}, $F_{1,65} = 9.74$, $P = 0.002$) but not between naïve and experienced larvae (LBT_{pref}, $F_{1,65} = 2.16$, $P = 0.15$; UBT_{pref}, $F_{1,65} = 0.15$, $P = 0.69$; Fig. 2). Overall, *I. alpestris* larvae maintained a narrower range of body temperatures than *L. vulgaris*.

Acute plasticity of behavioral thermoregulation

In vertical thermal gradients, body temperatures were affected by experimental grouping in both species (*I. alpestris*, $F_{3,89} = 6.63$, $P < 0.001$; *L. vulgaris*, $F_{3,92} = 12.55$, $P < 0.001$). Specifically, experienced *I. alpestris* larvae maintained higher body temperatures in the presence of heterospecifics than naïve larvae in all groups (vs. single, $t_{45} = 3.04$, $P = 0.005$; vs. conspecifics, $t_{48} = 3.47$, $P = 0.002$; vs. naïve-heterospecifics, $t_{46} = 4.86$, $P < 0.001$; Fig. 3a). In *L. vulgaris*, naïve larvae lowered their body temperatures in heterospecific pairs relative to other groups (vs. single, $t_{40} = 4.07$, $P < 0.001$; vs. conspecifics, $t_{48} = 4.65$, $P < 0.001$; vs. experienced-heterospecifics, $t_{45} = 4.24$, $P < 0.001$; Fig. 3a).

Social interactions affected the d_b index in both species (*I. alpestris*, $F_{3,90} = 3.58$, $P = 0.02$; *L. vulgaris*, $F_{3,89} = 10.34$, $P < 0.001$). Experienced *I. alpestris* larvae thermoregulated more accurately than larvae in other groups (vs. single, $t_{45} = 2.59$, $P = 0.01$; vs. conspecifics, $t_{50} = 2.77$, $P = 0.007$; vs. naïve-heterospecifics, $t_{46} = 4.86$, $P < 0.001$; Fig. 3b). Naïve *L. vulgaris* larvae from heterospecific pairs reduced their thermoregulatory efforts in comparison with single larvae ($t_{40} = 3.67$, $P < 0.001$) and conspecific pairs ($t_{45} = 4.20$, $P < 0.001$). According to d_e index values during interaction trials, thermal conditions were consistently more suitable for *L. vulgaris* (1.5 ± 0.1[SD] °C) than for *I. alpestris* larvae (2.7 ± 0.1 °C).

The *E* index reflected variation in d_b (Fig. 3c). Social interaction affected *E* in both species (*I. alpestris*, $F_{3,90} = 3.58$, $P = 0.02$; *L. vulgaris*, $F_{3,89} = 10.34$, $P < 0.001$). In *I. alpestris*, experienced larvae thermoregulated more effectively than larvae in conspecific ($t_{50} = 2.34$, $P = 0.02$) and heterospecific pairs ($t_{46} = 2.88$, $P = 0.006$). Naïve *L. vulgaris* larvae from heterospecific pairs decreased their thermoregulatory effectiveness relative to singles ($t_{40} = 3.17$, $P = 0.002$) and conspecific pairs ($t_{45} = 3.54$, $P = 0.002$).

Thermal dependence of growth rates

Larval growth rates increased with maximum temperatures of diel thermal fluctuations (see above) in both species ($F_{2,10} = 56.88$, $P < 0.001$; Fig. 4). *Ichthyosaura alpestris* larvae grew faster than *L. vulgaris* across thermal regimes ($F_{1,5} = 46.30$, $P < 0.001$). According to these results, the acute shift in mean body temperature due to interspecific social interactions should maximally increase larval growth rates by 8% in *I. alpestris* and decrease growth rates by 9% in *L. vulgaris* provided that thermal conditions in water allows for behavioral thermoregulation during daytime.

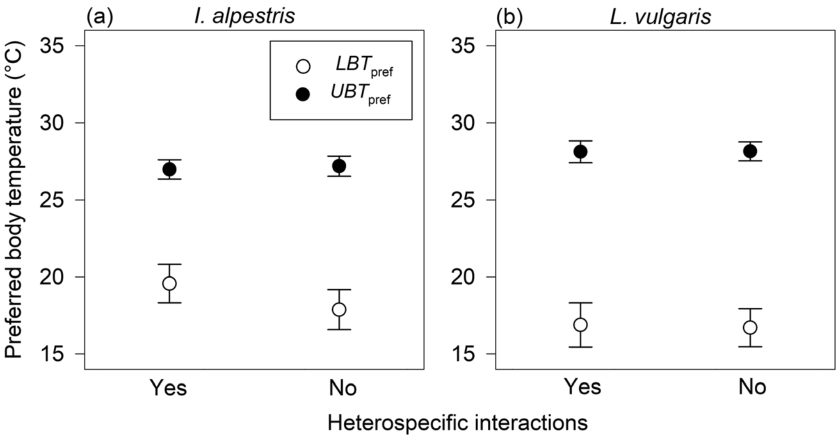

Fig. 2 Lower (LBT_{pref}) and upper (UBT_{pref}) boundaries of preferred body temperature ranges in larvae of (**a**) *I. alpestris* and (**b**) *L. vulgaris* reared under the presence or absence of heterospecific interactions. Note that *I. alpestris* larvae maintained their body temperatures within a narrower range than *L. vulgaris*. Values are means with 95% CIs

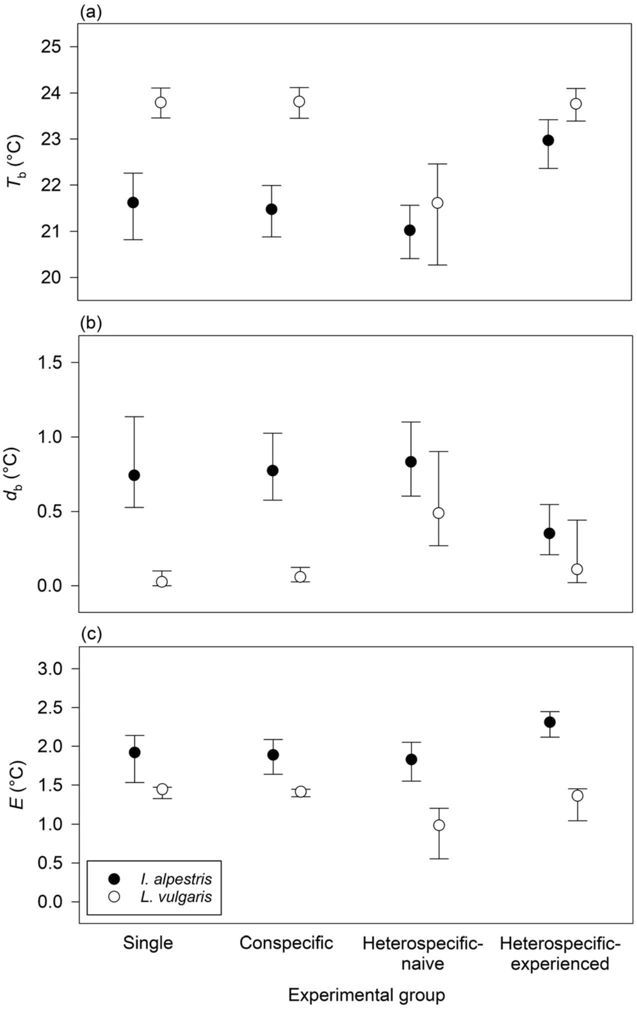

Fig. 3 Body temperatures (T_b), the index of accuracy of thermoregulation (d_b), and the index of effectiveness of thermoregulation (E) of newt larvae in a vertical thermal gradient under the presence or absence (single) of con- and heterospecific social interactions. Heterospecific interactions were performed using larvae with or without (naïve) previous exposure to the other species. Note the effect of heterospecific interactions on these traits. Values are means with 95% CIs

Discussion

Theory predicts that species interactions could affect the plasticity of behavioral thermoregulation in two ways, i.e., through developmental or seasonal plasticity of the T_{pref} range and through an acute shift in body temperature distribution (Gvoždík 2012; Little and Seebacher 2016; Fig. 1). Our results show that interspecific interactions during development had a minor influence on T_{pref} in newt larvae. Contrary to the prediction, social interactions induced a plastic shift in behavioral thermoregulation not only in the subordinate smaller species, but in both taxa. In the subordinate species, the acute thermoregulatory response was induced solely by an immediate reaction to heterospecifics in non-experienced (naïve) larvae. However, in the dominant species, previous experience with interspecific interactions, i.e., developmental plasticity, modified acute thermoregulatory responses to heterospecifics only. Social interaction-induced developmental and acute plasticity affected the distribution of body temperatures and, accordingly, the accuracy and effectiveness of behavioral thermoregulation, in this system. Given the similar influence of ecologically realistic thermal regimes on growth rates in both taxa, these thermoregulatory modifications may potentially affect the life history of larvae developing in the same aquatic habitat.

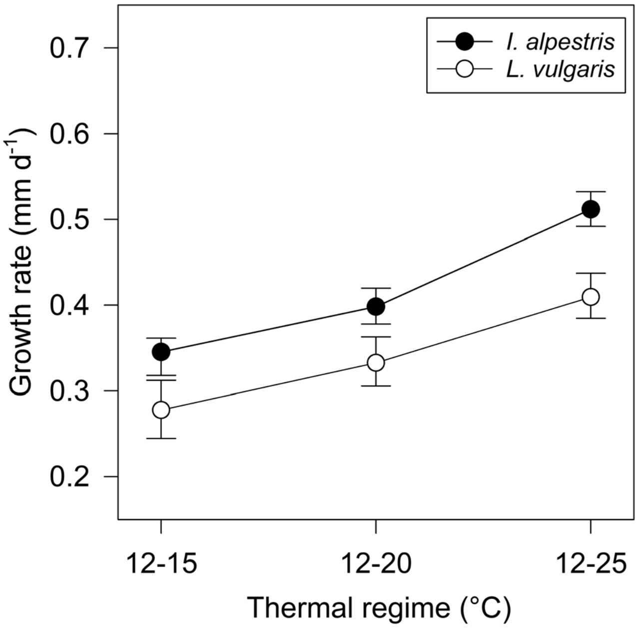

Fig. 4 Somatic growth rates in larvae of two newt species exposed to three thermal regimes. *Ichthyosaura alpestris* larvae grow faster than *Lissotriton vulgaris* consistently across temperatures. Values are means with 95% CIs

Preferred body temperatures were unaffected by previous experience with heterospecific interactions. Although this trait can be modified by various factors in alpine newts, such as thermal acclimatization (Hadamová and Gvoždík 2011), digestion (Gvoždík and Kristín 2017), or reproduction (Toufarová and Gvoždík 2016), our results suggest that it is rather insensitive to species interactions (Smolinský and Gvoždík 2012; this study). Given the importance of T_{pref} in characterizing species' thermal requirements (Magnuson et al. 1979; Labra et al. 2009; Buckley et al. 2014), this finding suggests good intraspecific trait stability, irrespective of diverse species' interactions within its distribution range.

The acute thermoregulatory response was affected by heterospecific, but not conspecific, social interactions. This is surprising, because published studies have largely demonstrated the effect of conspecific interactions on body temperatures or thermoregulatory efforts (Magnuson et al. 1979; Downes and Bauwens 2002; Tattersall et al. 2012; Rusch and Angilletta 2017). However, the previously studied taxa, i.e., crayfish and lizards, are well-known for their aggressive behavior among conspecifics, especially in males. Higher conspecific aggression is assumed also in theory because of a higher resource overlap within rather than between species (Peiman and Robinson 2010). Although newt larvae are aggressive towards each other, the presence of heterospecifics influenced their growth and injury rates more than with only conspecifics (Hloušková et al. 2018). Our results suggest that heterospecific, not conspecific, social interactions are also a relevant factor affecting thermoregulatory effort in newt larvae.

Previous exposure to interspecific social interactions increased body temperatures and the accuracy of thermoregulation in larvae of the dominant species. The most likely explanation for this is that in the presence of heterospecifics, experienced larvae of the dominant species thermoregulate more carefully to maintain their body temperatures close to thermal optima. This should provide an advantage in behavioral interference traits (Huey and Slatkin 1976). Alternatively, the experienced larvae of the dominant species may follow larvae of the inferior species to warmer water, because they are more aggressive against heterospecifics than the inferior species (Hloušková et al. 2018). However, this scenario suggests it would also be beneficial for the inferior species to relax its thermoregulatory efforts and, thereby, avoiding the risk of injuries (Downes and Bauwens 2002). Finally, larvae of the larger species may chase smaller species to warmer water, because they feed on their body parts. This explanation also seems unlikely because these species do not eat each other (Babik 1998). The increase in injury frequency has non-detectable positive or negative effects on the dominant and inferior species, respectively (Hloušková et al. 2018), which is inconsistent with the definition of predation or intraguild predation (Polis et al. 1989). In addition, newt larvae respond to real predation risks by shifting their position within water columns (Gvoždík et al. 2013).

Naïve larvae of the competitively inferior species decreased their body temperatures and the accuracy of thermoregulation. This concurs with previous findings in fish and lizards (Magnuson et al. 1979; Downes and Bauwens 2002), where inferior species avoid aggressive interference with dominant species by moving to the colder end of a thermal gradient. It should be noted that our results pertain only to naïve individuals. The co-occurrence of both species in the same habitat precludes acute heterospecific interactions between naïve larvae. However, this result suggests the examined species may vary in their behavioral responses to human introductions of other newt species at the larval stage. This issue should be considered in conservation management, i.e., reintroduction, translocation, relocation, and repatriation (Dodd and Seigel 1991; Semlitsch 2002), of amphibian populations.

Dominant *I. alpestris* larvae grew faster across the studied temperatures than the larvae of the subordinate species, *L. vulgaris*. Although larval growth rates should be used as a fitness proxy with caution (Earl and Whiteman 2015), body size is a major determinant of species interactions, because its variation reduces resource overlap and predation risk (Werner and Gilliam 1984; Boukal 2014). Indeed, larval body size influences both predator-induced selection and ecological competition in newts (Van Buskirk 2007; Gvoždík and Smolinský 2015; Hloušková et al. 2018). Our results suggest that if larvae of the dominant species maintain elevated body temperatures in the presence of the inferior species, the thermoregulatory shift will be beneficial for them in terms of accelerated growth rates. However, at least two factors must be considered before adopting this conclusion. First, the opportunity for thermoregulation varies substantially in newt aquatic habitats depending on the maximum depth of water columns and the degree of canopy cover (Hadamová and Gvoždík 2011). Second, although heterospecific social interactions increases mean body temperatures and thermoregulatory accuracy ($d_b = 0.4$ [0.2, 0.5] °C; this study) in the dominant species, its common predator, dragonfly larvae, affects behavioral thermoregulation to a greater magnitude and in the opposite direction ($d_b = 3.4$ [2.8, 4.1] °C; Gvoždík et al. 2013). Accordingly, in the presence of both predators and competitors, the effect of predators on behavioral competition should prevail, resulting in fact in reduced growth rates. Hence, the potential of behavioral thermoregulation in affecting larval life history is likely to be highly context-dependent in this system.

Our results showed the complex influence of interspecific social interactions on behavioral thermoregulation. Instead of independent effects in developmental plasticity of thermal preferences and acute plasticity on body temperature distribution, developmental exposure to conspecifics altered the acute plastic response to conspecifics. This is noteworthy on several levels. First, it provides necessary feedback for developing new models of biotic influences on behavioral thermoregulation, which should incorporate both developmental and acute physio-behavioral responses. Compared with other species interactions, social influences have received only limited attention from theoretical viewpoints. Second, behavioral thermoregulation has been considered an important mechanism for reducing exposure to local climatic change, especially in terrestrial ectotherms (Williams et al. 2008; Kearney et al. 2009; Huey et al. 2012). However, the evaluation of thermoregulatory effectiveness in coping with climate change is complicated by its sensitivity to species interactions, which may affect behavioral thermoregulation asymmetrically and in opposite directions. Finally, the ecological implications of interaction-modified thermoregulatory responses are still assumed rather than empirically tested. Examining the ecological relevance of thermoregulatory plasticity provides interesting research agenda for further field or mesocosm studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the use of animals were followed. All experimental procedures were approved by the Expert Committee for Animal Conservation of the Institute of Vertebrate Biology AS CR (research protocol no. 135/2016). The Agency for Nature Conservation and Landscape Protection of the Czech Republic issued permission to capture the newts (1154/ZV/2008).

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