Local adaptation and the potential effects of a contaminant on predator avoidance and antipredator responses under global warming: a space-for-time substitution approach

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Abstract

The ability to deal with temperature-induced changes in interactions with contaminants and predators under global warming is one of the outstanding, applied evolutionary questions. For this, it is crucial to understand how contaminants will affect activity levels, predator avoidance and antipredator responses under global warming and to what extent gradual thermal evolution may mitigate these effects. Using a space-for-time substitution approach, we assessed the potential for gradual thermal evolution shaping activity (mobility and foraging), predator avoidance and antipredator responses when *Ischnura elegans* damselfly larvae were exposed to zinc in a common-garden warming experiment at the mean summer water temperatures of shallow water bodies at southern and northern latitudes (24 and 20°C, respectively). Zinc reduced mobility and foraging, predator avoidance and escape swimming speed. Importantly, high-latitude populations showed stronger zinc-induced reductions in escape swimming speed at both temperatures, and in activity levels at the high temperature. The latter indicates that local thermal adaptation may strongly change the ecological impact of contaminants under global warming. Our study underscores the critical importance of considering local adaptation along natural gradients when integrating biotic interactions in ecological risk assessment, and the potential of gradual thermal evolution mitigating the effects of warming on the vulnerability to contaminants.

Introduction

A key challenge for ecotoxicology is to understand how global warming will interact with contaminants to shape the *in situ* persistence of populations (Noyes et al. 2009; Hooper et al. 2013; Moe et al. 2013). Higher temperatures typically increase the toxicity of contaminants (Noyes et al. 2009; but see pyrethroids: Harwood et al. 2009) such as metals (Sokolova and Lannig 2008). Yet, a largely unresolved applied evolutionary question in this context is whether gradual thermal evolution will reduce or enlarge (through genetic trade-offs) the impacts of contaminants under global warming (Moe et al. 2013). To persist locally under global warming, animals will not only have to be able to deal with the temperature increase and with temperature-induced changes in interactions with anthropogenic stressors such as contaminants (Noyes et al. 2009), but also with the changed interactions with natural enemies such as predators (Gilman et al. 2010). At higher temperatures, prey may become more vulnerable to predators if the attack efficiency of the predator is increased relative to the escape efficiency of the prey (Kruse et al. 2008; De Block et al. 2013). An important consideration thereby is that contaminants may shape the prey’s vulnerability to predation by affecting its activity levels, predator avoidance and antipredator responses (Mogren and Trumble 2010). Therefore, it is crucial to understand how contaminants will affect these behaviours under global warming and to what extent gradual thermal evolution may mitigate these effects.

Considerable progress has been made in the study of the effect of contaminants on activity levels, predator avoidance...
and antipredator responses (Mogren and Trumble 2010; Sornom et al. 2012; Cothran et al. 2013). Yet, how a temperature increase may modulate the effect of a contaminant on these fitness-related traits has rarely been studied (but see e.g. Broomhall 2004). The few experiments that focused on this used a ‘step-increase’ temperature experiment (De Frenne et al. 2013) at one latitude and therefore do not allow assessing the role of long-term gradual thermal evolution in mediating the impact of a temperature increase and the associated changes in sensitivity to contaminants. A key tool to evaluate the potential of long-term thermal evolution mitigating the effects of global warming at high latitudes is the space-for-time substitution approach (Dunne et al. 2004; Fukami and Wardle 2005; De Frenne et al. 2013). In this approach, animals from different latitudes are tested at two temperatures, whereby the projected temperature increase in the high-latitude sites matches the current temperature in the low-latitude sites (De Block et al. 2013). Such approach may inform us whether local thermal adaptation makes animals differentially vulnerable to contaminants, a key question for risk assessment at high latitudes under future climate scenarios (Moe et al. 2013).

In the current study, we assessed the vulnerability of an aquatic insect to a metal with regard to mobility, foraging, predator avoidance and antipredator responses under global warming. To test whether gradual thermal evolution may mitigate these effects at high latitudes, we applied a space-for-time substitution approach using a common-garden warming experiment with replicated populations from low and high latitudes spanning > 1500 km. At each latitude, two randomly chosen populations were sampled, namely Salette (+45°43′30.58″ N, +5°22′23.92″E) and Arandon (+45°42′35.64″N, +5°25′47.28″E) for France; and Kalmar Dämme (56°40′9.84″N, 16°17′48.48″E) and Längviken (+56°39′11.88″N, +16°20′27.64″E) for Sweden. All populations were situated at shallow water bodies containing large dragonfly larvae (Anax imperator, Aeshnidae) as predators. Zinc concentrations in these populations were below detection limit (water: <2.5 µg/L, sediment: <146 µg/g dw sediment) as measured using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo scientific, ICAP 6300 Duo, Waltham, MA, USA).

In June 2010, we collected 10 mated females per population and placed them individually in small plastic vials with wet filter paper for oviposition. Eggs of each female were kept separately and were transferred to the laboratory in Belgium. Throughout the rearing experiment, eggs were incubated and larvae were reared at a water temperature of 20 or 24°C and a photoperiod of 16:8 h light/dark. Temperatures of 20 and 24°C reflect the mean summer water temperatures in shallow ponds in southern Sweden and southern France, respectively (De Block et al. 2013). Importantly, the 4°C temperature difference also corresponds with the predicted temperature increase by 2100 under IPCC scenario A1FI (IPCC 2007). Larvae were kept in group for the first 10 days to enhance survival (De Block and Stoks 2003). Thereafter, they were allocated individually to plastic vials (7.5 cm height, 3.5 cm diameter) filled to a height of 6 cm with dechlorinated tap water. Vials were placed in one of the three temperature-controlled water baths per rearing temperature and were regularly redistributed. All six water baths were placed in the same
room to ensure equal condition such as light regimes. Larvae were fed *Artemia* nauplii *ad libitum* (459 ± 48, mean ± SE, n = 10) 5 days per week. When larvae moulted into the final instar, larvae from each rearing temperature were introduced in the exposure experiment at their respective temperature. Note that by doing so, all larvae had been fully acclimated to their experimental temperature (starting from the egg stage) before we tested the treatment effects.

**Experimental design**

To test whether the effects of zinc exposure on activity, predator avoidance and antipredator responses depend on temperature and latitude of origin, we set up a full factorial experiment with 2 populations per latitude × 2 latitudes × 2 temperatures (20 and 24°C) × 3 zinc concentrations (0, 50, 150 mg zinc/L). Throughout the experiment, we did not keep track of female identity of the larvae but we randomly, and as equally as possible, distributed larvae of each female across the six combinations of temperature and zinc concentration. This avoided that potential differences in the responses among families (Hopkins et al. 2013) would interfere with the interpretation of the treatment effects. The zinc concentrations were based on a previous study on another coenagrionid damselfly (*Argia* sp.) that reported an LC₅₀ 48 h of 320 mg/L (Wurtz and Bridges 1961). The chosen zinc concentrations are very high but have been observed in natural water bodies in contaminated areas in Europe (e.g. Nieto et al. 2007). Note that damselfly larvae are very resistant to zinc and that similar effects as the ones reported here are likely to occur at much lower zinc levels in other aquatic organisms that are more sensitive to zinc. We daily prepared the zinc solution based on a stock solution of ZnCl₂ (5 g zinc/L dissolved in MQ water), which was stored in the dark at 4°C. We diluted the stock solution with synthetic pond water (for details see Janssens and Stoks 2013a). Each larval activity test was run on day six of the exposure period and closely followed the protocol by Janssens and Stoks (2012). We observed the larval activity without predator cues first to avoid any lag effect of predator cues during the second observation period. Coenagrionid damselfly larvae do not show food saturation during the second observation period (Stoks and Johansson 2000; Stoks et al. 2005b; Janssens and Stoks 2012). Therefore, any difference in larval activity levels between the two observation periods can be attributed to predation risk. Prior to the activity test, each larva was weighted to the nearest 0.01 mg to correct for differences in individual body mass.

For the first observation period, each larva was acclimated for 7 min in a plastic container (15 cm × 10 cm × 12.5 cm) filled with 600 mL of medium corresponding to the zinc treatment of a given larva. As prey, *Artemia* nauplii (3560 ± 289, mean ± SE, n = 5) were then added to the container. We scored three larval activities for 7 min: general mobility (walking) and two traits directly linked to foraging (head orientations towards the prey and feeding strikes; Janssens and Stoks 2012). Walking was defined as the movement of a larva when at least one leg changed position; a head orientation was the movement of the head towards a prey item without moving the legs and a feeding strike was a quick extension of the labium towards a prey item. After 7 min, the larva was transferred to another container with the same medium, but with the addition of 1 mL predator medium. Again, a 7-min acclimation period was followed by a 7-min observation period.

We daily prepared the predator medium in a standardized way to keep the concentration of the chemical predator cues constant throughout the experiment. We had two plastic holding tanks (7.5 × 7.5 × 10 cm, filled with 400 mL of dechlorinated tap water), which each contained one larval *Anax imperator* dragonfly predator of the same size (ca. 30 mm). The water of these tanks was used to prepare the chemical cues. Two hours before the water extraction from these tanks, we fed each dragonfly one *I. elegans* nauplii (3560 ± 289, mean ± SE, n = 5) were then added to the container. We scored three larval activities for 7 min: general mobility (walking) and two traits directly linked to foraging (head orientations towards the prey and feeding strikes; Janssens and Stoks 2012). Walking was defined as the movement of a larva when at least one leg changed position; a head orientation was the movement of the head towards a prey item without moving the legs and a feeding strike was a quick extension of the labium towards a prey item. After 7 min, the larva was transferred to another container with the same medium, but with the addition of 1 mL predator medium. Again, a 7-min acclimation period was followed by a 7-min observation period.

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damsel fly larva which was always eaten within the first 10 min. After 2 h, we extracted ten ml water from each holding tank. In the pooled 20 mL volume, we homogenized one I. elegans larva and the resulting cocktail made up the predator medium that was added to the experimental damselfly larvae. Afterwards, we renewed the water in the holding tanks. Large aeshnid dragonfly larvae are important predators of Ischnura damselfly larvae (Stoks et al. 2005a), occur across the entire geographical distribution from France to Sweden (Dijkstra 2006) and were present in all four study populations. This way, damselfly larvae received a natural cocktail of predator kairomones and conspecific alarm cues to which they are known to react by reducing their activity levels (Stoks et al. 2003; Janssens and Stoks 2012).

Escape swimming speed was quantified directly after the activity test closely following the protocol of Stoks and McPeek (2006) using high-speed imaging. After the activity test, each larva was acclimated for 1 min in a white tray (20 cm × 15 cm × 4 cm) filled with 600 mL of dechlorinated fresh water at the rearing temperature of the larva. The larva was poked gently on the abdomen using a plastic pipette to mimic a predatory attack. Three successful swimming bouts were recorded using a high-speed camera (200 Hz, Basler AG, Ahrensburg, Hamburg, Germany) connected to a computer and controlled by Streampix 3 (NorPix Inc., Montreal, Quebec, Canada). Escape swimming speed (expressed in cm/s) was quantified based on the distance a larva swam during the first 0.2 s using the program Image-Pro Plus 5 (Media Cybernetics Inc., Warrendale, PA, USA). This initial response is most relevant for damselfly larvae to escape an attack from a sit-and-wait predator such as dragonfly larvae that do not pursue their prey after attacking it (Dayton et al. 2005). We used the average speed of three swimming bouts per larva for statistical analyses.

Sample sizes per response variable varied between 34 and 42 for each combination of latitude, zinc concentration and temperature, except for the treatment combinations with Swedish larvae tested at 24°C where sample sizes were lower (n = 13–14) due to higher mortality during the pre-exposure and exposure periods. Exact sample sizes per treatment combination are given in the Figures; the total number of larvae tested was 406 for activity levels and 379 for escape swimming speed.

Statistical analyses
To test for effects of the zinc treatment, temperature and latitude on escape swimming speed, we ran an ANCOVA using the mixed procedure of SAS v9.3 (SAS Institute Inc., Cary, NC, USA). Wet mass was included as a covariate. The analyses of the behavioural response variables were similar besides the fact that we considered the same behaviour measured in the absence and in the presence of predator chemical cues as repeats of that larva. Hence, we ran separate repeated-measures ANCOVAs per behavioural response variable (walking, head orientations and feeding strikes). In these analyses, predation risk was the within-subject factor. All behaviours were log(x+1)-transformed to meet ANOVA assumptions. Population nested in latitude was initially included as a random factor, but as it had no significant effect on any of the response variables, we removed it from the final models.

Results
Activity levels
Overall, French larvae were more mobile and foraged more actively than Swedish larvae, and all three behaviours occurred more frequently at 24°C than at 20°C (main effects Latitude and Temperature, Table 1, Fig. 1). Both mobility and foraging activity were reduced with increasing zinc concentration (main effect Zinc, Table 1, Fig. 1). Moreover, at 24°C, these zinc-induced activity reductions were stronger in Swedish than in French larvae (Latitude × Temperature × Zinc, Table 1).

Larvae reduced both their mobility and foraging activity in the presence of predator cues (main effect Predator cue, Table 1, Fig. 1). These predator-induced activity reductions depended upon latitude, temperature and zinc concentration. French larvae showed a more substantial predator-induced reduction in head orientations than Swedish larvae (Predator cue × Latitude, Table 1). All three behavioural antipredator responses depended upon temperature (Predator cue × Temperature, Table 1, Fig. 1) but not in a consistent way: the response in walking activity (trend) and feeding strikes was stronger at 20°C, while the response in head orientations tended to be stronger at 24°C. Overall, the predator-induced reductions in walking activity and head orientations were weaker in the presence of zinc (Predator cue × Zinc, Table 1, Fig. 1). For walking activity, this interactive effect was not present in Swedish larvae at 20°C generating a Predator cue × Latitude × Temperature × Zinc interaction (Table 1, Fig. 1A,B).

Escape swimming speed
French larvae swam faster than Swedish larvae (main effect Latitude, Table 1, Fig. 2). Exposure to zinc reduced swimming speed (main effect Zinc, Table 1, Fig. 2). This zinc-induced reduction was about twice as large in Swedish larvae (26%, control compared to 150 mg/L) than in French larvae (12%), as also indicated by a Latitude × Zinc interaction (Table 1).
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related traits likely negatively affect fitness as they will result
in a lower food intake, hence may contribute to a zinc-
reduced all three scored behaviours when exposed to zinc,
that metals have in general a stronger
negative impact at higher temperatures (Sokolova and Lan-
may offset the pattern that metals have in general a stronger
mortality except in Swedish larvae at 24°C (Dinh Van et al.
the zinc to have a stronger effect on the activity levels at the
tial local thermal adaptation across latitudes likely caused
pre-exposure period at 24°C in Swedish larvae in current
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°C than at 20°C, reflecting the general pattern of
higher temperatures in aquatic ecto-
In agreement with studies on other taxa, damsel-fly larvae
reduced all three scored behaviours when exposed to zinc,
which can be explained by tissue damage, impairment of
signal transduction, and/or the lowered energy content
(Mogren and Trumble 2010). Reductions in the foraging-
related traits likely negatively affect fitness as they will result
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A slower life history in the presence of zinc may further enlarge the zinc effects in nature by extending the larval exposure period (e.g. Snodgrass et al. 2005).

A key finding was the stronger zinc-induced activity reduction for all three behaviours in Swedish larvae compared with French larvae when tested at 24°C. This pattern is consistent with differential thermal adaptation across latitudes. Indeed, the mean summer water temperature in shallow ponds in southern Sweden is 20°C, and therefore, larvae likely perceive 24°C, the mean summer water temperatures in shallow ponds in southern France, as a suboptimal temperature (De Block et al. 2013). We have shown before in this species that French larvae outperform Swedish larvae at 24°C in terms of growth rate and that this is genetically determined (Shama et al. 2011; Stoks et al. 2012). In line with this, we also observed a higher mortality during the pre-exposure period at 24°C in Swedish larvae in current study (see Materials and methods). This pattern of differential local thermal adaptation across latitudes likely caused the zinc to have a stronger effect on the activity levels at the higher temperature in Swedish larvae but not in French lar-

Discussion

General activity levels
In line with the fact that multivoltine French larvae have
less time per generation to complete larval development than semivoltine Swedish larvae, French larvae were more
mobile and foraged more actively. In accordance, French
larvae show a higher growth rate and have a shorter larval
development compared with Swedish larvae (Stoks and De
Block 2011; Stoks et al. 2012). Activity levels of larvae were
higher at 24°C than at 20°C, reflecting the general pattern of
higher activities at higher temperature in aquatic ecto-

Table 1. The results of ANCOVAs testing for the effects of latitude, temperature and zinc on activity levels and escape swimming speed of Ischnura elegans larvae. The successive behaviours scored in the absence and presence of predator cues were treated as repeats; ‘predator cue’ was the repeated factor.

| Effect           | Walks                     | Head orientations | Feeding strikes | Escape swimming speed |
|------------------|---------------------------|-------------------|----------------|-----------------------|
|                  | df1, df2 F P              | df1, df2 F P      | df1, df2 F P   | df1, df2 F P          |
| Latitude (Lat)   | 1, 393 3.15 0.073 <0.0001 | 1, 393 81.00 17.32 | 1, 393 80.02 17.32 | 1, 393 47.36 <0.001   |
| Temperature (Temp) | 1, 393 88.66 <0.0001 | 1, 393 82.68 <0.0001 | 1, 393 81.00 17.32 | 1, 393 47.36 <0.001   |
| Zinc (Zn)        | 2, 393 7.93 <0.0001 | 2, 393 14.10 <0.0001 | 2, 393 33.31 <0.0001 | 2, 393 16.65 <0.001   |
| Lat × Temp       | 1, 393 0.29 0.61 0.021   | 1, 393 5.56 0.021 | 1, 393 1.69 0.22 | 1, 393 2.32 0.13      |
| Lat × Zn         | 2, 393 1.21 0.29 0.61    | 2, 393 0.41 0.61 0.61 | 2, 393 4.09 0.016   | 2, 393 3.34 0.037     |
| Temp × Zn        | 2, 393 1.44 0.23 0.61    | 2, 393 1.30 0.23 0.61 | 2, 393 0.28 0.74    | 2, 393 1.11 0.33      |
| Lat × Temp × Zn  | 2, 393 5.33 0.006        | 2, 393 3.82 0.028 0.028 | 2, 393 4.02 0.021   | 2, 393 0.15 0.86      |
| Mass             | 1, 393 0.59 0.46 0.37    | 1, 393 0.73 0.37 0.37 | 1, 393 11.03 0.001  0.00065 | 1, 393 16.80 <0.001   |
| Predator cues    | 1, 393 8.47 0.040 0.019 0.019 | 1, 393 5.68 0.019 | 1, 393 11.69 0.00065 | 1, 393 16.80 <0.001   |
| Predator cues × Lat | 1, 393 0.91 0.34 0.00085 | 1, 393 11.18 0.00085 | 1, 393 11.03 0.001  0.00065 | 1, 393 16.80 <0.001   |
| Predator cues × Temp | 1, 393 3.48 0.063 0.059 | 1, 393 3.55 0.059 0.059 | 1, 393 8.68 0.034   | 2, 393 17.90 0.16     |
| Predator cues × Zn | 2, 393 3.75 0.024 0.0001 | 2, 393 9.30 0.0001 0.0001 | 2, 393 1.79 0.16     | 2, 393 1.79 0.16      |
| Predator cues × Lat × Temp | 1, 393 0.13 0.72 0.13 | 1, 393 2.29 0.13 0.13 | 1, 393 1.85 0.18    | 2, 393 1.85 0.18      |
| Predator cues × Lat × Zn | 2, 393 0.10 0.90 0.02 | 2, 393 0.84 0.02 0.02 | 2, 393 2.15 0.14    | 2, 393 2.15 0.14      |
| Predator cues × Temp × Zn | 2, 393 1.71 0.20 0.07 | 2, 393 0.23 0.07 0.07 | 2, 393 1.24 0.33    | 2, 393 1.24 0.33      |
| Predator cues × Lat × Temp × Zn | 2, 393 3.09 0.045 | 2, 393 0.92 0.045 0.045 | 2, 393 1.14 0.29    | 2, 393 1.14 0.29      |

Significant P values (P < 0.05) are indicated in bold.
Predator avoidance and antipredator responses

Zinc exposure impaired key predator avoidance and antipredator responses in damselfly larvae: the predator-induced reductions in walking and head orientations were much less pronounced in the presence of zinc, and zinc caused a reduction in escape swimming speed. Reductions in activity levels and high escape speeds are widespread adaptive predator avoidance and antipredator responses to avoid being detected (e.g. Gyssels and Stoks 2005; Stoks et al. 2012) and has been explained by a reduced capacity to detect predators, an increased energy demand coupled with the inhibition of aerobic ATP production, and damage of neuromuscular transmission (e.g. Lurling and Schef-fer 2007; Sokolova and Lannig 2008; Sornom et al. 2012). Such reduced responsiveness to chemical predator cues in the presence of metals may make prey more vulnerable to predation as shown for example in juvenile coho salmon exposed to copper (McIntyre et al. 2012). As the larvae exposed to zinc still had the lowest activity levels in the presence of predation risk, the reduced predator avoidance is unlikely to contribute to a higher vulnerability to predation. Yet, reduced escape speeds will result in a higher chance of being killed by attacking predators such as dragonfly larvae (Strobbe et al. 2009, 2010).

Another key finding was that the zinc-induced reduction in escape speed was more pronounced in Swedish larvae. This magnifies the latitudinal difference in escape speed with higher escape speeds in French larvae; the latter pattern matches the higher predator densities at low latitudes (R. Stoks P. Lambret and E. Svensson, unpublished data; see also Laurila et al. 2008). Possibly, the stronger predation pressures in French populations selected for better physiological defence mechanisms to avoid a too strong zinc-induced reduction in escape speed. Importantly, our findings indicate that zinc exposure would increase the vulnerability to attacking predators stronger in Swedish larvae compared with French larvae.

Evolutionary perspectives with regard to global warming and ecological risk assessment

How organisms will cope with the changed interactions with contaminants as well as with predators will be a major...
factor in shaping the in situ persistence of their populations under global warming (Millennium Ecosystem Assessment 2005; Gilman et al. 2010; Sih et al. 2011; Urban et al. 2012). Moreover, as confirmed by our results, warming and contaminants may interact (Noyes et al. 2009) and contaminants such as metals may increase the vulnerability to predation. In a logical step forward, our study addressed the combined impact of warming and contaminants on activity, predator avoidance and antipredator responses at different latitudes. We will further highlight the here identified roles of local adaptation as a potential mediator of the effects of global warming and as an important factor to include in ecological risk assessment.

Our results tentatively suggest that in the absence of thermal evolution, hence under a scenario where Swedish larvae do not develop thermal adaptation to 24°C, and assuming all else staying equal, the predicted 4°C temperature increase by 2100 under IPCC scenario A1FI will make high-latitude populations more sensitive to contaminants such as zinc in terms of mobility and foraging activity compared with the current ambient summer water temperatures at the high latitude. Note that this, however, will critically depend on how warming will affect the susceptibility to zinc of the food and predators of the damselfly larvae. Moreover, it should be noted that increasing the temperature may also beneficially affect other traits of the Swedish populations. While it is highly unlikely (based on thermal degree days needed to complete a generation) that a 4°C warming will result in a switch from semivoltinism towards univoltinism at the high latitude, such temperature increase will reduce the exposure duration to the contaminant by shortening larval development times. A final aspect that needs consideration is the migration of low-latitude I. elegans genotypes to high-latitude populations. This is to be expected because odonates are among the taxa showing the strongest northward range shifts under global warming (Hickling et al. 2006), and strong gene flow occurs within the range of coenagrionid damselflies (Johansson et al. 2013). Our results suggest that invading low-latitude genotypes may perform better under global warming, especially in contaminated high-latitude sites, compared with the local high-latitude animals both during intraspecific competitive interactions as they show higher foraging levels and during interactions with invertebrate predators as they have a better escape swimming speed.

The used space-for-time substitution approach where the predicted 4°C temperature increase matches the current latitudinal difference in mean summer water temperatures of shallow water bodies inhabiting I. elegans between southern France and southern Sweden suggests that gradual thermal evolution may help the high-latitude populations to persist locally. This is based on the observation that the French larvae currently living at mean summer water temperatures of 24°C were less sensitive to zinc at 24°C than the Swedish larvae at 24°C, and on the assumption that the responses of the French currently living at 24°C are a good proxy for the expected responses of the Swedish larvae when they have enough time to adapt to a 4°C temperature increase by 2100.

Our findings highlight two implications for the role of local adaptation in ecological risk assessment. First, there is increasing awareness that impaired antipredator mechanisms may increase the negative effects of contaminants that are not captured in the typical single-species toxicity tests carried out in isolation (e.g. Brooks et al. 2009). The impairment of antipredator mechanisms has even been suggested as an index of heavy metal pollution (Lefcort et al. 2000). Our results add an important insight into these studies by demonstrating latitude-specific effects on the contaminant-induced impairment of antipredator mechanisms. This illuminates the critical importance of considering local adaptation along natural gradients when integrating biotic interactions in ecological risk assessment.

Second, whether local thermal adaptation makes animals differentially vulnerable to contamination is a key question for risk assessment of contaminants under future climatic scenarios (Moe et al. 2013). We identified large-scale latitudinal differentiation in the vulnerability of I. elegans damselfly larvae to zinc for the studied ecologically relevant traits with high-latitude populations being more sensitive in terms of swimming speed at both rearing temperatures and in terms of activity levels at the higher temperature. This pattern is opposite to the expectation that genetic adaptations to a warmer climate will come at the cost of a reduced tolerance to contaminants (Moe et al. 2013). This indicates that the assumed trade-off between tolerance to the warmer low-latitude climate and tolerance to contaminants may not be general. Importantly, the considerable latitude-dependent differences in vulnerability that we observed were repeatable across the two studied populations within a given latitude and occurred, while none of the populations had a history of metal contamination. While interpopulation differences in the vulnerability of animals to metals are well documented with regard to differences in local metal concentrations (reviewed in Morgan et al. 2007; Khan et al. 2011), little is known with regard to pristine habitats. In the only similar study, Cherkasov et al. (2010) documented the opposite pattern where oysters from two low-latitude populations were more sensitive to cadmium in terms of mitochondrial respiration than oysters from a high-latitude population. This study, together with ours, underscores the need to test contaminants at a large geographical scale and to evaluate their temperature-dependent impact across natural temperature gradients (Clements et al. 2012).
To conclude, our study illuminated the additional insights that can be obtained by assessing the impact of contaminants under global warming by using a space-for-time substitution approach. Combining a latitudinal gradient with a common-garden warming experiment not only revealed the potential of local adaptation to mitigate the effects of global warming in a contaminated world but also the need to consider local adaptation along natural gradients in ecological risk assessment. Space-for-time substitution studies have largely been neglected in ecotoxicology (Moe et al. 2013), and this despite the increasing awareness of the importance to consider evolution (Coutellec and Barata 2011; Jansen et al. 2011; Hammond et al. 2012; Cothran et al. 2013; Hopkins et al. 2013). More general, these substitution studies still are an underutilized tool in global change ecology (De Frenne et al. 2013). Our study illustrates the potential of this approach to explore topics at the interplay of both disciplines, and we advocate their addition to the standard toolbox of methods to study the role of evolution in ecotoxicology.

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Data archiving statement

Data for this study are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.tn715.

Literature cited

Brix, K. V., D. K. DeForest, and W. J. Adams 2011. The sensitivity of aquatic insects to divalent metals: a comparative analysis of laboratory and field data. Science of the Total Environment 409:4187–4197.
Bronmark, C., and L. A. Hansson 2002. Environmental issues in lakes and ponds: current state and perspectives. Environmental Conservation 29:290–307.
Brooks, A. C., P. N. Gaskell, and L. L. Maltby 2009. Sublethal effects and predator-prey interactions: implications for the ecological risk assessment. Environmental Toxicology and Chemistry 28:2449–2457.
Broomhall, S. D. 2004. Egg temperature modifies predator avoidance and the effects of the insecticide endosulfan on tadpoles of an Australian frog. Journal of Applied Ecology 41:105–113.
Cherkasov, A. S., C. Taylor, and I. M. Sokolova 2010. Seasonal variation in mitochondrial responses to cadmium and temperature in eastern oysters Crassostrea virginica (Gmelin) from different latitudes. Aquatic Toxicology 97:68–78.
Clements, W. H., C. W. Hickey, and K. A. Kidd 2012. How do aquatic communities respond to contaminants? It depends on the ecological context. Environmental Toxicology and Chemistry 31:1932–1940.
Cothran, R. D., J. M. Brown, and R. A. Relyea 2013. Proximity to agriculture is correlated with pesticide tolerance: evidence for the evolution of amphibian resistance to modern pesticides. Evolutionary Applications 6:832–841.
Coutellec, M. A., and C. Barata 2011. An introduction to evolutionary processes in ecotoxicology. Ecotoxicology 20:493–496.
Dayton, G. H., D. Saenz, K. A. Baum, R. B. Langerhans, and T. J. DeWitt 2005. Body shape, burst speed and escape behavior of larval anurans. Oikos 111:582–591.
De Block, M., and R. Stoks 2003. Adaptive sex-specific life history plasticity to temperature and photoperiod in a damselfly. Journal of Evolutionary Biology 16:986–995.
De Block, M., and R. Stoks 2004. Cannibalism-mediated life history plasticity to combined time and food stress. Oikos 106:587–597.
De Block, M., K. Pauwels, M. Van Den Broeck, L. De Meester, and R. Stoks 2013. Local genetic adaptation generates latitude-specific effects of warming on predator-prey interactions. Global Change Biology 19:689–696.
De Frenne, P., B. J. Graae, F. Rodriguez-Sanchez, A. Kolb, O. Chabriere, G. Decocq, H. De Kort et al. 2013. Latitudinal gradients as natural laboratories to infer species’ responses to temperature. Journal of Ecology 101:784–795.
Dijkstra, K. D. B. 2006. Field Guide to the Dragonflies of Britain and Europe. British Wildlife Publishing, Gillingham, Dorset, UK.
Dinh Van, K., L. Janssens, S. Debecker, M. De Jonge, P. Lambret, V. Nilsson-Orman, L. Bervoets, et al. 2013. Susceptibility to a metal under global warming is shaped by thermal adaptation along a latitudinal gradient. Global Change Biology 19:2625–2633.
Dunne, J. A., S. R. Saleska, M. L. Fischer, and J. Harte 2004. Integrating experimental and gradient methods in ecological climate change research. Ecology 85:904–916.
Fukami, T., and D. A. Wardle 2005. Long-term ecological dynamics: reciprocal insights from natural and anthropogenic gradients. Proceedings of the Royal Society B-Biological Sciences 272:2105–2115.
Gilman, S. E., M. C. Urban, J. Tewksbury, G. W. Gilchrist, and R. D. Holt 2010. A framework for community interactions under climate change. Trends in Ecology & Evolution 25:325–331.
Gyselens, F. G. M., and R. Stoks 2005. Threat-sensitive responses to predator attacks in a damselfly. Ethology 111:411–423.
Hammond, J. I., D. K. Jones, P. R. Stephens, and R. A. Relyea 2012. Phylogeny meets ecotoxicology: evolutionary patterns of sensitivity to a common insecticide. Evolutionary Applications 5:593–606.
Harwood, A. D., J. You, and M. J. Lydy 2009. Temperature as a toxicity identification evaluation tool for pyrethroid insecticides: toxico-kinetic confirmation. Environmental Toxicology and Chemistry 28:1051–1058.
Hassall, C., and D. J. Thompson 2008. The effects of environmental warming on Odonata: a review. International Journal of Odonatology 11:131–153.
Evolution of metal tolerance under global warming

Moe, S. J., K. De Schamphelaere, W. H. Clements, M. T. Sorensen, P. J. Van den Brink, and M. Liess 2013. Combined and interactive effects of global climate change and toxicants on populations and communities. Environmental Toxicology and Chemistry 32:49–61.

Mogren, C. L., and J. T. Trumble 2010. The impacts of metals and metalloids on insect behavior. Entomologia Experimentalis et Applicata 135:1–17.

Morgan, A. J., P. Kille, and S. R. Sturzenbaum 2007. Microevolution and ecotoxicology of metals in invertebrates. Environmental Science & Technology 41:1085–1096.

Nieto, J. M., A. M. Sarmiento, M. Olias, C. R. Canovas, I. Riba, J. Kalman, and T. A. Delvalls 2007. Acid mine drainage pollution in the Tinto and Odile rivers (Iberian Pyrite Belt, SW Spain) and bioavailability of the transported metals to the Huelva Estuary. Environmental International 33:445–455.

Noyes, P. D., M. K. McElwee, H. D. Miller, B. W. Clark, L. A. Van Tiem, K. C. Walcott, K. N. Erwin et al. 2009. The toxicology of climate change: environmental contaminants in a warming world. Environment International 35:971–986.

Shama, L. N. S., M. Campero-Paz, K. M. Wegner, M. De Block, and R. Stoks 2011. Latitudinal and voltinism compensation shape thermal reaction norms for growth rate. Molecular Ecology 20:2929–2941.

Sih, A., M. C. O. Ferrari, and D. J. Harris 2011. Evolution and behavioral responses to human-induced rapid environmental change. Evolutionary Applications 4:367–387.

Snodgrass, J. W., W. A. Hopkins, B. P. Jackson, J. A. Baionno, and J. Broughton 2005. Influence of larval period on responses of overwintering green frog (Rana clamitans) larvae exposed to contaminated sediments. Environmental Toxicology and Chemistry 24:1508–1514.

Sokolova, I. M., and G. Lannig 2008. Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. Climate Research 37:181–201.

Sornom, P., E. Gismondi, C. Vellinger, S. Devin, J. F. Ferrard, and J. N. Beisel 2012. Effects of sublethal cadmium exposure on antipredator behavioural and antitoxic responses in the invasive amphipod Diokerogammarus villosus. PLoS ONE 7:e42435.

Stoks, R., and A. Cordoba-Aguilar 2012. Evolutionary ecology of Odonata: a complex life cycle perspective. Annual Review of Entomology 57:249–265.

Stoks, R., and M. De Block 2000. The influence of predator species and prey age on the immediate survival value of antipredator behaviours in a damselfly. Archiv für Hydrobiologie 147:417–430.

Stoks, R., and M. De Block 2011. Rapid growth reduces cold resistance: evidence from latitudinal variation in growth rate, cold resistance and stress proteins. PLoS ONE 6:e16935.

Stoks, R., and F. Johansson 2000. Trading off mortality risk against foraging effort in damselflies that differ in life cycle length. Oikos 91:559–567.

Stoks, R., and M. A. McPeek 2003. Antipredator behavior and physiology determine Leptes species turnover along the pond-permanence gradient. Ecology 84:3327–3338.

Stoks, R., and M. A. McPeek 2006. A tale of two diversifications: reciprocal habitat shifts to fill ecological space along the pond permanence gradient. American Naturalist 168:550–572.

Stoks, R., M. A. McPeek, and J. L. Mitchell 2003. Evolution of prey behavior in response to changes in predation regime: damselflies in fish and dragonfly lakes. Evolution 57:574–585.
Evolution of metal tolerance under global warming

Janssens et al.

Stoks, R., M. De Block, and M. A. McPeek 2005a. Alternative growth and energy storage responses to mortality threats in damselflies. Ecology Letters 8:1307–1316.

Stoks, R., M. De Block, F. Van de Meutter, and F. Johansson 2005b. Predation cost of rapid growth: behavioural coupling and physiological decoupling. Journal of Animal Ecology 74:708–715.

Stoks, R., I. Swillen, and M. De Block 2012. Behaviour and physiology shape the growth accelerations associated with predation risk, high temperatures and southern latitudes in Ischnura damselfly larvae. Journal of Animal Ecology 81:1034–1040.

Stoks, R., A. N. Geerts, and L. De Meester 2014. Evolutionary and plastic responses of freshwater invertebrates to climate change: realized patterns and future potential. Evolutionary Applications. doi:10.1111/eva.12108.

Strobbe, F., M. A. McPeek, M. De Block, and R. Stoks 2010. Survival selection imposed by predation on a physiological trait underlying escape speed. Functional Ecology 24:1306–1312.

Urban, M. C., L. De Meester, M. Vellend, R. Stoks, and J. Vanoverbeke 2012. A crucial step toward realism: responses to climate change from an evolving metacommunity perspective. Evolutionary Applications 5:154–167.

Wellborn, G. A., D. K. Skelly, and E. E. Werner 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27:337–363.

Woodward, G., D. M. Perkins, and L. E. Brown 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Philosophical Transactions of the Royal Society B-Biological Sciences 365:2093–2106.

Wurtz, C. B., and C. H. Bridges 1961. Preliminary results from macroinvertebrate bioassays. Proceeding of the Pennsylvania Academy of Science 35:51–56.