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Strong Delayed Interactive Effects of Metal Exposure and Warming: Latitude-Dependent Synergisms Persist Across Metamorphosis

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S Supporting Information

ABSTRACT: As contaminants are often more toxic at higher temperatures, predicting their impact under global warming remains a key challenge for ecological risk assessment. Ignoring delayed effects, synergistic interactions between contaminants and warming, and differences in sensitivity across species' ranges could lead to an important underestimation of the risks. We addressed all three mechanisms by studying effects of larval exposure to zinc and warming before, during, and after metamorphosis in *Ischnura elegans* damselflies from high- and low-latitude populations. By integrating these mechanisms into a single study, we could identify two novel patterns. First, during exposure zinc did not affect survival, whereas it induced mild to moderate postexposure mortality in the larval stage and at metamorphosis, and very strongly reduced adult lifespan. This severe delayed effect across metamorphosis was especially remarkable in high-latitude animals, as they appeared almost insensitive to zinc during the larval stage. Second, the well-known synergism between metals and warming was manifested not only during the larval stage but also after metamorphosis, yet notably only in low-latitude damselflies. These results highlight that a more complete life-cycle approach that incorporates the possibility of delayed interactions between contaminants and warming in a geographical context is crucial for a more realistic risk assessment in a warming world.

INTRODUCTION

Current approaches to assess the risk of contaminants to aquatic ecosystems fail to adequately protect biodiversity.^{1–3} This may not be surprising as these approaches are developed for single stressors while stressors may interact,⁴ often do not include delayed postexposure effects,^{5,6} and ignore geographic variation in the sensitivity to pollutants.⁷ However, studies integrating these three key aspects are lacking, yet crucial to arrive at a more realistic ecological risk assessment. This is furthermore important in the context of global warming, which represents a serious threat that may strongly magnify the impact of contaminants.^{8–11} Integrating warming into risk assessment of contaminants has therefore been identified as a major challenge for ecotoxicology.^{12,13}

Delayed postexposure effects of contaminants can be equally strong or even stronger than the initial effects during exposure (e.g., refs 5, 6, 14–17). In animals with a so-called complex life cycle, such delayed effects may be revealed or magnified at metamorphosis, which has been identified as a sensitive, stressful event (“stressful metamorphosis hypothesis”).¹⁸ This is because the process of metamorphosis involves substantial tissue construction and reorganization, and requires a considerable amount of energy.^{19,20} Recent studies demon-

strated severe lethal effects of contaminants in aquatic insects during metamorphosis despite the absence of any effects during the preceding larval exposure period.^{21–23} Yet, also after successful metamorphosis, there is still potential for delayed effects.^{14,23,24} While studies are more frequently considering such delayed effects of single stressors across metamorphosis, and despite the increasing concern that interactions between stressors also occur when they are encountered sequentially,^{25,26} delayed postmetamorphic effects of combined larval exposure to contaminants and other stressors, particularly warming, are very limited (but see refs 27, 28). Understanding how aquatic stressors affect aquatic insect emergence and their postmetamorphic survival is important for managing both aquatic and riparian ecosystems as aquatic insects subsidize the diets of terrestrial insectivores such as spiders and birds.^{29,30}

Populations from the same species may differ widely in their sensitivity to contaminants^{31–33} and to warming^{34,35} because of geographic differences in the evolution of adaptation to these

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69 stressors. While adaptation to one stressor may ameliorate the
70 negative consequences of exposure to another stressor,^{36–38}
71 this has been poorly studied in the context of warming and
72 contaminants, yet crucial to arrive at a better risk assessment in
73 a warming world.⁸ Studies focusing on how differential thermal
74 adaptation in populations from different latitudes creates a
75 geographic signal in the interactive effects of warming and
76 contaminants may be especially revealing. The comparison of
77 contaminant effects in high-latitude populations with low-
78 latitude populations currently experiencing the higher temper-
79 atures that are predicted to occur at the high latitude under
80 global warming, may inform about the impact of gradual
81 thermal evolution on the toxicity of contaminants in a warming
82 world (“space-for-time substitution”).³⁹

83 To address these issues in an integrated way we investigated
84 the effects of larval exposure to a metal and warming on larval
85 and adult fitness-related traits in replicated high- and low-
86 latitude populations of an aquatic insect. More specifically, we
87 documented the combined effects of a metal and warming
88 during and after the metal-exposure period in the larval stage
89 (survival, growth rate, development time), during emergence
90 (emergence ratio), as well as in the terrestrial adult stage (size
91 at emergence, lifespan) in a common garden experiment with
92 the damselfly *Ischnura elegans*. This allowed testing for delayed
93 mortality caused by larval metal exposure and interactive effects
94 with warming before, during and after metamorphosis. High-
95 latitude populations were collected in southern Sweden, where
96 the species is semivoltine (one generation per two years),
97 whereas low-latitude populations were collected in southern
98 France, where the species is multivoltine (three to four
99 generations per year).⁴⁰ This difference in life-history strategy
100 is expected to translate into a different susceptibility to
101 contaminant exposure, with the more fast-lived southern
102 populations being more susceptible, because of a trade-off
103 between growth and investment in detoxification and repair
104 mechanisms.⁴¹ We further tested for a signal of thermal
105 adaptation in how damselflies from both latitudes differed in
106 their responses to the metal and temperature treatment (which
107 matched the IPCC 4 °C warming scenario RCP8.5⁴² at the
108 higher latitude). By comparing the performance of the high-
109 latitude populations at the local cooler and predicted warmer
110 temperatures, predictions can be made about how high-latitude
111 would perform under global warming in the absence of thermal
112 evolution. Comparing them with the low-latitude populations
113 (which have been locally adapted to warmer temperatures),
114 however, simulates a global-warming scenario that also takes
115 into account the performance change due to gradual thermal
116 evolution.

117 ■ MATERIALS AND METHODS

118 **Sampling and Rearing.** Using a common garden rearing
119 experiment, we quantified key life-history traits before, during
120 and after metamorphosis in the damselfly *Ischnura elegans* from
121 two latitudes spanning more than 1500 km. Eggs were collected
122 at three high-latitude locations in southern Sweden (56–57°N)
123 and three low-latitude locations in southern France (43°N)
124 (see [Supporting Information, Appendix S1](#) for details).⁴³ At
125 each location between 9 and 22 mated females were collected
126 and placed individually in small jars with wet filter paper for
127 oviposition. The eggs were transferred to the laboratory in
128 Belgium where they hatched. Larvae were placed individually in
129 200 mL vials with dechlorinated tap water and assigned to a
130 rearing temperature treatment of 20 or 24 °C. These

temperatures reflect the mean water temperatures in shallow
water bodies during summer in southern Scandinavia and
southern France, respectively.⁴⁴ The 4 °C temperature
difference between these latitudes matches the predicted
temperature increase in southern Scandinavia by 2100 under
IPCC scenario RCP8.5.⁴² Therefore, the experimental setup
allows a space-for-time substitution whereby the low-latitude
populations reared at 24 °C provide a proxy for the
evolutionary response of the high-latitude populations to global
warming. Temperature was manipulated using water baths,
across which rearing vials were regularly reshuffled. Throughout
the experiment, the larvae were kept at the assigned rearing
temperature and a constant photoperiod of 14:10 h light:dark.
During the pre-exposure period, the larvae were fed *Artemia*
nauplii ad libitum 5 days per week.

Experimental Setup. When a larva moulted into the final
instar, it was randomly assigned to a six-day zinc exposure
treatment (control or 100 mg L⁻¹ zinc). This resulted in a
nested full factorial design with three sampling locations per
latitude × 2 latitudes (high/Sweden and low/France) × 2
temperatures (20 and 24 °C) × 2 zinc treatments (zinc present
and absent). The chosen zinc concentration was previously
shown to reduce growth rate in *I. elegans*.³² Because of the low
metal sensitivity of damselflies⁴⁵ and the strictly aqueous
exposure route in our experiment (the dominant exposure
route for metals is generally through the diet),^{46,47} this
concentration strongly exceeds commonly occurring environ-
mentally concentrations (but see ref 48). Note, however, that
our aim was not to mimic a realistic field situation, but to test
for a proof-of-principle of the idea that susceptibility to a
contaminant across metamorphosis can be shaped by temper-
ature and latitude. Zinc solutions were prepared from a stock
solution of ZnCl₂ (5 g zinc L⁻¹ dissolved in milli-Q water) that
was stored in the dark at 4 °C and that was renewed monthly.
To prepare exposure media, the stock solution was further
diluted with synthetic pond water, which was also used as
control medium (for the composition see ref 49). The larvae
were exposed to 100 mL of the medium in the same vials as
they had been reared in. To minimize concentration changes
due to evaporation, the medium was renewed every 2 days. The
measured zinc concentrations in the experimental vials when
the medium was freshly renewed was 93.10 ± 1.65 mg L⁻¹
(mean ± SD, *n* = 5 pooled start samples, taken throughout the
experiment); after 48 h (just before renewal of the medium)
the concentration was 95.31 ± 2.84 mg L⁻¹, as verified with
ICP-OES. The associated water quality parameters were (mean
± 1 SD, *n* = 10 samples) the following: conductivity: 387 ± 17
μS/cm, pH: 6.7 ± 0.1, dissolved oxygen: 8.0 ± 0.2 mg L⁻¹,
hardness: 132 ± 4 mg L⁻¹ CaCO₃ and dissolved organic
carbon: 4.9 ± 1.3 mg L⁻¹. After the six-day zinc exposure, all
larvae were transferred to synthetic pond water and kept at
their rearing temperature until emergence. This assured that all
larvae were exposed to zinc for the same duration. Throughout
the final instar, larvae were daily fed *Artemia nauplii* ad libitum,
supplemented with 3 larvae of *Chironomus riparius* per week.

After emergence, the damselflies were kept overnight in their
rearing vials to ensure complete hardening of the exoskeleton
and wings before handling. Adults that emerged successfully
were individually marked by writing a number on one of the
wings with a permanent marker (Staedtler Lumocolor
permanent F), after which they were transferred to insectaries
(36 × 30 × 30 cm³) to record lifespan; for logistic reasons this
was only done for a subset of the adults. Males and females

194 were kept in separate insectaries with maximum 10 adults of
195 similar ages per insectary and were provided with ad libitum
196 fruit flies. The insectaries were kept in a room at $22\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$
197 with an air humidity between 70% and 80% and were sprayed
198 twice per day with dechlorinated tap water. By keeping all
199 adults at this intermediate temperature (between the two larval
200 rearing temperatures) any effects of temperature on adult
201 lifespan could be attributed to differences in larval rearing
202 temperature (hence, carry-over effects) without confounding
203 with the temperature experienced in the adult stage.

204 Initial sample sizes varied between 112 and 133 animals per
205 combination of latitude, temperature and zinc treatment (total
206 =969). Between 44 and 102 animals emerged successfully per
207 treatment combination (total =583). Sample sizes for the
208 subset on which lifespan was recorded varied between 18 and
209 52 per combination of latitude, temperature and zinc treatment
210 (total =291). The imbalance in sample size was due to
211 differential mortality and emergence success in the different
212 treatment combinations (see Results). Sample sizes per
213 treatment combination for each end point are presented in
214 Appendix S2.

215 **Response Variables.** We quantified larval growth rates
216 both during the six-day exposure period and during the
217 subsequent first 4 days of the postexposure period. To quantify
218 growth rates, each larva was weighed to the nearest 0.01 mg at
219 the start and at the end of the zinc exposure and again 4 days
220 later using an electronic balance (AB135-S, Mettler Toledo,
221 Zaventem, Belgium). Individual growth rates were calculated as
222 $(\ln \text{mass}_{\text{final}} - \ln \text{mass}_{\text{initial}})/\text{number of days}$ (see ref 50).
223 Rearing vials were checked twice per day for survival and adult
224 emergence. To obtain a detailed picture on when larval
225 mortality occurred we separately considered larval survival
226 during the six-day exposure period and during the remaining
227 postexposure period (until emergence). In addition, we
228 calculated the emergence ratio as the percentage of larvae
229 surviving until the end of the final instar that emerged
230 successfully (i.e., complete emergence without wing malforma-
231 tions). To obtain an integrated picture of larval survival and
232 emergence ratio we also quantified the total emergence success
233 as the percentage of individuals initially in the experiment that
234 emerged successfully. Development time was calculated as the
235 number of days between molting to the final instar and adult
236 emergence. Adults that emerged successfully were weighed to
237 the nearest 0.01 mg and sexed. Insectaries were checked twice
238 per day to record adult lifespan.

239 **Statistical Analyses.** Effects of latitude, rearing temper-
240 ature and zinc exposure on the response variables were
241 analyzed with (generalized) linear mixed-models (GLMMs/
242 LMMs) using the lme4 package⁵¹ in R.⁵² In each model all
243 interactions between latitude, rearing temperature and zinc
244 exposure (fixed factors) were included, as well as sampling
245 location nested in latitude as random effect (but see below for
246 survival). Effects on larval survival were tested with a GLMM
247 with binomial error distribution and a logit link function that
248 also included the fixed factor period (before/after zinc
249 exposure) and its interaction with the other fixed factors. For
250 the analysis of emergence ratio and total emergence success, we
251 ran GLMMs with binomial error distribution and a logit link
252 function. The two successive larval growth rates were modeled
253 with a repeated measures LMM that also included the fixed
254 factor period and its interaction with the other fixed factors, and
255 individual as random effect. The models for development time,
256 mass at emergence, and lifespan also included sex as fixed

factor, together with its interactions with the other fixed factors. 257
However, as this is not the focus of this manuscript, results on 258
sex differences are not reported. For lifespan a GLMM with a 259
Poisson error distribution and a log link function was used. 260
Because of nonconvergence of the model for larval survival 261
when including sampling location as a random factor, we 262
reduced the complexity of this model by including this factor 263
here as a nested fixed (instead of a random) effect (following 264
refs^{53, 54}). Whenever an interaction between stressors was 265
detected, we calculated the interaction effect size (estimated as 266
Hedges'd) with its 95% confidence interval following Jackson et 267
al.⁵⁵ An interaction effect size larger than zero indicates a 268
synergistic interaction among stressors. 269

RESULTS AND DISCUSSION 270

Latitude-Wide Delayed Effects of Zinc. While zinc 271
exposure barely affected the survival of the larvae during the six- 272
day exposure period (−1.5%), it considerably reduced survival 273
after the exposure period (−12.4%; period × Zinc; Table 1; 274 t1f1

Table 1. Results of the (G)LMMs Testing for the Effect of
Latitude, Rearing Temperature, and Zinc Exposure on
Larval Survival and Growth Rate of *Ischnura elegans* in the
Final Instar^a

effect	larval survival		larval growth rate	
	χ^2_1	P	χ^2_1	P
latitude (lat)	0.52	0.47	202.34	<0.001
temperature (temp)	5.35	0.021	13.47	<0.001
zinc (Zn)	26.13	<0.001	46.01	<0.001
lat × temp	0.59	0.44	70.75	<0.001
lat × Zn	12.12	<0.001	12.38	<0.001
temp × Zn	3.37	0.066	0.35	0.55
lat × temp × Zn	4.39	0.036	6.35	0.012
period (per)	104.38	<0.001	102.46	<0.001
per × lat	3.77	0.052	8.83	0.0030
per × temp	14.31	<0.001	6.02	0.014
per × Zn	16.43	<0.001	5.48	0.019
per × lat × temp	0.72	0.40	13.26	<0.001
per × lat × Zn	4.33	0.037	3.79	0.052
per × temp × zinc	5.21	0.022	2.38	0.12
per × lat × temp × Zn	0.49	0.48	0.21	0.65

^aBoth life-history traits were quantified during and after a six-day
exposure to zinc, indicated by the (repeated) factor Period. Significant
P values ($P < 0.05$) are printed in bold.

Figure 1a,b). Zinc furthermore decreased larval growth rate 275 f1
(main effect Zinc, Table 1). Again, this zinc effect was stronger 276
after than during the exposure period (period × Zinc; Table 1; 277
Figure 1c,d). Zinc neither induced a latitude-wide effect on 278
emergence ratio, nor on total emergence success (Table 2; 279 t2
Figure 2a,b), although there were pronounced latitude-specific 280 f2
effects of zinc on these end points (see below). Previous zinc 281
exposure did not lead to a longer development time (Table 2; 282
Figure 3a), but did result in a lower mass at emergence (main 283 f3
effect Zinc; Table 2; Figure 3b). Finally, adult lifespan was 284
strongly reduced (i.e., by ca. 7 days) by larval zinc exposure 285
(main effect Zinc; Table 2; Figure 3c). 286

These results show the presence of strong delayed carry-over 287
effects of larval metal exposure, not only in the larval stage after 288
the exposure period (survival, growth rate) but also after 289
emergence in the adult stage (adult mass and lifespan), and this 290
despite zinc imposing no biologically significant effects on 291

Figure 1. Percentage survival (a,b) and mean (+1 SE) growth rate (c,d) of *Ischnura elegans* larvae in the final instar during and after a six-day exposure to zinc as a function of latitude, rearing temperature and zinc exposure.

Table 2. Results of the (G)LMM Testing for Effects of Latitude, Temperature and Zinc on the Emergence Ratio (% Larvae Surviving until the End of the Final Instar That Emerged Successfully), Total Emergence Success (% Individuals Initially in the Experiment That Emerged Successfully), Development Time (Final Instar Duration), Mass at Emergence, and Adult Lifespan of *Ischnura elegans*^a

effect	emergence ratio		total emergence success		development time		mass at emergence		adult lifespan	
	χ^2_1	P	χ^2_1	P	χ^2_1	P	χ^2_1	P	χ^2_1	P
latitude (lat)	35.59	<0.001	28.74	<0.001	59.03	<0.001	189.53	<0.001	15.59	<0.001
temperature (temp)	35.88	<0.001	36.08	<0.001	65.55	<0.001	41.76	<0.001	5.60	0.018
zinc (Zn)	2.98	0.084	18.44	<0.001	0.026	0.87	14.46	<0.001	445.86	<0.001
lat × temp	1.31	0.25	4.37	0.037	372.09	<0.001	4.68	0.030	0.72	0.40
lat × Zn	20.88	<0.001	32.57	<0.001	0.43	0.51	2.11	0.15	2.25	0.13
temp × Zn	0.10	0.75	1.93	0.16	0.13	0.72	1.24	0.26	0.23	0.63
lat × temp × Zn	0.60	0.44	6.09	0.014	0.030	0.86	0.042	0.84	4.26	0.039

^aSignificant *P* values (*P* < 0.05) are printed in bold.

survival during the six-day exposure period. These delayed effects and particularly the lethal effects during metamorphosis of larval exposure to metals (see below) confirm the important role of metamorphosis as a survival bottleneck in metal-contaminated environments.^{21,29,56} Besides the important effects on survival, also the sublethal effect on mass at emergence may result in a lower fitness, as body size is positively related with fecundity in damselflies.⁵⁷ Yet more importantly, we showed that larval zinc exposure also caused a remarkably strong reduction in adult lifespan, a crucial fitness measure in damselflies.⁵⁸ This reduction in lifespan would have

particularly strong fitness implications as it reduced adult lifespan to ca. 2 days. Given that sexual maturation takes ca. 6 days in the study species,⁵⁹ no reproduction would occur in the damselflies that emerged successfully from the zinc treatment. This strong postmetamorphic lethal effect of zinc complements the very few documented adverse metal effects across metamorphosis: lowered survival in bryozoans,¹⁴ lowered fecundity in mosquitoes,⁶⁰ lowered immune function and delayed reproduction in flies,²⁷ and reduced body condition in juvenile frogs.²⁸ Although some elimination of metals can happen during metamorphosis (e.g., via the shed larval skins),³¹³

Figure 2. Emergence ratio (% larvae surviving until the end of the final instar that emerged successfully) (a) and total emergence success (% individuals initially in the experiment that emerged successfully) (b) of *Ischnura elegans* larvae as a function of latitude and previous rearing temperature and zinc exposure in the larval stage.

Figure 3. Mean (+1 SE) development time (number of days in the final instar) (a), mass at emergence (b), and adult lifespan (log transformed) (c) of *Ischnura elegans* as a function of latitude, previous rearing temperature and zinc exposure in the larval stage. All adults were kept under identical conditions.

The negative impact of zinc thus strongly differed between latitudes with all effects being more pronounced (and sometimes only present) in French larvae. As French showed adaptation to higher temperatures (see below), this pattern of an increased vulnerability to contaminants has been predicted as a population-level trade-off between tolerance to warming and tolerance to toxicants by Moe et al.⁸ This might furthermore indicate a role of energy-based life-history trade-offs shaping sensitivity to contaminants.⁴¹ In such case, fast growth is traded off against a lower investment in detoxification and repair mechanisms, which would make fast-lived individuals more susceptible for contaminants. Furthermore, fast growth

often covaries with a higher food intake, a smaller size (hence a higher surface-to-volume ratio), and a faster metabolic rate,^{64–67} traits that can lead to a higher uptake or accumulation of contaminants.^{9,68,69}

Although zinc-induced effects were consistently stronger in French damselflies, even in Swedish adults, which showed only a low zinc-induced reduction in larval survival (6.2% postexposure) and no zinc-induced mortality during metamorphosis, zinc reduced adult lifespan by ca. 71%. This strong reduction happened despite a potentially long recovery period between the end of the exposure and emergence (i.e., on average 27 days at 20 °C and 43 days at 24 °C). This finding particularly underscores the importance of covering the full life cycle in ecological risk assessment of pollutants.^{70,71} If our study would have been limited to premetamorphic life stages (e.g., ref 32) or had ended just after metamorphosis (e.g., ref 21), we would have erroneously concluded that Swedish animals would only suffer negligible zinc-induced effects, whereas the strong delayed effect on lifespan implicates a complete failure to reproduce.

Latitude-Specific Delayed Synergistic Effects between Zinc and Warming. While in Swedish larvae the effect of zinc on survival was similar across temperatures, the survival of French larvae was more strongly reduced by zinc at 24 °C than at 20 °C (latitude × temperature × Zinc; Table 1; Figure 1a,b). This indicated a synergistic interaction between the temperature and zinc treatments in French larvae, which was supported by the positive interaction effect size both during (Hedges' $d = 0.049$, 95% CI: [0.030,0.068]) and especially after the exposure period (Hedges' $d = 0.36$, 95% CI: [0.34,0.38]). Similarly, only in French larvae growth rate was more strongly affected by zinc at 24 °C than at 20 °C (period × latitude × Zinc; Table 1; Figure 1c,d; interaction effect size during the exposure period: Hedges' $d = 0.023$, 95% CI: [0.0016,0.044]; after the exposure period: Hedges' $d = 0.037$, 95% CI: [0.014,0.060]). Likewise, only in French damselflies, total emergence success was more strongly reduced by zinc at 24 °C (ca. 52%) than at 20 °C (ca. 23%) (interaction effect size: Hedges' $d = 0.47$, 95% CI: [0.45,0.48]) (latitude × temperature × Zinc; Table 2; Figure 2b). Finally, the strong effect of zinc on adult lifespan was more pronounced at 24 °C, but again only in French damselflies (latitude × temperature × Zinc). This indicated a synergistic interaction between the temperature and zinc treatments in French adults (interaction effect size: Hedges' $d = 0.17$, 95% CI: [0.09,0.24]).

We thus observed the expected synergism of metals being more toxic at higher temperatures for larval survival and growth, emergence success and adult lifespan.^{9,10} Importantly, the synergistic interactions were mainly delayed (occurred after the exposure period ended) and had a strong geographic signal as these were only present in French damselflies. Despite the concern that trace metals become more toxic at higher temperatures^{9,10} and the potential of strong delayed effects of metals during and after metamorphosis (see above), delayed interactive effects between metals and warming have been largely ignored. The only exception are two studies showing delayed sublethal effects of previous exposure to metals being magnified under warming (for adult body mass: ref 27, for juvenile body condition: ref 28). Here, we showed that also across metamorphosis delayed effects of a metal can be magnified under warming, and even translated in a synergistic lethal effect by shortening adult lifespan. The strong geographic signal in the delayed synergistic interaction also indicated that

thermal adaptation of French damselflies to 24 °C (see below) did not alleviate the adverse effects of zinc. Possibly, at 24 °C the French damselflies allocated a higher percentage of resources to growth than at 20 °C, and this at the expense of investment in detoxification and repair, whereas the Swedish damselflies, which grew even slower at the higher temperature than at the lower temperature, might have been better able to maintain investment in detoxification and repair at an optimal level.

Signals of Thermal Adaptation. We found widespread evidence of latitude-associated thermal adaptation (e.g., refs 72–74), with the French damselflies being adapted to higher environmental temperatures than the Swedish damselflies. While French larvae had a higher growth rate at 24 °C (the current mean water temperature in southern France⁴⁴) than at 20 °C, Swedish instead had a reduced growth rate at 24 °C than at 20 °C (the current mean water temperature in Sweden.⁴⁴) (latitude × temperature; Table 1). French damselflies had a high total emergence success in the control treatment at both temperatures, but that of Swedish damselflies was strongly reduced (by ca. 43%) at the higher temperature (latitude × temperature × Zinc; Table 2; Figure 2b). This was partly due to a lower larval survival at the higher temperature, but mainly to a lower emergence ratio. Furthermore, while French damselflies had a ca. six-day shorter development time at 24 °C than at 20 °C, the Swedish increased their development time at the higher temperature by ca. 18 days (latitude × temperature; Table 2, Figure 3a). The differential thermal responsiveness of larval growth and development resulted in a lower mass at emergence in damselflies reared at 24 °C compared to 20 °C (main effect temperature), and this mass reduction at the higher temperature was stronger in French than in Swedish damselflies (latitude × temperature; Table 2, Figure 3b).

Implications for Risk Assessment in a Warming World. In conclusion, our integrated study not only identified three key mechanisms challenging current risk assessment (i.e., delayed postexposure effects,⁵ interactions between stressors,⁴ and geographic variation in the sensitivity to pollutants⁷), but more importantly, also documented interactions between these mechanisms. A key observation was that the effects of zinc were very mild during the exposure period, yet were strongly magnified after the exposure period and carried over to the adult stage. We further observed a strong geographic signal in the sensitivity to zinc, with the French damselflies consistently more strongly affected than the Swedish, especially when the larvae had experienced a higher rearing temperature. Moreover, the synergistic interaction between the metal and the warming treatment in the French damselflies bridged metamorphosis and strongly affected (post-) metamorphic traits. However, even though Swedish damselflies experienced only mild effects of zinc on larval and metamorphic end points, they did show a very strong reduction in adult lifespan when exposed to zinc in the larval stage.

Predicting and understanding the impact of contaminants under warming remains a key challenge at the interface of ecotoxicology and global change biology.^{8,10} Even though the zinc concentration used in our study is much higher than those that would be encountered in realistic scenarios, our study provided a proof of principle, adding two key related insights that may contribute to the much needed development of a more realistic risk assessment,^{1–3} especially with regard to the impact of contaminants in a warming world.^{12,13} First, our

476 results refined the well-known pattern that trace metals are
477 more toxic at higher temperatures by showing this synergistic
478 interaction may persist across metamorphosis and may show a
479 strong geographic signal (being only present in French
480 damselflies). This geographic signal was consistent with a
481 trade-off scenario between thermal adaptation and tolerance to
482 toxicants,⁸ illustrating the complexity of predicting the impact
483 of metals in a warming world. Using a space-for-time
484 substitution our results thereby tentatively suggest that
485 warming at the high latitude would not make zinc more toxic
486 to the study species, unless high-latitude populations show
487 gradual thermal evolution and convert to the current French
488 phenotype.

489 Second, our study refined the hypothesis of aquatic insect
490 emergence as a sensitive indicator of aquatic metal toxicity²¹ in
491 two important ways that are directly relevant for ecological risk
492 assessment. (i) Metamorphosis success may strongly depend
493 on latitude as we only observed a zinc-induced mortality during
494 metamorphosis in French animals and not in Swedish animals.
495 (ii) Postmetamorphic survival may even be more important
496 than larval survival and emergence success; this result again
497 showed the importance of the geographical context, as this
498 pattern was especially striking for the Swedish animals. Taken
499 together our results thereby challenge current water quality
500 standards (such as EQS, EU Directive 2008/105/EC) which
501 assume that protection of aquatic stages will translate into the
502 protection of adults (but see ref 71), and ignore geographical
503 patterns in sensitivity to contaminants. Aquatic insects are
504 important vectors of transfer of nutrients from aquatic to
505 terrestrial ecosystems,^{30,75} and aquatic ecosystems are especially
506 vulnerable to warming^{76,77} and pollutants.⁷⁸ Combined with
507 current findings this highlights that risk assessment for aquatic
508 systems can highly benefit from a more complete life-cycle
509 approach addressing delayed synergisms under warming in a
510 geographical context in order to obtain more ecologically
511 relevant water quality standards.

512 ■ ASSOCIATED CONTENT

513 ■ Supporting Information

514 The Supporting Information is available free of charge on the
515 ACS Publications website at DOI: 10.1021/acs.est.6b04989.

516 Information on the sampled locations (S1), sample sizes
517 (S2), and presentation of the results and discussion on
518 baseline life-history differences between the latitudes
519 (S3) (PDF)

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525 Notes

526 The authors declare no competing financial interest.

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539 ■ REFERENCES

- (1) Beketov, M. A.; Kefford, B. J.; Schäfer, R. B.; Liess, M. Pesticides 540
reduce regional biodiversity of stream invertebrates. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (27), 11039–11043. 541
- (2) Malaj, E.; von der Ohe, P. C.; Grote, M.; Kühne, R.; Mondy, C. 542
P.; Usseglio-Polatera, P.; Brack, W.; Schäfer, R. B. Organic chemicals 543
jeopardize the health of freshwater ecosystems on the continental 544
scale. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (26), 9549–9554. 545
- (3) Poteat, M. D.; Buchwalter, D. B. Four reasons why traditional 546
metal toxicity testing with aquatic insects is irrelevant. *Environ. Sci. 547
Technol.* **2014**, *48* (2), 887–888. 548
- (4) Segner, H.; Schmitt-Jansen, M.; Sabater, S. Assessing the impact 549
of multiple stressors on aquatic biota: the receptor's side matters. 550
Environ. Sci. Technol. **2014**, *48* (14), 7690–7696. 551
- (5) Zhao, Y.; Newman, M. C. Shortcomings of the laboratory-derived 552
median lethal concentration for predicting mortality in field 553
populations: Exposure duration and latent mortality. *Environ. Toxicol. 554
Chem.* **2004**, *23* (9), 2147–2153. 555
- (6) Beketov, M. A.; Liess, M. Acute and delayed effects of the 556
neonicotinoid insecticide thiacloprid on seven freshwater arthropods. 557
Environ. Toxicol. Chem. **2008**, *27* (2), 461–470. 558
- (7) Clements, W. H.; Hickey, C. W.; Kidd, K. A. How do aquatic 559
communities respond to contaminants? It depends on the ecological 560
context. *Environ. Toxicol. Chem.* **2012**, *31* (9), 1932–1940. 561
- (8) Moe, S. J.; De Schampelaere, K.; Clements, W. H.; Sorensen, M. 562
T.; Van den Brink, P. J.; Liess, M. Combined and interactive effects of 563
global climate change and toxicants on populations and communities. 564
Environ. Toxicol. Chem. **2013**, *32* (1), 49–61. 565
- (9) Sokolova, I. M.; Lannig, G. Interactive effects of metal pollution 566
and temperature on metabolism in aquatic ectotherms: implications of 567
global climate change. *Clim. Res.* **2008**, *37* (2–3), 181–201. 568
- (10) Noyes, P. D.; McElwee, M. K.; Miller, H. D.; Clark, B. W.; Van 569
Tiem, L. A.; Walcott, K. C.; Erwin, K. N.; Levin, E. D. The toxicology 570
of climate change: environmental contaminants in a warming world. 571
Environ. Int. **2009**, *35* (6), 971–986. 572
- (11) Heugens, E. H. W.; Hendriks, A. J.; Dekker, T.; Straalen, N. M. 573
v.; Admiraal, W. A review of the effects of multiple stressors on aquatic 574
organisms and analysis of uncertainty factors for use in risk assessment. 575
Crit. Rev. Toxicol. **2001**, *31* (3), 247–284. 576
- (12) Landis, W. G.; Durda, J. L.; Brooks, M. L.; Chapman, P. M.; 577
Menzie, C. A.; Stahl, R. G.; Stauber, J. L. Ecological risk assessment in 578
the context of global climate change. *Environ. Toxicol. Chem.* **2013**, *32* 579
(1), 79–92. 580
- (13) Noyes, P. D.; Lema, S. C. Forecasting the impacts of chemical 581
pollution and climate change interactions on the health of wildlife. 582
Curr. Zool. **2015**, *61* (4), 669–689. 583
- (14) Ng, T. Y. T.; Keough, M. J. Delayed effects of larval exposure to 584
Cu in the bryozoan *Watersipora subtorquata*. *Mar. Ecol.: Prog. Ser.* **2003**, *257*, 77–85. 585
- (15) Rohr, J. R.; Sager, T.; Sesterhenn, T. M.; Palmer, B. D. 586
Exposure, postexposure, and density-mediated effects of atrazine on 587
amphibians: breaking down net effects into their parts. *Environ. Health 588
Persp.* **2006**, *114* (1), 46–50. 589
- (16) Abel, P. D. Toxicity of γ -hexachlorocyclohexane (Lindane) to 590
Gammarus pulex. *Freshwater Biol.* **1980**, *10* (3), 251–259. 591
- (17) Liess, M.; Schulz, R. Chronic effects of short-term 592
contamination with the pyrethroid insecticide fenvalerate on the 593
caddisfly *Limnephilus lunatus*. *Hydrobiologia* **1996**, *324* (2), 99–106. 594
- (18) Campero, M.; De Block, M.; Ollevier, F.; Stoks, R. 595
Metamorphosis offsets the link between larval stress, adult asymmetry 596
and individual quality. *Funct. Ecol.* **2008**, *22* (2), 271–277. 597

- (19) Rodriguez, J. L.; Sedano, F. J.; Garcia-Martin, L. O.; Perez-Camacho, A.; Sanchez, J. L. Energy metabolism of newly settled *Ostrea edulis* spat during metamorphosis. *Mar. Biol.* **1990**, *106* (1), 109–111.
- (20) Shilling, F. M.; Hoegh-Guldberg, O.; Manahan, D. T. Sources of energy for increased metabolic demand during metamorphosis of the abalone *Haliotis rufescens* (Mollusca). *Biol. Bull.* **1996**, *191* (3), 402–412.
- (21) Wesner, J. S.; Kraus, J. M.; Schmidt, T. S.; Walters, D. M.; Clements, W. H. Metamorphosis enhances the effects of metal exposure on the mayfly. *Environ. Sci. Technol.* **2014**, *48* (17), 10415–10422.
- (22) Bots, J.; De Bruyn, L.; Snijders, T.; Van den Branden, B.; Van Gossom, H. Exposure to perfluorooctane sulfonic acid (PFOS) adversely affects the life-cycle of the damselfly *Enallagma cyathigerum*. *Environ. Pollut.* **2010**, *158* (3), 901–905.
- (23) Dinh, K. V.; Janssens, L.; Therry, L.; Bervoets, L.; Bonte, D.; Stoks, R. Delayed effects of chlorpyrifos across metamorphosis on dispersal-related traits in a poleward moving damselfly. *Environ. Pollut.* **2016**, *218*, 634–643.
- (24) Boone, M. D.; Hammond, S. A.; Veldhoen, N.; Youngquist, M.; Helbing, C. C. Specific time of exposure during tadpole development influences biological effects of the insecticide carbaryl in green frogs (*Lithobates clamitans*). *Aquat. Toxicol.* **2013**, *130–131*, 139–148.
- (25) Segner, H. Moving beyond a descriptive aquatic toxicology: the value of biological process and trait information. *Aquat. Toxicol.* **2011**, *105* (3), 50–55.
- (26) Gunderson, A. R.; Armstrong, E. J.; Stillman, J. H. Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. *Annu. Rev. Mar. Sci.* **2016**, *8*, 357–378.
- (27) Pölkki, M.; Kangassalo, K.; Rantala, M. J. Effects of interaction between temperature conditions and copper exposure on immune defense and other life-history traits of the blow fly *Protophormia terraenovae*. *Environ. Sci. Technol.* **2014**, *48* (15), 8793–8799.
- (28) Hallman, T. A.; Brooks, M. L. Metal-mediated climate susceptibility in a warming world: Larval and latent effects on a model amphibian. *Environ. Toxicol. Chem.* **2016**, *35* (7), 1872–1882.
- (29) Kraus, J. M.; Schmidt, T. S.; Walters, D. M.; Wanty, R. B.; Zuellig, R. E.; Wolf, R. E. Cross-ecosystem impacts of stream pollution reduce resource and contaminant flux to riparian food webs. *Ecol. Appl.* **2014**, *24* (2), 235–243.
- (30) Nakano, S.; Murakami, M. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98* (1), 166–170.
- (31) Cherkasov, A. S.; Taylor, C.; Sokolova, I. M. Seasonal variation in mitochondrial responses to cadmium and temperature in eastern oysters *Crassostrea virginica* (Gmelin) from different latitudes. *Aquat. Toxicol.* **2010**, *97* (1), 68–78.
- (32) Dinh Van, K.; Janssens, L.; Debecker, S.; De Jonge, M.; Lambret, P.; Nilsson-Örtman, V.; Bervoets, L.; Stoks, R. Susceptibility to a metal under global warming is shaped by thermal adaptation along a latitudinal gradient. *Glob. Chang. Biol.* **2013**, *19* (9), 2625–2633.
- (33) Bridges, C. M.; Semlitsch, R. D. Variation in pesticide tolerance of tadpoles among and within species of *Ranidae* and patterns of amphibian decline. *Conserv. Biol.* **2000**, *14* (5), 1490–1499.
- (34) Eliason, E. J.; Clark, T. D.; Hague, M. J.; Hanson, L. M.; Gallagher, Z. S.; Jeffries, K. M.; Gale, M. K.; Patterson, D. A.; Hinch, S. G.; Farrell, A. P. Differences in thermal tolerance among sockeye salmon populations. *Science* **2011**, *332* (6025), 109–112.
- (35) Munday, P. L.; McCormick, M. I.; Nilsson, G. E. Impact of global warming and rising CO₂ levels on coral reef fishes: what hope for the future? *J. Exp. Biol.* **2012**, *215* (22), 3865–3873.
- (36) Sinclair, B. J.; Ferguson, L. V.; Salehipour-shirazi, G.; MacMillan, H. A. Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integr. Comp. Biol.* **2013**, *53* (4), 545–556.
- (37) Mitchell, S. N.; Stevenson, B. J.; Müller, P.; Wilding, C. S.; Egyir-Yawson, A.; Field, S. G.; Hemingway, J.; Paine, M. J. I.; Ranson, H.; Donnelly, M. J. Identification and validation of a gene causing cross-resistance between insecticide classes in *Anopheles gambiae* from Ghana. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (16), 6147–6152.
- (38) Bulbuli, O. A.; Loeschcke, V. Correlated responses to selection for stress resistance and longevity in a laboratory population of *Drosophila melanogaster*. *J. Evol. Biol.* **2005**, *18* (4), 789–803.
- (39) Fukami, T.; Wardle, D. A. Long-term ecological dynamics: reciprocal insights from natural and anthropogenic gradients. *Proc. R. Soc. London, Ser. B* **2005**, *272* (1577), 2105–2115.
- (40) Corbet, P. S.; Suhling, F.; Soendergerath, D. Voltinism of Odonata: a review. *Int. J. Odonatol.* **2006**, *9* (1), 1–44.
- (41) Congdon, J. D.; Dunham, A. E.; Hopkins, W. A.; Rowe, C. L.; Hinton, T. G. Resource allocation-based life histories: A conceptual basis for studies of ecological toxicology. *Environ. Toxicol. Chem.* **2001**, *20* (8), 1698–1703.
- (42) IPCC. *Climate Change 2013: The Physical Science Basis: Working Group I contribution to the Fifth assessment report of the Intergovernmental Panel on Climate Change*; 110705799X; Cambridge University Press: Cambridge, U.K., 2014.
- (43) Gosden, T. P.; Stoks, R.; Svensson, E. I. Range limits, large-scale biogeographic variation, and localized evolutionary dynamics in a polymorphic damselfly. *Biol. J. Linn. Soc.* **2011**, *102* (4), 775–785.
- (44) De Block, M.; Pauwels, K.; Van Den Broeck, M.; De Meester, L.; Stoks, R. Local genetic adaptation generates latitude-specific effects of warming on predator–prey interactions. *Glob. Chang. Biol.* **2013**, *19* (3), 689–696.
- (45) Wogram, J.; Liess, M. Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna*. *Bull. Environ. Contam. Toxicol.* **2001**, *67* (3), 360–367.
- (46) Mathews, T.; Fisher, N. S. Dominance of dietary intake of metals in marine elasmobranch and teleost fish. *Sci. Total Environ.* **2009**, *407* (18), S156–S161.
- (47) Timmermans, K. R.; Spijkerman, E.; Tonkes, M.; Govers, H. Cadmium and zinc uptake by two species of aquatic invertebrate predators from dietary and aqueous sources. *Can. J. Fish. Aquat. Sci.* **1992**, *49* (4), 655–662.
- (48) Nieto, J. M.; Sarmiento, A. M.; Olías, M.; Canovas, C. R.; Riba, I.; Kalman, J.; Deltalls, T. A. Acid mine drainage pollution in the Tinto and Odiel rivers (Iberian Pyrite Belt, SW Spain) and bioavailability of the transported metals to the Huelva Estuary. *Environ. Int.* **2007**, *33* (4), 445–455.
- (49) Janssens, L.; Stoks, R. Synergistic effects between pesticide stress and predator cues: Conflicting results from life history and physiology in the damselfly *Enallagma cyathigerum*. *Aquat. Toxicol.* **2013**, *132*, 92–99.
- (50) Stoks, R.; Swillen, I.; De Block, M. Behaviour and physiology shape the growth accelerations associated with predation risk, high temperatures and southern latitudes in *Ischnura* damselfly larvae. *J. Anim. Ecol.* **2012**, *81* (5), 1034–1040.
- (51) Bates, D.; Maechler, M.; Bolker, B.; Walker, S. *lme4: Linear Mixed-Effects Models Using Eigen and S4*. R Package Version 1.0–6, 2014.
- (52) R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2013.
- (53) Bolker, B. M.; Brooks, M. E.; Clark, C. J.; Geange, S. W.; Poulsen, J. R.; Stevens, M. H. H.; White, J.-S. S. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* **2009**, *24* (3), 127–135.
- (54) Schielzeth, H.; Nakagawa, S. Nested by design: model fitting and interpretation in a mixed model era. *Methods Ecol. Evol.* **2013**, *4* (1), 14–24.
- (55) Jackson, M. C.; Loewen, C. J. G.; Vinebrooke, R. D.; Chimimba, C. T. Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. *Glob. Chang. Biol.* **2016**, *22* (1), 180–189.
- (56) Bergeron, C. M.; Hopkins, W. A.; Todd, B. D.; Hepner, M. J.; Unrine, J. M. Interactive effects of maternal and dietary mercury exposure have latent and lethal consequences for amphibian larvae. *Environ. Sci. Technol.* **2011**, *45* (8), 3781–3787.

- (57) Sokolovska, N.; Rowe, L.; Johansson, F. Fitness and body size in mature odonates. *Ecol. Entomol.* **2000**, *25* (2), 239–248.
- (58) Thompson, D. J.; Hassall, C.; Lowe, C. D.; Watts, P. C. Field estimates of reproductive success in a model insect: behavioural surrogates are poor predictors of fitness. *Ecol. Lett.* **2011**, *14* (9), 905–913.
- (59) Cordero, A.; Carbone, S. S.; Utzeri, C. Mating opportunities and mating costs are reduced in androchrome female damselflies, *Ischnura elegans* (Odonata). *Anim. Behav.* **1998**, *55* (1), 185–197.
- (60) Perez, M. H.; Noriega, F. G. Sublethal metal stress response of larvae of *Aedes aegypti*. *Physiol. Entomol.* **2014**, *39* (2), 111–119.
- (61) Timmermans, K. R.; Walker, P. A. The fate of trace metals during the metamorphosis of chironomids (Diptera, Chironomidae). *Environ. Pollut.* **1989**, *62* (1), 73–85.
- (62) Kraus, J. M.; Walters, D. M.; Wesner, J. S.; Stricker, C. A.; Schmidt, T. S.; Zuellig, R. E. Metamorphosis alters contaminants and chemical tracers in insects: Implications for food webs. *Environ. Sci. Technol.* **2014**, *48* (18), 10957–10965.
- (63) Unrine, J. M.; Jagoe, C. H.; Hopkins, W. A.; Brant, H. A. Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenoccephala*) larvae. *Environ. Toxicol. Chem.* **2004**, *23* (12), 2964–2970.
- (64) Glazier, D. S. Is metabolic rate a universal ‘pacemaker’ for biological processes? *Biol. Rev.* **2015**, *90* (2), 377–407.
- (65) Réale, D.; Garant, D.; Humphries, M. M.; Bergeron, P.; Careau, V.; Montiglio, P.-O. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos. Trans. R. Soc., B* **2010**, *365* (1560), 4051–4063.
- (66) Biro, P. A.; Stamps, J. A. Are animal personality traits linked to life-history productivity? *Trends Ecol. Evol.* **2008**, *23* (7), 361–368.
- (67) Debecker, S.; Sanmartín-Villar, I.; de Guinea-Luengo, M.; Cordero-Rivera, A.; Stoks, R. Integrating the pace-of-life syndrome across species, sexes and individuals: covariation of life history and personality under pesticide exposure. *J. Anim. Ecol.* **2016**, *85*, 726–738.
- (68) Rubach, M. N.; Ashauer, R.; Buchwalter, D. B.; De Lange, H. J.; Hamer, M.; Preuss, T. G.; Töpke, K.; Maund, S. J. Framework for traits-based assessment in ecotoxicology. *Integr. Environ. Assess. Manage.* **2011**, *7* (2), 172–186.
- (69) Canli, M.; Atli, G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environ. Pollut.* **2003**, *121* (1), 129–136.
- (70) Preston, B. L.; Snell, T. W. Full life-cycle toxicity assessment using rotifer resting egg production: implications for ecological risk assessment. *Environ. Pollut.* **2001**, *114* (3), 399–406.
- (71) Schmidt, T. S.; Kraus, J. M.; Walters, D. M.; Wanty, R. B. Emergence flux declines disproportionately to larval density along a stream metals gradient. *Environ. Sci. Technol.* **2013**, *47* (15), 8784–8792.
- (72) Ragland, G. J.; Kingsolver, J. G. Influence of seasonal timing on thermal ecology and thermal reaction norm evolution in *Wyeomyia smithii*. *J. Evol. Biol.* **2007**, *20* (6), 2144–2153.
- (73) Seiter, S.; Kingsolver, J. Environmental determinants of population divergence in life-history traits for an invasive species: climate, seasonality and natural enemies. *J. Evol. Biol.* **2013**, *26* (8), 1634–1645.
- (74) Nilsson-Örtman, V.; Stoks, R.; De Block, M.; Johansson, F. Generalists and specialists along a latitudinal transect: patterns of thermal adaptation in six species of damselflies. *Ecology* **2012**, *93* (6), 1340–1352.
- (75) Baxter, C. V.; Fausch, K. D.; Carl Saunders, W. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biol.* **2005**, *50* (2), 201–220.
- (76) Heino, J.; Virkkala, R.; Toivonen, H. Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. *Biol. Rev.* **2009**, *84* (1), 39–54.
- (77) Woodward, G.; Perkins, D. M.; Brown, L. E. Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philos. Trans. R. Soc., B* **2010**, *365* (1549), 2093–2106.
- (78) Brönmark, C.; Hansson, L.-A. Environmental issues in lakes and ponds: current state and perspectives. *Environ. Conserv.* **2002**, *29* (03), 807–808.