Journal of Environmental Science and Health Part A (2009) **44**, 955–962

ISSN: 1093-4529 (Print); 1532-4117 (Online)
DOI: 10.1080/10934520902996849

Taylor & Francis
Taylor & Francis Group

Combined effects of chemical and temperature stress on *Chironomus riparius* populations with differing genetic variability

MATTHIAS OETKEN, LUCAS S. JAGODZINSKI, CHRISTIAN VOGT, ADRIENNE JOCHUM and JÖRG OEHLMANN

Institute for Ecology, Evolution and Diversity, Goethe University, Frankfurt am Main, Germany

Exposure to pollutants under multiple environmental stressors (e.g., climate change and global warming) and the genetic diversity of populations are suspected to have serious impacts on populations and ecosystems but have only rarely been analysed. In the present study, we investigated the effects of the biocide tributyltin (TBT) within a temperature gradient (17, 20 and 23°C) on life history parameters of a genetically diverse (GEN+) and a highly inbred population (GEN−) of the midge *Chironomus riparius*. While endpoints, mortality and reproduction parameters were considered, the population growth rate as an integrative endpoint was determined. We found severe effects for GEN−, indicating that populations with lower genetic diversity are more endangered by combined stressors such as increasing temperature and chemical pollution compared to genetically diverse populations.

Keywords: Environmental stressors, climate change, tributyltin, Chironomidae, life history parameters, genetic impoverishment, extinction risk.

Introduction

Biodiversity affects all terrestrial, marine and freshwater organisms: microbes, plants and animals ranging from the gene pool of species (genetic diversity), to the number of species within an ecosystem (species diversity) and the number of ecosystem types (ecosystem diversity). Habitat destruction, excessive use by wildlife and human encroachment such as the introduction of chemical, biological (e.g., alien species) and physical stressors (e.g. global warming) are identified as key factors for loss of biodiversity. Recently, 19 scientists emphasized in *Nature* that the biodiversity decreases rapidly and "that there is clear scientific evidence that we are on the verge of a major biodiversity crisis".[1]

Although man-made chemicals are evaluated according to their (eco)-toxicological effects under the new European Union legislation (REACH), these studies do not provide any information about their effects on the gene pool of pollutant-exposed populations. In this context, laboratory experiments with the organotin compound, tributyltin (TBT) and the midge *Chironomus riparius* have shown that the exposure to environmentally relevant concentrations of TBT results in a decrease of genetic variability across several generations in exposed populations compared to a control population.[2]

Consequences of the emission of greenhouse gases, climate change and global warming represent further stressors affecting the occurrence of species and hence, lead to a reduction of biodiversity. The IPCC (International Panel of Climate Change) draft report *Climate Change 2007*[3] points out that approximately 20–30% of plant and animal species will be at higher risk of extinction if increases in global average temperatures exceed 1.5–2.5°C. Different scenarios predicted a rise of the average global temperatures by 2.0–4.5°C by the year 2100 with the most probable increase reaching approximately 3°C. However, effects of global warming merit discussion on a smaller scale since they can differ between ecosystems and regions. According to a recent projection of the Max Planck Institute for Meteorology (Hamburg, Germany), the average temperature in Germany will likely increase around 2.75–3°C in the north and up to 3.75°C in the south. This will be accompanied by a 30% increase of the annual winter precipitation within the next 30 years. Temperature affects not only toxicokinetics[4] but also metabolism[5] and physiological state[6] of poikilothermic animals.

Address correspondence to Matthias Oetken, Institute for Ecology, Evolution and Diversity, Goethe University, Siesmayerstrasse 70, D-60323 Frankfurt am Main, Germany. E-mail: oetken@bio.uni-frankfurt.de
Received March 4, 2009.

In the present study, the combined effects of chemical (TBT) and temperature stress (the selected temperature range from 17 to 23°C representing the European isotherms and corresponding to recent projections on temperature increases due to global warming) on life-cycle traits of *Chironomus riparius* were assessed. The experiment was additionally designed to investigate if genetically impoverished populations are more sensitive to temperature stress than genetic diverse populations. In particular, we focused on three main questions:

(i) Can we observe an increased response in life-cycle parameters of *C. riparius* when both stressors act simultaneously?
(ii) Which stressor is mainly responsible for the observed effects?
(iii) Is a genetically impoverished population more sensitive in the presence of sychronously acting stressors compared to a genetically diverse population?

Materials and methods

Test organism

The midge *Chironomus riparius* (Insecta, Chironomidae) is the test organism in this study. Briefly, the Chironomidae represent the largest family within the order Diptera. It possesses the richest species group of the invertebrates in freshwater environments[7] with approximately 1000 different taxa in Central Europe. *C. riparius* is widely distributed in small streams, ditches, ponds and puddles throughout the holarctic. In organically polluted and muddy habitats, the species may reach high densities and it plays a key role in freshwater ecosystems for nutrient cycling and energy flux.[7] Moreover, *C. riparius* is an important food source for predatory fish and insect species.[7,8] As detritus feeders living in the upper sediment layers, chironomids have instant contact to sediment-associated toxicants, such as heavy-metals, PCBs or organotin components.

Two midge-populations of different genetic diversity were used for the present study. One population became genetically impoverished due to isolated laboratory breeding conditions (GEN−). The second population was established in 2004 with egg ropes of 11 different laboratory stocks from seven countries (Bulgaria, Germany, France, Finland, Great Britain, the Netherlands and the USA). To allow for random allelic distribution at the beginning of the experiment for GEN+, the study started with the F_2-generation after the cross-breeding. Genetic variety was determined using a microsatellite analysis approach[9], which measured individual length differences of highly variable satellite DNA-fragments. In total, 36 individuals of each population and temperature were checked. The cultures of the two genetically different populations were maintained in a climate room with a constant temperature of 20 ± 1°C, 70% relative humidity and a light:dark rhythm of 16:8h.

Experimental procedures

Static life-cycle experiments were conducted following the OECD Guideline 218[10] in 600 mL glass beakers containing 100 g quartz sand (grain size 0.1–0.4 mm) and 400 mL reconstituted water (pH-value 7.9–8.4; conductivity 540 μS/cm.[11]) The beakers were covered with gauze and aerated. The experiment was distributed to 3 climate chambers (Sorvall Heraeus, Kendro Laboratory Products, UC 600), adjusted to 17, 20 and 23°C. In each climate chamber seven control replicates and an equal number of TBT contaminated sediments were placed. To compensate possible temperature differences inside the climate chamber, the position of the beakers was randomized daily. At the beginning of the experiment, the sediment of the TBT treatments was contaminated with 195 μg TBT/kg sediment dw, corresponding to a heavy metal (as Sn) concentration of 80 μg Sn/kg sediment dw.[12]

Due to low water solubility, TBT was solved in ethanol before the compound was spiked on the sediment. Ethanol was also applied on the solvent control sediment (30 mL per beaker). The solvent control at this point is referred to as *control* because the solvent was completely evaporated overnight and, therefore, no effect on the mortality and reproduction of the midges could be expected.[12] Test beakers were gently aerated (40 A, Die Pumpe, Holm, Germany). Three days before the start of the experiment, freshly laid (≤ 24 h) egg ropes were taken from the corresponding cultures and transferred into 24-microwell plates for hatching (2 mL reconstituted water per cavity). Newly hatched larvae from different egg ropes were combined to randomly selected 20 first instar larvae per beaker. The aeration was stopped for 24 h in order to enable the larvae to dig into the sediment. Larvae were fed with a ground TetraMin® suspension (Tetra GmbH, Melle, Germany) according to OECD guideline 218.[10]

As endpoints, mortality, mean emergence time (EmT_{50}) of females, number of eggs per clutch, number of fertile clutches per female, male dry body weight as well as the sex ratio were considered. Successfully emerged midges were collected with an exhauster and transferred into a breeding container, which consisted of a glass aquarium (30 × 20 × 20 cm) closed with a stainless steel gauze (mesh size 0.5 mm). Each breeding container included a plastic dish (11.5 × 11.5 × 5.5 cm), filled with 400 mL of reconstituted water for oviposition. All breeding containers were maintained at a constant temperature of 20°C. The egg ropes were removed daily out of the breeding container and the number of eggs was counted.[13] Subsequently, each egg mass was placed in a tube of a 24-microwell plate. After 3 days the hatchability of larvae was determined.

Based on the laboratory life history data (larval mortality, sex ratio, number of eggs per egg mass, mean emergence time (EmT_{50}) of females and the number of fertile egg masses per female), the population growth rate (PGR) was calculated according to Forbes and Cold[14] and Sibley

et al.[15] The PGR allows the integration of effects on different life-cycle traits on the population level. At the end of the experiment, dead males were collected and dried at 34°C for 3 days. The dry body weight of each animal was determined to the nearest μg using a sensitive laboratory balance (Sartorius 4401, Germany). Females were not considered because of the high variability of their body weight, which depended on whether they had deposited egg masses or not.

Statistical analyses

Statistical analyses were performed with the software program GraphPad Prism®, Version 5.00 (GraphPad Software Inc., San Diego, CA, USA). All data were tested for normality by the Kolmogorov–Smirnov test. Data sets were checked for significant differences using one-way analysis of variances (ANOVA) followed by Tukey's post hoc test in case of normally distributed data. Otherwise, the Kruskal–Wallis test followed by Dunn's post hoc test was applied. Differences in the number of clutches (total/fertile) and in the sex ratio (expressed as female fraction) were tested with Fisher's exact test. For the fertile clutches per female, no statistical test was possible because only one breeding container was used per treatment.

The number of eggs per egg mass was determined according to Vogt et al.[12] Based on the time and the number of emerged females, the EmT_{50} was calculated. PGR was calculated according to a simplified Euler-Lotka calculation based on: mortality rate, mean emergence time of females, female fraction, number of eggs per egg-mass and number of fertile egg-masses per female (for details see Vogt et al.[12]). The population size after 30 days was estimated using the experimentally determined PGR of each treatment group. As in the experiment, the modeling of each group also started with 140 larvae per treatment.

For calculating the EmT_{50} it was necessary to transform the data. The natural logarithm of time (in days) was taken and the number of emerged midges was cumulated and normalised to percentages for each replicate. The normalisation was performed to correct for non-substance mediated differences in emerged midges between replicates within a treatment. Thereafter, non-linear regression was performed using the logistic curve containing the EmT_{50} as a parameter. Significant effects of the combined stressors, temperature and genetic diversity were checked using two-way ANOVA. The significance level was set at $P < 0.05$ and is indicated in the graphs by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Results and discussion

Genetic diversity of the populations

As shown in Table 1, the two *Chironomus riparius* populations exhibit clear differences in heterozygosity. As a result of inbreeding in the laboratory, the genetically impoverished population (GEN−) attains a value of 0.263, whereas the GEN+ population shows a heterozygosity level of 0.509. Thus, nearly 50% of the individuals exhibit two different alleles for the examined loci, in contrast to GEN− with only 26% of the midges being heterozygous. The loss of genetic variation is associated with inbreeding which has often been reported in agricultural stock-breeding as well as in captive-breeding programs for species conservation purposes.[16] The measured heterozygosity of the laboratory stock of GEN+ and of field populations are in the same range. Nowak et al.[17] determined a heterozygosity of 0.596 in a *C. riparius* field population taken from a small ditch close to Weinheim (Germany).

Table 1. Observed heterozygosity levels of the *Chironomus riparius* populations GEN+ and GEN− (Nowak et al.[17]).

	GEN+	GEN−
Heterozygosity	0.509	0.263

Mortality and mean emergence time (EmT_{50})

Effects of the biocide TBT on chironomids at sublethal, environmentally relevant concentrations have already been described at an exposure temperature of 20°C before.[12,18,19] We could confirm these findings in our study. Figure 1A shows the mortality of the GEN+ population at the three test temperatures. At 17°C, we observed a moderate increase of mortality in the TBT-treatment compared to the corresponding control for which the percentage of dead animals was in the same range at 20°C with 12.1 (control) and 11.4% (TBT). At 23°C, the mortality of control individuals was significantly higher than in the biocide exposed population (one-way ANOVA with Tukey's post hoc test, $P < 0.05$).

In the genetically impoverished population GEN−, the mortality of the TBT-treated population was higher at the lowest temperature compared to the control (Figure 1B). At 20°C the mortality of the control and the TBT group occurred at the same level (18.3 and 19.3%). At 23°C in both treatments, a clear and significant increase (one-way ANOVA with Tukey's post test) in mortality was found (38.6 and 45.7%, respectively). There was no significant difference in mortality between the control and the TBT-exposed population at 23°C. An increasing mortality at higher temperatures is already described for the genus *Chironomus* by Frouz et al.[20] and Stevens.[21] In our study, these findings were corroborated for GEN−. Overall, the results are in good agreement with former studies in our group. For example, Nowak et al.[22] found that genetically impoverished *C. riparius* populations react more sensitively to the heavy metal cadmium compared to populations possessing higher levels of genetic variation. This observation is also confirmed by a

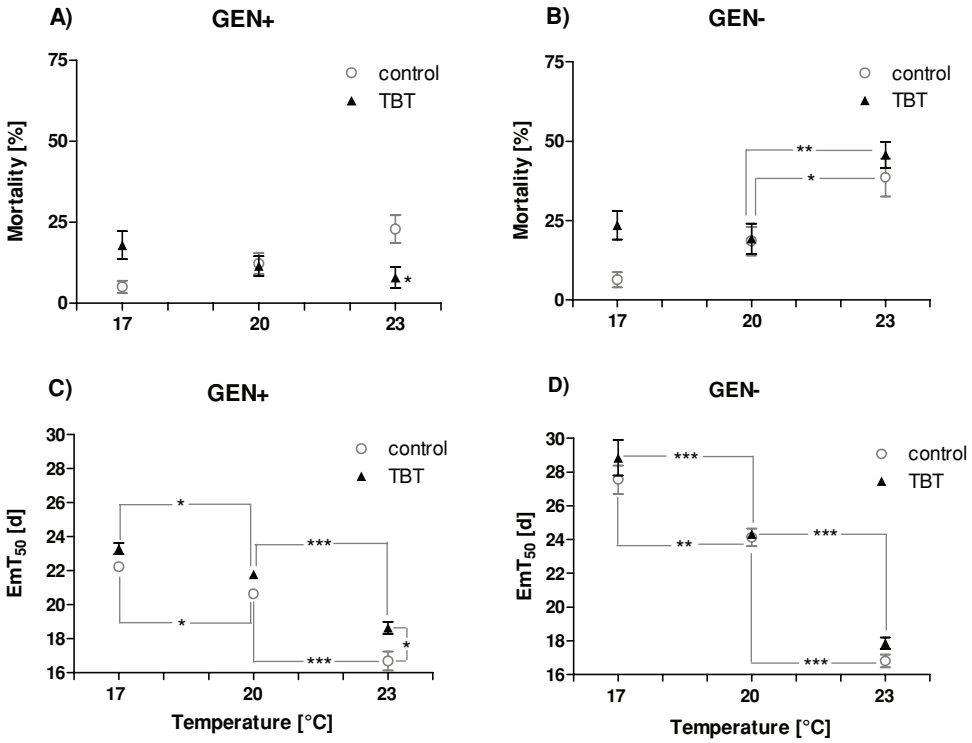

Fig. 1. *Chironomus riparius* – mortality (A and B) and EmT_{50} of females (C and D) of the GEN+ and GEN− population at different temperatures (mean ± SEM, one-way-ANOVA with Tukey's post hoc test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n = 7).

meta-analysis of 34 data sets on various animal and plant species.[23]

The 20°C experiment fulfilled the validity criteria for mortality and emergence according to OECD guideline 218.[9] The observed mortality in the control groups was always below 30% and emergence occurred between day 12 and 23, independently of the level of heterozygosity. While GEN+ demonstrated a low mortality of the control and the TBT-treatments was observed at all temperatures and survival was affected by the significant interaction between TBT and exposure temperature (30.4% of variation within the data, Table 2). The number of dead animals was higher for GEN−, especially in the 23°C group, whereby 48.9% of the total variation in the data can be explained by temperature (Table 2, two-way ANOVA, $P < 0.0001$).

It is well known that temperature plays an important role for growth and development of chironomids.[7,24,25] The EmT_{50} of the females of both populations is shown in Figures 1C-D. The values decreased significantly with increasing temperatures in both control and TBT-exposed groups. At 17 and 20°C a delay of the emergence time of GEN− compared to GEN+ can be shown. In contrast, at 23°C the controls of GEN+ and GEN− developed comparably (16.7 and 16.8 days) and a TBT effect was observed within the 23°C group for GEN+ only. Other studies document the decrease of the EmT_{50} with increasing temperatures[26] and explain this observation with increased food intake[27] and increased metabolism.[28] In general, the emergence time is strongly temperature-dependent. 79.0 and 88.5% of the total variation can be explained by temperature for GEN+ and GEN−, respectively (Table 2, two-way ANOVA, $P < 0.0001$).

Reproduction

The number of clutches (total and fertile) produced by GEN+ exhibited a decreasing trend in both the control and the TBT treatment with increasing temperatures (Table 3). The differences between the 20°C group and the other temperature treatments as well as those between the TBT treatments and the corresponding controls were not statistically significant. The control groups of GEN− (Table 3) also showed a reduced number of clutches with increasing temperatures. In contrast to GEN+, we observed a marked and statistically significant (Fisher's exact test, $P < 0.05$) decline of fertile clutches in the TBT treatment at 23°C with only 40% of the clutches being fertile compared to 80% in the associated control group. Moreover, a significant decrease in number of clutches (total and fertile) between the 20 and the 23°C TBT treatment was found (Fisher's exact test, $P < 0.001$).

The size of fertile clutches of GEN+ also showed a decreasing trend with increasing temperatures independently from the TBT treatment. Almost all TBT groups of GEN+ and GEN− have larger egg masses of fertile clutches in comparison to the controls. However, no significant

Table 2. Two-way ANOVA for six life history traits of the genetically variable (GEN+) and genetically impoverished (GEN−) *Chironomus riparius* population with *F*-values (*F*) and significance levels (*P*).

		GEN+		*GEN−*	
Trait	*Cause*	*F*	*P*	*F*	*P*
Mortality	TBT	0.21	0.7362	5.92	0.0292
	Temperature	2.96	0.4554	48.9	< 0.0001
	TBT × temperature	30.4	0.0011	3.89	0.1977
EmT_{50}	TBT	8.34	< 0.0001	0.79	0.1070
	Temperature	79.0	< 0.0001	88.5	< 0.0001
	TBT × temperature	0.69	0.3633	0.25	0.6537
Eggs per fertile clutch	TBT	3.78	0.0086	0.15	0.5850
	Temperature	3.61	0.0364	4.55	0.0123
	TBT × temperature	0.61	0.5658	0.34	0.7131
Sex ratio	TBT	0.01	0.9410	0.54	0.6179
	Temperature	0.99	0.8239	11.06	0.0903
	TBT × temperature	7.88	0.2247	10.97	0.0920
Male dry body weight	TBT	0.08	0.43242	0.24	0.4153
	Temperature	49.4	< 0.0001	17.4	< 0.0001
	TBT × temperature	4.54	< 0.0001	0.34	0.6174
Population growth rate	TBT	18.5	0.0026	6.22	0.0450
	Temperature	14.9	0.0222	16.8	0.0065
	TBT × temperature	3.12	0.4209	25.1	0.0008

EmT_{50} (mean emergence time), PGR (population growth rate).

Table 3. *Chironomus riparius* produced clutches (total/fertile), eggs per fertile clutch (mean ± SD), fertile clutches per female, sex ratio (female fraction) and male dry body weight (mean ± SD, in mg) of the GEN+ and GEN− population (C – (solvent) control, TBT – tributyltin).

Endpoint	*Treatment*	17°C	20°C	23°C
		GEN+		
Number of clutches (total/fertile)	C	44/32	44/28	28/18
	TBT	45/30	31/17	33/14
Eggs/fertile clutch (mean ± SD)	C	489 (± 90)	446 (± 143)	411 (± 80)
	TBT	513 (± 115)	490 (± 77)	481 (± 139)
Fertile clutches per female	C	0.604	0.509	0.367
	TBT	0.556	0.334	0.286
Sex ratio (female fraction)	C	0.414	0.462	0.495
	TBT	0.495	0.421	0.419
Male dry body weight [mg]	C	0.368 (± 0.033)	0.365 (± 0.033)	0.318 (± 0.026)[a]
	TBT	0.386 (± 0.027)	0.379 (± 0.034)	0.293 (± 0.034)[b]
		GEN−		
Number of clutches (total/fertile)	C	40/34	33/23	25/20
	TBT	38/32	43/38[c]	15/6[d]
Eggs/fertile clutch (mean ± SD)	C	445 (± 171)	424 (± 134)	388 (± 130)
	TBT	480 (± 128)	428 (± 131)	383 (± 69)
Fertile clutches per female	C	0.462	0.489	0.645
	TBT	0.681	0.603	0.240
Sex ratio (female fraction)	C	0.520	0.439	0.388
	TBT	0.452	0.578	0.379[e]
Male dry body weight [mg]	C	0.386 (± 0.051)[f]	0.345 (± 0.045)	0.315 (± 0.033)[f]
	TBT	0.376 (± 0.048)	0.332 (± 0.024)	0.319 (± 0.048)

Statistically significant to: [a](control 20°C, $P < 0.001$); [b](TBT 20°C, $P < 0.001$); [c](corresponding control, $P < 0.05$, and 23°C TBT, $P < 0.001$); [d](corresponding control, $P < 0.05$); [e](TBT 20°C, $P < 0.05$),[f](control 20°C, $P < 0.01$).

differences were found either between the controls and the corresponding TBT treatments nor between the 17°C/23°C group and the 20°C group. Péry and Garric[27] observed a slight decrease of clutch sizes of *C. riparius* when the midges were exposed to a temperature range between 15.0 and 26.7°C. This phenomenon was also found for other chironomids.[29] Surprisingly, in the case of GEN− control, the number of fertile clutches per female shows an increase with increasing temperatures. However, no statistical test was possible because only one breeding container was used per treatment. As presented by two-way ANOVA, only 3.61 (GEN+) and 4.55% (GEN−) of the total variance within the data could be explained by temperature (Table 2).

The sex ratio of GEN+ was not affected in the controls nor in the TBT-treatments, while the genetically impoverished population GEN− showed a skewed sex ratio in favour of males in the TBT/23°C group compared to the TBT/20°C group (Fisher's exact test, $P < 0.05$). A shift in the sex ratio independent of the temperature is also documented for other arthropods. Voordouw and Anholt[30] investigated sex determination in the copepod species *Tigriopus californicus* at 15 and 22°C. They found that higher temperature induces masculinization in this species. Two-way ANOVA clarifies that sex ratio is most likely affected by temperature in the GEN− population although, the *F*-value is not significant (Table 2).

Dry body weight of males

The mean male dry weight varied between 0.386 (GEN+, 17°C, TBT) and 0.293 mg (GEN+, 23°C, TBT). With increasing temperatures, male dry body weight decreased in both the control and the chemical treatment (Table 3). While the GEN+ population showed the strongest loss (Kruskal–Wallis statistics with Dunn's post hoc test) of average body weight between 20 and 23°C both in the control (−13.5%, $P < 0.001$) and in the TBT treatment (−23.7%, $P < 0.001$), the decrease of male dry body weight of GEN− was evenly distributed over the entire temperature range. As already discussed, the animals developed faster with increasing temperatures. During the shorter development period the increase of food intake and metabolism may not be sufficient to attain a comparable weight to midges being exposed to lower temperatures. The fact that shorter larval development due to higher temperatures leads to smaller imagines is in line with results from other studies on chironomids.[20,31,32] As shown in Table 2, only 0.08 (GEN+) and 0.24% (GEN−) of the total variability of the data are induced by chemical exposure, while 49.4 (GEN+) and 17.4% (GEN−) are attributable to temperature (two-way ANOVA).

Population growth rate (PGR) λ

Growth rates of each population are shown in Figure 2. For the genetically diverse population GEN+, a growth advantage is obvious in the control compared to the TBT-treatment over the whole temperature range. While the PGR of the control increased with increasing temperatures moderately ($\lambda = 1.239$, 1.245 and 1.275 d^{-1}), the PGR remain approximately on the same level for the TBT treatments (Figure 2A). A hypothetical daily increase of 28–29% ($\lambda = 1.28$ and 1.29 d^{-1}) of untreated *C. riparius* populations is already described for an ambient temperature of 21°C.[33,34] As demonstrated in Table 3, the temperature and the treatment were significantly responsible for the variation within the data set (14.9 and 18.5%, respectively).

The control group of the genetically impoverished population GEN− revealed considerable smaller PGR compared to GEN+ with exception of the 23°C control group (Figure 2B). Here the PGR of both populations were nearly the same (1.275 for GEN+ and 1.276 d^{-1} for GEN−). While exposure temperatures of 17 and 20°C caused no effect in the TBT treatment compared to the respective

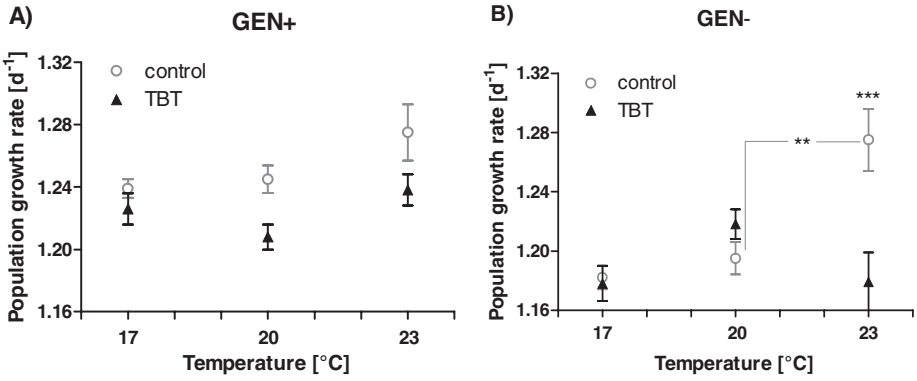

Fig. 2. Population growth rate [d^{-1}] of the genetically variable GEN+ (A) and the impoverished *Chironomus riparius* population GEN− (B) at different temperatures with and without TBT exposure. Significant differences were calculated using one-way-ANOVA (Tukey's post hoc test) comparing TBT treatment and corresponding control as well as 17 and 23°C with the standard temperature 20°C (mean ± SEM, ** $P < 0.01$, *** $P < 0.001$, n = 7).

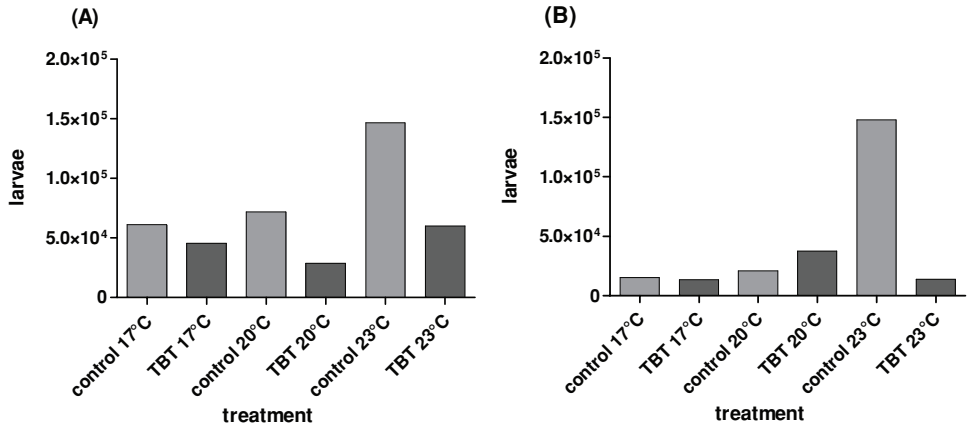

Fig. 3. Predicted number of individuals of different *Chironomus riparius* populations with and without TBT exposure within a temperature gradient (17, 20 and 23°C). The number of individuals of the genetically variable population GEN+ (A) and the genetically impoverished population GEN− (B) are represented for a 30-day experiment.

control, we observed a significant increase of PGR (one-way ANOVA with Tukey's post hoc test, $P < 0.01$) in the 23°C control compared to the 20°C control group. The PGR of the TBT group decreases at 23°C significantly ($\lambda = 1.179$) compared to the 23°C control ($\lambda = 1.276$, one-way ANOVA with Tukey's post hoc test, $P < 0.001$). Overall, the two-way ANOVA demonstrated a significant impact of both TBT treatment and temperature, which are responsible for 6.22 and 16.8% of the observed data variation (Table 2). Moreover, the analysis indicates for GEN− a significant ($P < 0.001$) interaction between both factors.

The ecological relevance of the PGR becomes particularly clear if the number of individuals in the different treatment groups is calculated after 30 days (Figure 3). With the exception of GEN− at 20°C, all TBT treated groups produced at all temperatures fewer individuals than the control, independent of the level of heterozygosity. At an exposure temperature of 23°C, the TBT-treatment of GEN− produces 90.6% fewer individuals than the control. Although this exposed population produces still $1.4*10^4$ individuals within 30 days and is, thus, not acutely endangered, an increased extinction risk is conceivable for this r-strategists under the influence of additional stressors prevalent in the natural environment.

Conclusion

We present the first study investigating the combined effects of temperature and chemical stress considering genetic variability of an ecotoxicological model species under standardized conditions. In a similar experiment, Airas et al.[35] investigated the effects of multiple stressors (temperature, low oxygen concentration, chemicals) acting simultaneously on *C. riparius*. They found that individual stressors had no effect. In combination, however, significant effects were observed.

Our study demonstrates that a decrease in genetic variability has important consequences on the fitness of populations under the most moderate temperature stress. Overall, the decreased EmT_{50} of the GEN− TBT group at 23°C (Figure 1D) is not able to compensate for the high mortality and the very low reproduction resulting in a dramatic decrease of the PGR. However, the PGR was above 1.0 per day indicating that the population still grows at optimal laboratory conditions due to the high number of eggs per clutch (Table 3). This result suggests that global temperature change could have serious effects on the distribution and survival of locally distributed or rare species.

Acknowledgments

We would like to thank the staff of the working groups Ecology and Evolution and Aquatic Ecotoxicology of the Goethe University Frankfurt am Main for technical assistance. This research has been supported by the programme "Environmental Quality and its Security and Sustainment" (BW-Plus) with funding of the state of Baden-Württemberg (project number BWR 22018) and by the LOEWE Research Centre Biodiversity and Climate Frankfurt (BiK^F).

References

[1] Loreau, M.; Oteng-Yeboah, A.; Arroyo, M.T.K.; Babin, D.; Barbault, R.; Donoghue, M.; Gadgil, M.; Hauser, C.; Heip, C.; Larigauderie, A.; Ma, K.; Mace, G.; Mooney, H.A.; Perrings, C.; Raven, P.; Sarukhan, J.; Schei, P.; Scholes, R. J.; Watson, R. T. Diversity without representation. Nature **2006**, *442*, 245–246.

[2] Nowak, C.; Vogt, C.; Pfenninger, M.; Oehlmann, J.; Streit, B.; Schwenk, K.; Oetken, M. Rapid genetic erosion in pollutant-exposed experimental chironomid populations. Environ. Pollut. **2009**, *157*, 881–887.

[3] IPCC. Climate Change 2007: The physical science basis – summary for policymakers Intergovernmental Panel on Climate Change 2007. http://www.ipcc.ch/SPM2feb07.pdf (accessed March 2009).
[4] Heinonen, J.; Honkanen, J.; Kukkonen, J.V.K.; Holopainen, I.J. Bisphenol A accumulation in the freshwater clam *Pisidium amnicum* at low temperatures. Arch. Environ. Contam. Toxicol. **2002**, *43*, 50–55.
[5] Hamburger, K.; Dall, P.C.; Lindegaard, C. Energy metabolism of *Chironomus anthracinus* (Diptera: Chironomidae) from the profundal zone of Lake Esrom, Denmark, as a function of body size, temperature and oxygen concentration. Hydrobiologia **1994**, *294*, 43–50.
[6] Heugens, E.H.W.; Jager, T.; Creyghton, R.; Hendriks, A.J.; van Straalen, N.M.; Admiraal, W. Temperature-dependent effects of cadmium on *Daphnia magna*: accumulation versus sensitivity. Environ. Sci. Technol. **2003**, *37*, 2145–2151.
[7] Armitage, P.D.; Cranston, P.S.; Pinder, L.C.V. The Chironomidae. The biology and ecology of non-biting midges. Chapman & Hall: London, 1995.
[8] Stief, P.; Holker, F. Trait-mediated indirect effects of predatory fish on microbial mineralization in aquatic sediments. Ecology **2006**, *87*, 3152–3159.
[9] Nowak, C.; Hankeln, T.; Schmidt, E.R.; Schwenk, K. Development and localization of microsatellite markers for the sibling species *Chironomus riparius* and *Chironomus piger* (Diptera: Chironomidae). Mol. Ecol. Notes. **2006**, *6*, 915–917.
[10] Organisation for Economic Development and Cooperation (OEDC). Guideline for Testing Chemicals No 218. Sediment-Water Chironomid Toxicity Test Using Spiked Sediment, Original guideline 218; OEDC: Paris, France, 2004.
[11] Duft, M.; Schulte-Oehlmann, U.; Tillmann, M.; Markert, B.; Oehlmann, J. Toxicity of triphenyltin and tributyltin to the freshwater mudsnail *Potamopyrgus antipodarum* in a new sediment biotest. Environ. Toxicol. Chem. **2003**, *22*, 145–152.
[12] Vogt, C.; Belz, D.; Galluba, S.; Nowak, C.; Oetken, M.; Oehlmann, J. Effects of cadmium and tributyltin on development and reproduction of the non-biting midge *Chironomus riparius* (Diptera)—baseline experiments for future multi-generation studies. J. Environ. Sci. Health Pt. A, **2007**, *42*, 1–9.
[13] Benoit, D.A.; Sibley, P.K.; Juenemann, J.L.; Ankley, G.T. *Chironomus tentans* life-cycle test: design and evaluation for use in assessing toxicity of contaminated sediments. Environ. Toxicol. Chem. **1997**, *16*, 1165–1176.
[14] Forbes, V.E.; Cold, A. Effects of the pyrethroid esfenvalerate on life cycle traits and population dynamics of *Chironomus riparius*—importance of exposure scenario. Environ. Toxicol. Chem. **2005**, *24*, 78–86.
[15] Sibley, P.K.; Benoit, D.A.; Ankley, G.T. The significance of growth in *Chironomus tentans* sediment toxicity test: Relationship to reproduction and demographic endpoints. Environ. Toxicol. Chem. **1997**, *16*, 336–345.
[16] Woodworth, L.M.; Montgomery, M.E.; Briscoe, D.A.; Frankham, R. Rapid genetic deterioration in captive populations: Causes and conservation implications. Conserv. Genet. **2002**, *3*, 277–288.
[17] Nowak, C.; Vogt, C.; Barateiro, J.; Schwenk, K. Genetic impoverishment in laboratory cultures of the test organism *Chironomus riparius*. Environ. Toxicol. Chem. **2007**, *26*, 188–122.
[18] Hahn, T.; Schulz, R. Ecdysteroid synthesis and imaginal disc development in the midge *Chironomus riparius* as biomarkers for endocrine effects of tributyltin. Environ. Toxicol. Chem. **2002**, *21*, 1052–1057.
[19] Fargasova, A. Comparison of tributyltin compound effects on the alga *Scenedesmus quadricauda* and the benthic organisms *Tubifex tubifex* and *Chironomus plumosus*. Exotox. Environ. Safe. **1998**, *41*, 222–230.
[20] Frouz, J.; Ali, R.; Lobinske, R.J. Influence of temperature on developmental rate, wing length, and larval head capsule size of pestiferous midge *Chironomus crassicaudatus* (Diptera: Chironomidae). J. Econ. Entomol. **2002**, *95*, 699–705.
[21] Stevens, M.M. Development and survival of *Chironomus tepperi* Skuse (Diptera: Chironomidae) at a range of constant temperatures. Aquat. Insect. **1998**, *20*, 181–188.
[22] Nowak, C.; Jost, D.; Vogt, C.; Oetken, M.; Schwenk, K.; Oehlmann, J. Consequences of inbreeding and reduced genetic variation on tolerance to cadmium stress in the midge *Chironomus riparius*. Aquat. Toxicol. **2007**, *85*, 278–284.
[23] Armbruster, P.; Reed, D.H. Inbreeding depression in benign and stressful environments. Heredity **2005**, *95*, 235–242.
[24] Rossaro, B. Chironomids and water temperature. Aquat. Insects **1991**, *13*, 87–98.
[25] Pinder, L.C.V. Biology of freshwater Chironomidae. Ann. Rev. Entomol. **1986**, *31*, 1–23.
[26] Vogt, C.; Pupp, A.; Nowak, C.; Jagodzinski, L.S.; Baumann, J.; Jost, D.; Oetken, M.; Oehlmann, J. Interaction between genetic diversity and temperature stress on life-cycle parameters and genetic variability of *Chironomus riparius* populations. Climate Res. **2007**, *33*, 207–214.
[27] Péry, A.R.R.; Garric, J. Modelling effects of temperature and feeding level on the life cycle of the midge *Chironomus riparius*: an energy-based modelling approach. Hydrobiologia **2006**, *553*, 59–66.
[28] Sankarperumal, G.; Pandian, T.J. Effect of temperature and *Chlorella* density on growth and metamorphosis of *Chironomus circumdatus* (Kieffer) (Diptera). Aquat. Insect. **1991**, *13*, 167–177.
[29] Xue, R.D.; Ali, A. Relationship between winglength and fecundity of a pestiferous midge, *Glyptotendipes paripes* (Diptera, Chironomidae). J. Am. Mosq. Control. Assoc. **1994**, *10*, 29–34.
[30] Voordouw, M.J.; Anholt, B.R. Environmental sex determination a splash pool copepod. Biol. J. Linn. Soc. **2002**, *76*, 511–520.
[31] Atkinson, D. Temperature and organism size: a biological law for ectotherms. Adv. Ecol. Res., **1994**, *25*, 1–58.
[32] Surakarn, R.; Yano, K. Development of a paddy-dwelling chironomid, *Chironomus kiiensis* (Diptera, Chironomidae) under different temperatures. Jpn. J. Entomol. **1995**, *63*, 389–398.
[33] Charles, S.; Ferreol, M.; Chaumot, A.; Péry, A.R.R. Food availability effect on population dynamics of the midge *Chironomus riparius*: a Leslie modelling approach. Ecol. Model. **2004**, *175*, 217–229.
[34] Lopes, C.; Péry, A.R.R.; Chaumot, A.; Charles, S. Ecotoxicology and population dynamics: Using DEBtox models in a Leslie modelling approach. Ecol. Model. **2005**, *188*, 30–40.
[35] Airas, S.; Leppanen, M.; Kukkonen, J.V.K. Effects of temperature and oxygen concentration in sediment toxicity testing. Ecotoxicol. Environ. Safe. **2008**, *70*, 475–482.