PERIODICUM BIOLOGORUM
VOL. 124, No 1–2, 29–40, 2022
DOI: 10.18054/pb.v124i1-2.11623

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Original Research Article

Fitness recovery and two-generation phenotypic plasticity in the freshwater snail *Planorbarius corneus* L. exposed to hyperosmotic solutions

NEDA MAŽURAN[1]
VLADIMIR HRŠAK[2]
GORAN KOVAČEVIĆ[3]*

[1] Zagreb, Croatia

[2] Faculty of Science, University of Zagreb, Division of Biology, Department of Botany, Zagreb, Croatia

[3] Faculty of Science, University of Zagreb, Division of Biology, Department of Zoology, Zagreb, Croatia

***Correspondence:**
Goran Kovačević
E-mail address: goran.kovacevic@biol.pmf.hr

Keywords: chronic exposure; hyperosmotic salts; calcium chloride; calcium bromide; F1 offspring acclimation; fecundity; growth

Received December 8, 2021
Revised May 25, 2022
Accepted May 26, 2022

Abstract

Background and purpose: *Hyperosmotic solutions of calcium chloride and calcium bromide, extensively used in the oil industry, can be a source of aquatic pollution.*

Materials and methods: *In the present study, we investigated whether the freshwater snail* Planorbarius corneus *L., previously chronically exposed to toxic concentrations of these chemicals, can recover fecundity and growth in clean water. We also examined if a higher tolerance could be induced in F1 offspring after parental and developmental chronic exposure to the chemicals.*

Results: *The fitness-recovery study showed a high compensatory potential of the snails to recover fitness through increased reproduction and growth. In the tolerance study, we observed increased tolerance in F1 offspring in hyperosmotic solutions of calcium chloride. However, there was no significant improvement of reproduction performance in bromide-containing solutions.*

Conclusions: *Increased tolerance was associated with a redirection of resources from growth to maintenance and survival.*

INTRODUCTION

Phenotypic plasticity is the ability of a genotype to produce more than one phenotype when exposed to different environments *(1,2)*. If it is adaptive, phenotypic plasticity is beneficial, allowing a genotype to have wider tolerance and higher fitness across variable environments *(3)*. Plasticity may be expressed at behavioral, biochemical, physiological or developmental levels *(2)*. One type of phenotypic plasticity is physiological acclimation, the ability of an individual organism to alter its performances in response to environmental changes *(4-6)*. Environmental factors affect development and can cause the modification of developmental events, so phenotypic plasticity is also defined as developmental plasticity *(4,7-9)*. Environmental factors can affect development even before the formation of the zygote as an environmental effect on the parental generation and a parental effect on the offspring generation, i.e., as a parental or maternal effect *(4,10)*. For instance, strong evidence for gamete plasticity in response to osmotic stress in marine tubeworm *Hydroides diramphus* has been demonstrated: parents were able to adjust the salinity tolerance of their gametes so that the highest success of fertilization, gamete performance, development and larval survival was observed when matched the parental salinity environment *(11)*. A maternal or parental effect is the influence exerted on the offspring pheno-

type resulting from the phenotype of the mother or from the environment she experiences *(12)*. A maternal effect can induce developmental novelties in offspring in accordance with the environmental conditions present in the parental generations *(13)*. For example, parental exposure to chronic hypoxia for 2-4 weeks can induce increased hypoxia resistance in zebrafish (*Danio rerio*) larvae *(14)*. Maternal plastic effects in marine stickleback (*Gasterosteus aculeatus*) F1 offspring of mothers acclimated to ambient and elevated temperatures (17 °C and 21 °C) were found *(15)*. Thirty days after hatching they had a significantly larger body size (a key component of fitness) when reared in the same environment as their mothers. The benefits were stronger and lasted longer under stressful conditions. Moreover, maternally inherited mitochondrial respiration capacities in adult F1 offspring matched the pattern of juvenile body size. Parentally induced plasticity is preferred when there is a temporal or spatial fluctuation in environments across generations. Environmentally induced phenotypic plasticity and maternal effect can initiate evolutionary novelties and influence the evolution of traits that could improve offspring fitness, even on an ecological timescale *(16,17)*, through nongenetic inheritance, i.e., transgenerational developmental plasticity *(18,19)*.

Evolutionary changes in phenotypic plasticity can occur after anthropogenic disturbances *(20)*. Anthropogenic stress factors can have evolutionary consequences on a population exposed to pollution *(21,22)*. When chemical stressors affect fitness parameters, such as survival and reproduction, genetic resistance can evolve in less sensitive animals *(23)* or there can be an adaptive response, i.e., tolerance *(5,24,25)*. The acquisition of higher tolerance or resistance to chemical stressors in multigeneration studies has been reported: a higher tolerance after eleven generations *(26)*, a rapid increased resistance after only one generation *(27)*, acclimation and/or adaptation of freshwater planorbid snails over the course of exposure over three generations *(28)*, acclimation to copper (Cu) and adaptation to tributyltin oxide (TBTO) in *Tigriopus californicus (29)*, acclimation to high salinity and high temperatures after nine generations in *Daphnia magna (30)*, cadmium acclimation in *Daphnia* clones *(31)*, and increased Cd tolerance in *Lymnaea stagnalis* hatchlings from parents exposed to cadmium *(32)*.

The pulmonate freshwater planorbid snail *Planorbarius corneus* L. is a common species occupying various aquatic habitats *(33)*. It is a suitable experimental animal, which is why it has been subject to a tremendous amount of biochemical, neurobiological, embryological, endocrinological and parasitological research. Ecotoxicological assessment has also been done on this species in Europe where it is distributed. *P. corneus* is a simultaneous hermaphrodite, in field populations the egg-laying period occurs from May to November *(34)*; in laboratory cultures reproduction continuously lasts throughout the year. The eggs are enclosed in jelly capsules, i.e., egg masses. The number of egg masses and eggs per egg mass reported in literature is very variable. Fecundity is strongly affected by experimental conditions *(35)*.

Freshwater snails are almost completely restricted to shallow waters where environmental conditions are unstable and where there are broad fluctuations in the diurnal temperature, acidity of the water, water level, dissolved minerals and concentration of gases, pollution caused by decaying organic matter and the availability of calcium *(36)*. The snails have wide ecological and environmental tolerances that has evolved with a capacity to be passively transferred to other habitats with new ecological and environmental conditions *(36,37)*. Living in these variable and transitory habitats, many species of freshwater pulmonates are strongly selected for the possession of adaptive plasticity *(38)*. Pulmonate snails are important to aquatic community structure and function *(39)*. They are considered good pollution and water quality indicators. Freshwater gastropods account for ~20% of recent mollusk extinctions, despite constituting only ~5% of the world's gastropod fauna, reasons for these declines are anthropogenic effects including habitat loss and degradation, introduced species, water pollution and others *(37)*. The predicted increase in temperature due to climatic change may have dramatic consequences for the presence and composition of freshwater pulmonates in north-western Europe *(40)*. Populations of the great ramshorn, *P. corneus* L., have been declining in recent years *(33,41,42)*. Therefore, it is important to investigate the capacity of snails to recover fitness impaired by different environmental pollutants and to assess if an adaptive response (tolerance or resistance) to the long-term or permanent presence of different pollutants can occur.

This study is the continuation of the earlier published investigation *(43)* of the sublethal effects of calcium chloride and calcium bromide on the fecundity of *P. corneus* L. Saturated solutions of calcium chloride and calcium bromide and mixtures, defined as clear brines or high-density brines, are used extensively in the oil and gas industry *(44)*. They can be a source of aquatic environment pollution in oil and gas extraction areas. When working operations are finished, these saturated solutions are released as waste and can pollute fresh and ground waters in various concentrations. In north-western Croatia, most gas and oil exploration and production activities have been located in regions rich in stagnant and slow-running waters, where *P. corneus* L. is one of the most common and indigenous species. As was previously shown, sublethal concentrations of both chemicals showed a statistically significant dose-response decrease in the survival and fecundity (reduction of egg mass number) of *P. corneus* L. in long-term tests. Calcium bromide proved to be more toxic than calcium chloride *(43)*.

To assess the toxic effect of calcium chloride and calcium bromide on *P. corneus,* we performed additional

laboratory tests to estimate how successfully exposed animals can recover from the toxic effect after the stressor has been withdrawn by measuring the life-cycle parameters of reproduction, survival, and growth. In order to test the hypothesis that parental and developmental exposure to the chemicals would lead to increased tolerance, we also investigated the effect of the chemicals on the next generation life history parameters, submitting the progeny of the exposed animals to the same toxic conditions. Here we consider tolerance synonymous with "acclimation" and "adaptive phenotypic plasticity" as stress response induced by environmental conditions or transferred from mother, while resistance is synonymous with "adaptation" or heritable change resulting from selection *(5,29)*.

MATERIALS AND METHODS

Study population and experimental protocol

We used a population of *P. corneus* L. maintained in the laboratory for several generations. They originated from samples collected at one time from ponds connected with Sava River 40 km south of Zagreb. In the laboratory the snails were reared in a 35 L aquarium. Two weeks before the experiments they were acclimated to experimental conditions.

In the previous study *(43)* we selected snails of equal ages (3.5 months) with a shell length of 8-9 mm from a laboratory mass culture. In both studies (previous and present) the experimental protocols were the same: for each chemical concentration we used 50 snails of equal size and ages and placed them in five 1000 ml glass beakers 10 snails each. There were three replicates (beakers with 10 animals each) for each chemical concentration and two replicates (beakers with 10 animals each) for the control for each experimental solution. We fed the animals daily on washed organic lettuce leaves ad libitum. They were kept under a natural photoperiod, the temperature was 23°C ± 1, and the pH was 6.8-7.3. We discarded dead snails from the experiment on the same day when they were found dead. The beakers were covered with Petri dishes to prevent an increase in concentration through evaporation. The experiments were run for 6 weeks. The dilution water, as well as control water, was dechlorinated tap water containing 80 mg/L Ca^{2+} and 35 mg/L Cl^{-}. We measured the actual concentrations of the chemicals at the start of each week after preparing fresh solutions and at the end of the week just before changing the solutions. The initial concentrations of the chemicals measured each week after preparation in the test beakers varied ± 0.1-5% from the nominal values (Ca^{2+} concentration varied ± 0.1-5%, Br^{-} varied ± 0.3-1.3%, Cl^{-} varied ± 0.3-3.6%). The calcium concentrations in the diluted test solution samples and in the control water samples were measured with a Varian Techron AA5 atomic absorption spectrophotometer (ASTM D 512-93 1995). The bromide concentrations were measured by the spectrophotometric method. The chloride concentrations were determined by the silver nitrate volumetric method ASTM D 512-89 1995.

Fitness recovery study

In the fitness recovery study, we assessed if the animals exposed to 10 hyperosmotic solutions proved toxic in previous experiment *(43)* can recover from the toxic effect. Immediately after the six-week exposure to the tested chemicals ($CaCl_2$ 1203 mg/L, 2406 mg/L and 4813 mg/L, $CaBr_2$ 1066 mg/L, 2664 mg/L and 5329 mg/L, 1:1 Ca/$BrCl_2$ volume mixture 774 mg/L, 1934 mg/L, 3868 mg/L and 5801 mg/L) we transferred the snails to the control water for observation of recovery for the next six weeks in the same laboratory conditions as during the chemical testing. The controls were the same control snails from the previous exposure study.

In order to establish the capacity of the snails to recover fitness parameters (fecundity and growth) after exposure to the chemicals, we removed and counted the egg masses produced in the recovery treatment weekly just as in the controls. To estimate the shell growth rate (the growth recovery), measurements of the shells were taken at the beginning of the experiments, after the six-week exposure to the chemicals, and after the six-week recovery period in the clean control water. The maximum diameter of the shell (width from the edge of the peristome across the umbilicus to the opposite periphery of the shell) was measured to 0.1 mm with calipers.

Tolerance study

In the tolerance experiment, we investigated the potential of the next generation snails to acclimate to the previously tested toxic solutions and to acquire increased tolerance. Therefore, we selected young adult snails (3.5 months old and 8 mm wide, when they started to breed) from a laboratory culture as parental (P) snails and started a six-week experiment with the same substance concentrations and under conditions described above. Each week we removed all the collected egg masses from the parental beakers to new beakers. After 14-21 days of hatching time, the young (F1) snails were raised to the adult stage in the same solution concentrations and under the same conditions (three chemical solution replicates and two control replicates) as the parental snails. Environmental conditions in both (P and F1) parts of experiment were identical. When the F1 snails started to reproduce, (six months after hatching) we started the experiment. The F1 snails' egg masses were removed and counted weekly during the six-week exposure to the same laboratory conditions as the parental snails. Control snails were specimens of the same age as F1 snails, obtained from the laboratory culture. Statistical comparisons with the control snails and the parental snails were made. Measurements of the shells were also taken after six weeks of experiment in both parental and filial snails and equal

statistical comparisons were made. Thus, we compared the reproduction and growth of the same life stage period (six weeks period after beginning of the reproduction).

Statistical analysis

Statistical analyses for recovery and tolerance study were performed by using PAST 2.17 software (http://folk.uio.no/ohammer/past/). Prior to statistical analysis, the data were tested for normality by the Kolmogorov-Smirnov normality test and homogeneity of variances was tested by Bartlett's test. To determine the significance of the differences in the production of egg masses among all groups, eggs per egg mass and shell growth between the experimental groups and the controls, between the chemical and recovery treatments, and between parental and filial snails, one way ANOVA followed by Tukey HSD post-hoc tests were used ($p<0.001$). The effect of time on the weekly production of egg masses was analysed by Wilcoxon matched pairs test and the Mann-Whitney U-test.

RESULTS

Fitness recovery study

After six weeks of recovery in control water for all groups of snails previously exposed to chemicals, the number of egg masses increased in all but one recovery treatment group in comparison to the egg mass number in the chemical treatments (Figures 1a, 1b, 1c). The average number of 22.01±2.82 eggs per egg mass was not affected by the chemicals since it was not significantly different from the controls (21.78±2.78) during and after the chemical treatment. In the recovery treatment of the snails previously exposed to three $CaCl_2$ concentrations, the increase in the egg mass number after the recovery treatment was not statistically significant compared to the egg mass number in the chemical exposure treatment. In the recovery treatment of the snails previously exposed to the highest $CaCl_2$ concentration (4813 mg/L), the number of egg masses was three times higher than in the chemical exposure. However, a statistical significance was not found due to high variability arising from the differences in the number of egg masses between weekly counts (Figure 1a). In all other recovery treatments, except in the 774 mg/L mixture, the increased egg mass number was statistically significant (ANOVA and Tukey post hoc test) and several times higher (2.4 to 17 times higher) than the number in the chemical exposure treatments. In two cases ($CaBr_2$ 1066 mg/L and 2664 mg/L), it was even higher than in the control (Figure 1b). The highest increase in egg mass production was observed in the recovery groups of snails which produced the lowest number

Table 1. *Mean shell growth rate and mortality of* Planorbarius corneus *L. in control, chemical exposure and recovery treatment groups in six weeks (results are expressed in millimeters as mean ± SE).*

Chemical concentrations	Control	Chemical exposure	Recovery treatment	Mortality in control (%)	Mortality in chemical exposure (%)	Mortality in recovery treatment (%)
$CaCl_2$ 1203 mg/L	3.0 ± 0.14	1.2 ± 0.08***	1.6 ± 0.23	0	6.6	3.3
$CaCl_2$ 2406 mg/L	3.3 ± 0.17	1.1 ± 0.12***	1.8 ± 0.21	3.3	13.3	0
$CaCl_2$ 4813 mg/L	3.3 ± 0.19	0.7 ± 0.11***	2.2 ± 0.21	3.3	3.3	3.3
$CaBr_2$ 1066 mg/L	3.2 ± 0.17	0.4 ± 0.10***	3.5 ± 0.21	3.3	43.3	13.3
$CaBr_2$ 2664 mg/L	1.7 ± 0.18	0.4 ± 0.09***	2.2 ± 0.22	0	13.3	3.3
$CaBr_2$ 5329 mg/L	2.6 ± 0.19	0.3 ± 0.12***	1.4 ± 0.23	0	20	10
$Ca/BrCl_2$ 1:1 774 mg/L	1.7 ± 0.21	1.0 ± 0.13	1.1 ± 0.21	0	0	0
$Ca/BrCl_2$ 1:1 1934 mg/L	2.8 ± 0.22	0.3 ± 0.12***	1.5 ± 0.22	0	13.3	0
$Ca/BrCl_2$ 1:1 3868 mg/L	2.3 ± 0.11	0.4 ± 0.11***	0.9 ± 0.19	3.3	6.6	6.6
$Ca/BrCl_2$ 1:1 5801 mg/L	1.8 ± 0.16	0.1 ± 0.10***	1.0 ± 0.20	0	30	3.3

There were two replicates for each control and three replicates for each chemical exposure.

*** significant difference between chemical exposure groups and the controls (ANOVA post hoc p<0.001)

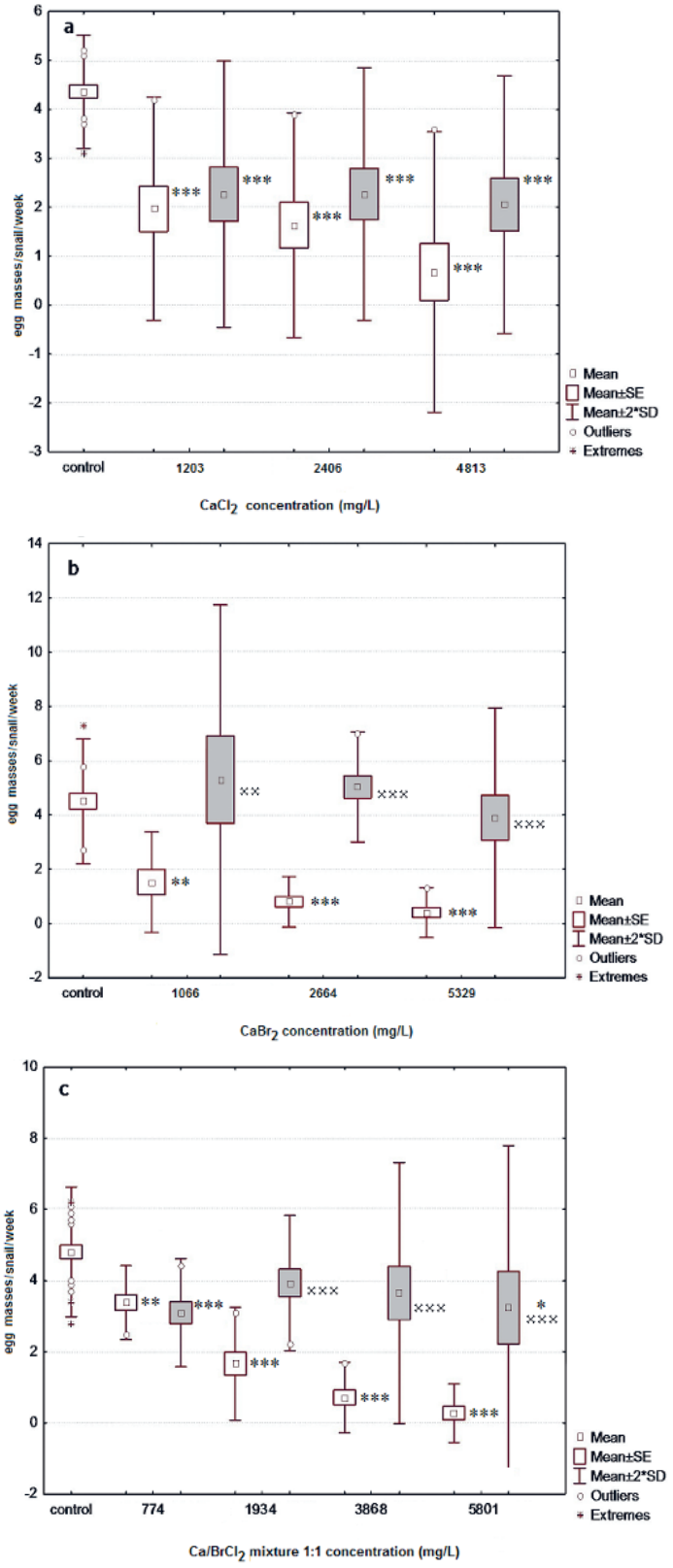

Figure 1. ***Planorbarius corneus* L., fitness recovery study.** *Mean number of egg masses per snail per week in control, chemical exposure (white bars) and recovery treatment in clear water (gray bars); a) $CaCl_2$, b) $CaBr_2$ and c) $Ca/BrCl_2$ 1:1 mixture. * statistical significance compared to the control (ANOVA, post hoc *** p<0.001, ** p<0.01, * p<0.05); × significant difference between chemical exposure concentration and recovery (ANOVA, post hoc ××× p<0.001, ×× p<0.01). Number of replicates in chemical exposures and recovery treatments were 3 for each concentration, and number of control replicates were 2 for each chemical concentration and recovery treatment.*

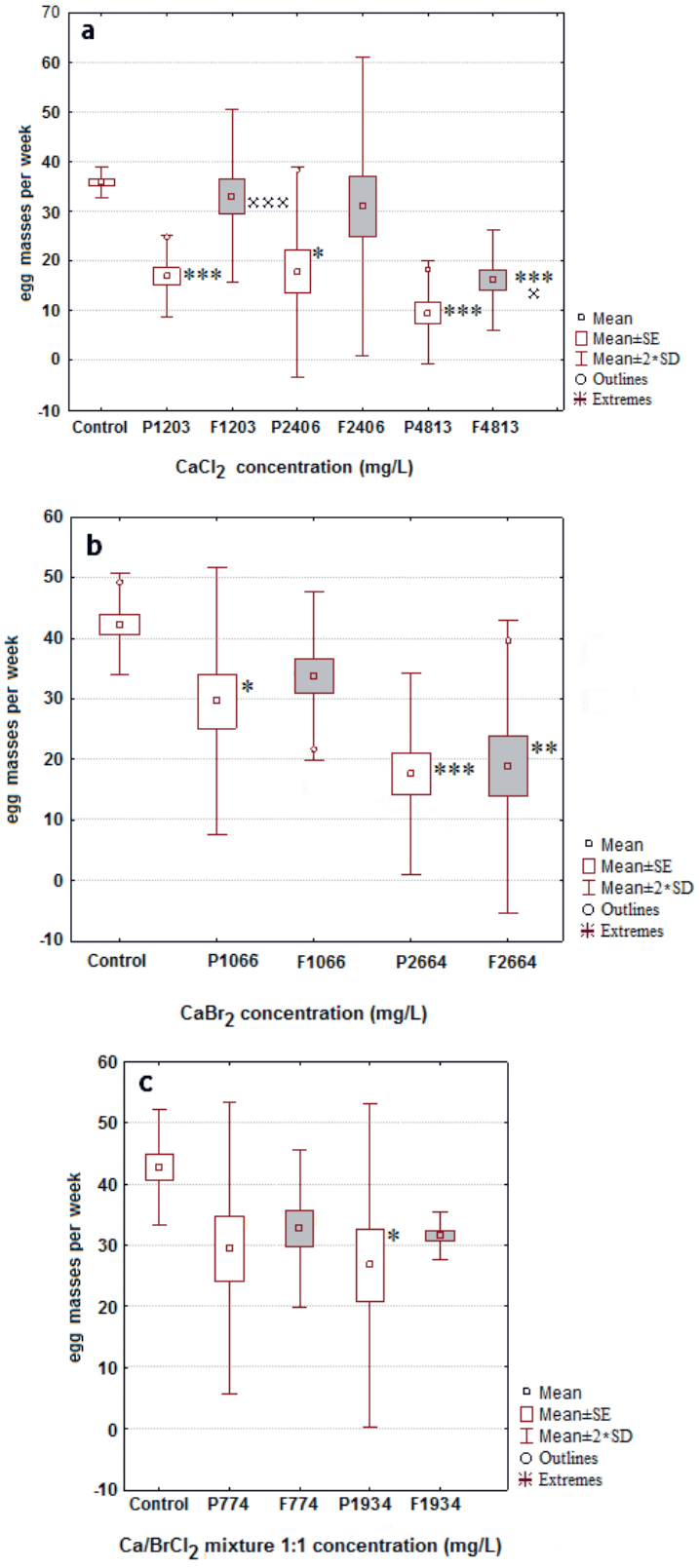

Figure 2. ***Planorbarius corneus* L., acclimation study.** *Mean number of egg masses per week in control, parental snails (white bars) and filial snails (gray bars); a) $CaCl_2$, b) $CaBr_2$ and c) $Ca/BrCl_2$ 1:1 mixture. P – parental snails; F1 – filial snails; * statistical significance compared to the control (ANOVA, post hoc *** p<0.001, ** p<0.01, * p<0.05); × statistical significance between P and F1 (ANOVA, post hoc× p<0.05, ××× p<0.001). Number of replicates in chemical exposures were 3 for each concentration, and number of control replicates were 2 for each chemical concentration.*

of egg masses during chemical exposure (i.e., those previously exposed to more toxic higher concentrations containing bromide (Figures 1a, 1b, 1c). In most groups of recovery treatment, the highest increase in egg mass production was observed in the first two weeks of recovery in clean water (not shown). Since the egg mass number was counted weekly, we analysed the effect of time using the nonparametric Wilcoxon matched pairs test and the Mann-Whitney U-test (not shown), but the results showed no significant effect. No significant difference in egg mass production was observed among the control groups within each individual experiment (a, b, c), so the control values presented in Figure 1 are the mean control values for each experiment (one-way ANOVA and Tukey HSD post-hoc test).

Shell growth decreased significantly in all chemical exposures in six weeks compared to the controls (Table 1, ANOVA $p<0.001$). In the $CaCl_2$ solutions, growth declined proportionally with the rise in concentration, and in the bromide-containing solutions the growth rate was even lower, ranging from 0.1-1.0 mm. During the six-week recovery period in the control water, the mean growth in all groups was higher than in the chemical-exposed groups, up to 10 times higher. Survival during exposure to the chemicals was high in most groups, except for the two concentrations: 70% in the 5801 mg/L mixture and 57% in $CaBr_2$ 1066 mg/L (Table 1).

Tolerance study

Egg masses were produced by parental snails in all chemical concentrations, but in $CaBr_2$ 5329 mg/L and the mixture 5801 mg/L the mortality of the embryos was 100%. The average number of eggs per egg mass (22.90±2.64) was not statistically different from the control (22.27±2.96) so it was not affected by the chemicals. After the 14-21 day hatching time, the same as in the controls, the mortality of young F1 snails in all solutions was very high (80-90%) so that in the mixture 3868 mg/L there were not enough young snails to continue the experiment. The surviving young snails in the chemical solutions grew very slowly and started depositing egg masses at the age of 5.5-6 months, attaining an 8.5-13 mm shell diameter.

A comparison of the number of egg masses produced by filial snails in six weeks with the number produced by parental snails in the same period showed a higher egg mass production by the filial snails in all chemical concentrations (Figures 2a, 2b, 2c). The egg mass number of the parental snails dropped significantly in all three $CaCl_2$ concentrations compared to the controls. In the filial snails in two lower concentrations of $CaCl_2$ (1203 mg/L and 2406 mg/L), egg mass production was not significantly different from the production in the control snails. In 1203 mg/L, the egg mass number of the filial snails was significantly higher than that of the parental snails

Table 2. *Mean shell growth of* Planorbarius corneus *L. in six weeks in controls and in chemical exposures of parental and filial snails (results are expressed in millimeters as mean ± SE).*

Chemical concentration	Parental control	Parental snails	Filial snails (F1)
$CaCl_2$ 1203 mg/L	3.6 ± 0.19	1.7 ± 0.25***	0.7 ± 0.13
$CaCl_2$ 2406 mg/L	3.7 ± 0.22	1.6 ± 0.18***	1.2 ± 0.13
$CaCl_2$ 4813mg/L	3.6 ± 0.16	1.2 ± 0.14***	0.4 ± 0.27
$CaBr_2$ 1066 mg/L	3.7 ± 0.23	1.8 ± 0.14***	2 ± 0.17
$CaBr_2$ 2664 mg/L	3.6 ± 0.21	1.3 ± 0.12***	1.0 ± 0.21
$Ca/BrCl_2$ 1:1 774 mg/L	3.5 ± 0.17	1.4 ± 0.23***	1.5 ±0.17
$Ca/BrCl_2$ 1:1 1934 mg/L	2.8 ± 0.26	1.7 ± 0.18*	0.9 ± 0.16

There were two replicates for each control and three replicates for each chemical exposure.
*** significant differences between chemical exposure groups and the controls (ANOVA post hoc $p<0.001$); * $p<0.05$).

($p<0.001$). Almost equal increase in 2406 mg/L as that in 1203 mg/L was not significant, probably due to the high variability of the egg mass count between weeks. In the highest $CaCl_2$ concentration, 4813 mg/L, the difference between the filial and parental egg mass number was also significant ($p<0.05$, Figure 2a).

In the case of $CaBr_2$ solutions, the egg mass production of parental snails was two times smaller than in the control in 2664 mg/L ($p<0.001$) and 1.6 times smaller than in the control in 1066 mg/L ($p<0.05$). In the filial snails, the number of egg masses in 2664 mg/L also dropped significantly compared to the control ($p<0.01$), but in 1066 mg/L it was not significantly different from the control and the difference between parental and filial snails was not significant in either concentration (Figure 2b).

In the lowest mixture concentration, 774 mg/L, the decline in the egg mass number of the parental and filial snails in comparison to the control was not significant and there was no significant difference between the parental and filial egg mass number. In mixture 1934 mg/L in parental snails, the egg mass number was significantly lower compared to the control ($p<0.05$), but in the filial snails the decline in the egg mass number was not significantly different from the control. However, there was no significant difference between P and F1 snails (Figure 2c). Again, the significant effect of time was not found using the nonparametric Wilcoxon matched pairs test and the Mann-Whitney U-test (not shown). As there was not significant difference among the control groups within

each experiment, the control values in Figure 2 are the mean of the control groups within particular experiment.

The shell growth in the parental snails was significantly lower in all $CaCl_2$ concentrations compared to the controls ($p<0.001$) and decreased proportionally with the increase in concentration. In $CaBr_2$ and in the mixture concentrations, the decrease in shell growth was also significant (Table 2). Some mortality was observed in $CaBr_2$ 2664 mg/L and $CaCl_2$ 4813 mg/L (20%, not shown). In F1 snails, growth in six weeks was lower than that of the parental snails in most groups (Table 2), but there was no mortality in any F1 group.

DISCUSSION

Recovery study

Field investigations have shown that variations in breeding and in the growth rate of freshwater pulmonates are characterized by a high degree of ecophenotypic plasticity resulting from environmental influences, and that this adaptive plasticity is of fundamental selective value for populations to compensate for annual variations in unstable and unpredictable transient habitats *(38,45)*. The plastic compensatory potential of snails is supported by a number of studies. For example, the results of laboratory experiments with *Planorbis contortus* (*Bathyomphalus contortus*) in which the growth rates increased after a temperature switch from lower to higher temperatures, and increased even more with snails transferred to a normal food regime after starvation in comparison to the continuously fed group, indicated the compensatory regulation capacity of freshwater snails *(46)*. The rapid growth of a single surviving planorbid snail *Helisoma duryi* when placed under normal conditions after a 14-day period of starvation, and the highest egg laying capacities in a few survivors after 21 or 28 days of starvation were observed *(47)*.

Similar results were obtained in this study which showed a strong capacity of *P. corneus* to recover from hyperosmotic and toxic stress and compensate for decreased growth and reproduction during the exposure. The growth rate of snails treated with toxic concentrations of chemicals which were then transferred to control water increased after 6 weeks of recovery in all experimental groups with some treatments showing 5, 8 and 10 times more growth than during exposure to the chemicals, and in some of the groups even more than in the control groups, indicating the reversibility of the chemical effect.

During the recovery period egg mass production was higher in the groups of snails previously exposed to higher chemical concentrations, especially those containing bromide where consequently egg mass production during chemical exposure was lower: the mean number of egg masses during recovery was 2.4-17 times higher than the number produced in the chemical solutions. In the recovery groups previously exposed to lower concentrations of salt and lower concentrations of bromide ($CaCl_2$ 1203 mg/L and 2406 mg/L and mixture 774 mg/L), egg mass production during the recovery period was not significantly higher in comparison to the previous production in the chemicals. This indicated that the compensation mechanism was not triggered by milder egg mass loss. The reduced growth and fecundity in hyperosmotic salt solutions could be the consequence of osmotic stress caused by elevated ion concentrations and metabolic work used for osmotic regulation. At higher external concentrations of calcium, there is a net influx of calcium into the mollusk body *(48,49)* and the net uptake increases with an increase in the concentrations in the medium *(49)*. At high environmental concentrations, the animals have to do metabolic work to remove excess calcium, and the necessity for homeostasis could result in an increased metabolic rate so that there might be fewer metabolic resources for egg laying and growth *(50)*. The majority of freshwater pulmonates allocate a very large proportion of assimilated energy to reproduction, and fecundity is directly related to size and the level of energy assimilation *(45)*. The energy budget in the marine snail *Chorus gigan-teus* was determined and it was found that under optimal food and temperature conditions energy allocated to growth and/or reproduction was 75.8% and metabolic demand was 18.9%. Under poor feeding and temperature conditions, metabolic demand in these marine snails increased to 217.9% of energy absorbed, leaving no energy available for growth and/or reproduction *(51)*. Since all the resistance and repair processes (e.g., active transport and others) are energy demanding and increase the metabolic rate *(52)*, it is possible that the snails exposed to very high salt concentrations spent most of their energy on osmotic regulation. This might have caused the observed decreased growth and reproduction. Mollusks, particularly pulmonate snails, are amongst the most sensitive of the freshwater invertebrates to increases in salinity *(53)*.

The results of the fitness recovery study showed that the toxic effect of the chemicals was reversible. After transferring the snails to normal osmotic conditions, they were able to compensate for the losses of reproduction and growth. The increased reproduction and growth in the recovery period can be explained as a plastic physiological reaction to a period of inability to reproduce and grow in a contaminated environment, a compensatory mechanism that can sustain population level after extreme environmental changes. These results indicate that the strong reproductive potential of the snails could compensate for losses after temporary and reversible toxicant impacts. Investigations have shown that populations with higher reproduction rates could overcome the effects of toxicants *(54)*.

Tolerance study

The tolerance study experiments involved rearing F1 snails in toxic solutions to maturity and comparing their

reproduction and growth rates with the parental ones. The mortality of newly hatched snails was 80–90% in all solutions except in $CaBr_2$ 5329 mg/L and mixture 5801 mg/L where a 100% mortality of embryos was recorded. After hatching, the juvenile snails had a reduced growth rate, especially in $CaBr_2$ solutions and began to breed at the age of 6 months which is almost 3 months later than the snails in the control water. The toxic effect of bromide on reproduction and on embryos was also found in other species. In long-term tests with *Poecilia reticulata* and *Daphnia magna*, the No Observed Effect Concentration (NOEC) for reproduction was only 7.8 mg/L Br^-, and bromide also proved embryotoxic for both species and caused degenerative changes of the reproductive organs in *Poecilia (55)*.

The results on reproduction in this study show a statistically significant decrease in egg mass production in parental snails and an increase in egg mass production in filial snails in comparison to parental snails in all chemical exposures. Based on comparison of the results between the P and F1 generations of snails in this study, it can be concluded that in the F1 generation tolerance to $CaCl_2$ increased at least in 1203 mg/L where the difference between P and F1 reproduction was significant and the difference between reproduction in F1 and control snails was insignificant. In a higher $CaCl_2$ concentration, 2406 mg/L, the F1 snails' egg mass number was almost the same as in 1203 mg/L, and was insignificant compared to the control, however, due to high inherent variability, no significant difference was found between reproduction in P and F1 snails. In the highest salt concentration, $CaCl_2$ 4813 mg/L, a significant difference between P and F1 snails' reproduction was also found. In all exposures to bromide-containing chemicals, except the highest $CaBr_2$ 2664 mg/L, the F1 snails' egg mass production was not significantly different from that of the control snails, but also not significantly different from the P snails' production. The results indicate that tolerance of the snails reared in the tested chemicals, could be limited to lower salt concentrations that would be even lower in the bromide-containing solution. In the long-term toxicity test on sodium bromide with three generations of *Daphnia magna*, it was found that exposing three generations of animals to the chemical which strongly reduced reproduction capacity did not lead to a change in NOEC values. Comparing the NOEC (E=reproduction) and the NOLEC (L=mortality) in the P generation, the authors noted a remarkable difference by a factor of about 400 *(55)*.

There are reports on acclimation and resistance in freshwater organisms and in snails: resistance in *Daphnia* to metals by pre-exposing the daphnids to a sublethal concentration of toxicant *(56)*, and physiological acclimation and adaptive response to trace metal pollution in land snails *(57)*. There were found *(28)* strain specific differences in response to multi-generation cadmium exposure in the freshwater gastropod *Biomphalaria glabrata* and that acclimation and/or adaptation to cadmium may have occurred. Multi-generation studies provide evidence of the acquisition of higher tolerance to a stressor. A multi-generation study (eleven generations) on *Chironomus reparius (26)* showed clear differences between a single generation and multi-generation exposure at a low sublethal concentration of biocide tributyltin, as in later generations evolutionary alteration (life-cycle changes and higher tolerance) was found. Increased tolerance to cadmium as a result of prior three-generation exposure was found in the freshwater planorbid snail *Biomphalaria glabrata (58)*. Four generations of copepod *Tigriopus japonicus* were exposed to different mercuric chloride treatments and all treatments underwent recovery in a clean environment for one generation *(59)*. In the recovery generation, none of the traits differed from the control, so it was concluded that the obtained Hg tolerance could be explained as physiological acclimation, i.e., phenotypic plasticity.

The results of our study show increased tolerance to hyperosmotic calcium chloride solutions and improved reproduction performances in calcium bromide and mixture solutions in F1 generation snails and indicate that tolerance was associated with fitness-cost as evidenced by slower growth and delayed maturation in comparison with the growth rate and maturation time in control snails. There is a common opinion that adaptation, resistance, and increased tolerance to stress bear costs, because some detoxification mechanisms are energetically expensive so that energy allocated to detoxification and somatic maintenance cannot be spent on growth and reproduction *(60,61)*. Energy assimilated from food is allocated to reproduction, somatic growth, maintenance metabolism and storage. As all the resistance and repair processes are energy-demanding, the metabolic rate should increase with an increasing concentration of toxin or with exposure time *(52)*. An increase in the metabolic rate diverted to somatic maintenance and stress resistance might reduce energy content for growth and reproduction *(25,62)*. Resource allocation trade-offs are phenotypically plastic (they depend on the environment) and there are priority rules for energy allocation under stressful or nutrient poor conditions among the traits: maintenance>reproduction>growth *(63,64)*. The concept of trade-offs based on energy allocation as a result of toxicant stress is supported by some studies *(27,65)*. However, other studies conclude that the concept is not well supported *(60)*. Some recent studies support the energy-cost hypothesis of tolerance. For example, the physiological cost of increased tolerance to copper in *Daphnia longispina* was found in the higher respiration rate of the most resistant clone *(66)*.

The observed increased tolerance of F1 snails in this study could have been induced by maternal effect inheritance and/or as a response to the environment during development. The F1 snails were exposed to the stressor during the entire development (from embryo to the adult stage) so they experienced a specific developmental input about their environment which could have induced phy-

siological changes *(4,6,9)*. Parental effect and developmental plasticity together were studied in a freshwater snail, and it was found that both processes can have an impact on offspring toxicant tolerance and that the effects of parental exposure on offspring toxicant tolerance may be eclipsed by the effect of offspring exposure during development *(24)*. Meta-analysis of experimental studies showed that parental effects are subtle compared with the direct effect of offspring environment *(67)*.

Increased tolerance in F1 generation snails in the present study could be a consequence of the elimination of the most sensitive animals in the juvenile stage. Since only 10-20% of the hatched snails survived and grew to maturity, they were probably more tolerant to hyperosmotic conditions and the toxic effect of bromide. Prolonged growth, late sexual maturity and delayed reproduction might be explained as physiological acclimation to a permanently increased energy demand due to maintaining osmotic balance and possibly detoxification. According to physiological energetic models, in the presence of toxic stress some tolerant animals deploy resistance mechanisms and increase their metabolism which decreases the energy available for production (growth and reproduction), but their chances of survival increase *(62)*.

This study has shown that the sublethal toxic effects of calcium chloride and calcium bromide on *Planorbarius corneus* L. are reversible. More resistant animals that survived were able to compensate for losses in reproduction and growth and fully recover their fitness traits such as growth and fecundity. These results suggest that the *P. corneus* population can recover fitness in the case of temporary pollution with high density brines.

In the tolerance study, increased tolerance, i.e., physiological acclimation, although at the expense of high juvenile mortality, prolonged growth, and increased time to reproductive size, was observed in $CaCl_2$ hyperosmotic solutions. As *Planorbarius corneus* has an annual life cycle, prolonged growth, and time to reach reproductive size followed by delayed reproduction and increased mortality could have an impact on the population size in natural habitats polluted with high density brines.

Future research on induced higher chemical tolerance in freshwater snails should consider possible consequential changes in the population life cycle due to impaired growth and reproduction dynamics.

***Acknowledgements**: This study has received no financial support as it was performed within the regular workload.*

REFERENCES

1. PIGLIUCCI M 2005 Evolution of phenotypic plasticity: where are we going now? Trends Ecol Evol 9: 481-486. https://doi.org/10.1016/j.tree.2005.06.001
2. PIGLIUCCI M, MURREN CJ, SCHLICHTING CD 2006 Phenotypic plasticity and evolution by genetic assimilation. J Exp Biol 209: 2362-2367. https://doi.org/10.1242/jeb.02070
3. GHALAMBOR CK, MCKAY JK, CARROLL SP, REZNICK DN 2007 Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecol 21: 394-407. https://doi.org/10.1111/j.1365-2435.2007.01283.x
4. GARLAND T JR, KELLY SA 2006 Phenotypic plasticity and experimental evolution. J Exp Biol 209: 2344-2361. https://doi.org/10.1242/jeb.02244
5. MORGAN AJ, KILLE P, STÜRZENBAUM SR 2007 Microevolution and Ecotoxicology of Metals in Invertebrates. Environ Sci Technol 41: 1085-1096. https://doi.org/10.1021/es061992x
6. FUSCO G, MINELLI A 2010 Phenotypic plasticity in development and evolution: facts and concepts. Philos Trans R Soc Lond B Biol Sci 365: 547-556. https://doi.org/10.1098/rstb.2009.0267
7. MINELLI A, FUSCO G 2010 Developmental plasticity and the evolution of animal complex life cycles. Philos Trans R Soc Lond B Biol Sci 365: 631-640. https://doi.org/10.1098/rstb.2009.0268
8. MOCZEK AP, SULTAN S, FOSTER S, LEDÓN-RETTIG C, DWORKIN I, NIJHOUT HF, ABOUHEIF E, PFENNIG DW 2011 The role of developmental plasticity in evolutionary innovation. Proc Biol Sci 278: 2705–2713. https://doi.org/10.1098/rspb.2011.0971
9. NETTLE D, BATESON M 2015 Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? Proc Biol Sci 282: 20151005. https://doi.org/10.1098/rspb.2015.1005
10. RITCHIE H, MARSHALL DJ 2013 Fertilisation is not a new beginning: sperm environment affects offspring developmental success. J Exp Biol 216: 3104-3109. https://doi.org/10.1242/jeb.087221
11. JENSEN N, ALLEN RM, MARSHALL DJ 2014 Adaptive maternal and paternal effects: gamete plasticity in response to parental stress. Functional Ecol 28: 724-733. https://doi.org/10.1111/1365-2435.12195
12. MARSHALL D., ULLER T 2007 When is a maternal effect adaptive? Oikos 116: 1957-1963. https://doi.org/10.1111/j.2007.0030-1299.16203.x
13. BADYAEV A, ULLER T 2009 Parental effects in ecology and evolution: mechanisms, processes and implications. Philos Trans R Soc Lond B. Biol Sci 364: 1169-1177. https://doi.org/10.1098/rstb.2008.0302
14. HO DH, BURGGREN, WW 2012 Parental hypoxic exposure confers offspring hypoxia resistance in zebrafish (*Danio rerio*). J Exp Biol 215: 4208-4216. https://doi.org/10.1242/jeb.074781
15. SHAMA LNS, STROBEL A, MARK FC, WEGNER M 2014 Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean. Functional Ecol 28: 1482-1493. https://doi.org/10.1111/1365-2435.12280
16. WEST-EBERHARD MJ 2005. Developmental plasticity and the origin of species differences. PNAS 102: 6543-6549. https://doi.org/10.1073/pnas.0501844102
17. CAROLL SP, HENDRY AP, REZNICK DN, FOX CW 2007 Evolution on ecological time-scales. Functional Ecol 21: 387–393. https://doi.org/10.1111/j.1365-2435.2007.01289.x
18. BONDURIANSKY R, DAY T 2009 Nongenetic Inheritance and its Evolutionary Implications. Ann Rev Ecol Evol Systematics 40: 103–25. https://doi.org/10.1146/annurev.ecolsys.39.110707.173441
19. ENGLISH S, PEN I, SHEA, N, ULLER T 2015 The information Value of Non-Genetic Inheritance in Plants and Animals. PLoS ONE 101: 1-17 e0116996. https://doi.org/10.1371/journal.pone.0116996
20. CRISPO E, DIBATTISTA, JD, CORREA C, THIBERT-PLANTE X, MCKELLAR AE, SCHWARTZ AK, BERNER D, DE LEÓN LF, HENDRY AP 2010 The evolution of phenotypic plas-

ticity in response to anthropogenic disturbance. Evol Ecol Res 12: 47-66. https://doi.org/10.1.1.713.3370

21. COUTELLEC MA, BARATA C 2011 An introduction to evolutionary processes in ecotoxicology. Ecotoxicology 20: 493-496. https://doi.org/10.1007/s10646-011-0637-x
22. MESSIAEN M, JANSSEN CR, THAS O, DE SCHAMPHELAERE KAC 2012 The potential for adaptation in a natural *Daphnia magna* population: broad and narrow-sense heritability of net reproductive rate under Cd stress at two temperatures. Ecotoxicology 21: 1899-1910. https://doi.org/10.1007/s10646-012-0923-2
23. KLERKS PL, XIE L, LEVINTON JS 2011 Quantitative genetics approaches to study evolutionary processes in ecotoxicology; a perspective from research on the evolution of resistance. Ecotoxicology 20: 513-523. https://doi.org/10.1007/s10646-011-0640-2
24. PLAUTZ SC, SALICE CJ 2013 Plasticity in offspring contaminant tolerance traits: developmental cadmium exposure trumps parental effects. Ecotoxicology 22: 847-853. https://doi.org/10.1007/s10646-013-1076-7
25. PLAUTZ SC, GUEST T, FUNKHOUSER MA, SALICE CJ 2013 Transgenerational cross-tolerance to stress: parental exposure to predators increases offspring contaminant tolerance. Ecotoxicology 22: 854-861. https://doi.org/10.1007/s10646-013-1056-y
26. VOGT C, NOWAK C, DIOGO JB, OETKEN M, SCHWENK K, OEHLMANN J 2007 Multi-generation studies with *Chironomus riparius* – Effects of low tributyltin concentrations on life history parameters and genetic diversity. Chemosphere 67: 2192-2200. https://doi.org/10.1016/j.chemosphere.2006.12.025
27. XIE L, KLERKS PL 2003 Response to selection for cadmium resistance in the least killifish, *Heterandria formosa*. Environ Toxicol Chem 22: 313-320. https://doi.org/10.1002/etc.5620220211
28. SALICE CJ, MILLER TJ, ROESIJADI G 2009 Demographic Responses to Multigeneration Cadmium Exposure in Two Strains of the Freshwater Gastropod, *Biomphalaria glabrata*. Arch Environ Contam Toxicol 56: 785-795. https://doi.org/10.1007/s00244-008-9203-9
29. SUN YP, FOLEY HB, HANDSCHUMACHER L, SUZUKI A, KARAMANUKYAN T, EDMANDS S 2014 Acclimation and adaptation to common marine pollutants in the copepod *Tigriopus californicus*. Chemosphere 112: 465–471. https://doi.org/10.1016/j.chemosphere.2014.05.023
30. LOUREIRO C, CUCO AP, CLARO MT, SANTOS JI, PEDROSA MA, GONÇALVES F, CASTRO BB 2015 Progressive acclimation alters interaction between salinity and temperature in experimental *Daphnia* populations. Chemosphere 139: 126-132. https://doi.org/10.1016/j.chemosphere.2015.05.081
31. HAAP T, SCWARTZ S, KÖHLER HR 2016 Metallothionein and Hsp70 trade-off against one another in *Daphnia magna* cross-tolerance to cadmium and heat stress. Aquat Toxicol 170:112-119. https://doi.org/10.1016/j.aquatox.2015.11.008
32. REÀTEGUI-ZIRENA EG, FIDDER BN, OLSON AD, DAWSON DE, BILBO TR, SALICE CJ 2017 Transgenerational endpoints provide increased sensitivity and insight into multigenerational responses of *Lymnaea stagnalis* exposed to cadmium. Environ Pollut 224: 572-580. https://doi.org/10.1016/j.envpol.2017.02.040
33. JOPP F 2006 Comparative studies on the dispersal of the Great Ramshorn (*Planorbarius corneus* L.): A modelling approach. Limnologica 36: 17-25. https://doi.org/10.1016/j.limno.2005.10.003
34. COSTIL K, DAGUZAN J 1995 Comparative life cycle and growth of two freshwater gastropod species, *Planorbarius corneus* L. and *Planorbis planorbis* L. Malacologia 37: 53-68.
35. COSTIL K, DAGUZAN J 1995 Effect of temperature on reproduction in *Planorbarius corneus* (L.) and *Planorbis planorbis* (L.) throughout the life span. Malacologia 36: 79-89.
36. HUBEDINCK B 1958 Factors conditioning the habitat of freshwater snails. Bull World Health Organ 18: 1072-1080.
37. STRONG EE, GARGOMINY O, PONDER, WF, BOUCHED P 2008 Global diversity of gastropods (Gastropoda; Mollusca) in freshwater. Hydrobiologia 595: 149-166. https://doi.org/10.1007/S10750-007-9012-6
38. RUSSEL HUNTER W 1961 Life cycles of four freshwater snails in limited populations in Loch Lomond, with a discussion of infraspecific variation. Proc Zool Soc Lond 137: 135-171. https://doi.org/10.1111/j.1469-7998.1961.tb06166.x
39. DILLON RT JR 2000 The Ecology of Freshwater Molluscs. Cambridge University Press, Cambridge
40. CORDELLIER M, PFENNINGER A, STREIT B, PFENNINGER M 2012 Assessing the effects of climate change on the distribution of pulmonate freshwater snail biodiversity. Marine Biol 159: 2519-253. https://doi.org/10.1007/s00227-012-1894-9
41. WIES V 2005 Nonmarine mollusca of Schleswig-Holstein (northernmost Germany). MOLLBASE-National Database of German Land- and Freshwater-Mollusks. http://www.mollbase.org/sh/ Last accessed 20-07-2022
42. LOMBARDO P, MICCOLI FP, GIUSTINI M, CICOLANI V 2011 Planarian (*Dugesia polychroa*) predation on freshwater gastropod eggs depends on prey species, clutch morphology, and egg size. Fundamental Applied Limnol 178: 235-339. https://doi.org/10.1127/1863-9135/2011/0178-0325
43. MAŽURAN N, HRŠAK V, TOMIĆ M, PAPEŠ D 1999 Effects of $CaCl_2$ and $CaBr_2$ on the fecundity of *Planorbarius corneus* L. Chemosphere 10: 2345-2355. https://doi.org/10.1016/S0045-6535(98)00438-X
44. HEMEIDA AM, GAWISH A 2008 Evaluation the potassium bromide and zinc bromide brines for workover operations. Oil and Gas Business 1: 1-10.
45. BYRNE RA, REYNOLDS JD, MCMAHON, RF 1989 Shell growth, reproduction and life cycles of *Lymnaea peregra* and *L. palustris* (Pulmonata: Basommatophora) in oligotrophic turloughs (temporary lakes) in Ireland. J Zool London 217: 321-339. https://doi.org/10.1111/j.1469-7998.1989.tb02491.x
46. CALOW P 1973 On the regulatory nature of individual growth: some observations from freshwater snails. J Zool London 170: 415-428. https://doi.org/10.1111/j.1469-7998.1973.tb05056.x
47. EL-EMAM MA, MADSEN H 1982 The effect of temperature, darkness, starvation and various food types on growth, survival and reproduction of *Helisoma duryi, Biomphalaria alexandrina* and *Bulinus truncatus* (Gastropoda: Planorbidae). Hydrobiologia 88: 265-275. https://doi.org/10.1007/BF00008506
48. GREENWAY P 1971 Calcium regulation in the freshwater mollusc, *Lymnaea stagnalis* (L.) (Gastropoda: Pulmonata) I. The effect of internal and external calcium concentration. J Exp Biol 54: 199-214. https://doi.org/10.1242/jeb.54.1.199
49. THOMAS JD, LOUGH A 1974 The effects of external calcium concentration on the rate of uptake of this ion by *Biomphalaria glabrata* (Say). J Animal Ecol 43: 861-871. https://doi.org/10.2307/3540
50. DUSSART G, KAY R 1980 Relationships between water chemistry and respiration rate in several populations of *Lymnaea perega* Müller (Gastropoda: Mollusca). Hydrobiologia 69: 57-65. https://doi.org/10.1007/BF00016536
51. NAVARRO JM, LEIVA GE, GALLARDO CS, VARELA C 2002 Influence of diet and temperature on physiological energetics of *Chorus giganteus* (Gastropoda: Muricidae) during reproductive conditioning. New Zealand J Marine Freshwater Res 36: 321-332. https://doi.org/10.1080/00288330.2002.9517089
52. CALOW P 1988 Physiological ecotoxicology; Theory, Practice and Application. In: Lokke H, Tyle H, Bro-Rasmussen F (eds) Proceedings of the First European Conference an Ecotoxicology. Conference Organizing Committee, Lyngby, Copenhagen, pp 23-35

53. BAILEY, PCE, JAMES K 2000 Riverine & Wetland Salinity Impacts – Assessment of R & D Needs LWRRDC Occasional Paper 25/99

54. STARK JD, TANIGOSHI L, BOUNFOUR M, ANTONELLI A 1997 Reproductive Potential: Its Influence on the Susceptibility of a Species to Pesticides. Ecotoxicol Environ Saf 37: 273-279. https://doi.org/10.1006/eesa.1997.1552

55. CANTON JH, WESTER PW, MATHIJSSEN-SPIEKMAN EAM 1983 Study on the toxicity of sodium bromide to different freshwater organisms. Food Chem Toxicol 21: 369-378. https://doi.org/10.1016/0278-6915(83)90090-X

56. STUHLBACHER A, BRADLEY MC, NAYLOR C, CALOW P 1993 Variation in the development of cadmium resistance in *Daphnia magna* Straus; Effect of temperature, nutrition, age and genotype. Environ Pollut 80: 153-158. https://doi.org/10.1016/0269-7491(93)90141-A

57. FRITSCH C, COEURDASSIER M, GIMBERT F, CRINI N, SCHEIFLER R, VAUFLEURY A 2011 Investigations of responses to metal pollution in land snail populations (*Cantareus aspersus* and *Cepaea nemoralis*) from a smelter-impacted area. Ecotoxicology 20: 739-759. https://doi.org/10.1007/s10646-011-0619-z

58. SALICE CJ, ANDERSON TA, ROESIJADI G 2010 Adaptive responses and latent costs of multigeneration cadmium exposure in parasite resistant and susceptible strains of a freshwater snail. Ecotoxicology 19: 1466-1475. https://doi.org/10.1007/s10646-010-0532-x

59. LI H, SHI L, WANG D, WANG M 2015 Impacts of mercury exposure on life history traits of *Tigriopus japonicus*: Multigeneration effects and recovery from pollution. Aquat Toxicol 166: 42-49. https://doi.org/10.1016/j.aquatox.2015.06.015

60. VAN STRAALEN NM, TIMMERMANS MJTN 2002 Genetic Variation in Toxicant-Stressed Populations: An Evaluation of the „Genetic Erosion" Hypothesis. Hum Ecol Risk Assess 8: 983-1002. https://doi.org/10.1080/1080-700291905783

61. HARSHMAN LG, ZERA AJ 2007 The cost of reproduction: the devil in the details. Trends Ecol Evol 22: 80-86. https://doi.org/10.1016/j.tree.2006.10.008

62. CALOW P, SIBLY RM 1990 A physiological basis of population processes: Ecotoxicological implications. Functional Ecol 4: 283-388. https://doi.org/10.2307/2389587

63. JOKELA J, MUTIKAINEN P 1995 Phenotypic plasticity and priority rules for energy allocation in a freshwater clam: a field experiment. Oceologia 104: 122-132. https://doi.org/10.1007/BF00365570

64. ZERA AJ, HARSHMAN LG 2001 The Physiology of Life History Trade-Offs in Animals Ann Rev Ecol Syst 32: 95-126. https://doi.org/10.1146/annurev.ecolsys.32.081501.114006

65. BRULLE F, MITTA G, LEROUX R, LEMIERE S, LEPRETRE A, VANDENBULCKE F 2007 The strong induction of metallothionein gene following cadmium exposure transiently affects the expression of many genes in *Eisenia fetida*: A trade-off mechanism? Comparative Biochem Physiol Part C 144: 34-341. https://doi.org/10.1016/j.cbpc.2006.10.007

66. AGRA AR, SOARES AMWM, BARATA C 2011 Life-history consequences of adaptation to pollution. *Daphnia longispina* clones historically exposed to copper. Ecotoxicology 20: 552-562. https://doi.org/10.1007/s10646-011-0621-5

67. ULLER T, NAKAGAWA S, ENGLISH S 2013 Weak evidence for anticipatory parental effects in plants and animals. J Evol B 26: 2161-2170. https://doi.org/10.1111/jeb.12212