



Research Article

Hot and cold exposure triggers distinct transcriptional and behavioral responses in laboratory-inbred pond snails

Veronica Rivi^{a,*}, Anuradha Batabyal^{b,c}, Cristina Benatti^{a,d}, Fabio Tascetta^{d,e,f}, Johanna Maria Catharina Blom^{a,f}, Ken Lukowiak^b^a Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy^b Department of Physiology and Pharmacology, Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Alberta, Canada^c Department of Physical and Natural Sciences, FLAME University, Pune, India^d Centre of Neuroscience and Neurotechnology, University of Modena and Reggio Emilia, Modena, Italy^e CIB, Consorzio Interuniversitario Biotecnologie, Trieste, Italy^f Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

ARTICLE INFO

Keywords:

Temperature sensitivity

Lymnaea stagnalis

Garcia effect

HSPs

CREB

Energy

ABSTRACT

Animals exhibit remarkable behavioral and molecular adaptations to cope with thermal stressors, which are crucial for survival in variable environments that are exacerbated by climate change. Aquatic poikilotherms like our model organism—the pond snail *Lymnaea stagnalis*—face significant challenges due to their dependence on external temperatures. Our study provides valuable insights into the different behavioral and molecular responses of lab-inbred snails to cold and heat shock stressors (i.e., 4 °C and 30 °C), particularly in the context of learning and memory formation. We found that while short-term (1 h) cold exposure transiently upregulated the expression levels of HSP70 and HSP40 in the snail's central ring ganglia, prolonged cold exposure (24 h) resulted in a significant downregulation of LymMIPII and an upregulation of LymMIPR. These data suggest, albeit at the transcriptional level, the existence of a negative feedback loop necessary for sustaining cellular functions when metabolic demands might shift towards conserving energy during prolonged cold exposure. At the behavioral level, we found that, compared to heat shock, cold exposure did not result in a Garcia effect (i.e., a “special form” of conditioned taste aversion). The difference in memory outcomes was associated with changes in the expression levels of selected targets involved in neuronal plasticity and the stress response. While both cold and heat shock upregulated the HSP levels in the snail's central ring ganglia, cold exposure did not affect the expression levels of the neuroplasticity genes LymGRIN1 and LymCREB1, contrasting with heat shock's neurogenic effects. Overall, this study provides insights into *L. stagnalis*'s adaptive responses to thermal stressors, emphasizing different molecular strategies for coping with heat versus cold challenges in aquatic environments. These findings contribute to our understanding of thermal biology and stress physiology in aquatic organisms, underscoring the importance of molecular mechanisms in shaping species' resilience in dynamic environments.

1. Introduction

Climate change is resulting in extreme temperature fluctuations from heat waves and cold waves to incessant drought and floods across the globe, which is threatening species' existence (Duffy et al., 2022; Rocha et al., 2022; Soravia et al., 2021; Wilson and Nicoll, 2001). To cope with such ecological stressors, organisms either acclimate or show behavioral plasticity to local environmental changes (Piyaphongkul et al., 2014; Prahlad and Morimoto, 2009; Quan et al., 2023; Rensing and Ruoff, 2002; Scott et al., 1997), with thermal adaptation being one of the most

common strategies observed in species living in diverse temperatures (Fontúrbel et al., 2021; Quan et al., 2022; Tian et al., 2020; van Heerwaarden et al., 2016; Vasseur et al., 2014; Zhou et al., 2018). Poikilotherm organisms, in particular, are vulnerable as their internal temperature depends on external conditions (Guschina and Harwood, 2006; Malik et al., 2023; Wagner et al., 2023). Thermal stress across animal taxa induces the expression of heat shock proteins (HSPs)—molecular chaperones that protect cells from misfolded proteins under stress (Sørensen et al., 2007; Tedeschi et al., 2015, 2016; Tomanek and Sanford, 2003)—whose differential expression is linked to variation in thermal

* Corresponding author.

E-mail address: veronica.rivi@unimore.it (V. Rivi).<https://doi.org/10.1016/j.watbs.2024.100315>

Received 26 July 2024; Received in revised form 30 August 2024; Accepted 21 October 2024

Available online 22 October 2024

2772-7351/© 2024 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

tolerance within and among species (J. Gao et al., 2014; Woodruff et al., 2022).

We use the great pond snail *Lymnaea stagnalis* as a model species for investigating the effects of different stressors occurring in aquatic environments and as a candidate bioindicator (Lukowiak et al., 2010, 2014; Rivi et al., n.d.; Rivi et al., 2023a; Rivi, Batabyal, Benatti, Blom, et al., 2023b; Rivi, Batabyal, Lukowiak, Benatti, et al., 2023d). Pond snails inhabit stagnant or slow-flowing ponds and lakes in northern North America and Eurasia, experiencing broad temperature fluctuations daily and seasonally (Amorim et al., 2019). In Alberta and Saskatchewan, where we collected wild snails, temperatures in water bodies ranged from about 4 °C in April–May to nearly 35 °C in July–August (Brown, 1979). As *L. stagnalis* is a poikilotherm, cold and hot temperatures affect both its physiology and behavior (Bowler, 2018), as shown by the fact that *Lymnaea* grow between 11 and 28 °C, growth does not occur below 10 °C, and the upper lethal limit is around 32–33 °C (Batabyal et al., 2022; Rivi et al., 2022c). *L. stagnalis* overwinter in the wild by moving to the deepest location or the bottom of lakes where the temperature is maintained at 4 °C while the water at the surface freezes (Fernell et al., 2021). Inbred, laboratory-reared snails—like those used in this study—are kept at around 20 °C, rarely experiencing temperature fluctuations greater than 1–2 °C annually. These snails have been maintained at standardized conditions for more than 250 generations (Fodor et al., 2020). Thus, in inbred snails, the exposure to an acute heat shock (1 h at 30 °C), resembling typical mid-summer conditions experienced by wild snails, acutely up-regulates mRNA levels of HSP40 and HSP70 (Rivi et al., 2021a,b; Sunada et al., 2016a; Foster et al., 2015; Teskey et al., 2012) as a response to stressful conditions. Although prolonged exposure to 30 °C is lethal for lab-inbred snails, an acute exposure (for 1 h) after the presentation of a novel appetitive taste results in a sickness-like state and, therefore, in a taste-specific and long-lasting form of conditioned taste aversion known as the Garcia effect (Batabyal et al., 2024; Rivi et al., 2021; Rivi et al., 2022d; Rivi et al., 2022c; 2023b, 2023a). At a behavioral level, we observed long-term memory of the novel food aversion, whereas at the molecular level, the heat shock exposure resulted in a significant upregulation of the expression levels of HSP70 and HSP40 whose inhibition prevents the formation of the Garcia effect, suggesting a key role of these targets in the Garcia effect formation (Batabyal et al., 2021; Rivi et al., 2021a,b, 2024; Sunada et al., 2016a). That is, the Garcia effect offers a measure of learning and memory abilities and allows to study how heat tolerance and HSP induction occurs in snails from different thermal conditions, including the cold ones.

On the other hand, cold shock can be used to block the snail's memory consolidation following the operant conditioning of aerial respiration, and prolonged exposure to temperatures lower than 10 °C blocks the snail's growth (Orr et al., 2009; Sangha et al., 2003; Martens et al., 2007; Takahashi et al., 2013). Thus, cold and heat shock exposures differentially affect the pond snail's homeostatic behaviors and cognitive functions. Understanding responses to cold and heat shock and tolerance limits is crucial in our changing world, as animal distribution and diversity are closely linked to their stress responses. Animals can navigate temperature fluctuations by altering physiological, psychological, behavioral, and cognitive functions as well as through range shifts to suitable locations (Yin et al., 2024). However, models like *L. stagnalis*, which are found in stagnant water bodies and at higher latitudes and altitudes, have fewer dispersal opportunities and are more at risk.

In this complex scenario, *L. stagnalis* serves as an optimal model to explore the consequences of water temperature fluctuations on their homeostatic behaviors and cognitive functions, as they have been used for ecotoxicology and neuroscience research since the 1970s and exhibit natural cognitive ability differences (Kagan et al., 2023; Kagan et al., 2023; Rivi, Batabyal, Benatti, Blom, et al., 2023a; Rivi et al., 2023e).

This study consisted of two experiments. *Experiment 1* focused on transcriptional effects induced by cold shock exposure (1 h or 24 h) in the inbred snail's central ring ganglia. Previous studies showed that cold, food-scarce winter conditions create a significant bottleneck on animal's

poleward persistence, leading to a reversible seasonal phenotype of inactivity, low body temperature, fasting, and low metabolic rate as a survival tactic (Reeve et al., 2022; Speers-Roesch et al., 2018). However, mechanisms underlying winter dormancy remain poorly understood, especially in poikilotherm species like *L. stagnalis*. To elucidate the role of HSPs in the central ring ganglia of *L. stagnalis* under different cold regimens (1 h or 24 h), we assessed transcriptional changes in targets involved in energy homeostasis and neuroplasticity: LymHSP70 and LymHSP40, molluscan insulin peptide II (LymMIP-II) and its receptor (LymMIPR), glutamate ionotropic receptor NMDA type subunit 1 (LymGRIN1), and cAMP response element-binding protein 1 (LymCREB1) (Murakami et al., 2013; Rivi et al., 2023e). We hypothesized that short-term exposure to 4 °C would upregulate LymHSP70 and LymHSP40 expression, while long-term exposure would downregulate the insulin pathway to promote an 'energy saving' mode. Given that cold exposure can induce the blockage of memory formation, we tested the hypothesis that exposing snails to 4 °C downregulates neuroplasticity targets like LymGRIN1 and LymCREB1. *Experiment 2* investigated whether a cold shock (4 °C for 1 h) could induce a Garcia effect similar to heat shock (Rivi et al., 2022d) by examining transcriptional effects of these two procedures (cold vs. heat shock) on HSPs and neuroplasticity targets in the snail's central ring ganglia.

This is the first study to investigate both short-term (i.e., 1 h) and long-term responses to cold shock while comparing the biological implications of opposite temperature extremes. Understanding how species like *L. stagnalis* respond to thermal stress is crucial for predicting and mitigating the impacts of climate change on aquatic ecosystems. This is essential for preserving biodiversity and maintaining the stability of ecosystem services (A. k et al., 2023).

The cross-talk between targets involved in stress response, energy balance, and neuroplasticity in mediating thermal tolerance and cognitive functions may help to unravel the critical molecular mechanism through which aquatic organisms cope with temperature variation, offering biomarkers for assessing the health and stress levels of aquatic populations. Furthermore, exploring the neurobiological aspects of thermal stress, particularly its impact on learning and subsequent memory formation, may contribute to enhancing our understanding of how environmental factors influence neural and behavioral plasticity. This knowledge is vital for predicting how aquatic species might adapt to rapid environmental changes. As climate change exacerbates temperature fluctuations and extreme weather events, maintaining the health of aquatic ecosystems becomes increasingly important. This study provides insights into the resilience of aquatic organisms, which can inform conservation efforts and guide the management of water resources (Trégarot et al., 2024; Venegas et al., 2023). Overall, this study underscores the interconnectedness of water biology, neurobiology, and climate change. It calls for integrated approaches to research and conservation that consider the multifaceted impacts of global temperature changes on aquatic organisms and ecosystems.

2. Material and methods

2.1. Snails and their maintenance

In this study, we used a laboratory-inbred strain (W-strain) of *L. stagnalis* maintained at the University of Calgary Biology Department. This strain originated from an inbred stock kept at the Vrije University of Amsterdam, originally bred from animals collected in the 1950s in polders near Utrecht, The Netherlands (Koene, 2006). The snails were housed in artificial pond water, which was made from deionized water supplemented with Instant Ocean (0.25 g/L) and calcium carbonate (CaCO₃) to maintain calcium concentrations above 50 mg/L (Dalesman and Lukowiak, 2010). Snails were kept at 20 ± 1 °C with a light-dark cycle of 16 h light and 8 h dark. Six-month-old snails with shell lengths of 20–25 mm were used for the experiments. While experiments on pond snails do not require ethics committee approval, we ensured the

well-being of the snails throughout the behavioral procedures.

2.2. Experiment 1: transcriptional effects of short- and long-term cold exposure in *L. stagnalis*'s central ring ganglia

To investigate the transcriptional effects of short- and long-term cold exposure, 16 naive snails were placed in a 1-L beaker filled with 500 mL of artificial pond water cooled to 4 °C. The beaker was maintained in a water bath at 4 °C for either 1 h (N = 8) or 24 h (N = 8). Control naive snails (N = 8) were placed in a 1-L beaker with 500 mL of 20 °C (i.e., room temperature) artificial pond water. After exposure, the snails were returned to their home aquaria for 3 h before being sacrificed. The central ring ganglia were extracted for analysis 3 h post-cold exposure.

2.3. Experiment 2: impact of cold and heat shock on the behavioral and molecular outcomes of a Garcia effect procedure

To investigate whether a cold shock, similar to a heat shock, could induce a Garcia effect in snails, 18 naive snails were divided into three groups. We examined their feeding response to a carrot slurry (C), a novel appetitive stimulus eliciting robust rasping behavior, before and 3 h after exposure to different thermal conditions. The carrot slurry was prepared by blending two organic carrots with 500 mL of artificial pond water. Rasping behavior was monitored by placing snails in a 14 cm Petri dish partially submerged in carrot slurry (Rivi et al., 2021). The dishes were positioned on a clear Plexiglas stand elevated above a mirror for better visibility (Rivi et al., 2022f). After a 3-min acclimation, the number of rasps was recorded over 2 min. Snails were then returned to their aquaria for 1 h before being exposed to 30 °C (heat shock), 4 °C (cold shock), or 20 °C (room temperature) for 1 h. Three hours later, the rasping behavior was recorded again after a 3-min acclimation period. During the 3-h interval, snails were kept in their home tanks without food.

Behavioral experiments were performed in the morning, as learning scores are better at this time. After testing for the Garcia effect, snails were euthanized by placing them on ice for 10 min, and the central ring ganglia were dissected and stored at -80 °C for analysis.

2.4. Total RNA extraction, reverse transcription, and Real-Time polymerase Chain reaction

Before sacrifice, snails were anesthetized on ice for 10 min. The central ring ganglia were dissected and stored at -80 °C. Total RNA extraction and DNase treatment were performed using the GenElute™ Total RNA Miniprep Kit and DNASE70-On-Column DNase I Digestion Set (Merck Millipore). Each central ring ganglion was used for RNA extraction and a 200-ng RNA sample was reverse transcribed using a High-

Capacity cDNA Reverse Transcription Kit (ThermoFisher). Real-time quantitative PCR was conducted on 20 ng mRNA using a Bio-Rad® CFX Connect™ Real-Time PCR Detection System with SYBR Green Master Mix (Bio-Rad). The cycling parameters were 95 °C for 120 s, 95 °C for 10 s, and 60 °C for 30 s for 40 cycles. Cycle threshold (Ct) values were determined using CFX Maestro™ Software (Bio-Rad) (Rivi et al., 2022g). Primers were 19–23 nucleotides long, with a melting temperature between 58 and 62 °C, and a guanine-cytosine (GC) content between 40% and 60%, generating an amplicon of 75–200 bp at a final concentration of 300 nM (Table 1). mRNA levels of each target were normalized to the mean of two housekeeping genes, elongation factor 1 α , and tubulin, which were stable across groups as confirmed by the analysis using NormFinder (Wang et al., 2012) and were unaffected by any procedure. For quantitative evaluation of changes, the comparative 2^{- $\Delta\Delta$ Ct} method was used, with control animals (exposed to room temperature) as the calibrator.

2.5. Statistical analyses

Behavioral data were analyzed using a paired Student's *t*-test to compare the number of rasps before and after heat or cold exposure (Fig. 2). For gene expression analyses, normality was assessed using the Kolmogorov-Smirnov one-sample test. One-way ANOVA was used to compare the expression levels of each target and significant differences were determined by Tukey's post hoc test. All tests were defined as significant at *p* < 0.05. Data are presented as mean \pm standard error (SEM). Statistical analyses were conducted, and graphs were generated using GraphPad Prism v. 9.0.0e for Mac® (GraphPad Software, Inc.).

3. Results

3.1. Transcriptional effects of short- and long-term cold exposure in *Lymnaea*'s central ring ganglia

The aim of Experiment 1 was to answer the following question: *what are the transcriptional effects induced by exposure to a cold shock (4 °C) for 1 h or 24 h in the central ring ganglia of lab-bred snails?* We focused our attention on the expression levels of selected targets involved in energy homeostasis, response to stressors, and memory formation in the central ring ganglia of *L. stagnalis* (Murakami et al., 2013; Nakai et al., 2022). A one-way ANOVA demonstrated that there was a significant effect of the cold treatment on the expression levels of LymHSP70 ($F_{2,21} = 46.23$, *p* < 0.001) (Fig. 1A), LymHSP40 ($F_{2,21} = 11.07$, *p* = 0.0005) (Fig. 1B), LymMIP-II ($F_{2,21} = 7.88$, *p* = 0.003) (Fig. 1E), and LymMIPR ($F_{2,21} = 18.66$, *p* < 0.0001) (Fig. 1F). Tukey's post hoc test revealed that short-term exposure to 4 °C significantly upregulated LymHSP70 and

Table 1

The forward (FW) and reverse (RV) primer nucleotide sequences utilized in qRT-PCR, along with the accession number for each target and the size (bp) of the PCR product obtained through the amplification of cDNA (mRNA).

Gene bank accession	Target	Product length (bp)	Type sequence
DQ206432.1	<i>Lymnaea stagnalis</i> heat-shock protein 70LymHSP70	199 bp (134–333)	5' - AGGCAGAGATTGGCAGGAT -3' 3' - CCATTTTCATTGTGTCGTTCG -5'
DQ278442.1	<i>Lymnaea stagnalis</i> heat-shock protein 40LymHSP40	186 bp (152–338)	5' - AAGGCTCTGAATCCTGATG - 3' 3' - GTGTTTGGTCCACCTTCTTT - 5'
X59302.1	<i>Lymnaea stagnalis</i> molluscan insulin-related peptideLymMIP II	186 bp (152–338)	5' - CCAATCATCTTGCAGTTTA - 3' 3' - GTCGTCAGATCTGTTTCT - 5'
X84994.1	<i>Lymnaea stagnalis</i> putative molluscan insulin-related peptide receptorLymMIPR	78 bp (4137–4215)	5' - ATGGAGACTTTGGTATGAC - 3' 3' - ACACCTCCATCTTTGAGAGAC - 5'
AY571900.1	<i>Lymnaea stagnalis</i> NMDA-type glutamate receptorLymGRIN1	140 bp (831–917)	5' - AGAGGATGCATCTACAATTT - 3' 3' - CCAITTTACTAGGTGAACCTCC - 3'
AB041522.1	<i>Lymnaea stagnalis</i> cAMP responsive element binding proteinLymCREB1	180 bp (49–229)	5' - GTCAGCAGGGAATGGTCTCG - 3' 3' - AACCGCAGCAACCCCTAACAA - 5'
X15542.1	Snail, beta-tubulinLymTUB	100 bp (92–192)	5' - GAAATAGCACCCCATCC - 3' 3' - CGCCTCTGTGAACCTCCATCT - 5'
DQ278441.1	<i>Lymnaea stagnalis</i> elongation factor 1-alphaLymEF1 α	150 bp (7–157)	5' - GTGTAAGCAGCCCTCGAACT - 3' 3' - TTCCTCATCAATACCACCA - 5'

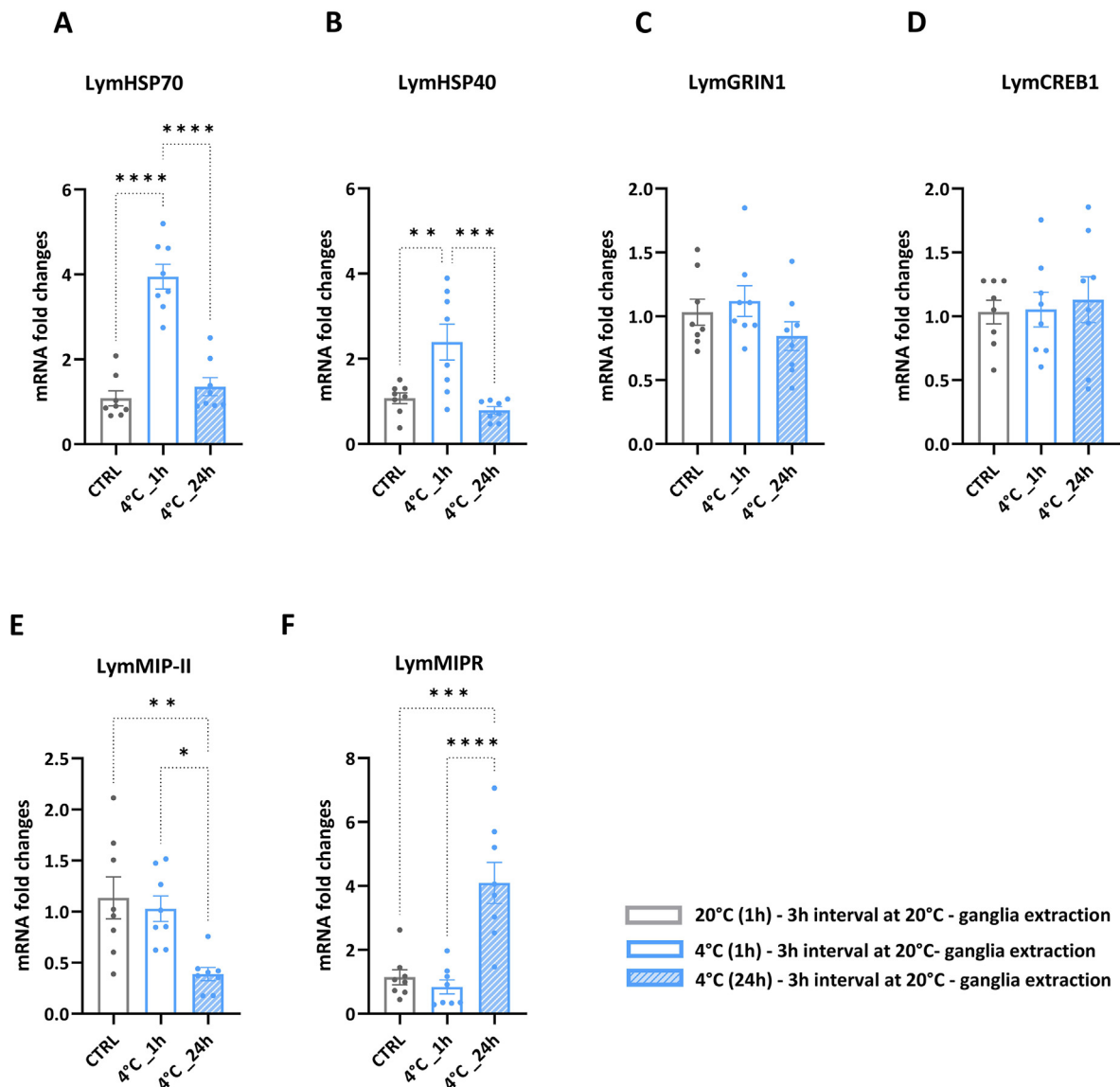


Fig. 1. Transcriptional effects induced by the exposure to 4 °C for 1 h or 24 h in the central ring ganglia of lab-inbred snails. The expression levels of LymHSP70 (A), LymHSP40 (B), LymMIP-II (C), LymMIP-R (D), LymGRIN1 (E), and LymCREB1 (F) were measured in the central ring ganglia of snails maintained at 20 °C (room temperature) (CTRL group: grey bars) or at 4 °C for 1 h (light blue bars) or 24 h (hatched blue bars) (N = 8 for each group). Data are presented as means \pm SEM and were analyzed with a one-way ANOVA followed by Tukey post hoc tests. **** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05.

LymHSP40 mRNA levels compared to control snails (p < 0.0011 and p = 0.0007, respectively) and snails exposed for 24 h (p < 0.0011 and p = 0.004, respectively). Notably, no significant differences in LymHSP70 and LymHSP40 mRNA levels were observed between control snails and those maintained at 4 °C for 24 h (p = 0.69 and p = 0.72, respectively). Moreover, we found that the prolonged exposure to 4 °C for 24 h resulted in a significant downregulation of LymMIP-II mRNA levels and a significant upregulation of LymMIP-R compared to control snails (LymMIP-II: p = 0.04 and LymMIP-R: p = 0.0002) and those exposed to the cold shock procedure for 1 h (LymMIP-II: p = 0.013 and LymMIP-R: p < 0.0001). No effects induced by the cold exposure (1 h or 24 h) on the expression levels of LymGRIN1 ($F_{2,21}$ = 1.56, p = 0.027) and LymnCREB1 ($F_{2,21}$ = 0.13, p = 0.88) were found (Fig. 1C and D).

3.2. Impact of cold and heat shock on the behavioral and transcriptional outcomes of a Garcia effect procedure

After demonstrating that a 1-h cold exposure significantly upregulated LymHSP70 and LymHSP40 expression, consistent with our previous

findings using a heat shock of 30 °C, we explored whether cold shock could induce a Garcia effect and compared the transcriptional effects with those induced by heat shock. Our hypothesis was based on previous research in mammals and *L. stagnalis*, suggesting that prolonged fasting would affect learning and memory differently than a single day of fasting, potentially enhancing memory performance. To investigate this, we assessed the learning abilities and memory performances of snails using the Garcia effect behavioral procedure. Initially, we replicated the Garcia effect by demonstrating that a single pairing of a novel taste (i.e., carrot slurry) followed by a subsequent heat shock (30 °C for 1 h) significantly suppressed the feeding response elicited by carrots for at least 3 h (paired t -test: t = 4.49, df = 5, p = 0.006) (Fig. 2A). In contrast, control snails exposed to room temperature pond water (20 °C) for 1 h (without thermal shock) did not exhibit a Garcia effect (paired t -test: t = 0.90, df = 5, p = 0.41) (Fig. 2C), consistent with our previous observations. Interestingly, we found that the number of rasps elicited by C at 3 h post cold shock (C-post 3 h) did not significantly differ from those recorded before the cold shock (C-pre) (Fig. 2B). This suggests that unlike heat shock, cold shock does not result in a sickness state that induces a Garcia effect in lab-

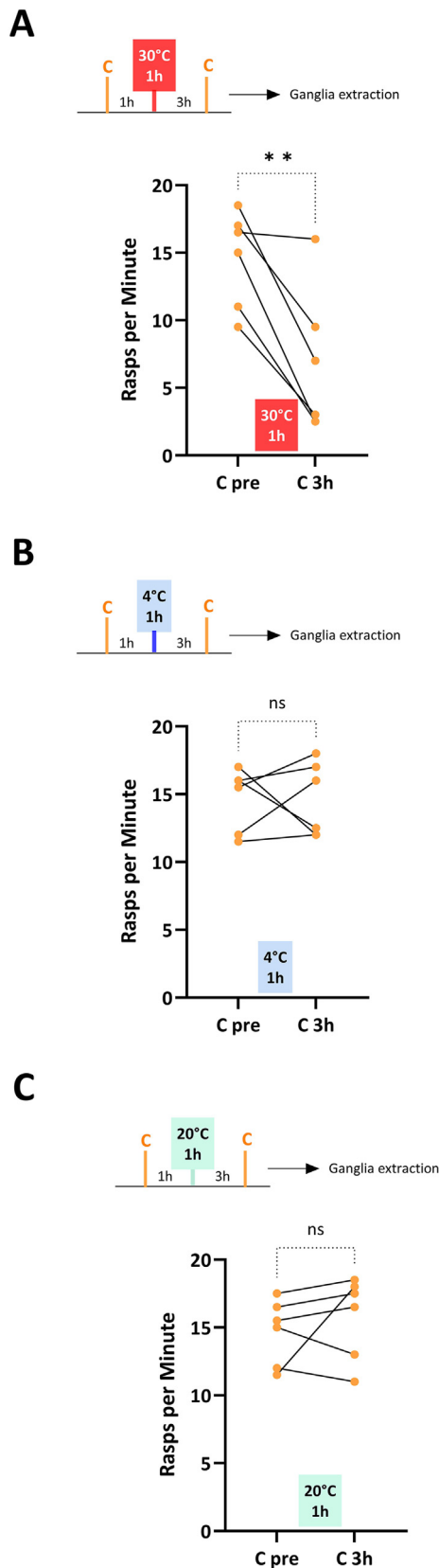


Fig. 2. Cold shock fails to elicit a Garcia effect in laboratory-inbred snails compared to heat shock. The timeline of each experiment is presented above the data. The number of rasps elicited by the carrot slurry (C) was counted for 2 min (C pre). One hour later, these snails experienced the heat (30 °C; 2A) or the cold (4 °C; 2B) shock stressor for 1 h. Control snails (2C) were maintained at room temperature (20 °C) for 1 h. Three hours later the number of rasps elicited by the carrot slurry (C 3h) was recorded again for 2 min. Exposure to carrot slurry followed by heat shock induced a Garcia effect, evidenced by a significant reduction in the number of rasps observed in snails tested 3 h after 30 °C exposure. In contrast, cold shock did not induce a Garcia effect, as no significant differences in rasping behavior in carrot slurry were observed when comparing C pre and C 3h. Data were analyzed using a paired *t*-test. The solid line is the mean, and the error bars are the SEM. ***p* < 0.001, ns = not significant as *p* > 0.05.

inbred snails. Having confirmed our prediction that cold and heat shock induce distinct behavioral responses, we sacrificed the snails following the memory test (C 3h) to analyze the transcriptional effects of the Garcia effect procedure on the mRNA levels of LymHSP70, LymHSP40, LymGRIN1, and LymCREB1 in their central ring ganglia (Fig. 3). A one-way ANOVA followed by Tukey's post hoc test revealed a significant effect of the behavioral procedure on the expression levels of LymHSP70 ($F_{2,18} = 45.68, p < 0.0001$) (Fig. 3A), LymHSP40 ($F_{2,18} = 9.09, p = 0.002$) (Fig. 3B), LymGRIN1 ($F_{2,18} = 9.13, p = 0.002$) (Fig. 3C), and LymCREB1 ($F_{2,18} = 29.49, p < 0.0001$) (Fig. 3D).

Specifically, following the Garcia effect procedure, both heat and cold shock stressors induced significant upregulation of LymHSP70 and LymHSP40 compared to control conditions (LymHSP70: heat shock: $p < 0.0001$, cold shock: $p = 0.0002$; LymHSP40: heat shock: $p = 0.003$, cold shock: $p = 0.009$). However, the increase in LymHSP70 expression in the central ring ganglia of snails subjected to heat shock was significantly higher compared to those exposed to cold shock ($p = 0.0009$), indicating that heat shock causes a more severe stress response in lab-inbred snails. Moreover, significant upregulation of key neuroplasticity targets such as LymGRIN1 and LymCREB1 was observed only in snails that formed the Garcia effect (i.e., heat shock-exposed) compared to those subjected to cold shock (LymGRIN1: $p = 0.01$, LymCREB1: $p < 0.0001$) and control conditions (LymGRIN1: $p = 0.002$, LymCREB1: $p < 0.0001$), highlighting different neuroplastic responses to thermal stressors in these snails.

4. Discussion

With rising global temperatures that may result in increased temperatures in lakes and ponds, aquatic poikilotherm species, like *L. stagnalis*, are likely to face unprecedented physiological stress in coping with thermal extremes (Benedetti et al., 2021; Caldutch-Giner et al., 2022; Fernández et al., 2022; Harvey et al., 2022; Reid et al., 2019). Climate change is causing an increase in the frequency and duration of heatwaves and cold spells across various landscapes where *Lymnaea* species are found (Marx et al., 2021; Meehl and Tebaldi, 2004; Neven, 2000; Rivi et al., 2022d). HSPs are conserved players in the response to thermal stress (both heat and cold) across animal taxa (Mayer, 2010), underscoring their adaptive significance (Anderson and Bell, 2009; Jeyachandran et al., 2023; Oksala et al., 2014; Pluess et al., 2023; Setti et al., 2022).

In this study, we used the pond snail *L. stagnalis*, a model species for ecological and neuroscience studies, to investigate the behavioral and transcriptional effects induced by a cold stressor and compared it with a heat shock stressor. In laboratory settings, pond snails experience stable temperatures around 20 °C, contrasting sharply with the natural environment's temperature variability (Fernell et al., 2021). While heat shock has been extensively studied in various species, including *L. stagnalis*, cold shock's behavioral and transcriptional effect on learning and memory abilities remained relatively unexplored until *Experiment 1* of this study, where we demonstrated that short-term exposure to 4 °C significantly upregulated the mRNA levels of LymHSP70 and LymHSP40 in the

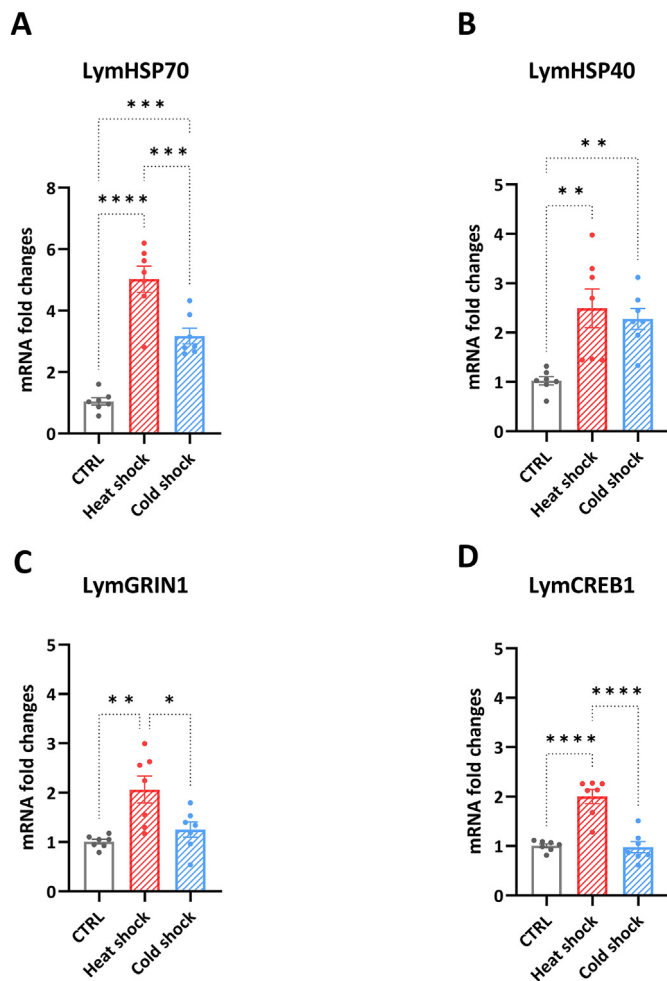


Fig. 3. Transcriptional effects induced by the Garcia effect procedure in snails exposed to 4 °C or 30 °C. The expression levels of LymHSP70 (A), LymHSP40 (B), LymGRIN1 (C), and LymCREB1 (D) were measured in the central ring ganglia of *L. stagnalis* trained for the Garcia effect using the heat shock (red hatched bars) or the cold (blue hatched bars) stressors. Control snails (CTRL: grey open bars) were maintained at 20 °C (room temperature) for 1 h. N = 7 for each group. Data are represented as means \pm SEM and were analyzed with a one-way ANOVA followed by a Tukey post hoc test. **** p < 0.0001, *** p < 0.001, ** p < 0.01 and * p < 0.05.

central ring ganglia of *L. stagnalis*.

This suggests that these HSPs are upregulated as part of an immediate response mechanism to cold stress, potentially aiding in the stabilization of cellular proteins and the maintenance of cellular homeostasis under adverse thermal conditions (Harada and Goto, 2017; Jiang et al., 2021; Li et al., 2012; Ma et al., 2021; Matz et al., 1995; Rinehart et al., 2007). This rapid upregulation of HSPs aligns with their role as molecular chaperones that prevent protein misfolding and aggregation, which are common consequences of thermal stress (Lindquist and Craig, 1988; Rinehart et al., 2007). Interestingly, no significant changes in the expression levels of LymHSP70 and LymHSP40 were observed in snails exposed to 4 °C for 24 h, suggesting a transient upregulation of these genes in response to short-term cold exposure. Snails exposed to prolonged cold conditions may have evolved mechanisms to minimize the energetic costs associated with continuous HSP production, thereby optimizing their response to cold stress while mitigating potential fitness costs linked to HSP synthesis (Sørensen et al., 2003).

HSPs are the major physiological marker of thermal (both heat and cold) stress and have a high energy demand, which can impair growth and reduce fitness (Sørensen et al., 2003). Furthermore, the findings

suggest that *L. stagnalis* may prioritize different molecular responses depending on the nature and duration of the thermal stress encountered.

Short-term cold exposure triggers an immediate but transient HSP response, likely aimed at acute stress management and cellular protection. In contrast, prolonged cold exposure may necessitate other adaptive mechanisms beyond HSP induction to maintain cellular function and overall metabolic homeostasis over extended periods (Jin et al., 2019, 2020; Lewis et al., 2016). This hypothesis is strengthened by the results of the expression levels of LymMIP-II and LymMIPR. Although short-term cold exposure *per se* did not affect the expression levels of LymMIP-II and its receptor, prolonged cold exposure was associated with a significant downregulation of LymMIP-II and an upregulation of LymMIPR. These effects, albeit only at the transcriptional levels, support the existence of a negative feedback loop necessary for maintaining glucose homeostasis and sustaining cellular functions despite challenging environmental conditions (Rivi et al., 2023e). We hypothesize that the downregulation of LymMIP-II results in a decrease in insulin production or secretion under prolonged cold stress. Similar results have been obtained in severely food-deprived snails, suggesting a common underlying pathway involved in modulating stress responses (Rivi et al., 2023e). Insulin is pivotal in promoting glucose uptake and storage, processes that may be temporarily less critical during extended periods of cold when metabolic demands might shift towards conserving energy rather than actively utilizing glucose (Matsunaga et al., 2016; Nakai et al., 2020; Sim and Denlinger, 2008). This downregulation could also indicate a state of reduced metabolic activity or even a metabolic depression strategy employed by *L. stagnalis* to conserve energy resources during prolonged cold exposure. As previous studies in *L. stagnalis* showed that insulin secretion plays a significant role in memory formation (Murakami et al., 2013), the downregulation of LymMIP-II may also to some extent explain why long-term memory persists in the cold (Fernell et al., 2021) as forgetting in *L. stagnalis* requires altered gene activity and new protein synthesis (Sangha et al., 2005).

Conversely, the upregulation of LymMIPR suggests an increased sensitivity or responsiveness of cells to insulin signals. This adaptive change may enhance the efficiency of glucose utilization in tissues that remain metabolically active despite the cold stress. By upregulating insulin receptors, cells can maintain sensitivity to insulin signaling, optimizing glucose uptake when conditions permit or when metabolic demands increase again (X. Gao et al., 2022; Partonen, 2013; Zhang et al., 2024). The observed changes in LymMIP-II and LymMIPR expression levels likely contribute to a negative feedback loop aimed at stabilizing glucose levels within a narrow physiological range. We hypothesize that this feedback mechanism is crucial for preventing hyperglycemia or hypoglycemia, both of which can disrupt cellular functions and overall metabolic balance. Thus, those transcriptional adjustments in insulin-related genes underscore the adaptive flexibility of *L. stagnalis* in response to prolonged cold stress, highlighting the intricate interplay between environmental cues and molecular responses in maintaining metabolic homeostasis.

Finally, we found that cold exposure (short and long-term) did not induce a change in the expression levels of LymGRIN1 and LymCREB1, which are associated with neuroplasticity, memory formation, and forgetting (Abrams, 2012; Bartsch et al., 1998; Batabyal et al., 2021; Fernell et al., 2021). The absence of significant effects on the expression levels of these targets suggests several intriguing insights into the neurobiological responses of *L. stagnalis* to cold stress.

Thus, cold shock, even when applied for prolonged periods, may not elicit the same level of molecular activation in these pathways, possibly due to different metabolic demands or cellular responses required to cope with cold stress (Sørensen et al., 2003). Snails may prioritize immediate physiological responses, such as the activation of HSPs to maintain protein stability under cold conditions, over long-term neuroplasticity-related changes. The lack of significant changes in LymGRIN1 and LymCREB1 expression could indicate that snails allocate resources differently in response to cold stress, focusing more on survival

mechanisms rather than enhancing cognitive functions or synaptic plasticity.

It is also possible that *L. stagnalis* has evolved specific adaptive strategies to cope with cold stress that do not rely heavily on neuroplasticity-related pathways. These adaptations may involve metabolic adjustments, alterations in membrane fluidity, or changes in ion channel activity rather than modifications in synaptic plasticity genes like GRIN1 and CREB1 (Acutain et al., 2021; Avery & Krichmar, 2015).

Understanding the molecular responses of *L. stagnalis* to cold stress, particularly in terms of neuroplasticity and memory formation, provides crucial insights into the adaptive mechanisms of aquatic organisms facing environmental fluctuations amidst ongoing climate changes affecting animal distributions and biodiversity (Zhang et al., 2024).

Thus, in *Experiment 2*, we investigated at the behavioral and transcriptional levels whether cold shock (4 °C for 1 h) could elicit a Garcia effect comparable to heat shock. The Garcia effect procedure is a well-founded learning paradigm (Garcia et al., 1955) that offers a behavioral metric to assess thermal tolerance and HSP induction in response to various thermal environments (Rivi et al., 2021a,b). First, we confirmed that a single exposure to a novel taste (carrot slurry) followed by a heat shock induces a Garcia effect, shown by a significant suppression of feeding response to carrot slurry for at least 3 h. This effect was absent in control snails that did not experience thermal shock, which is consistent with previous observations. On the other hand, cold shock (4 °C for 1 h) did not lead to a Garcia effect, as there was no significant difference in feeding response to carrots before and after the cold shock.

This behavioral distinction highlights that different thermal stressors exert different effects on memory consolidation processes in *L. stagnalis*. The absence of a Garcia effect with cold shock aligns with the transcriptional findings that showed distinct molecular responses between heat and cold shock, particularly in the expression levels of neuroplasticity-related genes like LymGRIN1 and LymCREB1. Heat shock, by inducing a robust upregulation of stress response genes like HSPs, not only triggers learning and memory formation associated with the Garcia effect in lab-bred snails (Rivi et al., 2022h), but also enhances long-term memory formation for the operant conditioning of aerial respiratory behavior when applied before, during, or immediately after training (Alagar Boopathy et al., 2022; Ecroyd et al., 2023; Stetler et al., 2010; Teskey et al., 2012). In contrast, cooling lab-bred snails for 1 h immediately after operant conditioning training is sufficient to block memory formation, whereas the same cooling procedure performed 15 min after training prevents forgetting (Sangha et al., 2003). Moreover, our previous work showed that exposing lab-bred snails to a cold spell for 4 weeks following training extended the persistence of long-term memory for operant conditioning of aerial respiration for at least 4 weeks (Fernell et al., 2021). This finding aligns with recent studies in *Caenorhabditis elegans*, where memories are retained only if the worms are cooled quickly, whereas if they acclimatize to the cold by spending the night in cool conditions before training and then are placed on ice, they forget the information as fast as usual (Landschaft et al., 2024).

Thus, our current and previous studies suggest that the intensity, timing, exposure duration, and nature of the stressor are critical in shaping behavioral responses and memory consolidation in *L. stagnalis* and other invertebrates. These findings pave the way for future research into the underlying mechanisms behind the different effects of heat and cold shock on memory consolidation.

This could include exploring the role of specific neurotransmitter systems, synaptic plasticity mechanisms, and other molecular pathways involved in memory formation in response to thermal stressors. Additionally, comparative studies across different populations or species of aquatic organisms could elucidate variation in thermal stress responses and their adaptive significance in natural environments. These findings contribute to our understanding of the adaptive strategies employed by aquatic organisms to cope with environmental challenges and underscore the importance of considering the specific nature of stressors in shaping behavioral and cognitive responses. At the transcriptional level, we found

a significant upregulation of stress response genes (LymHSP70 and LymHSP40) both in snails that formed the Garcia effect (i.e., those exposed to the heat shock) and those that did not form the Garcia effect (i.e., those exposed to the cold shock) compared to the control snails maintained at room temperature. However, the upregulation in LymHSP70 was notably higher in snails exposed to heat shock compared to those exposed to cold shock, indicating a more pronounced stress response to heat.

Furthermore, genes associated with neuroplasticity (LymGRIN1 and LymCREB1) showed significant upregulation only in snails that exhibited the Garcia effect (heat shock-exposed), suggesting that heat shock induces more substantial molecular changes linked to memory formation compared to cold shock or control conditions. This differential gene expression profile highlights the nuanced neurobiological responses to varying thermal stressors in these snails, with implications for understanding adaptive responses to environmental challenges. Understanding how poikilotherm species like *L. stagnalis* respond at the molecular level to temperature fluctuations is crucial for predicting their resilience to environmental changes, particularly in the context of climate change (Alexander et al., 2006; Angilletta Jr, 2009). These insights can inform conservation efforts and management strategies aimed at preserving aquatic biodiversity and ecosystem stability in a changing climate.

This study provides valuable insights into the transcriptional adaptations of aquatic organisms to temperature fluctuations, which is essential for understanding their resilience to changing environmental conditions.

Future studies could explore the broader implications of these molecular and behavioral responses across different populations or species of aquatic organisms. Investigating how genetic variability influences these responses and whether adaptive differences exist in natural populations could provide deeper insights into the evolutionary potential of aquatic species facing climate-driven challenges. Additionally, further investigation is needed into how *Lymnaea* detects water temperature changes. Many animals can detect shifts in temperature through afferent nerve fibers in the skin (Scheepers and Ringkamp, 2010). However, whether such receptors exist in *L. stagnalis* and how they work has been largely unexplored. Identifying the molecular identity of these receptors could reveal how *L. stagnalis* interprets thermal signals and comparing these mechanisms with those in other mollusks may uncover evolutionary adaptations that help species thrive in diverse environments. Research on *L. stagnalis*'s thermoreceptors could deepen our understanding of the adaptive responses of our model species to both cold and hot stressors and pave the way for comparative studies.

Overall, these findings can also contribute to our understanding of thermal biology and stress physiology in aquatic organisms, underscoring the importance of molecular mechanisms in shaping species' resilience in dynamic environments.

5. Conclusions

Our study offers important insights into the adaptive responses of *L. stagnalis* to thermal stressors, amidst the backdrop of escalating climate change. We demonstrated that short-term cold exposure triggers an immediate but transient upregulation of key HSPs, underscoring their role in acute stress management. In contrast, prolonged cold exposure led to a strategic shift towards energy conservation and metabolic homeostasis. These findings reveal the nuanced molecular adaptations of *L. stagnalis*, balancing the high energy demands of stress response with long-term survival strategies. Moreover, our behavioral experiments highlighted the different impacts of heat and cold shock on memory formation. While heat shock induced a robust Garcia effect, indicative of enhanced associative learning, cold shock failed to elicit a similar response, aligning with the absence of significant changes in neuroplasticity-related genes. This distinction suggests that the nature and intensity of thermal stressors critically influence the cognitive and behavioral adaptations in aquatic organisms. These findings underscore the complexity of thermal

adaptation in *L. stagnalis*, emphasizing the importance of context-specific responses to environmental stressors. Understanding these mechanisms is pivotal for predicting the resilience of aquatic poikilotherms in the face of climate change, providing a foundation for conservation strategies aimed at preserving biodiversity and ecosystem stability. Future research should delve deeper into the molecular pathways and genetic variability underlying these adaptive responses, offering a broader perspective on the evolutionary potential of aquatic species amid global environmental fluctuations.

CRedit authorship contribution statement

Veronica Rivi: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Anuradha Batabyal:** Writing – review & editing, Investigation, Conceptualization. **Cristina Benatti:** Writing – review & editing, Supervision, Resources, Methodology. **Fabio Tascetta:** Writing – review & editing, Supervision, Resources. **Johanna Maria Catharina Blom:** Writing – review & editing, Supervision, Resources. **Ken Lukowiak:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Data curation, Conceptualization.

Ethics statement

Lymnaea stagnalis are invertebrate animals; thus, the approval of IACUC (Institutional Animal Care and Use Committee) was not required (Italian Legislative Decree D.L. 4 marzo 2014, n. 26 “Attuazione della Direttiva n. 2010/63/UE sulla protezione degli animali utilizzati a fini scientifici”). However, every effort was made to minimize the number of animals used, ensuring adequate food, clean oxygenated water, and low-density conditions.

Data availability

Further information and requests for resources and reagents should be directed to and will be fulfilled by Dr. Veronica Rivi.

Fundings

The current work was funded by Regione Emilia Romagna L.R. N. 20/2002 PROGETTI DI RICERCA SUI METODI ALTERNATIVE ALL'UTILIZZO DI ANIMALI; FAR 2016 Department of Life Sciences, University of Modena and Reggio Emilia, and the Natural Sciences and Engineering Research Council of Canada.

Declaration of competing interest

The authors disclose no commercial or financial conflicting interests upon publication of this article.

Acknowledgments

The authors would like to thank Consorzio Interuniversitario Biotecnologie (CIB, Trieste, Italy) for the research grant awarded to Dr. Veronica Rivi to work as a visiting researcher at the Hotchkiss Brain Institute (University of Calgary, AB, Canada).

References

A. K. P., M. M., Rajamanickam, S., Sivarethinamohan, S., Gaddam, M.K.R., Velusamy, P., R. G., Ravindiran, G., Gurugubelli, T.R., Muniasamy, S.K., 2023. Impact of climate change and anthropogenic activities on aquatic ecosystem – a review. *Environ. Res.* 238, 117233. <https://doi.org/10.1016/j.envres.2023.117233>.

Abrams, T.W., 2012. Studies on *Aplysia* neurons suggest treatments for chronic human disorders. *Curr. Biol.* 22 (17), R705–R711. <https://doi.org/10.1016/j.cub.2012.08.011>.

Acutain, M.F., Griebler Luft, J., Vazquez, C.A., Popik, B., Cercato, M.C., Epstein, A., Salvetti, A., Jerusalinsky, D.A., de Oliveira Alvares, L., Baez, M.V., 2021. Reduced

expression of hippocampal GluN2A-NMDAR increases seizure susceptibility and causes deficits in contextual memory. *Front. Neurosci.* 15. <https://www.frontiersin.org/articles/10.3389/fnins.2021.644100>.

Alagar Boopathy, L.R., Jacob-Tomas, S., Alecki, C., Vera, M., 2022. Mechanisms tailoring the expression of heat shock proteins to proteostasis challenges. *J. Biol. Chem.* 298 (5), 101796. <https://doi.org/10.1016/j.jbc.2022.101796>.

Alexander, L.V., Zhang, X., Peterson, T.C., Caesar, J., Gleason, B., Klein Tank, A.M.G., Haylock, M., Collins, D., Trewin, B., Rahimzadeh, F., Tagipour, A., Rupa Kumar, K., Revadekar, J., Griffiths, G., Vincent, L., Stephenson, D.B., Burn, J., Aguilar, E., Brunet, M., et al., 2006. Global observed changes in daily climate extremes of temperature and precipitation. *J. Geophys. Res. Atmos.* 111 (D5). <https://doi.org/10.1029/2005JD006290>.

Amorim, J., Abreu, I., Rodrigues, P., Peixoto, D., Pinheiro, C., Saraiva, A., Carvalho, A.P., Guimarães, L., Oliva-Teles, L., 2019. *Lymnaea stagnalis* as a freshwater model invertebrate for ecotoxicological studies. *Sci. Total Environ.* 669, 11–28. <https://doi.org/10.1016/j.scitotenv.2019.03.035>.

Anderson, B.G., Bell, M.L., 2009. Weather-related mortality: how heat, cold, and heat waves affect mortality in the United States. *Epidemiology* 20 (2), 205–213. <https://doi.org/10.1097/EDE.0b013e318190e08>.

Angilletta, Jr.M.J., 2009. Looking for answers to questions about heat stress: researchers are getting warmer. *Funct. Ecol.* 23 (2), 231–232. <https://doi.org/10.1111/j.1365-2435.2009.01548.x>.

Avery, M.C., Krichmar, J.L., 2015. Improper activation of D1 and D2 receptors leads to excess noise in prefrontal cortex. *Front. Comput. Neurosci.* 9, 31. <https://doi.org/10.3389/fncom.2015.00031>.

Bartsch, D., Casadio, A., Karl, K.A., Serodio, P., Kandel, E.R., 1998. CREB1 encodes a nuclear activator, a repressor, and a cytoplasmic modulator that forms a regulatory unit critical for long-term facilitation. *Cell* 95 (2), 211–223. [https://doi.org/10.1016/S0092-8674\(00\)81752-3](https://doi.org/10.1016/S0092-8674(00)81752-3).

Batabyal, A., Chau, D., Rivi, V., Lukowiak, K., 2022. Risk in one is not risk in all: snails show differential decision-making under high- and low-risk environments. *Anim. Behav.* 190, 53–60. <https://doi.org/10.1016/j.anbehav.2022.05.013>.

Batabyal, A., Rivi, V., Benatti, C., Blom, J.M.C., Lukowiak, K., 2021. Long-term memory of configural learning is enhanced via CREB upregulation by the flavonoid quercetin in *Lymnaea stagnalis*. *J. Exp. Biol.* 224 (13). <https://doi.org/10.1242/jeb.242761>. Article 13.

Batabyal, A., Rivi, V., Benatti, C., Blom, J.M.C., Tascetta, F., Lukowiak, K., 2024. Snails go on a fast when acetylsalicylic acid comes along with heat stress: a possible effect of HSPs and serotonergic system on the feeding response. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 276, 109805. <https://doi.org/10.1016/j.cbpc.2023.109805>.

Benedetti, F., Vogt, M., Elizondo, U.H., Righetti, D., Zimmermann, N.E., Gruber, N., 2021. Major restructuring of marine plankton assemblages under global warming. *Nat. Commun.* 12 (1), 5226. <https://doi.org/10.1038/s41467-021-25385-x>.

Bowler, K., 2018. Heat death in poikilotherms: is there a common cause? *J. Therm. Biol.* 76, 77–79. <https://doi.org/10.1016/j.jtherbio.2018.06.007>.

Brown, K.M., 1979. The adaptive demography of four freshwater pulmonate snails. *Evolution* 33 (1), 417–432. <https://doi.org/10.2307/2407631>.

Calduch-Giner, J., Holhorea, P.G., Ferrer, M.A., Naya-Català, F., Rosell-Moll, E., Vega García, C., Prunet, P., Espmark, Å.M., Leguen, I., Kolarevic, J., Vega, A., Kerneis, T., Goardon, L., Afonso, J.M., Pérez-Sánchez, J., 2022. Revisiting the impact and prospects of activity and ventilation rate bio-loggers for tracking welfare and fish-environment interactions in salmonids and mediterranean farmed fish. *Front. Mar. Sci.* 9. <https://doi.org/10.3389/fmars.2022.854888>.

Dalesman, S., Lukowiak, K., 2010. Effect of acute exposure to low environmental calcium on respiration and locomotion in *Lymnaea stagnalis* (L.). *J. Exp. Biol.* 213 (Pt 9), 1471–1476. <https://doi.org/10.1242/jeb.040493>.

Duffy, K., Gouhier, T.C., Ganguly, A.R., 2022. Climate-mediated shifts in temperature fluctuations promote extinction risk. *Nat. Clim. Change* 12 (11), 1037–1044. <https://doi.org/10.1038/s41558-022-01490-7>.

Ecroyd, H., Bartelt-Kirbach, B., Ben-Zvi, A., Bonavita, R., Bushman, Y., Casarotto, E., Cecconi, C., Lau, W.C.Y., Hibshman, J.D., Joosten, J., Kimonis, V., Klevit, R., Liberek, K., McMenimen, K.A., Miwa, T., Mogk, A., Montepietra, D., Peters, C., Rocchetti, M.T., et al., 2023. The beauty and complexity of the small heat shock proteins: a report on the proceedings of the fourth workshop on small heat shock proteins. *Cell Stress & Chaperones* 28 (6), 621–629. <https://doi.org/10.1007/s12192-023-01360-x>.

Fernández, I., Mozanzadeh, M.T., Hao, Y., Gisbert, E., 2022. Editorial: physiological impacts of global warming in aquatic organisms. *Front. Physiol.* 13, 914912. <https://doi.org/10.3389/fphys.2022.914912>.

Fernell, M., Rivi, V., Batabyal, A., Lukowiak, K., 2021. The temperature sensitivity of memory formation and persistence is altered by cold acclimation in a pond snail. *J. Exp. Biol.* 224 (11), jeb242513. <https://doi.org/10.1242/jeb.242513>.

Fodor, I., Hussein, A.A., Benjamin, P.R., Koene, J.M., Pirger, Z., 2020. The unlimited potential of the great pond snail, *Lymnaea stagnalis*. *Elife* 9, e56962. <https://doi.org/10.7554/eLife.56962>.

Foster, N.L., Lukowiak, K., Henry, T.B., 2015. Time-related expression profiles for heat shock protein gene transcripts (HSP40, HSP70) in the central nervous system of *Lymnaea stagnalis* exposed to thermal stress. *Commun. Integr. Biol.* 8 (3), e1040954. <https://doi.org/10.1080/19420889.2015.1040954>.

Fontúrbel, F.E., Nespolo, R.F., Amico, G.C., Watson, D.M., 2021. Climate change can disrupt ecological interactions in mysterious ways: using ecological generalists to forecast community-wide effects. *Clim. Change Ecology* 2, 100044. <https://doi.org/10.1016/j.ecoche.2021.100044>.

Gao, J., Zhang, W., Dang, W., Mou, Y., Gao, Y., Sun, B.-J., Du, W.-G., 2014. Heat shock protein expression enhances the heat tolerance of reptile embryos. *Proc. Biol. Sci.* 281 (1791), 20141135. <https://doi.org/10.1098/rspb.2014.1135>.

- Gao, X., Cai, T., Lin, Y., Zhu, R., Hao, W., Guo, S., Hu, G., 2022. The function of glucose metabolism in embryonic diapause of annual killifish. *Comp. Biochem. Physiol. Genom. Proteonomics* 42, 100965. <https://doi.org/10.1016/j.cbcd.2022.100965>.
- Garcia, J., Kimeldorf, D.J., Koelling, R.A., 1955. Conditioned aversion to saccharin resulting from exposure to gamma radiation. *Science* 122 (3160), 157–158. <https://doi.org/10.1126/science.122.3160.157>.
- Guschina, I.A., Harwood, J.L., 2006. Mechanisms of temperature adaptation in poikilotherms. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 580 (23), 5477–5483. <https://doi.org/10.1016/j.febslet.2006.06.066>.
- Harada, E., Goto, S.G., 2017. Upregulation of heat-shock proteins in larvae, but not adults, of the flesh fly during hot summer days. *Cell Stress & Chaperones* 22 (6), 823–831. <https://doi.org/10.1007/s12192-017-0812-y>.
- Harvey, B.P., Marshall, K.E., Harley, C.D.G., Russell, B.D., 2022. Predicting responses to marine heatwaves using functional traits. *Trends Ecol. Evol.* 37 (1), 20–29. <https://doi.org/10.1016/j.tree.2021.09.003>.
- Jeyachandran, S., Chellapandian, H., Park, K., Kwak, I.-S., 2023. A review on the involvement of heat shock proteins (extrinsic chaperones) in response to stress conditions in aquatic organisms. *Antioxidants* 12 (7), 1444. <https://doi.org/10.3390/antiox12071444>.
- Jiang, F., Chang, G., Li, Z., Abouzaid, M., Du, X., Hull, J.J., Ma, W., Lin, Y., 2021. The HSP/co-chaperone network in environmental cold adaptation of *Chilo suppressalis*. *Int. J. Biol. Macromol.* 187, 780–788. <https://doi.org/10.1016/j.jbiomac.2021.07.113>.
- Jin, J., Li, Y., Zhou, Z., Zhang, H., Guo, J., Wan, F., 2020. Heat shock factor is involved in regulating the transcriptional expression of two potential hsps (AhHsp70 and AhHsp21) and its role in heat shock response of agasicles hygrophila. *Front. Physiol.* 11. <https://doi.org/10.3389/fphys.2020.562204>.
- Jin, J., Zhao, M., Wang, Y., Zhou, Z., Wan, F., Guo, J., 2019. Induced thermotolerance and expression of three key hsp genes (Hsp70, Hsp21, and sHsp21) and their roles in the high-temperature tolerance of agasicles hygrophila. *Front. Physiol.* 10, 1593. <https://doi.org/10.3389/fphys.2019.01593>.
- Kagan, D., Batabyal, A., Lukowiak, K., 2023a. Remember the poke: microRNAs are required for long-term memory formation following operant conditioning in *Lymnaea*. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* 209 (3), 403–410. <https://doi.org/10.1007/s00359-022-01604-8>.
- Kagan, D., Rivi, V., Benatti, C., Tascadda, F., Blom, J.M.C., Lukowiak, K., 2023b. No food for thought: an intermediate level of food deprivation enhances memory in *Lymnaea stagnalis*. *J. Exp. Biol.* 226 (10), jeb245566. <https://doi.org/10.1242/jeb.245566>.
- Koene, J.M., 2006. Tales of two snails: sexual selection and sexual conflict in *Lymnaea stagnalis* and *Helix aspersa*. *Integr. Comp. Biol.* 46 (4), 419–429. <https://doi.org/10.1093/icb/iccj040>.
- Landschaft Berliner, D.L., et al., 2024. Preprint at bioRxiv. <https://doi.org/10.1101/2024.04.03.587909>.
- Lewis, M., Götting, M., Anttila, K., Kanerva, M., Prokkola, J.M., Seppänen, E., Kolari, I., Nikinmaa, M., 2016. Different relationship between hsp70 mRNA and hsp70 levels in the heat shock response of two salmonids with dissimilar temperature preference. *Front. Physiol.* 7, 511. <https://doi.org/10.3389/fphys.2016.00511>.
- Li, J., Moghaddam, S.H.H., Du, X., Zhong, B., Chen, Y.-Y., 2012. Comparative analysis on the expression of inducible HSPs in the silkworm, *Bombyx mori*. *Mol. Biol. Rep.* 39 (4), 3915–3923. <https://doi.org/10.1007/s11033-011-1170-y>.
- Lukowiak, K., Orr, M., de Caigny, P., Lukowiak, K.S., Rosenegger, D., Han, J.I., Dalesman, S., 2010. Ecologically relevant stressors modify long-term memory formation in a model system. *Behav. Brain Res.* 214 (1), 18–24. <https://doi.org/10.1016/j.bbr.2010.05.011>.
- Lukowiak, K., Sunada, H., Teskey, M., Lukowiak, K., Dalesman, S., 2014. Environmentally relevant stressors alter memory formation in the pond snail *Lymnaea*. *J. Exp. Biol.* 217 (Pt 1), 76–83. <https://doi.org/10.1242/jeb.089441>.
- Ma, C.-S., Ma, G., Pincebourde, S., 2021. Survive a warming climate: insect responses to extreme high temperatures. *Annu. Rev. Entomol.* 66, 163–184. <https://doi.org/10.1146/annurev-ento-041520-074454>.
- Malik, H.R., Bertolesi, G.E., McFarlane, S., 2023. TRPM8 thermosensation in poikilotherms mediates both skin color and locomotor performance responses to cold temperature. *Commun. Biol.* 6, 127. <https://doi.org/10.1038/s42003-023-04489-8>.
- Martens, K., Amarell, M., Parvez, K., Hittel, K., De Caigny, P., Ito, E., Lukowiak, K., 2007. One-trial conditioning of aerial respiratory behavior in *Lymnaea stagnalis*. *Neurobiol. Learn. Mem.* 88, 232–242.
- Marx, W., Haunschild, R., Bornmann, L., 2021. Heat waves: a hot topic in climate change research. *Theor. Appl. Climatol.* 146 (1–2), 781–800. <https://doi.org/10.1007/s00704-021-03758-y>.
- Matsunaga, Y., Honda, Y., Honda, S., Iwasaki, T., Qadota, H., Benian, G.M., Kawano, T., 2016. Diapause is associated with a change in the polarity of secretion of insulin-like peptides. *Nat. Commun.* 7 (1), 10573. <https://doi.org/10.1038/ncomms10573>.
- Matz, J.M., Blake, M.J., Tatelman, H.M., Lavoi, K.P., Holbrook, N.J., 1995. Characterization and regulation of cold-induced heat shock protein expression in mouse brown adipose tissue. *Am. J. Physiol.* 269 (1 Pt 2), R38–R47. <https://doi.org/10.1152/ajpregu.1995.269.1.R38>.
- Mayer, M.P., 2010. Gymnastics of molecular chaperones. *Mol. Cell* 39 (3), 321–331. <https://doi.org/10.1016/j.molcel.2010.07.012>.
- Meehl, G.A., Tebaldi, C., 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* 305 (5686), 994–997. <https://doi.org/10.1126/science.1098704>.
- Murakami, J., Okada, R., Sadamoto, H., Kobayashi, S., Mita, K., Sakamoto, Y., Yamagishi, M., Hatakeyama, D., Otsuka, E., Okuta, A., Sunada, H., Takigami, S., Sakakibara, M., Fujito, Y., Awaji, M., Moriyama, S., Lukowiak, K., Ito, E., 2013. Involvement of insulin-like peptide in long-term synaptic plasticity and long-term memory of the pond snail *Lymnaea stagnalis*. *J. Neurosci.: J. Soc. Neurosci.* 33 (1), 371–383. <https://doi.org/10.1523/JNEUROSCI.0679-12.2013>.
- Nakai, J., Chikamoto, N., Fujimoto, K., Totani, Y., Hatakeyama, D., Dyakonova, V.E., Ito, E., 2022. Insulin and memory in invertebrates. *Front. Behav. Neurosci.* 16. <https://www.frontiersin.org/articles/10.3389/fnbeh.2022.882932>.
- Nakai, J., Totani, Y., Kojima, S., Sakakibara, M., Ito, E., 2020. Features of behavioral changes underlying conditioned taste aversion in the pond snail *Lymnaea stagnalis*. *Invertebr. Neurosci.* 20 (2), 8. <https://doi.org/10.1007/s10158-020-00241-7>.
- Neven, L.G., 2000. Physiological responses of insects to heat. *Postharvest Biol. Technol.* 21 (1), 103–111. [https://doi.org/10.1016/S0925-5214\(00\)00169-1](https://doi.org/10.1016/S0925-5214(00)00169-1).
- Oksala, N.K.J., Ekmekçi, F.G., Özsoy, E., Kirankaya, Ş., Kokkola, T., Emecen, G., Lappalainen, J., Kaamiranta, K., Atalay, M., 2014. Natural thermal adaptation increases heat shock protein levels and decreases oxidative stress. *Redox Biol.* 3, 25–28. <https://doi.org/10.1016/j.redox.2014.10.003>.
- Orr, M.V., Hittel, K., Lukowiak, K., 2009. 'Different strokes for different folks': geographically isolated strains of *Lymnaea stagnalis* only respond to sympatric predators and have different memory-forming capabilities. *J. Exp. Biol.* 212 (14), 2237–2247. <https://doi.org/10.1242/jeb.031575>.
- Partonen, T., 2013. During winter the body rests insulin. *Hypertens. Res.* 36 (5), 390–391. <https://doi.org/10.1038/hr.2012.227>.
- Piyaphongkul, J., Pritchard, J., Bale, J., 2014. Effects of acclimation on the thermal tolerance of the brown planthopper *Nilaparvata lugens* (Stål). *Agric. For. Entomol.* 16 (2), 174–183. <https://doi.org/10.1111/afe.12047>.
- Pluess, M., Lionetti, F., Aron, E.N., Aron, A., 2023. People differ in their sensitivity to the environment: an integrated theory, measurement, and empirical evidence. *J. Res. Pers.* 104, 104377. <https://doi.org/10.1016/j.jrp.2023.104377>.
- Prahlad, V., Morimoto, R.I., 2009. Integrating the stress response: lessons for neurodegenerative diseases from *C. elegans*. *Trends Cell Biol.* 19 (2), 52–61. <https://doi.org/10.1016/j.tcb.2008.11.002>.
- Quan, Y., Mason, C.E., He, K., Wang, Z., Wei, H., 2023. Impact of heat waves on egg survival and biological performance across life stages in the Asian corn borer. *Entomol. Exp. Appl.* 171 (2), 129–137. <https://doi.org/10.1111/eea.13262>.
- Quan, Y., Wang, Z., Wei, H., He, K., 2022. Transcription dynamics of heat shock proteins in response to thermal acclimation in *Ostrinia furnacalis*. *Front. Physiol.* 13, 992293. <https://doi.org/10.3389/fphys.2022.992293>.
- Reeve, C., Rowsey, L.E., Speers-Roesch, B., 2022. Inactivity and the passive slowing effect of cold on resting metabolism as the primary drivers of energy savings in overwintering fishes. *J. Exp. Biol.* 225 (8), jeb243407. <https://doi.org/10.1242/jeb.243407>.
- Reid, G.K., Gurney-Smith, H.J., Marcogliese, D.J., Knowler, D., Benfey, T., Garber, A.F., Forster, I., Chopin, T., Brewer-Dalton, K., Moccia, R.D., Flaherty, M., Smith, C.T., De Silva, S., 2019. Climate change and aquaculture: considering biological response and resources. *Aquac. Environ. Int.* 11, 569–602.
- Rensing, L., Ruoff, P., 2002. Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. *Chronobiol. Int.* 19 (5), 807–864. <https://doi.org/10.1081/cbi-120014569>.
- Rinehart, J.P., Li, A., Yocum, G.D., Robich, R.M., Hayward, S.A.L., Denlinger, D.L., 2007. Up-regulation of heat shock proteins is essential for cold survival during insect diapause. *Proc. Natl. Acad. Sci. USA* 104 (27), 11130–11137. <https://doi.org/10.1073/pnas.0703538104>.
- Rivi, V., Batabyal, A., Benatti, C., Blom, J.M.C., Tascadda, F., Lukowiak, K., 2023a. Investigating the interactions between multiple memory stores in the pond snail *Lymnaea stagnalis*. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*. <https://doi.org/10.1007/s00359-023-01649-3>.
- Rivi, V., Batabyal, A., Benatti, C., Blom, J.M., Tascadda, F., Lukowiak, K., 2023b. Novel taste, sickness, and memory: lipopolysaccharide to induce a Garcia-like effect in inbred and wild strains of *Lymnaea stagnalis*. *Physiol. Behav.* 263, 114137. <https://doi.org/10.1016/j.physbeh.2023.114137>.
- Rivi, V., Batabyal, A., Benatti, C., Blom, J.M.C., Lukowiak, K., 2022c. Nature versus nurture in heat stress-induced learning between inbred and outbred populations of *Lymnaea stagnalis*. *J. Therm. Biol.* 103, 103170. <https://doi.org/10.1016/j.jtherbio.2021.103170>.
- Rivi, V., Batabyal, A., Benatti, C., Tascadda, F., Blom, J.M.C., Lukowiak, K., 2022d. Aspirin reverts lipopolysaccharide-induced learning and memory impairment: first evidence from an invertebrate model system. *Naunyn-Schmiedeberg's Arch. Pharmacol.* <https://doi.org/10.1007/s00210-022-02286-4>.
- Rivi, V., Batabyal, A., Benatti, C., Tascadda, F., Blom, J.M.C., Lukowiak, K., 2023a. A novel behavioral display in *Lymnaea* induced by quercetin and hypoxia. *Biol. Bull.* 244 (2), 115–127. <https://doi.org/10.1086/725689>.
- Rivi, V., Batabyal, A., Benatti, C., Tascadda, F., Blom, J.M.C., Lukowiak, K., 2023b. Comparison of behavioral and transcriptional responses to a heat stressor between freshly collected and an inbred strain of *Lymnaea*. *Can. J. Zool.* 101 (10), 904–912. <https://doi.org/10.1139/cjz-2023-0088>.
- Rivi, V., Batabyal, A., Benatti, C., Tascadda, F., Blom, J.M.C., Lukowiak, K., 2023c. Prey populations with different predation histories show differences in behavioral and transcriptional effects under acute predation threats. *Neurobiol. Learn. Mem.* 203, 107775. <https://doi.org/10.1016/j.nlm.2023.107775>.
- Rivi, V., Batabyal, A., Benatti, C., Tascadda, F., Blom, J.M., Lukowiak, K., 2022f. Too hot to eat: wild and lab-bred *Lymnaea stagnalis* differ in feeding response following repeated heat exposure. *Biol. Bull.* <https://doi.org/10.1086/720948>, 000–000.
- Rivi, V., Batabyal, A., Juego, K., Kakadiya, M., Benatti, C., Blom, J.M.C., Lukowiak, K., 2021a. To eat or not to eat: a Garcia effect in pond snails (*Lymnaea stagnalis*). *J. Comp. Physiol.* 207 (4), 479–495. <https://doi.org/10.1007/s00359-021-01491-5>.
- Rivi, V., Batabyal, A., Lukowiak, K., 2024. The multifaceted effects of flavonoids on neuroplasticity. *Restorative Neurol. Neurosci.* Preprint(Preprint) 1–19. <https://doi.org/10.3233/RNN-230150>.

- Rivi, V., Batabyal, A., Lukowiak, K., Benatti, C., Rigillo, G., Tascetta, F., Blom, J.M.C., 2023d. LPS-induced Garcia effect and its pharmacological regulation mediated by acetylsalicylic acid: behavioral and transcriptional evidence. *Biology* 12 (8), 1100. <https://doi.org/10.3390/biology12081100>.
- Rivi, V., Batabyal, A., Wiley, B., Benatti, C., Tascetta, F., Blom, J.M.C., Lukowiak, K., 2022g. Fluoride affects memory by altering the transcriptional activity in the central nervous system of *Lymnaea stagnalis*. *Neurotoxicology* 92, 61–66. <https://doi.org/10.1016/j.neuro.2022.07.007>.
- Rivi, V., Benatti, C., Actis, P., Tascetta, F., Blom, J.M.C., 2022h. Behavioral and transcriptional effects of short or prolonged fasting on the memory performances of *Lymnaea stagnalis*. *Neuroendocrinology*. <https://doi.org/10.1159/000527489>.
- Rivi, V., Benatti, C., Lukowiak, K., Colliva, C., Albani, S., Tascetta, F., Blom, J.M.C., 2021b. What can we teach *Lymnaea* and what can *Lymnaea* teach us? *Biol. Rev. Camb. Phil. Soc.* 96 (4), 1590–1602. <https://doi.org/10.1111/brv.12716>.
- Rivi, V., Rigillo, G., Batabyal, A., Lukowiak, K., Pani, L., Tascetta, F., Benatti, C., & Blom, J. M. C. (n.d.). Different stressors uniquely affect the expression of endocannabinoid-metabolizing enzymes in the central ring ganglia of *Lymnaea stagnalis*. *J. Neurochem.* *n/a*(n/a). <https://doi.org/10.1111/jnc.16147>.
- Rocha, J., Oliveira, S., Viana, C.M., Ribeiro, A.I., 2022. Chapter 8—climate change and its impacts on health, environment, and economy. In: Prata, J.C., Ribeiro, A.I., Rocha-Santos, T. (Eds.), *One Health*. Academic Press, pp. 253–279. <https://doi.org/10.1016/B978-0-12-822794-7.00009-5>.
- Sangha, S., McComb, C., Lukowiak, K., 2003. Forgetting and the extension of memory in *Lymnaea*. *J. Exp. Biol.* 206 (Pt 1), 71–77. <https://doi.org/10.1242/jeb.00061>.
- Sangha, S., Scheibenstock, A., Martens, K., Varshney, N., Cooke, R., Lukowiak, K., 2005. Impairing forgetting by preventing new learning and memory. *Behav. Neurosci.* 119, 787–796.
- Scott, M., Berrigan, D., Hoffmann, A.A., 1997. Costs and benefits of acclimation to elevated temperature in *Trichogramma carverae*. *Entomol. Exp. Appl.* 85 (3). <https://findanexpert.unimelb.edu.au/scholarlywork/1091798-costs-and-benefits-of-acclimation-to-elevated-temperature-in-trichogramma-carverae>.
- Setti, A., Lionetti, F., Kagari, R.L., Motherway, L., Pluess, M., 2022. The temperament trait of environmental sensitivity is associated with connectedness to nature and affinity to animals. *Heliyon* 8 (7), e09861. <https://doi.org/10.1016/j.heliyon.2022.e09861>.
- Schepers, R.J., Ringkamp, M., 2010. Thermoreceptors and thermosensitive afferents. *Neurosci. Biobehav. Rev.* 34 (2), 177–184.
- Sim, C., Denlinger, D.L., 2008. Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens*. *Proc. Natl. Acad. Sci. USA* 105 (18), 6777–6781. <https://doi.org/10.1073/pnas.0802067105>.
- Soravia, C., Ashton, B.J., Thornton, A., Ridley, A.R., 2021. The impacts of heat stress on animal cognition: implications for adaptation to a changing climate. *WIREs Climate Change* 12 (4), e713. <https://doi.org/10.1002/wcc.713>.
- Sørensen, J.G., Kristensen, T.N., Kristensen, K.V., Loeschcke, V., 2007. Sex-specific effects of heat-induced hormesis in Hsf-deficient *Drosophila melanogaster*. *Exp. Gerontol.* 42 (12), 1123–1129. <https://doi.org/10.1016/j.exger.2007.09.001>.
- Sørensen, J.G., Kristensen, T.N., Loeschcke, V., 2003. The evolutionary and ecological role of heat shock proteins. *Ecol. Lett.* 6 (11), 1025–1037. <https://doi.org/10.1046/j.1461-0248.2003.00528.x>.
- Speers-Roesch, B., Norin, T., Driedzic, W.R., 2018. The benefit of being still: energy savings during winter dormancy in fish come from inactivity and the cold, not from metabolic rate depression. *Proc. Biol. Sci.* 285 (1886), 20181593. <https://doi.org/10.1098/rspb.2018.1593>.
- Stetler, R.A., Gan, Y., Zhang, W., Liou, A.K., Gao, Y., Cao, G., Chen, J., 2010. Heat shock proteins: cellular and molecular mechanisms in the CNS. *Prog. Neurobiol.* 92 (2), 184–211. <https://doi.org/10.1016/j.pneurobio.2010.05.002>.
- Sunada, H., Riaz, H., de Freitas, E., Lukowiak, K., Swinton, C., Swinton, E., Protheroe, A., Shymansky, T., Komatsuzaki, Y., Lukowiak, K., 2016. Heat stress enhances LTM formation in *Lymnaea*: role of HSPs and DNA methylation. *J. Exp. Biol.* 219 (9), 1337–1345. <https://doi.org/10.1242/jeb.134296>.
- Takahashi, T., Takigami, S., Sunada, H., Lukowiak, K., Sakakibara, M., 2013. The critical period of memory enhancement during taste avoidance conditioning in *Lymnaea stagnalis*. *PLoS One* 8 (10), e75276. <https://doi.org/10.1371/journal.pone.0075276>.
- Tedeschi, J.N., Kennington, W.J., Berry, O., Whiting, S., Meekan, M., Mitchell, N.J., 2015. Increased expression of Hsp70 and Hsp90 mRNA as biomarkers of thermal stress in loggerhead turtle embryos (*Caretta caretta*). *J. Therm. Biol.* 47, 42–50. <https://doi.org/10.1016/j.jtherbio.2014.11.006>.
- Tedeschi, J.N., Kennington, W.J., Tomkins, J.L., Berry, O., Whiting, S., Meekan, M.G., Mitchell, N.J., 2016. Heritable variation in heat shock gene expression: a potential mechanism for adaptation to thermal stress in embryos of sea turtles. *Proc. Biol. Sci.* 283 (1822), 20152320. <https://doi.org/10.1098/rspb.2015.2320>.
- Teskey, M.L., Lukowiak, K.S., Riaz, H., Dalesman, S., Lukowiak, K., 2012. What's hot: the enhancing effects of thermal stress on long-term memory formation in *Lymnaea stagnalis*. *J. Exp. Biol.* 215 (24), 4322–4329. <https://doi.org/10.1242/jeb.075960>.
- Tian, C.B., Li, Y.Y., Huang, J., Chu, W.Q., Wang, Z.Y., Liu, H., 2020. Comparative transcriptome and proteome analysis of heat acclimation in predatory mite *neoseiulus barkeri*. *Front. Physiol.* 11, 426. <https://doi.org/10.3389/fphys.2020.00426>.
- Tomanek, L., Sanford, E., 2003. Heat-shock protein 70 (Hsp70) as a biochemical stress indicator: an experimental field test in two congeneric intertidal gastropods (genus: *Tegula*). *Biol. Bull.* 205 (3), 276–284. <https://doi.org/10.2307/1543291>.
- Trégarot, E., D'Olivo, J.P., Botelho, A.Z., Cabrito, A., Cardoso, G.O., Casal, G., Cornet, C.C., Cragg, S.M., Degia, A.K., Fredriksen, S., Furlan, E., Heiss, G., Kersting, D.K., Maréchal, J.-P., Meesters, E., O'Leary, B.C., Pérez, G., Seijo-Núñez, C., Simide, R., et al., 2024. Effects of climate change on marine coastal ecosystems – a review to guide research and management. *Biol. Conserv.* 289, 110394. <https://doi.org/10.1016/j.biocon.2023.110394>.
- van Heerwaarden, B., Kellermann, V., Sgrò, C.M., 2016. Limited scope for plasticity to increase upper thermal limits. *Funct. Ecol.* 30 (12), 1947–1956. <https://doi.org/10.1111/1365-2435.12687>.
- Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., McCann, K.S., Savage, V., Tunney, T.D., O'Connor, M.I., 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proc. Biol. Sci.* 281 (1779), 20132612. <https://doi.org/10.1098/rspb.2013.2612>.
- Venegas, R.M., Acevedo, J., Treml, E.A., 2023. Three decades of ocean warming impacts on marine ecosystems: a review and perspective. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 212, 105318. <https://doi.org/10.1016/j.dsr2.2023.105318>.
- Wagner, T., Schliep, E.M., North, J.S., Kundel, H., Custer, C.A., Ruzich, J.K., Hansen, G.J.A., 2023. Predicting climate change impacts on poikilotherms using physiologically guided species abundance models. *Proc. Natl. Acad. Sci. USA* 120 (15), e2214199120. <https://doi.org/10.1073/pnas.2214199120>.
- Wang, Q., Ishikawa, T., Michiue, T., Zhu, B.-L., Guan, D.-W., Maeda, H., 2012. Stability of endogenous reference genes in postmortem human brains for normalization of quantitative real-time PCR data: comprehensive evaluation using geNorm, NormFinder, and BestKeeper. *Int. J. Leg. Med.* 126 (6), 943–952. <https://doi.org/10.1007/s00414-012-0774-7>.
- Wilson, R.I., Nicoll, R.A., 2001. Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. *Nature* 410 (6828), 588–592. <https://doi.org/10.1038/35069076>.
- Woodruff, M.J., Zimmer, C., Ardia, D.R., Vitousek, M.N., Rosvall, K.A., 2022. Heat shock protein gene expression varies among tissues and populations in free-living birds. *Ornithology* 139 (3), ukac018. <https://doi.org/10.1093/ornithology/ukac018>.
- Yin, B., Fang, W., Liu, L., Guo, Y., Ma, X., Di, Q., 2024. Effect of extreme high temperature on cognitive function at different time scales: a national difference-in-differences analysis. *Ecotoxicol. Environ. Saf.* 275, 116238. <https://doi.org/10.1016/j.ecoenv.2024.116238>.
- Zhang, R., Tomasi, D., Shokri-Kojori, E., Manza, P., Demiral, S.B., Wang, G.-J., Volkow, N.D., 2024. Seasonality in regional brain glucose metabolism. *Psychol. Med.* 1–9. <https://doi.org/10.1017/S0033291724000436>.
- Zhou, J., Liu, Q., Han, Y., Dong, H., 2018. High-temperature tolerance and thermal-adaptability plasticity of Asian corn borer (*Ostrinia furnacalis* Guenée) after a single extreme heat wave at the egg stage. *J. Asia Pac. Entomol.* 21 (3), 1040–1047. <https://doi.org/10.1016/j.aspen.2018.07.024>.