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An automated modular heating solution for experimental flow-through stream mesocosm systems

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Abstract

Water temperature is a key environmental variable in stream ecosystems determining species distribution ranges, community composition, and ecological processes. In addition to global warming, direct anthropogenic impacts, for example through the influx of power plant cooling water or due to sun exposure after the removal of riparian vegetation, result in elevated water temperatures. However, temperature effects in stream ecosystems have mostly been tested in recirculating experimental systems, which can neither capture diurnal and seasonal variability in other environmental variables nor allow for entrainment of stream organisms. In contrast, open flow-through systems, which are constantly supplied with stream water, offer a more realistic setting for stream ecological experiments, yet are difficult to implement. Here, we outline a heating module for the purpose of differential temperature regulation in a flow-through mesocosm system by automatic control of warm water supply. We validated the functionality of the module in indoor trials as well as in an outdoor *ExStream* experimental mesocosm system. Furthermore, we tested the implications of different warm water temperatures for the survival of invertebrates drifting through the heating module to derive recommendations for the maximum warm water temperature for mixing with the natural water inflow. The module allows for controlled open flow-through experiments in the field and the key components are flexible and scalable. Therefore, the module can be easily integrated into existing experimental flow-through setups.

Water temperature is one of the main drivers of biotic community composition change in streams and strongly determines species distributions for macroinvertebrates (Daufresne et al. 2004), fishes (Trigal and Degerman 2015; Myers et al. 2017), and periphyton (Soininen et al. 2016; but also see Costello et al. 2018 for a counter example). The mechanisms by which temperature changes can impact aquatic organisms are numerous and involve physiological effects for instance by modification of oxygen availability (Deutsch et al. 2015), changes in life history and behavior (Hogg and Williams 1996; Geffroy and Wedekind 2020), as well as indirect effects through altered biological interactions (Traill et al. 2010).

As a consequence, global warming constitutes a serious threat to stream ecosystems. In this context, it is not only the rise in mean annual water temperature that is problematic (Kaushal et al. 2010), but also an increased frequency of extreme events such as heatwaves (Tassone et al. 2022), which can cause drastic and long-lasting changes in community

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Additional Supporting Information may be found in the online version of this article.

composition within a short time frame (Mouthon and Daufresne 2015). Furthermore, streams often have extensive contact zones with anthropogenically altered landscapes due to their importance for human infrastructure and as a resource for potable water. Therefore, they are exposed to a variety of anthropogenic disturbances: thermal pollution by power plants (Wright et al. 1999) or industrial effluents, and removal of riparian vegetation (Kail et al. 2021) intensify the problem locally by causing an increase in maximum water temperatures of up to 2 to 3°C. To make matters worse, the negative effects of contaminants such as heavy metals or pesticides are frequently amplified by warming (Holmstrup et al. 2009). Such synergistic interactions of multiple stressors are more prevalent in rivers than in lakes (Birk et al. 2020), stressing the vulnerability of streams to future anthropogenic change.

Therefore, research on effects of warming on stream communities and ecosystems in a multiple stressor context is crucial. Long-term monitoring and space-for-time substitution studies provide a substantial body of literature for the effects of warming (e.g., Langford 1990; Castella et al. 2001; Daufresne et al. 2004; Bonada et al. 2007; Durance and Ormerod 2007). However, such approaches are correlational and because other environmental variables can co-vary or overwrite the effects of warming, they cannot unambiguously establish cause-effect relationships. Instead, experimental approaches using whole-ecosystem manipulations or mesocosms are needed to pinpoint the effects of warming in a multiple stressor context. Whereas numerous mesocosm experiments have been conducted in lakes using warming as a stressor, only few have been carried out in lotic systems (Stewart et al. 2013). Clearly, the reasons for the paucity of such experiments are costs and logistic challenges, but not the lack of relevance.

One approach to simulate lotic systems and manipulate temperature is through closed recirculating experimental systems. While these systems facilitate the precise regulation of water temperature and need substantially less energy for heating than open systems, this approach oversimplifies the environmental complexity and heterogeneity in streams. Whereas diurnal temperature variations can be simulated indoors (Sardiña et al. 2017) or may arise from air temperature in an outdoor recirculating system (Arias Font et al. 2021), natural variation in other environmental variables such as salinity or nutrient concentrations cannot be adequately reflected in such studies. Flow-through experimental systems which are directly supplied with stream water are inclusive of this environmental variability, which may alter stress responses compared to an environmentally stable setting. Additionally, entrainment of stream organisms can only take place in such an open system with a constant supply of stream water. Macroinvertebrate drift and aerial migration, as well as microbial colonization alter the process of community assembly (Brown et al. 2018). For example, Hogg and Williams (1996) observed a strong shift in life history for a variety of insect taxa, whereas community composition remained unchanged during a 2-yr thermal manipulation within a stream reach. They explained this discrepancy with migration from unexposed source populations. Such insights may draw attention to more subtle indicators of thermal stress that also become evident in natural environments. In summary, flow-through systems are better suited to study the effects of long-term warming and heat waves on stream communities and ecosystems, because of their more biologically realistic setting.

The *ExStream* system (Piggott et al. 2015*b*) is an experimental flow-through system that allows for high sample sizes with typically 64 or 128 mesocosms and has been used for some of the rare examples of thermal manipulation in flow-through mesocosm systems (Piggott et al. 2015*a*,*b*,*c*; Macaulay et al. 2021; but see Carolli et al. 2012; Bondar-Kunze et al. 2021 for warming pulses). Since the *ExStream* system can be assembled from commercially available components, it has become a well-established setup and is now used worldwide for multiple stressor experiments (e.g., Germany: Beermann et al. 2018; Ireland: Davis et al. 2019; China: Juvigny-Khenafou et al. 2021; New Zealand: Macaulay et al. 2021). In the *ExStream* system, water is constantly supplied from the stream and redirected to header tanks (HTs) on a scaffold, which gravity-feed the circular mesocosms below. Colonization takes place by macroinvertebrates, algae, and microorganisms, which are transported with the stream water. In preceding studies, water temperature increases were achieved by heating a fraction of the stream water with propane heaters and supplying the heated water directly to the HTs (Piggott et al. 2015*a*; Macaulay et al. 2021). The discharge of heated water into the HTs has been so far adjusted manually and had to be tightly controlled to constantly achieve the targeted water temperature increase.

As a major advancement to the aforementioned approach, we present an automated heating module for differential water temperature regulation in a flow-through system by automatic control of warm water supply. The module achieves thorough mixing of heated and unheated stream water by creating turbulent flow conditions. A motorized ball valve regulates the inflow of warm water and is operated by a control system that can be set to the desired target water temperature increase. In this article, we (1) describe the design of the module and (2) demonstrate its functionality for maintaining the target water temperature increase in indoor trials, and when implemented outside in a typical *ExStream* experimental setup. Furthermore, we (3) test the implications of different warm water temperatures for the survival of invertebrates drifting through the heating module to derive recommendations for the temperature selection of warm water considering both technical heating feasibility and organism survival.

Materials and procedure

General design of the thermally regulated mixing module

The thermally regulated mixing module is designed to maintain a constant water temperature increase relative to the background temperature of flowing water by adding warm water as needed. There are two main components of the module: (1) A static mixer that facilitates the homogenous mixture of cold and warm water. (2) A control system that, according to the demands, engages or disengages the warm water supply to the static mixer. This control system can be customized with respect to the target water temperature increase, its permitted deviation and the maximum water temperature that should be realized.

The static mixer achieves intermixing of water with widely different temperatures by inducing turbulent flow conditions. It is composed of commonly available materials and constructed with U-PVC fittings: Cold and warm water coalesce in a Y-shaped fitting and the flow is directed first downwards and then upwards via four 90° elbow fittings (HT) (Fig. 1a). In the setup described here, an inner diameter of 63 mm was chosen for the static mixer to facilitate the passage of large volumes of water under ambient pressure conditions.

The control system adapts to the different boundary conditions to regulate the inflow of warm water to a warming treatment tank. For this purpose, a differential temperature controller with two measuring inputs and two control outputs was selected. The measuring inputs are given by resistance temperature detectors immersed in the cold water (background temperature, T_B) and in the mixed water. The control outputs can switch 16 A (maximum load 3600 W), one of which is to be used for the supply of warm water by operating a motorized ball valve (U.S. Solid 1″ brass electrical ball valve, 9–24 V AC/DC). The second output can be used to simultaneously supply a similar volume of unheated water for a separate control condition tank. Adding the desired increase (T_I) to the background temperature yields the set point water temperature (T_S): $T_S = T_B + T_I$. Given a user specified hysteresis (ΔT) and, if desired, a maximum water temperature (T_{max}), the control system automatically switches between non-heating and heating modes: When the water temperature of the mixed water is below $T_S - \Delta T$, the control system opens the valve. When it exceeds $T_S + \Delta T$ or T_{max}, the valve is closed.

The digital controller thermostat used in the given setup has a temperature calibration function for more accurate temperature control and its optimal control range is between −5°C and 60°C with a control accuracy of ± 0.5°C. The measurement sensors are two NTC-Sensors with 5 kΩ at 25°C. They have a measurement range between −50°C and + 150°C and a measurement accuracy of ± 0.5°C. The hysteresis is adjustable in 0.1°C steps, which allows for a uniform temperature curve.

Ideally, the static mixer is mounted in between two tanks under low-pressure conditions with a moderate downward slope toward its outlet. The first tank comprises the cold water tank and should be constantly supplied with water, whereas the second tank comprises the mixed water tank and should be constantly drained, for example, toward the mesocosms in an experiment. By using tanks as reservoirs instead of a pressurized pipe system, water temperature fluctuations between switching cycles are reduced. Warm water is supplied from a tank positioned on a platform above the static mixer to prevent backflow when the motorized valve is open (Fig. 1a,b,e).

Stratification experiment

Laboratory setup

In laboratory experiments, two 203 L rain barrels (70 × 70 cm, height: 82 cm) were connected with the static mixer. The upstream cold water tank was supplied with tap water at room temperature (mean temperature of tap water: 23.18 ± 0.17°C, $N = 1083$) and the downstream mixed water tank was equipped with 16 outlets (diameter: 13 mm) at a height of ∼ 10 cm from the bottom of the tank in a semicircular arrangement. Each outlet was connected to a shut-off valve, which was calibrated to a flow rate of 1 L min^{-1}. A 12 L bucket (warm water tank) was placed ∼ 1 m above the static mixer and supplied with tap water heated by an electric mobile heating system (triMobil EHZ36, maximum capacity: 36 kW). To prevent excessive heat loss, the warm water tank was wrapped with an insulation mat (Armaflex, Armacell). Its outlet (diameter: 25 mm) drained into the static mixer, with a motorized valve operated by the control system installed in between. The temperature sensors of the control system for background and actual mixed water temperature were immersed into the cold water and the mixed water tank, respectively, ∼ 20 cm above the tank bottom. Two 40 mm overflow pipes drained off excessive water from the warm water and the mixed water tank.

Data collection

The thermal stability of the mixed water was tested for three distinct gradients between background and warm water temperatures. Keeping the background temperature (23°C) and the settings of the control system ($T_I = +6.5$°C, no $T_{max,}$ $\Delta T = 0.2$°C) constant, the warm water was heated to 45°C, 55°C, or 65°C by the mobile heating unit and water temperature was monitored at 12 positions in the mixed water tank using temperature loggers (HOBO Pendant Temp/Light and HOBO Pendant MX Temp/Light with ± 0.5°C accuracy). The loggers were calibrated to log the current water temperature every 5 s. One logger was positioned at the outlet of the cold water tank and of the warm water tank, respectively, and three sets of four loggers measured water temperature at different depths within the mixed water tank (height in tank, top: 50 cm, center: 30 cm, bottom: 10 cm). The four loggers within a set were positioned equidistantly along the outer diameter of the mixed water tank (Fig. 1c). After a new temperature for warm water was set, there was a 30 min delay before data collection commenced to ensure complete exchange of water in the system.

Drift mortality experiment

In several biological experiments entrainment of stream organisms by drift plays an important role. However, heating up stream water usually requires a previous filtration to reduce the wear on heating devices. With low warm water temperatures, larger volumes of stream water have to be filtered and subsequently heated, posing technical challenges for the

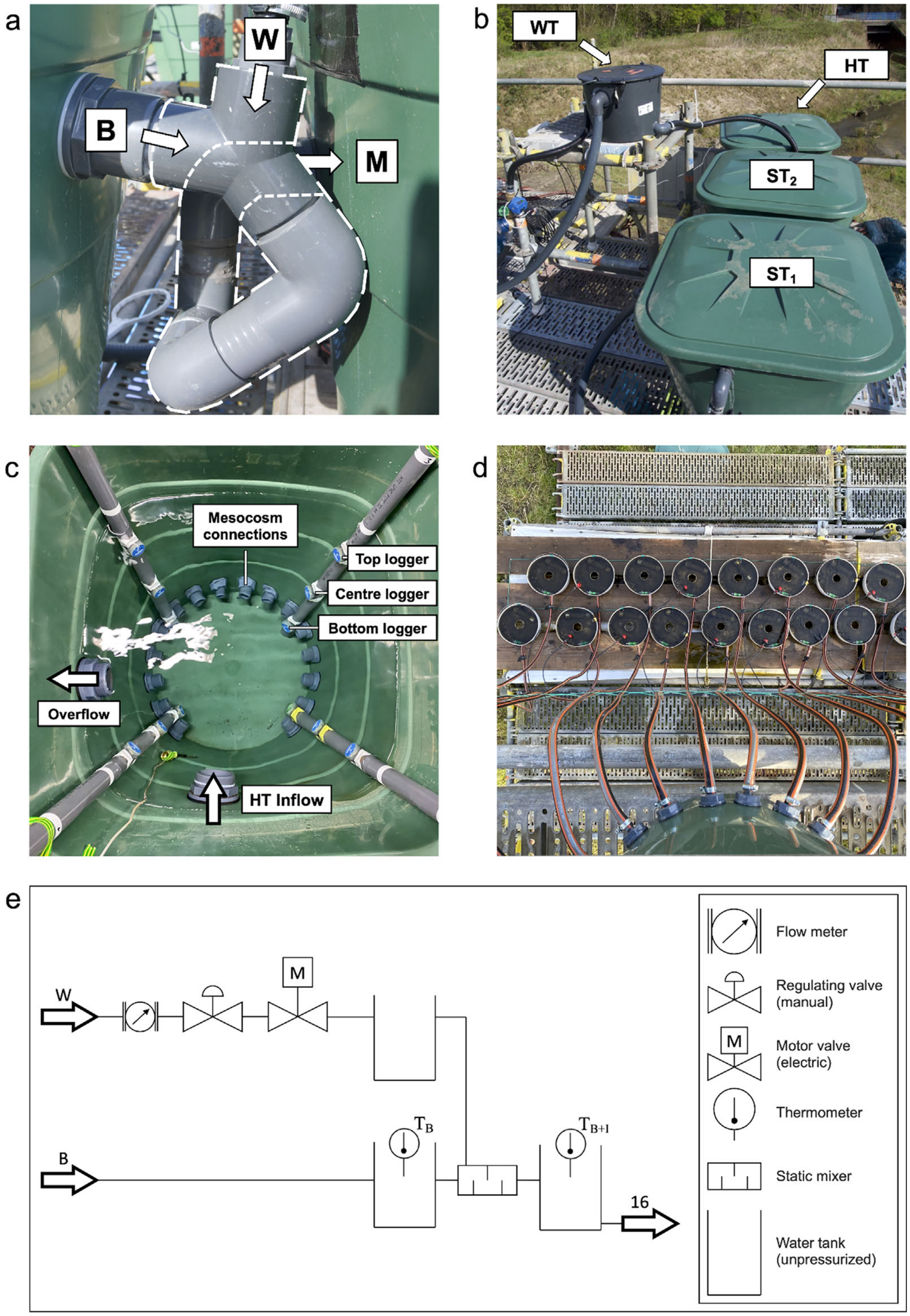

Fig. 1. Components of the thermally regulated mixing module in the *ExStream* setup. (**a**) Static mixer, highlighted by a dashed line. Water with background temperature (B) and warm water (W) are thoroughly mixed by passing through one Y-shaped fitting and four 90° elbow fittings before being drained into a tank with mixed water (M). (**b**) Stream water is pumped to the top of a scaffold and first passes through two settling tanks (ST1 and ST2), before entering the header tank (HT). Warm water is supplied from the higher positioned warm water tank (WT) to the static mixer between ST2 and HT. (**c**) Locations of the loggers in the HT. Three loggers are placed in all four corners of the tank. (**d**) From the HT, water is gravity-fed to 16 circular mesocosms per block. (**e**) Mechanical sketch of the laboratory experimental setup. Thermometers are connected to the control unit, which operates the motor valve. The valve opens when $T_{B+I} < T_S - \Delta T$ and closes when $T_{B+I} > T_S + \Delta T$. The water from the mixed water tank subsequently flows into 16 mesocosms. Additional details regarding the setup are available in the supplement, accompanied by a video showing the setup. Abbreviation: T_B = background temperature, T_I = desired water temperature increase, T_S = set point water temperature, ΔT = user specified hysteresis.

experimental system. Although warm and cold water mix rapidly within the static mixer, high warm water temperatures can cause fatal heat shock to freshwater organisms that drift through. Therefore, the second laboratory experiment assessed the maximum usable warm water temperature to avoid fatal heat shock to drifting freshwater invertebrates.

Here, *Gammarus* sp., a freshwater amphipod, was selected as a model organism. As *Gammarus* species are common in the Nearctic and Palearctic, are often highly abundant, play an important role as shredders in stream foodwebs, and are sensitive to high water temperatures (Gaufin and Hern 1971; Stewart et al. 2013). Therefore, they are well suited as a representative organism for freshwater invertebrates exposed to a potential heat shock by the supply of warm water. In the Boye catchment, where later the *ExStream* experiment was conducted, two species of this genus have been reported, *G. pulex* and *G. fossarum* (Prati et al. 2022). Total body length of adult specimens varies greatly, with a minimum length of 4.5 mm being reported in *G. fossarum* (Beracko et al. 2012) and a maximum length of 21 mm in *G. pulex* (Pinkster 1970).

Experimental setup

In total, 250 specimens were collected from the Boye river (longitude, latitude: 51.5574, 6.9365; North Rhine-Westphalia, Germany) and kept in groups of 10 individuals in glass jars, subjected to a light dark cycle of 8:16 h. The holding jars were filled with 300 mL water from the sampling location and equipped with 40 mL washed gravel, a PVC tube for shelter (length: 5 cm, diameter: 32 mm), and an airstone for aeration.

One day after sampling, the laboratory setup was prepared as described for the stratification experiment. However, the control system for the thermally regulated mixing module was disabled to keep the valve for the warm water inflow permanently open. Water temperature in the cold water tank was at $\sim 22°C$. Temperature in the warm water tank was left unchanged for the control groups or adjusted according to treatments with the mobile heating device. The targeted warm water temperature treatments were 45°C, 55°C, and 65°C. Although high mortality can be expected with 65°C warm water, a fast mixture of warm water (without organisms) with the cold water (with organisms) can result in considerably lower exposure temperatures for the drifting invertebrates. Because the experiment was conducted on two separate days, two control groups were required to rule out the possibility that differential water chemistry affected the results. The allocation of gammarid groups to treatments and the order of treatment application was randomized (Day 1: 65°C, control, 55°C; Day 2: 45°C, control).

Data collection

First, each group of 10 gammarids was transferred to a plastic container and gently poured into the cold water inlet of the mixing module. To recapture the gammarids and return them to their holding jars, a stocking was placed over the outlet. The jars were then replenished with fresh water from the sampling location. Each time a new temperature was set, a full exchange of water in the system was prepared before starting the next treatment. Mortality of the gammarids was determined after 24 h and specimens were preserved in 80% technical ethanol.

Statistical analysis

To analyze the suitability of warm water temperatures for experiments that allow macroinvertebrates to drift through the static mixer, Fisher's exact test was applied using R Statistical Software (R Core Team 2021). This test was used to evaluate if there was an association between gammarid mortality and heating treatments (control group, mixing with 45°C, 55°C, or 65°C warm water). Pairwise comparisons between the control group and the different warm water temperatures were conducted. Bonferroni-corrected *p*-values are presented along with 95% confidence intervals (CI) for the odds ratio of mortality.

Treatment accuracy experiment

To validate its applicability in field studies, the thermally regulated mixing module was deployed in the *ExStream* system (Piggott et al. 2015*a*). In the given experiment, four different temperature levels (control, + 2°C, + 4°C, and + 6°C) were applied with 16 replicates each. The experiment was conducted between 6 March and 11 April 2022 at the Boye river in North Rhine-Westphalia, Germany (latitude, longitude in decimal degrees: 51.5533, 6.9485). The Boye river is a carbonate-rich, sand-bottomed lowland river (www.elwasweb.nrw.de, last accessed 14 April 2023) that flows through heavily urbanized areas. It is characterized by turbid conditions due to a high sediment load and dissolved organic material.

General ExStream *setup*

Two centrifugal pumps (Pedrollo NGAm 1A-Pro, maximum capacity: 315 L min^{-1}) were equipped with a 5 mm filter at their inlet and conveyed the water on top of a two-level scaffolding system (upper level: 3 m, lower level: 1 m high). Here, a manifold partitioned the water among four spatial blocks. The high sediment load of the Boye river required additional mitigation measures to minimize clogging of the smaller apertures further downstream in the system: In each block, the water initially passed through two 203 L rain barrels connected in series, each of which served as a settling tank (Fig. 1b). Here, flow velocity was substantially reduced, and coarse-meshed fishing nets were positioned across the flow direction to facilitate sedimentation. Additionally, the connection to the subsequent tank tapped the water exclusively from the surface to further reduce the amount of sediment carried over. Such adjustments are not necessary when working in streams less affected by sediment influx (e.g., Piggott et al. 2015*a*; Beermann et al. 2018).

The static mixer described above connected the second settling tank with another rain barrel of the same type, the HT. From this point, the water was gravity-fed toward the lower level of the scaffolding system into 16 circular mesocosms per block (Fig. 1d). These 3.5 L mesocosms (outer diameter: 25 cm, central outflow: 6 cm) contained 1 L sieved fine sediment (grain size: < 1 mm) from the river under study with 100 mL fine particulate organic matter for inoculation, a 200 mL quartz stone patch (size: 6–8 mm) and three bigger quartz stones behind the stone patch (size: 40–80 mm). While the fine sediment represents the dominant substrate in the river under study, also stones occur occasionally and thus were added to increase habitat heterogeneity for benthic macroinvertebrates. Additionally, the arrangement of the stone patch close to the inflow had a stabilizing function for the fine sediment. Discharge was calibrated three times a day with shut-off valves to ~ 1.9 L min^{-1} and entered through a 90° L-piece, producing a clockwise flow direction. Water passing through the central outflow was collected and drained into the adjacent retention filter basin before being returned to the Boye further downstream from the system's inlet.

Piping specifications

The diameter of PVC hoses and connections was reduced from 40 mm at the pump's inlet to 25 mm downstream of the manifold to 13 mm for the connections to the mesocosms. In between settling tanks and HTs, the connections had an inner diameter of 63 mm, allowing even large volumes of water to easily flow through under ambient pressure conditions. Any excess water that did not pass through the mesocosms entered the system drain through a 40 mm overflow pipe at the top of the HTs or off the first settling tanks, instead.

Heating setup

Temperature treatments were applied in blocks by supplying heated stream water to the static mixer according to needs. Prior to heating, the stream water had to be fine-filtered to prevent damage to the heating equipment. As a by-product, the entrainment of living organisms into a HT decreased in proportion to the provided volume of warm water. To offset this effect, cool fine-filtered stream water was supplied to the control tank and lower-level temperature treatments, with the goal to supply each HT with equal volumes of filtered water.

Therefore, two additional centrifugal pumps (Onga 400 series, Melbourne, Australia, max. capacity 300 L min^{-1}) were equipped with a 1 mm mesh as a prefilter. One of those pumps supplied the heating line, the other the cool filtered water line. Both were split into four parallel 38 mm lines, each of them having one 125 μm stainless steel filter that was automatically flushed every 10 min for 15 s. The splitting of the heating and cool filter water lines reduced pressure loss during fine filtering and allowed for a continuous water supply while flushing, and the lines were reconnected further downstream, respectively.

For heating, a fuel oil powered mobile heater (Mobile Wärme 24, 350 kW, circulation pump: Wilo-Stratos 50/1–9) recirculated warm water in an internal circuit through a brazed plate heat exchanger (Cosmo, CWTG 85). Here, stainless steel plates facilitated heat transfer between two fluids, with the heating line supplying the filtered stream water as the second fluid. After passing through the heat exchanger, the line was split and discharged at a temperature of ~ 50°C into three 12 L buckets, one for each heating treatment (+ 2°C, + 4°C, and + 6°C). Similarly, the cool filtered water line was directed to three 12 L buckets (control, + 2°C, and +4°C). All buckets were positioned ~ 1 m above the static mixer of the associated block and connected to it using a 25 mm PVC hose (Fig. 1b). For the blocks which were supplied with both cool filtered water and warm water, the hoses of both buckets united into one before entering the static mixer. The motorized ball valves regulating the volume of warm or cool filtered water were installed at the inlet of a bucket. If all valves are closed at the same time, the pumps are at risk of damage. Therefore, an overflow pipe was installed for both lines and regulated to minimum discharge.

For the control system, the background temperature was measured at the first sediment trap of a heated block, while the actual mixed water temperature was taken in the associated HT. Temperature sensors were positioned at the outer diameter of a tank, ~ 20 cm above its bottom. While T_I was set according to the treatment (2.5°C, 4.5°C, and 6.5°C to account for heat loss from the HTs to the experimental units), ΔT was consistently set to 0.2°C. Three control systems operated one pair of valves each (Supporting Information Fig. S1): The heated water line for the + 6°C treatment block was coupled with the cool filtered water line for the control block, the heated water line of the + 4°C block was coupled with the cool filtered water line for the + 2°C block and vice versa.

Still, pressure differences between lines had to be compensated by manual adjustment with a brass gate valve and the aid of water counters. Behind each motorized valve, a mechanical water counter to estimate the flow rate and a gate valve was installed, which was manually adjusted to equilibrate filtered water volumes among blocks according to measurements. After initial adjustment, water counters were read out every 24 h and gate valves were fine-tuned, if necessary.

Data collection

For each block, temperature loggers (HOBO Pendant Temp/Light and HOBO Pendant MX Temp/Light with ± 0.5°C accuracy) were set to log water temperature every 5 min and positioned on the outer walls of two randomly chosen mesocosms, adjacent to the inlet of a mesocosm opposite the flow direction. This allowed evaluating how accurately the targeted water temperature increases (+ 2°C, + 4°C, and + 6°C) were achieved.

To control for the possibility that mesocosms within the same treatment show different temperature profiles, loggers were placed in all 16 mesocosms of the + 6°C treatment and were set to log temperature every 5 s for a 70-min time frame. The highest temperature treatment was chosen for this experiment because it is most prone to suffer from insufficient mixing and differential heat loss. The degree of heat loss can differ among mesocosms within a block due to differences in the lengths of hoses connecting them to the HT, or their positioning on the scaffold, which in turn affects exposure to sun or wind.

Statistical analysis

Statistical analyses were performed using R Statistical Software (R Core Team 2021). Temperature profiles across time were modeled with the *mgcv* package (version 1.8–41, Wood 2004) in R, applying a generalized additive mixed effects model (GAMM) with identity link and a continuous AR(1) correlation structure to correct for temporal autocorrelation. Penalized cubic regression splines were used, estimating the smoothing parameters with the generalized cross validation criterion. This approach was chosen because simply comparing means of water temperatures from each channel or treatment would result in overly optimistic confidence intervals (CI) that do not account for the temporal structure of the data.

To evaluate if the achieved water temperatures in the field experiment were equivalent to the target temperature increase for each treatment, modeled temperature profiles were based on 5 min logging intervals over a 3-d time frame. Temperature treatment was included as a parametric, factorial explanatory variable and the model specification can be written as follows:

$$\text{Temperature}_i = \beta_0 + f_1(\text{Time}_i) + \beta_1 \times \text{Treatment}_i + \varepsilon_i \qquad (1)$$

To show that mesocosms from the same treatment in the field application received water with equivalent temperatures, the temperature profiles were based on 5 s logging intervals over a time frame of 70 min for the highest warming

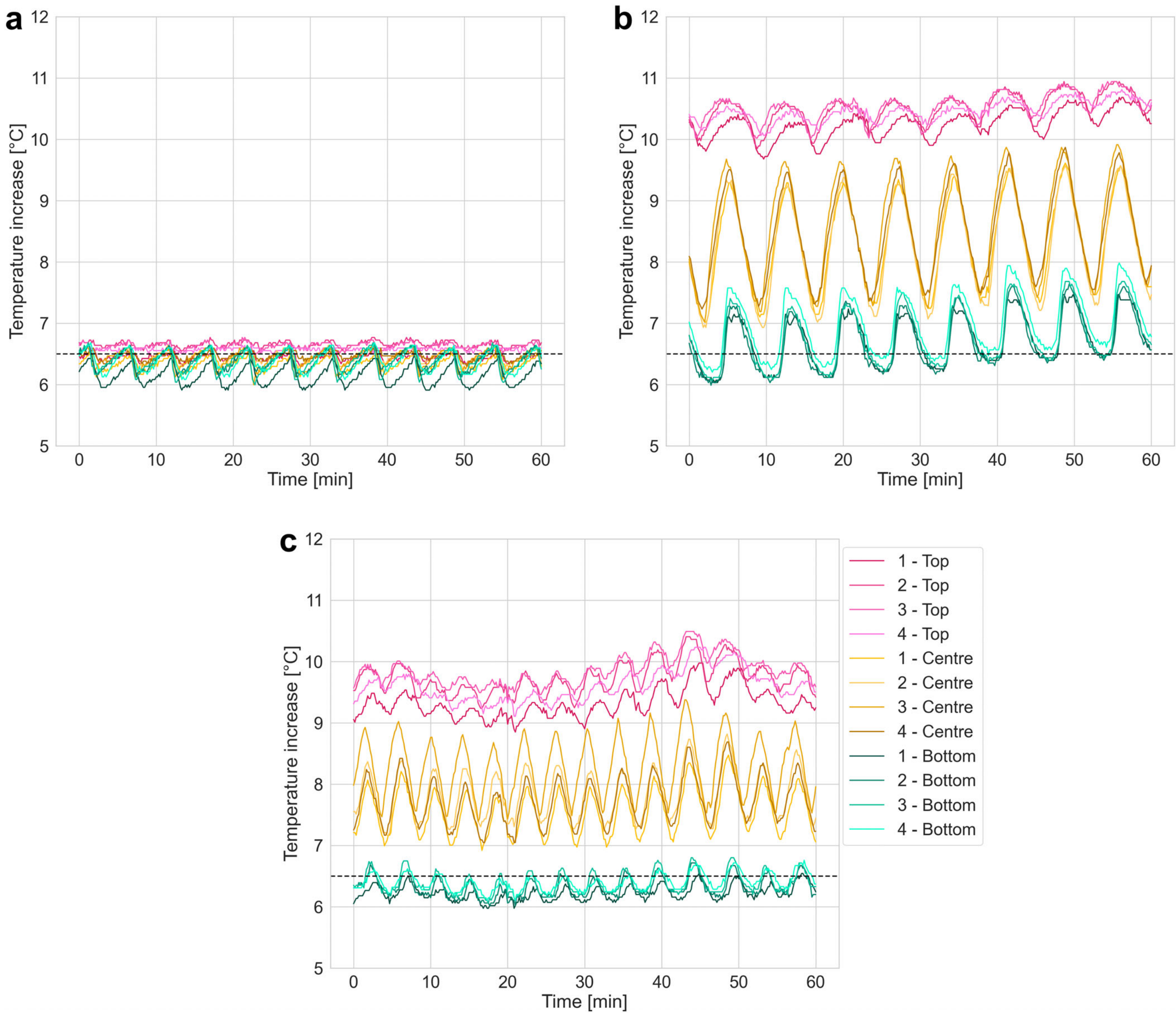

Fig. 2. Temperature curves showing the temperature difference to the background temperature during the one-hour lab experiments measured every 15 s. The mixing efficiency within the mixed water tank at three different warm water inflow temperatures was tested: (**a**) 45°C, (**b**) 55°C, and (**c**) 60°C. Dashed lines indicate the set temperature difference of + 6.5°C.

treatment (+ 6°C). The temperature variable was centered and the mesocosm position was included as a parametric, factorial explanatory variable with deviation coding. With this model specification, the effect of the mesocosm position can be interpreted as the deviation of the mesocosm's temperature from the average temperature of the treatment. The regression model can be written as follows:

$$\text{Centered temperature}_i = \beta_0 + f_1(\text{Time}_i) + \beta_1 \times \text{Mesocosm}_i + \varepsilon_i \quad (2)$$

As a test for equivalence, the two one-sided test procedure was applied (Lakens 2017). For each warming treatment (Eq. 1) or mesocosm (Eq. 2), the 90% CI for its deviation from

Table 1. Statistics for the drift mortality experiment. Unheated tap water was mixed with heated water (45°C, 55°C, and 65°C, respectively), while gammarids (*Gammarus* sp.) drifted through the static mixer. Mean and SD for the warm water temperature (*T*) is specified. The number of gammarids that survived (N_S) or did not survive (N_D) after 24 h is given. 95% confidence intervals for the odds ratio are given in brackets and the adjusted *p*-value (p_{adj}) is derived from Fisher's exact test and Bonferroni-corrected.

Treatment	Mean *T* (°C)	N_S	N_D	Mortality (%)	Odds ratio	p_{adj}
Control	22.9 ± 1.2	96	2	2.0		
Heated_{45}	43.6 ± 2.4	49	1	2.0	0.98 [0.03, 12.79]	1
Heated_{55}	54.0 ± 0.2	30	19	38.8	29.61 [6.55, 274.71]	<0.001
Heated_{65}	62.0 ± 1.5	7	42	85.7	259.83 [52.2, 2702.0]	<0.001

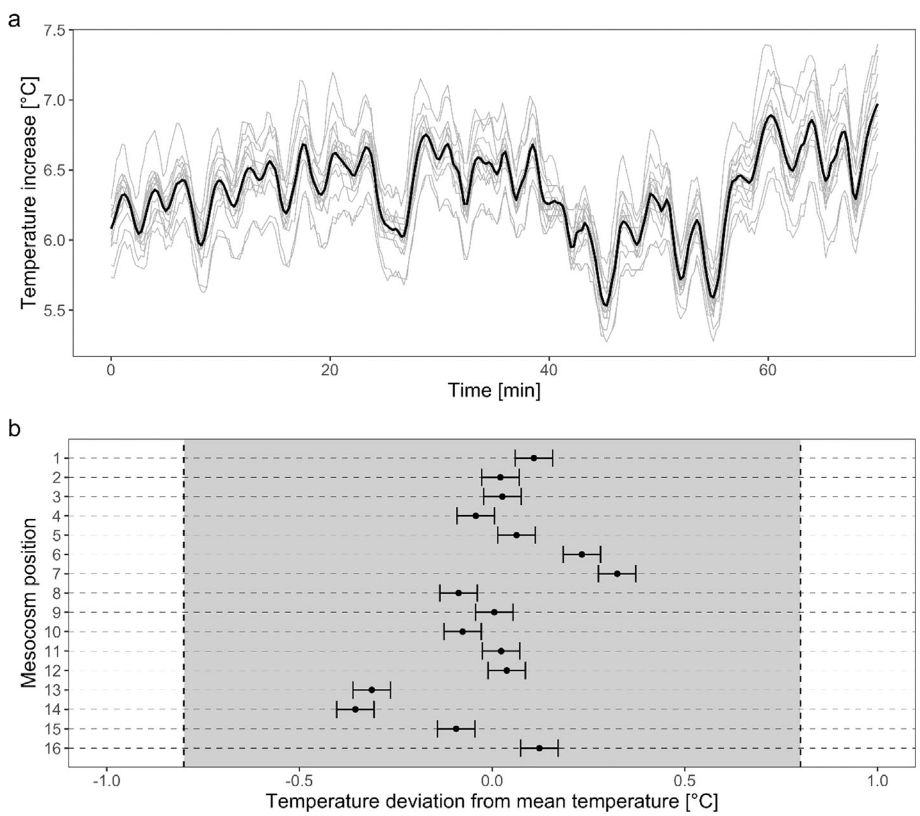

Fig. 3. Generalized additive mixed effects model results for the temperature profiles of the + 6°C treatment. (**a**) Smoother for temperature increase relative to background temperature over a 70 min time frame. In black, the cubic regression spline smoother for the grand mean of the data are shown. In gray, the actual temperature data for each mesocosm is displayed. (**b**) Equivalence tests for individual mesocosms. The 90% confidence intervals (CI) for the deviation of each temperature profile from the overall smoother are shown. The gray area marks the region of practical equivalence (ROPE).

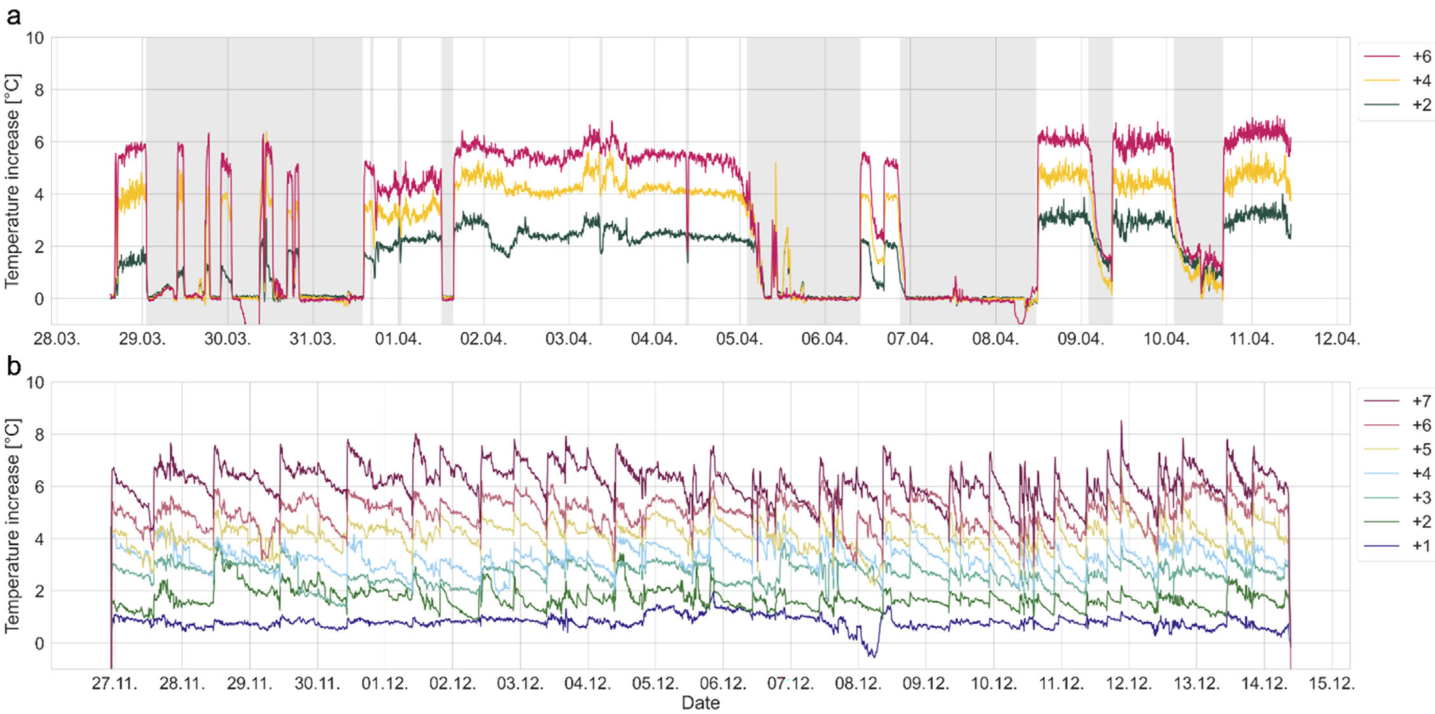

Fig. 4. Temperature curves showing the median logged temperatures taken at 5-min intervals within mesocosms relative to the logged background temperature during *ExStream* field experiments. (**a**) Two-week stressor phase of the *ExStream* system placed near the Boye river (North Rhine-Westphalia, Germany) in 2022. Gray areas mark downtime of the system due to initial frequent pump failures and two flooding events near the end of the experimental period and subsequent high sediment loads. (**b**) Three-week stressor phase of the *ExStream* system placed near the Kauru river (Otago, New Zealand) in 2009.

the target increase or overall mean was calculated using the *emmeans* package (version 1.8.8, Lenth 2023). Equivalence was assumed if this CI was entirely located within the region of practical equivalence (ROPE). The ROPE was predetermined to cover a deviation between -0.8°C and $+0.8$°C, corresponding to the sum of the measuring accuracy (± 0.5°C) of the temperature loggers and an allowed margin of error of 5% from the desired temperature increase (± 0.3°C). Residual plots were assessed to check model assumptions and are presented in the supplement.

Assessment

Stratification experiment

During laboratory experiments three different set temperatures of heated water (45°C, 55°C, and 65°C) were tested for determining temperature differences within the mixed water tank, however, the highest warm water temperature could not be achieved with the chosen heating device (mean temperatures of warm water $\pm$ SD: 45.24 ± 0.08°C, 54.09 ± 0.16°C, and 61.57 ± 0.74°C, $N = 1444$). For higher warm water supply temperatures, a thermal stratification within the mixed water tank was observed and the surface water was up to 5 °C higher than the target temperature.

However, water temperatures were stable for all experimental periods at the top, center, and bottom where the 16 outlets leading to the mesocosms were located (Fig. 2b,c). When a warm water supply temperature of 45°C was used, no thermal stratification inside the mixing tank was observed. The maximum recorded temperature difference between two positions inside the mixing tank here was 0.77°C and the target water temperature rise of $+6.5$°C was accurately maintained within the entire mixing tank (mean temperature increase $\pm$ SD: 6.44 ± 0.17°C; min 5.91°C, max 6.77°C, Fig. 2c).

Drift mortality experiment

The usage of water temperatures of 55°C or above to achieve the desired target water temperature increased mortality in gammarids after drifting through the static mixer (Fisher's exact test with Bonferroni-corrected *p*-values: $OR = 29.61$, $p < 0.001$; $OR = 259.83$, $p < 0.001$, Table 1). In contrast, when water was heated to 45°C or less, there was no evidence for a change in mortality relative to the addition of unheated water ($OR = 0.98$, $p > 0.999$).

Treatment accuracy experiment

Measurements of temperature fidelity for all mesocosms with a target increase of $+6$°C showed that the mean difference at each timepoint was 6.35 ± 0.29°C (Fig. 3). This temperature increase fluctuated over time with the largest range

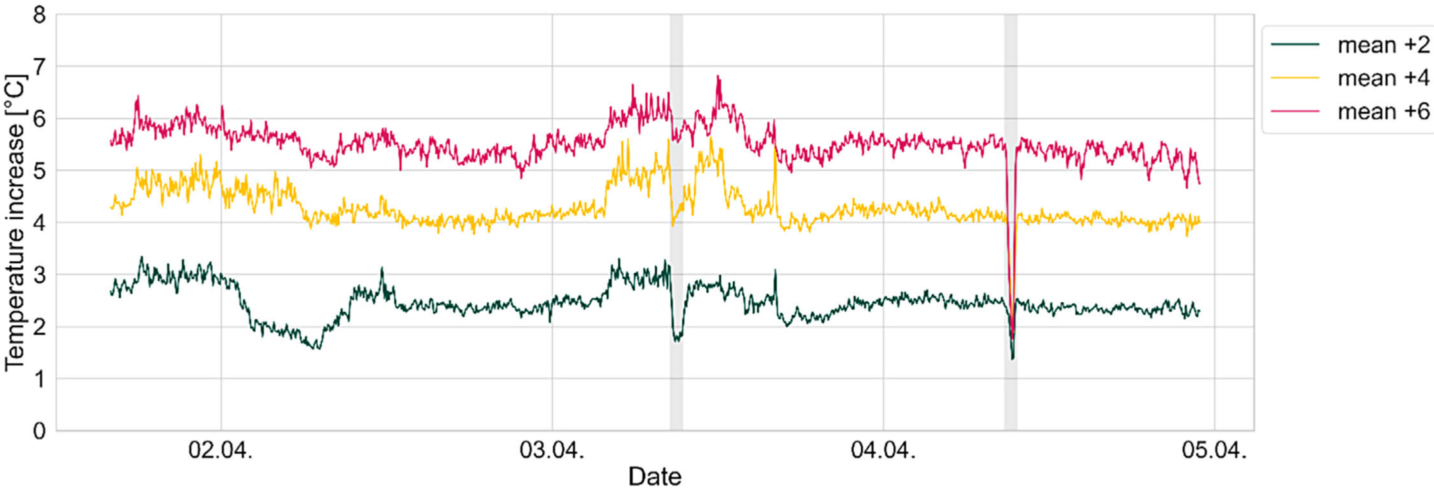

Fig. 5. Temperature curves showing the median of logged temperatures taken at 5-min intervals within mesocosms for 3 d during the *ExStream* field experiment ($N = 1392$). Gray areas mark downtime of the system during cleaning of the warm water filter system.

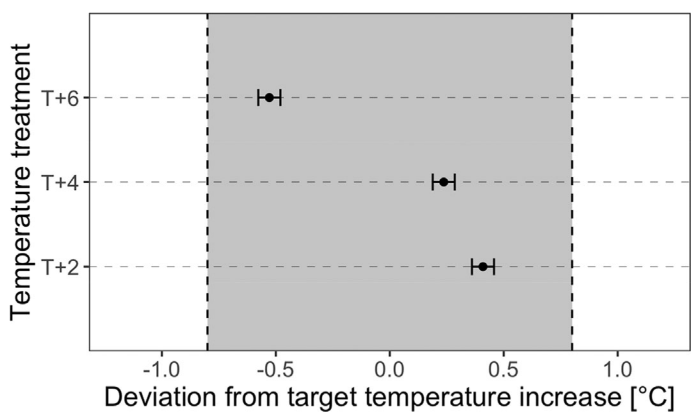

Fig. 6. Equivalence tests for warming treatments. The 90% confidence intervals (CI) for the deviation of a treatment from the target water temperature increase is shown. The gray area marks the region of practical equivalence (ROPE).

observed within one mesocosm encompassing 1.78°C. The individual water temperature profiles of the mesocosms were similar in shape (Fig. 3a) and could therefore be modeled reasonably well using one smoother (adjusted R^2 of the GAMM: 0.933). All profiles were equivalent over the observed period, as the 90% CI for the deviation of the mesocosm's water temperature profile from the overall mean smoother was within the predefined ROPE (Fig. 3b).

ExStream *long-term data*

The thermally regulated mixing module was able to automatically keep different experimental water temperature levels for several days in the field, even though setting up the *ExStream* experiment at the Boye river proved to be challenging. Initial frequent pump failures because of defective pumps and clogging of fine-filters upstream of the heat exchanger disrupted the warm water supply repeatedly. A combination of manual cleaning and automatic flushing was later used to prevent clogging of the filters. However, two major flooding events near the end of the experimental period drastically increased the sediment load and rendered the filtration of the stream water impractical (Fig. 4a). Still, the automatic thermally regulated mixing module performed well by maintaining constant water temperatures above ambient conditions for three consecutive undisturbed days of the experiment (Fig. 5). Here, all target water temperature levels (+ 2, + 4, and + 6°C) adjusted quickly after interruptions of the warm water supply caused by, for example, daily cleaning of the upstream filters and water temperature levels were kept constant and clearly separate in the long term (mean temperature increase ± SD: 2.46 ± 0.32°C, 4.29 ± 0.37°C, and 5.52 ± 0.39°C). The achieved water temperature was equivalent to the targeted temperature increase for all treatments (Fig. 6).

Discussion

The demonstrated heating module is capable of automatically controlling water temperature in experimental indoor and outdoor flow-through systems. In a controlled indoor environment, the system maintained the target temperature increase accurately for the entire experimental period. In a field setup, three different elevated target water temperatures were successfully maintained by the device against the strongly fluctuating background temperatures. Furthermore, water temperatures were equivalent in mesocosms of the same treatment.

The heating phase in the *ExStream* field setup presented here lasted for 2 weeks, but the pump for the warm water supply was malfunctioning and had to be replaced within the first few days. Later on, heavy rainfall and the resulting surge in sediment load of the river water meant that treatment had to be suspended. As water levels decreased, sediment load still remained high. As a consequence, the automatic flushing of the filters was not sufficient, and they needed to be manually cleaned and would become blocked overnight. Between the deployment of the new pump for the heating system and the start of flooding, there were 3 d in which the system operated under suitable conditions. While it needs to be stressed, that the heating system itself did not fail and would allow for longer running times under normal conditions, the collected data for 3 d is well-suited for a proof of principle for the use of the heating module in streamside outdoor mesocosm experiments. In the field setup, we adjusted the control system to maintain a water temperature increase in the HT that was 0.5°C higher than the actual target to account for heat loss. This accomplished the desired effect, and the achieved temperature increase was equivalent to the target temperature increase in all treatments.

Certainly, outdoor mesocosm experiments should be run over a longer time period than 3 d to provide a realistic estimation of the response of riverine communities to long-term warming or heat waves. Short-term experiments are less likely to accurately capture effects on diversity and ecosystem functions in the field (Cardinale et al., 2012) and also underestimate taxon-specific sensitivities (Iwasaki et al., 2018). For the simulation of a heat wave, a minimum duration of 5 d is desirable, aligning with the definition of a marine heatwave (Hobday et al., 2016) that has been adapted for lotic fresh waters (Tassone et al., 2022). Meanwhile, long-term warming effects might better be captured with a treatment duration of more than a month, depending on the life span of the studied organisms (Stewart et al., 2013, 2013). Hence, it is crucial to be able to maintain elevated water temperatures reliably over an extended time frame.

However, the issues encountered in the presented experiment stem from the requirement of water filtration combined with strong rainfall events and the high prevalence of fine sediments in the river. Filtration in streamside mesocosm experiments is often either restricted to a coarse-meshed filtration (> 4 mm, e.g., Beermann et al., 2018; Brasseur et al., 2022) or deliberately avoided (e.g., Jones et al., 2015; Iannino et al., 2023). Fine filtrations are necessary if the entrainment of invertebrates should be avoided. In this experiment, the lack of organisms in heated river water should be simulated also for control groups by an equivalent addition of filtered unheated water. In other experiments, fine filtration was supported for example by pressure-boosting pumps (0.5 mm, Piggott et al. 2015*a*,*b*,*c*) or a continuously self-cleaning filtration system (50 μm, Iannino et al., 2021), which may also suffer from clogging when a severe sediment load occurs in the river. The filtration of large amounts of turbid river water is logistically challenging; yet, it is not a problem specific to the proposed modular heating system, but rather in general for experiments that need to exclude invertebrate entrainment either altogether, or for a fraction of the supplied water.

Despite our efforts to maintain a consistent warm water supply in our experimental setup at the sand-dominated lowland river Boye, we frequently encountered disruptions due to clogging of the filter system even with it being automatically flushed every 10 min. To reduce the filters' propensity to clogging, a series of increasingly finer filters with a similar self-flushing system as used here or in Iannino et al. (2021) could be applied, the surface area of the filters could be increased, or a large sedimentation basin could be used. Water would first be pumped from the river into the basin and subsequently from the basin into the heating system. A bottom drain should allow removal of settled fine-particulate material. Another option can be the use of frequently cleaned or exchanged drift nets or fine in-line filters for the hoses leading to the mesocosms (e.g., Wellnitz, 2014), which completely excludes macroinvertebrate entrainment. This maximizes the filter area to filtered water volume ratio, but we strongly advocate combining it with settlement tanks in the upstream section of the system to minimize the risk of clogging and consequentially disrupted water supply in between manual maintenance periods. We expect such countermeasures not to be necessary for clean streams with low turbidity and negligible sediment load. Apart from considerations regarding stream type, high sediment loads or amount of transported organic material can also vary as significantly as stream flow depending on season (e.g., Canhoto et al. 2013). Therefore, we recommend choosing a timeframe for experiments that avoid seasonal peaks in sediment load and apply our suggested countermeasures to prevent clogging of filters if an experiment takes place at a sediment-rich site.

In a previous *ExStream* experiment conducted by Piggott et al. (2015*a*) during spring/summer in the southern hemisphere at the Kauru river in Otago, New Zealand, different heating levels were achieved by manually adjusting the flow rate of warm water into a mixing tank on a daily basis. While the Kauru river and the corresponding external conditions allowed for a less disturbed experimental period, the recorded water temperatures show a recurring decrease between manual adjustments of the gas heater (Fig. 4b). In contrast to that, the automatic regulation presented here keeps the temperature constant for longer periods of time and the temperature levels show a more distinct separation overall (Fig. 4a). In addition, manual manipulation is reduced considerably for the proposed solution and strict work safety regulations can be observed.

To achieve a target temperature increase in large-scale flow-through experiments through the supply of warm water, either the quantity of the added warm water or its temperature must be increased accordingly. The warm water volume is limited by the maximum pumping capacity, the feasibility of filtration, and the resistance of the experimental setup. The upper limit of the warm water temperature, on the other hand, should be chosen to prevent heat shock for the studied organismal groups drifting through the static mixer. We examined the survival rate of one keystone taxon (*Gammarus* sp.) from the studied river to warm water temperatures of 45°C, 55°C, and 65°C. Gammarids are among the most temperature-sensitive aquatic macroinvertebrate taxa (Gaufin and Hern 1971; Stewart et al. 2013) and the critical maximum temperatures for different *Gammarus pulex* populations range between 25.9°C and 30.9°C (Cottin et al. 2012). Nevertheless, we found no evidence for a lowered survival rate when using a warm water temperature of 43.6°C. Thus, the mixing of cold and warm water in the static mixer shows not only to be homogenous, but fast enough to not shock sensitive drifting specimens. Still, our results show a lower survival rate of drifting gammarids when using warm water temperature of 54°C or above. In the case that such high water temperatures have to be used, we recommend preventing the drift of specimens altogether. If organisms are allowed to enter the experimental system by drifting through the static mixer, we suggest using a lower warm water temperature (max. 45°C) than the ones tested here for mixing as a precaution. By using such low warm water temperatures along with drinking water approved fittings, the risk of release of pollutants such as plasticizers is considerably reduced. An additional benefit of reducing the warm water temperature instead of the volume of warm water supply is the prevention of thermal stratification inside the mixing tank.

Since the heating solution presented here is modular, existing experimental flow-through setups can be upgraded at low costs (about ~ 220 €/$ per set, including two static mixers, one control unit, and two automatic valves [~ 50 €/$ each]). The mixing module can be integrated into different heating systems and, depending on the energy demand of the system and availability, it can be powered by either electricity, gas, or heating oil. The flexibility and scalability of the static mixer as well as the various possible applications of the control system allow to overcome logistic challenges posed by heating flowing water and enable others to continue researching the understudied field of climate change and heat waves in lotic systems.

Data availability statement

The data needed to recreate the statistical analysis as well as the corresponding script are openly available within the article's supplementary material.

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Conflict of Interest

None declared.

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