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PRACTICAL TOOLS

Methods in Ecology and Evolution

All-in-one, versatile and low-cost experimental set-up to implement environmental stochasticity in mesocosms (PiStoch)

Chloé Souques | Jérémy Bacon | Ludovic Guillard | Pauline Eymard-Dauphin | Loïc Teulier | François-Xavier Dechaume-Moncharmont | Anne-Kristel Bittebiere | Yann Voituron

Universite Claude Bernard Lyon 1, CNRS, ENTPE, LEHNA, UMR 5023, Villeurbanne, France

Correspondence
Yann Voituron
Email: yann.voituron@univ-lyon1.fr

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Abstract

1. Environmental stochasticity in abiotic factors is inherent to ecosystems and is exacerbated by global change. However, experimental protocols typically cancel this factor by using cyclic or constant conditions, limiting the study of environmental variations. This simplification highlights the need for better methodological tools to control fluctuating environmental variables.
2. We present here a guidance and solution for generating and implementing stochastic environmental conditions through our Raspberry **Pi** System for environmental **Stoch**asticity (PiStoch). This low-cost, low-tech and scalable method for mesocosm experiments also has the potential to replicate any other form of variability, including cyclic patterns.
3. This system successfully reproduced stochastic time series in temperature and oxygen manipulation experiments. Testing with two biological case studies (macrophyte biomass and freshwater fish oxygen consumption), it demonstrated that thermal stochasticity had stronger effects than mean temperature, highlighting the importance of studying fluctuating conditions.
4. Developing accessible methods to study organismal responses to environmental stochasticity is essential for improving the realism of laboratory experiments and enhancing the accuracy of physiological and ecological predictions.

KEYWORDS
abiotic factor, biomass, global change, metabolic rate, oxygen, Raspberry-Pi, temperature

1 | INTRODUCTION

Variation is ubiquitous across living systems but also to time-dependent fluctuations in abiotic parameters, ranging from the most periodic (e.g. circadian and seasonal rhythms) to the least predictable (e.g. meteorological conditions) (Cornwall et al., 2013; Dobry et al., 2021; Fujiwara, 2009; Guadayol et al., 2014; Massetti, 2020). Variability is characterized by two key components: constancy (the stability of a variable) and contingency (the autocorrelation of variations in an abiotic parameter) through time (Abrahms et al., 2021;

Chloé Souques and Jérémy Bacon contributed equally.

Beissinger & Gibbs, 1993; Riotte-Lambert & Matthiopoulos, 2020). Thus, the degree of variability follows a continuum ranging from highly constant and contingent conditions (i.e. stable) to conditions of very low constancy and contingency (i.e. stochastic).

Climate change and pervasive anthropogenic activities are predicted to disrupt both environmental contingency and constancy, increasing the stochasticity of abiotic conditions (Chefaoui et al., 2024; Easterling et al., 2000; Helbling et al., 2024; Jenny et al., 2016; Oliver et al., 2018; Sampaio et al., 2021; Smucker et al., 2021). While predictable fluctuations favour the emergence of evolutionary adaptations, stochasticity provides fewer cues for organism response, imposing specific constraints on performance (Arnoldini et al., 2012; Bernhardt et al., 2020; Lovegrove, 2000). Empirical studies have also revealed context-dependent effects of variability (Bernhardt et al., 2018; Bozinovic et al., 2011; Colinet et al., 2015; Kingsolver et al., 2015; Noe, 2002; Slein et al., 2023), underlining the complexity of the mechanisms involved. Consequently, incorporating variability into experimental studies has become increasingly important for evolutionary ecologists seeking to predict organism responses and better understand evolutionary processes under global change (Franch-Gras et al., 2017; Morash et al., 2018; Vasseur et al., 2014; Verheyen & Stoks, 2019).

The impact of stochasticity on organisms, population dynamics and community structure has primarily been studied through theoretical models (Reed et al., 2010; Roughgarden, 1975; Shoemaker et al., 2020). Current experimental approaches typically reproduce deterministic patterns of variability, neglecting the limited contingency that characterizes real-world stochasticity (Burggren, 2019; Gerhard et al., 2023). Indeed, most laboratory-based studies address variability through cyclic (Braz-Mota et al., 2024; Foray et al., 2014; Massey et al., 2022; Pettersen et al., 2024; Salinas et al., 2019), gradual or stepwise patterns (Burggren, 2019), even if recent studies attempt to upgrade ecological realism by incorporating unpredictable aspects that are, for instance, carried in a field approach (Cicchino et al., 2024; von Schmalensee, 2023). This mismatch between environmental stochasticity and experimental conditions will likely increase due to global change, emphasizing the need to move beyond traditional contingency frameworks (Burggren, 2019; Ibáñez et al., 2013).

The limited consideration of proper stochasticity in experimental literature, despite its biological relevance, reveals a critical methodological limitation in controlling abiotic parameter variations. Experimenting with stochasticity requires generating appropriate stochastic time series (hereafter, *input*) and implementing them in experimental set-ups to match recorded parameter variations in mesocosms (hereafter, *output*) (Figure 1). This presents three main challenges: (i) generating appropriate stochastic time series that combine low contingency with experimenter-defined constraints for treatment comparison and protocol repeatability, (ii) coordinating regulatory systems to replicate given time series, which has led to accurate but costly commercial solutions (e.g. Memmert incubators, Campbell Scientific, Loligo Systems, Optoreg by Ern and Jutfelt (2024), Ecolab by Verdier and colleagues (2014)), and DIY systems that struggle to simulate intended stochastic conditions (Greenspan et al., 2016; Pisano et al., 2019) and (iii) achieving accurate input–output matching through frequent, bidirectional (i.e. positive and negative), and abrupt automatic parameter adjustments.

To address these methodological gaps, we present the Raspberry-Pi System for applying environmental Stochasticity (PiStoch). This all-in-one, low-cost and versatile system offers possibilities to generate, implement and apply any form of variable environmental conditions in experimental set-ups. While PiStoch can simulate any pattern along the variability continuum, we demonstrate its efficiency in controlling temperature and dissolved oxygen variations in two aquatic case studies investigating thermal stochasticity effects on a freshwater fish and pond macrophytes. This reliable and financially accessible method expands opportunities for ecologists to explicitly manipulate stochasticity in their experimental work, enabling more realistic replication of natural system dynamics and improving prediction accuracy.

2 | MATERIALS AND METHODS

2.1 | Modelling stochasticity

Being able to modulate both the constancy and contingency of parameter variation is crucial for addressing a wide range of biological questions (Figure 1, *input–issues*). Here, we introduce two algorithms that enable the simulation of parameter variation over time. These algorithms allowed for varying the two main components of stochasticity by modulating both the constancy and the contingency (degree of temporal autocorrelation) in the time series (Figure 1, *input–solutions*). The controlled parameters can be any abiotic parameter (e.g. temperature, dissolved oxygen saturation, pH and light intensity).

2.1.1 | Constrained random walk

To generate stochastic time series of parameter P that must however satisfy some specific properties (e.g. mean P_{mean}, variance P_{var}, and range of the parameter P_{min} and P_{max}, maximum variation rate per unit of time P_{rate}), an algorithm of constrained random walk algorithm is a straightforward method. The total duration of the series is discretized in fixed time steps *t*. Stochastic time series are generated based on a free one-dimensional and discrete random walk that changes through time, according to a Markov (memoryless) process $P(t) = P(t-1) + \epsilon$, where P is the value of the considered parameter, *t* is time step, and ϵ is the parameter variation simulated as an independent and identically (no overall trend) distributed (iid) random variable, drawn from a jump distribution at each time step. ϵ can rely on discrete jumps (e.g. {−0.3, 0.3}) or, for even lower contingency, on Brownian movement (e.g. $N(\mu = 0, \sigma)$). The controlled parameter can therefore either increase, decrease or remain stable between two consecutive time steps. Such unconstrained random walk can lead to stochastic time series which strongly differ in terms of properties (e.g. range, mean,

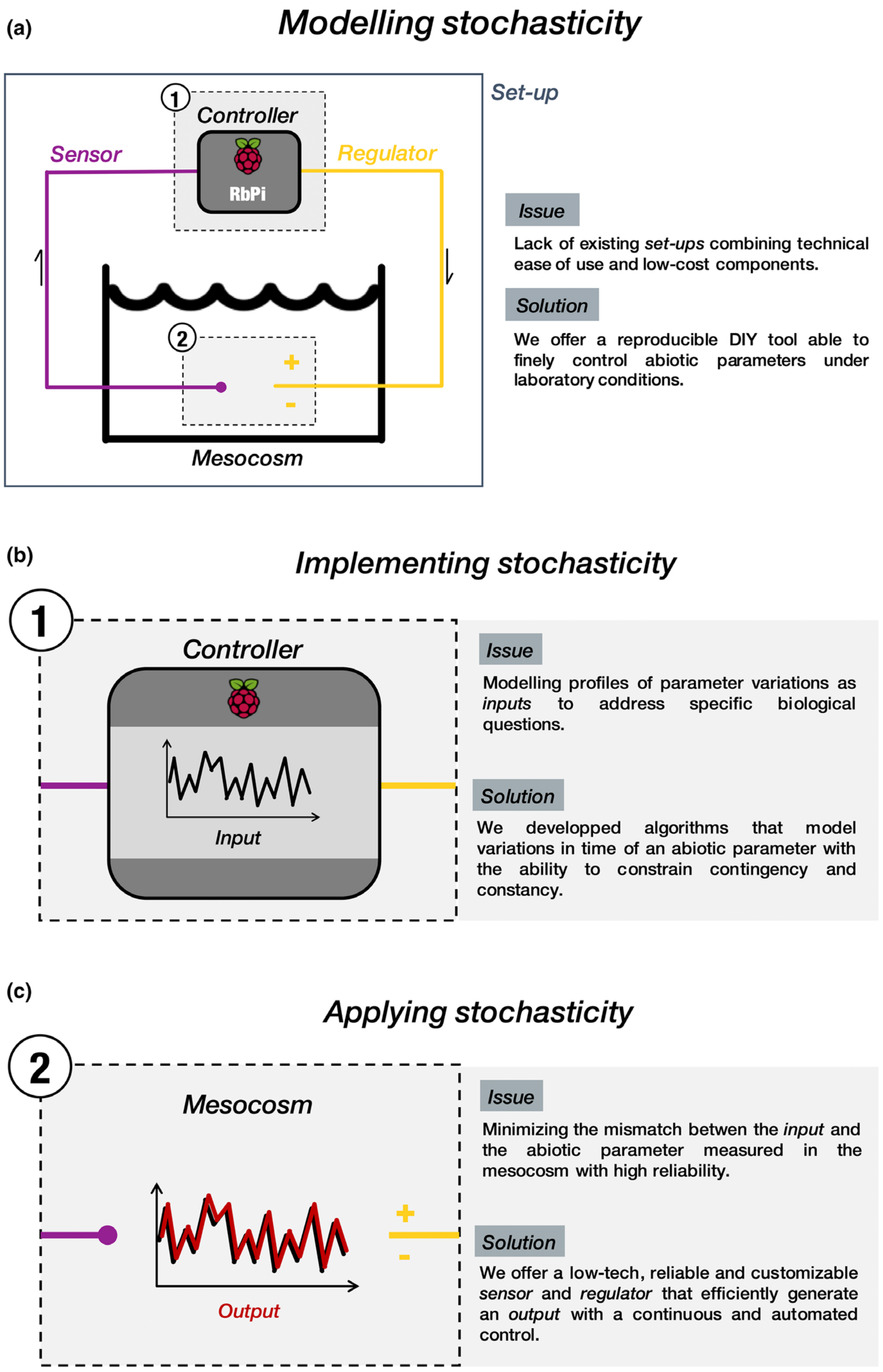

FIGURE 1 Conceptual scheme illustrating the different modules of PiStoch (see Section 2 for more details on each module).

variance and bridge condition). We used brute force to control these properties of the stochastic time series. Simulations of time series are repeated as often as necessary until satisfying properties are met (see source code on Zenodo; Souques et al., 2025).

This algorithm is highly versatile. Increasing the contingency in the stochastic time series is possible, with the addition of other constraints (e.g. fixing the starting and ending parameter values, generating cyclic patterns by forcing the parameter value at t to be higher or lower than at $t-1$). Modulating the properties related to amplitude (P_{min} and P_{max}) and variance (P_{var} and P_{rate}) of the time series allows to increase its constancy. To sum up, any wanted pattern can be modelled with this algorithm.

2.1.2 | Sinusoid method: Cyclic pattern with flexible degree of stochasticity

A different method than the constrained random walk algorithm to incorporate rhythmicity (e.g. circadian) while allowing for potential differences between each period, is to model stochastic time series based on a day-time sinusoid curve modulated by a random process The sine function is built on a 24-h interval, cut in 24 setpoints (1 per hour): 12 for daytime and 12 for night-time. On each setpoint, the value of the studied parameter is randomly weighted by a coefficient bounded between a maximum and a minimum, regarding the acceptableness of the variation the studied organism can handle, to preserve the general sine aspect.

2.2 | Experimental set-up: Implementing and applying stochasticity

2.2.1 | Sensor and controller

The stochastic time series (i.e. Figure 1—*input*) is implemented in Python (see source code Zenodo; Souques et al., 2025) and deployed on a monocard nano-computer Raspberry Pi (i.e. Figure 1—*controller*). The system's hysteresis threshold defines the tolerance limit between input and output values, determining when the regulatory pathways (i.e. Figure 1—*Regulator*) are activated. This threshold, combined with the regulator lag specific to each set-up, prevents system self-limitations and ensures successful achievement of target parameter values.

System monitoring and control is facilitated through the Virtual Network Computing software (RealVNC Viewer 7.12.0). The set-up can be expanded with additional components, such as a high-capacity SD card for increased storage or an external display for direct data visualization.

The Raspberry Pi interfaces with a digital probe (Figure 1—*Sensor*) that continuously measures the parameter of interest at regular intervals (e.g. every 10 seconds). We opted for a digital, rather than an analogue probe, to enable binary signal reading and eliminate signal degradation over long cable runs. Consequently, a 1-Wire protocol (Analog Devices, Inc.) was used to connect the sensor with the Raspberry Pi. Such connection protocol imposes rigorous constraints on the set-up (e.g. recommendation of a bus type connection, USB-1-Wire controller and converter to improve the reliability of the system by avoiding communication conflicts).

The Python control program operates by comparing sensor signals with input values at each time step. The system response follows two main paths: (i) When the sensor value matches the input, the system maintains its current state or (ii) when the sensor value deviates from the input, the positive/negative regulator (see Section 2.2.2) is activated to increase/decrease the parameter value. These regulatory actions continue until the sensor value aligns with the input target.

2.2.2 | Regulator

The regulatory system implementation requires a specific hardware configuration (Figure 1c). The Raspberry Pi connects to the regulator through two pins, featuring a dual on/off switch. This switch comprises an electrical relay connected to a 220-V power supply, ensuring adequate power delivery to each device. The system employs Transistor–Transistor Logic (TTL), with each pin independently controlling one relay to activate either the positive or negative pathway as needed.

2.2.3 | Set-up checking

PiStoch incorporates multiple checking systems to ensure operational reliability. Users can remotely monitor measured values and their corresponding setpoints in real-time via an online interface. The system stores data in InfluxDB (version 1.8.10), an open-source time-series database, and displays it through Grafana (version 8.4.3), an open-source visualization platform. In addition, the Python-based control program includes safety constraints that automatically deactivate (i.e. emergency stop) regulation pathways if values exceed specified thresholds and also send warning emails when abnormal conditions are detected.

2.3 | Testing the efficiency of the experimental set-up

We demonstrate the system's functionality using two water parameters.

First, the system's responsiveness has been tested with three experiments manipulating water temperature using modelling methods presented above (see Section 2.1): (i) Stochastic time series, (ii) Stochastic time series incorporating a cyclic pattern. All experiments have been conducted in accordance with animal care guidelines and have been approved by the ethics committees of Lyon (France), as well as the Ministry of Research and Higher Education (APAFIS #51425-202410151503521 v2).

We implemented and applied these time series using the material listed in Table S1 (columns 'Module', 'Item' and 'Price').

(i) Stochastic time series—The input modelled with a constrained random walk met the following conditions: mean temperature = 21°C ±0.1, minimal temperature = 18°C reached at least once, maximal temperature = 24°C also reached at least once, 0.3°C variation per 10-min time step (i.e. the temperature variation could not exceed 1.8°C per hour). Additionally, the top and lower 33% temperature values had to represent less than 30% of the whole time series to ensure that temperatures would not remain disproportionately close to bound values. A 'bridge condition' has been implemented to ensure that the initial and the final values would remain close to the mean temperature value. This experiment lasted for 6 days.

(ii) Stochastic time series incorporating a cyclic pattern—The input was based on daytime sinusoid. The instructions given to the system were: mean = 13°C, minimal temperature = 8°C, maximal temperature = 18°C measurements of water temperature in the mesocosm. The variable temperature regime was simulated with a sine function divided into 24 times steps, to vary the temperature setpoint every hour. Each time step was multiplied by a randomly generated value ranging from 0.01 to 5°C each day and −0.01 to −5°C each night. This experiment lasted for 10 weeks (only 5 days are shown in Figure 2c,d).

A robustness test to a challenging time series has been added in supporting information (Appendix S1). The aim was to estimate the minimum time lag required for the system to maintain accurate responsiveness when confronted with a time series where the target temperature followed variations of 10°C amplitude with increasing frequency (Figure S1).

To control temperature variations in water, a submersible water heater was used as a positive regulation pathway and a closed circuit initiated with a water pump as a negative regulation pathway. The cooling system circulates water through a metal coil immersed in a temperature-controlled cool box (maintained at 8°C). The controller manages the pump's activation duration (longer operation periods resulting in greater cooling effect). The design keeps aquarium and cool box water separate, allowing multiple aquariums to share a single cool box through individual coils. A tutorial presenting the steps used in our mesocosm experiments to regulate temperature using PiStoch is available in a public repository (see zenodo; Souques et al., 2025).

Second, we tested system responsiveness with an experiment manipulating water saturation in dissolved oxygen for 24h following a stochastic time series modelled based on the above-described constrained random walk method. The simulated model met the following conditions: mean O_2 content=80%, minimal O_2 content=10% reached at least once, maximal O_2 content=110% also reached at least once, 5% variation per 5min time step (i.e. the variation could not exceed 60% per hour).

To control dissolved oxygen saturation variations in water, we used a bubbler as positive regulation pathway and a dinitrogen bottle as negative regulation pathway. The controller manages both the bubbler activation and solenoid valve opening.

2.4 | Statistical analyses

We assessed the matching between the input and output time series (i.e. the reliability of the system) of temperature and dissolved oxygen, by quantifying the strength of the linear regression between the two time series (response variable: input; predictor variable: output) as R^2 coefficients. For the data presented in Box 1, Cohen's *d* was computed as an effect size index for comparison between thermal treatments (Nakagawa & Cuthill, 2007) using the package 'effsize' (Torchiano, 2020). All the tests were performed using R version 4.4.1 (R Core Team, 2024). R and Python code source is accessible on Zenodo (Souques et al., 2025).

3 | RESULTS

3.1 | Matching between temperature input and output in PiStoch

The output time series of temperature showed very high correlation with the input time series generated by both the constrained random walk (R^2=0.978) and the sinusoid method (R^2=0.995) (Figure 2), demonstrating the reliability of PiStoch to control temperature.

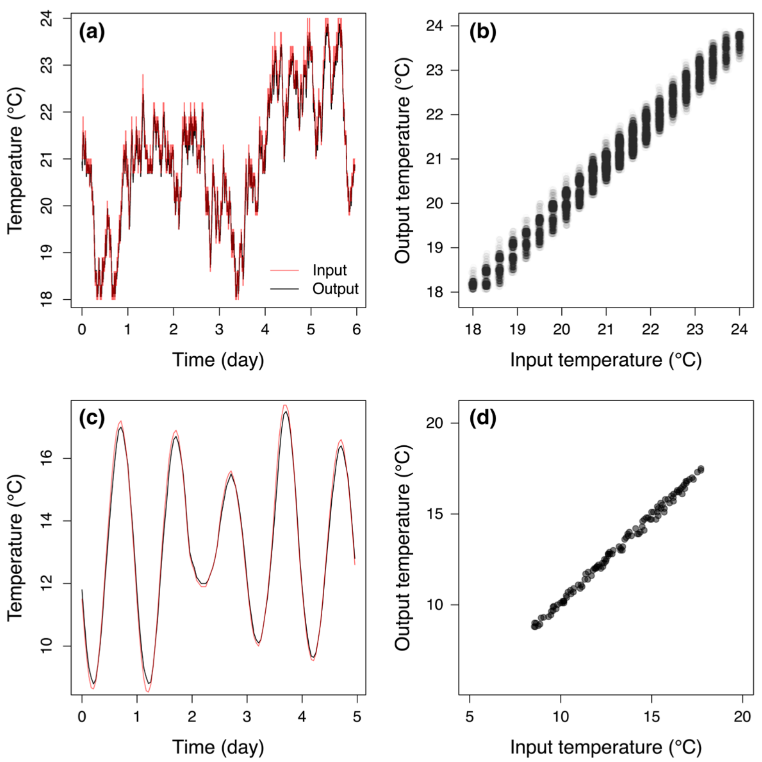

FIGURE 2 System responsiveness to (a) a stochastic temperature series simulated during 6days thanks to the constrained random walk, and (b) a cyclic stochastic sinusoid simulated during 5days thanks to the sinusoid method. The black curve corresponds to the output and the red curve refers to the input. Panels (c) and (d) show respective regression scatterplots. The input values have been jittered to improve visualization.

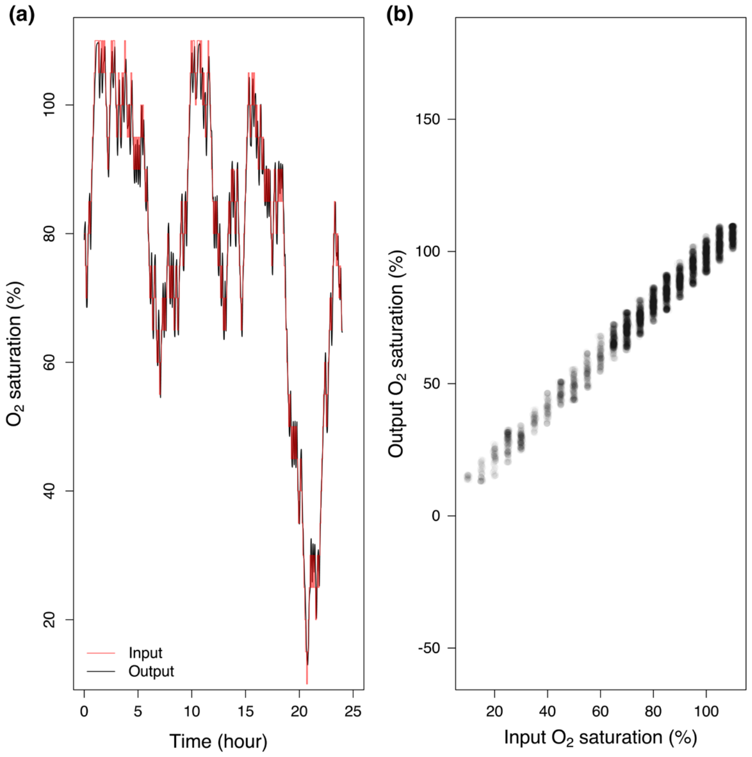

FIGURE 3 (a) System responsiveness during 24 h, to highly stochastic variations in percent of saturation of dissolved oxygen in water through time, simulated thanks to the constrained random walk algorithm. The black curve corresponds to the output and the red curve refers to the input. Panel (b) shows the associated regression scatterplot. The input values have been jittered to improve visualization.

3.2 | Matching between dissolved oxygen input and output in PiStoch

The input and the output time series describing the level of dissolved oxygen in water showed a very strong correlation ($R^2 = 0.974$) (Figure 3), indicating high performance of PiStoch to experimentally manipulate this parameter.

4 | DISCUSSION

We offer a reproducible method able of precisely controlling the variance of changing abiotic parameters to more accurately reflect environmental stochasticity under experimental conditions.

PiStoch demonstrated high effectiveness in generating and reliably applying stochastic time series of environmental parameters in indoor mesocosms, representing a significant advancement in mimicking natural conditions in experimental studies. The strong matching between inputs and outputs time series validates this DIY approach, offering researchers a methodological opportunity to implement ecologically relevant abiotic conditions, thereby enhancing the realism of mesocosms studies. Indeed, experimental designs only focussing on stable temperatures or regular sinusoid may misestimate organisms' responses *in natura*, particularly problematic when attempting to make robust predictions in a changing world (Bernhardt et al., 2020; Burggren, 2019; Gerhard et al., 2023; Wolkovich et al., 2012).

A primary objective of our study was to develop a cost-effective set-up with maximum versatility. The functional Raspberry Pi computer, while being the most expensive component (approximately 50€), proves highly suitable for this application (Table S1). Depending on the coupled accessories, the Raspberry Pi computer presents high flexibility in parameter control, allowing multiple experimental applications and reuses (Jolles, 2021) with unlimited data storage thanks to external disks. It avoids restrictions in memory capacity as for HOBO devices, for instance. Moreover, a cloud data recording is feasible via VPN connectivity, as we used in both our case studies. For instance, the set-up used in the experiments involving fish in four mesocosms and plants in five mesocosms (for plants, stochastic treatment only) (see Box 1) cost less approximately 1400 € and 1500 €, respectively (aquarium chiller and cooler included) (Table S1). The total system cost varies based on the number of connected mesocosms, primarily due to sensor requirements. While each mesocosm requires dedicated sensors, control equipment (i.e. Raspberry Pi, heater, cooling system) can be shared across multiple units. Thus, the more mesocosms are added, the lower the cost per experiment.

The system's adaptability extends beyond our described configuration. However, it must be kept in mind that although the controlled water volume of mesocosms is flexible, larger water volumes require more powerful (and *in fine*, expensive) controllers. Similarly,

BOX 1 Biological applications: Responses to thermal stochasticity of the oxygen consumption of freshwater fish and of the aerial biomass of pond plants

Background. We propose here two examples of biological applications of the PiStoch set-up. We investigated the responses of both an animal and a plant species of aquatic ecosystems to stochastic variations in the thermal conditions of their habitat, using the PiStoch set-up to control for water temperatures in mesocosms. We quantified the oxygen consumption associated with thermal stochasticity in a cyprinid fish, the spirlin (*Alburnoides bipunctatus*), and the individual aerial biomass in a macrophyte species, *Limosella australis* R.Br. (Scrophulariaceae).

Methods. Stochastic time series were generated as presented before using either the constrained random walk algorithm for fishes (Figure 2a), or the sinusoid method showing daily variations with unpredictable amplitudes for plants (Figure 2b showing a 5-day extract of the total 10-week time series).

For fish, this design aimed to reproduce an extreme climatic event through thermal shocks resulting in significant daily variations. For plants, the experimental design aimed to mimic pond ecosystems of the sub-Antarctic region at small scale, to assess changes in individual performances in response to extreme thermal events. In both cases, the tested thermal window reflected the ecology of the species and temperature data for the catchment area where it is found (Beaufort et al., 2020; Douce et al., 2023). Thus, in both cases, organisms were exposed either to a stochastic or to a constant temperature treatment, which were equivalent on average (i.e. 21°C for fish and 13°C for plants) but with contrasted variances.

Sixteen spirlins were randomly assigned to four 15-L experimental aquariums (two replicate tanks per treatment, four fish per tank) containing dechlorinated water (dimensions 35 cm × 20 cm × 25 cm). Each tank featured gravel substrate, Easy Plant Superfish artificial vegetation, and cylindrical shelters (diameters of 4 and 9.5 cm). Water quality was preserved through continuous full aeration and filtration via EHEIM professional 4+ pumps. The fish experienced a balanced photoperiod of 12-h darkness and 12-h light. Their diet consisted of defrosted chironomid larvae (Europrix) provided daily, ensuring feeding to satiation excepted the day before the respirometry trials, on which they were fasted for 24 h to reach postabsorptive states. The stochastic temperature time series of each tank was controlled independently by PiStoch. After 6 days of exposure to the stochastic time series, fish oxygen uptake (MO_2) was continuously measured overnight for 18 h in individual static respirometers and using an intermittent stop flow respirometry protocol (Chabot et al., 2016; Clark et al., 2013; Svendsen et al., 2016) conducted at the mean temperature of the time series. The bridge condition ensured that fish were close to 21°C when taken out for respirometry trials. Respirometry data were collected using Oxygen Logger software (Pyroscience, Aachen, Germany). Fish Standard Metabolic Rate (SMR), which is exclusively linked to the maintenance of vital functions in ectotherms, was calculated as the 10 lowest per cent of all recorded MO_2 values.

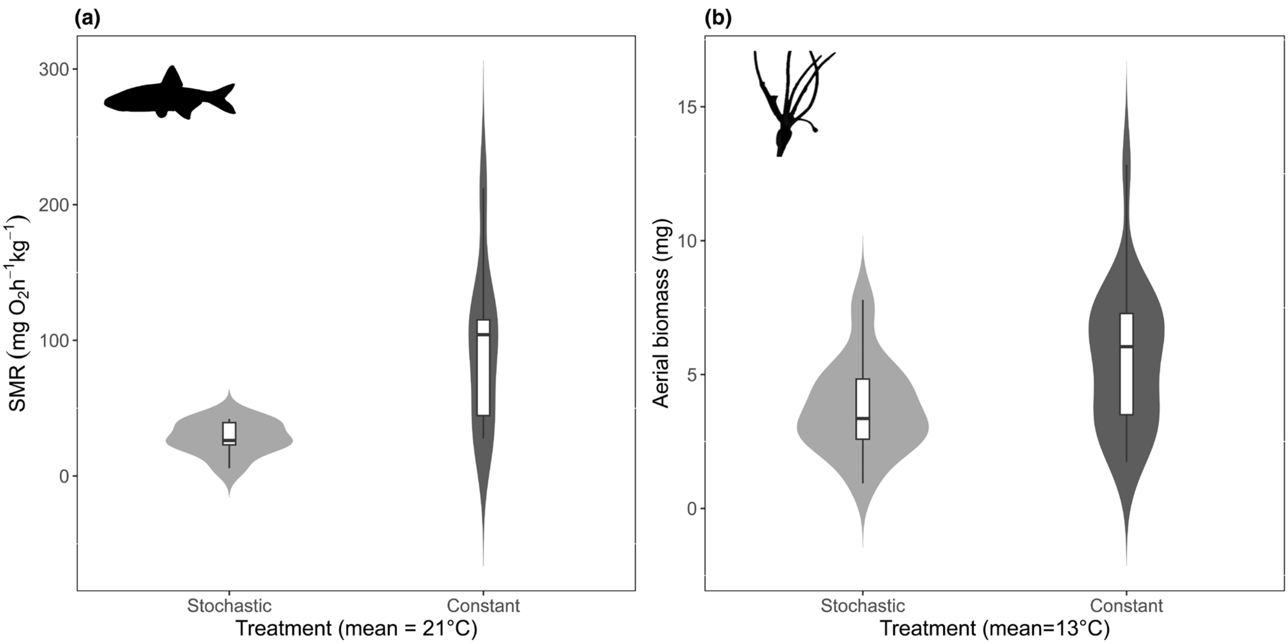

FIGURE 4 Responses to stochastic and stable temperatures in two aquatic organisms. (a) *A. bipunctatus* Standard Metabolic Rate (SMR), and (b) *L. australis* individual aerial biomass.

(Continues

Box 1 (Continued)

Fifteen *L. australis* individuals were cultivated separately in square pots replicates (10×10×11 cm) distributed between 10 mesocosms (of which five are controlled by PiStoch) filled with 42 L of water (dimensions 78.7 cm×58.6 cm×15 cm). A recirculating water pump (EHEIM Compact ON 300) homogenized temperature distribution. Cultures were conducted on homogenized mixture of 50% of sand and 50% of compost (N: 250 g.m^{-3}, P_2O_5: 120 g.m^{-3}, K_2O: 80 g.m^{-3}) with 12 h/12 h light regime. Mesocosm water was entirely renewed one a week to avoid nutrient leaching from the pot substrate. After 10 weeks of clonal growth under exposure to thermal treatments, three newly produced individuals per replicate were randomly harvested and aerial biomass was measured after drying them for 48 h in oven at 65°C. All plants were cleaned before any measurement.

Results. Results revealed a decrease in studied response variables in organisms exposed to stochastic temperatures compared to stable ones. Fishes exposed to fluctuating temperatures (Figure 4a) exhibited significantly lower SMR (linear model, $F_{1,14}=11.726$, $p=0.004$, Cohen's $d=1.55$, 95% CI = [0.323; 2.766]) than fishes that had experienced the constant temperature. Similarly, in the plant species *L. australis* (Figure 4b), individuals exhibited a lower aerial biomass under fluctuating temperatures than under constant ones (linear mixed model, $\chi^2_1=7.74$, $p=0.005$, Cohen's $d=0.83$, 95% CI = [0.199; 1.454]).

Conclusion. In the two biological applications of PiStoch tested, thermal stochasticity significantly impacted physiological or morphological traits across distant taxa, suggesting broader implications for aquatic ecosystem responses to temperature variations.

applying very low contingency and constancy to a controlled parameter will be more energy consuming.

The system's versatility enables simultaneous control of multiple parameters within individual mesocosms, giving concrete advances for the development of multifactor experimentation. Such perspective allows to investigate the additivity or potential complex interaction dynamics (i.e. positive or negative covariance) between concurrently changing stressors, which could modulate organismal responses (Koussoroplis et al., 2017; Koussoroplis & Wacker, 2016; Wu et al., 2011). Users should note that each parameter has inherent physical constraints independent of the system, such as dissolved oxygen's slower response time compared to temperature due to gas diffusion lag.

The flexibility of our method is not only inherent to the multiple abiotic parameters controllable by adapting the sensor and the controller but also associated to the diversity of time series (i.e. gradient of stochasticity) that can be generated and the opportunity of adapting or upgrading our algorithms based on our open-source codes. PiStoch accommodates field-recorded data implementation as an alternative to algorithm-generated time series. Therefore, real-time data transmission from field stations could be a promising and time-saving perspective to add more automatization and realism to indoors experiments. If real-time data transmission is too restrictive, it is possible to use data previously recorded by an electronic data-logger from the field as instructions as if a modelled input was implemented.

While the Raspberry Pi platform may initially appear challenging for users with limited programming experience, we provide all the scripts and descriptions necessary to reproduce this set-up very easily without such skills. As the use of Large Language Models (LLM) is now easily accessible, we encourage the use of artificial intelligence to help beginners with Python programming or electronic set-ups. Using a Raspberry Pi platform connected to an open access Python program also offers various perspectives (e.g. a graphical user interface—GUI, use of a server) driven by the needs of the users' community and contributing to the upgradeable aspect of our system.

While our focus has been on aquatic ecosystems, the 'kernel system' (i.e. control centre, nano-computer and operating system) remains applicable to terrestrial mesocosms with appropriate modifications (Stewart et al., 2013). If affordable, adapting a wine cellar or a greenhouse into a hermetic terrestrial mesocosm could offer a solution to precisely control abiotic parameters in non-aquatic experimental mesocosms using PiStoch.

In conclusion, PiStoch demonstrates potential for integration with diverse mesocosm configurations, encompassing multidisciplinary scientific questions. It represents a cost-effective alternative to commercial systems while maintaining high efficiency for physiological and ecological studies. We hope this system will support the development of experimental protocols incorporating environmental parameters with stochastic variations. In an era of global changes, stimulating our knowledge of organisms' responses to such variations is crucial to refine predictions about species and ecosystems persistence and adaptability to an increasingly unpredictable world.

AUTHOR CONTRIBUTIONS

Anne-Kristel Bittebiere, Loïc Teulier, François-Xavier Dechaume-Moncharmont and Yann Voituron conceived the original idea and developed the protocol. Ludovic Guillard designed the experimental set-up. Pauline Eymard-Dauphin, Chloé Souques and Loïc Teulier ran the experiments. Anne-Kristel Bittebiere, François-Xavier Dechaume-Moncharmont, Jérémy Bacon and Chloé Souques performed the statistical analyses. Chloé Souques and Jérémy Bacon led the writing of the manuscript, largely supported by Yann Voituron, Anne-Kristel Bittebiere, Loïc Teulier and François-Xavier Dechaume-Moncharmont. All authors analysed the data, contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/2041-210X.70094.

DATA AVAILABILITY STATEMENT

The datasets, Python and R scripts for the analysis, guidance for non-technical users and set-up controls described herein are available on Zenodo https://doi.org/10.5281/zenodo.15480013 (Souques et al., 2025).

STATE OF INCLUSION

We conducted the study exclusively within a single regional context. We perhaps constrained our research by limiting intellectual diversity and missed opportunities to incorporate perspectives, insights, and alternative methodological approaches that external stakeholders might have contributed.

ORCID

Chloé Souques https://orcid.org/0009-0004-4482-5829
Jérémy Bacon https://orcid.org/0009-0001-2406-2287
Ludovic Guillard https://orcid.org/0009-0005-4383-5459
Pauline Eymard-Dauphin https://orcid.org/0009-0004-4910-4895
Loïc Teulier https://orcid.org/0000-0001-7779-7634
François-Xavier Dechaume-Moncharmont https://orcid.org/0000-0001-7607-8224
Anne-Kristel Bittebiere https://orcid.org/0000-0002-9882-968X
Yann Voituron https://orcid.org/0000-0003-0572-7199

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: Robustness test.

Figure S1: Robustness testing, with the input (red curve) switching from 10°C to 20°C at increasing frequency (every 120, 60, 30, 15, 8, 4 and 2 min), and the corresponding output recorded (black curve).

Appendix S2: Material used and price.

Table S1: List of used materials. Prices reflect those we paid during the experiments, they are subject to change since then. * While the bubbler can be replaced with a pure dioxygen bottle (see *Optoreg* (Ern & Jutfelt, 2024)), this alternative requires additional safety measures due to fire and explosion risks. ** For setups with multiple mesocosms, multiple solenoid valves (negative pathway) can be connected to the nitrogen bottle.

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Supporting Information

Supporting information can be found online at the end of the article.

Appendix S1: Robustness test

In the two modelled and implemented temperature stochastic time series (Fig. 2), the temperature variation is largely auto-correlated, with small differences in temperatures between two consecutive time steps. We also assessed the robustness of PiStoch by deliberately simulating intense thermal conditions, involving a very inconstant time series and in which the target temperature switched between 10°C and 20°C at increasing frequency (every 120, 60, 30, 15, 8, 4 and 2 minutes). The conditions simulated in this test are beyond normal operational capacity. Our aim was to assess the limits of heating and cooling abilities of our system, until it is unable to cope with extreme regime of temperature change in freshwater. We measured the response time as the average time to reach the target temperature after each switch. This experiment lasted for 24 hours.

We used the same sensor and controller modules as for the two temperature stochastic time series (see Table S1). As for regulators, we used a submersible heater TETRA HT 300W (Aquastore, ref T710940) and a TK2000 (TECO, Ravenna, Italia) for the positive and negative pathway respectively. We used a 52L glass tank as mesocosm. The mean heating duration (from 10°C to 20°C) was 102.7 +/- 2.1 minutes and the mean cooling duration (from 10°C to 20°C) corresponded to 98.3 +/- 1.6 minutes (Fig.S1). The shorter the period, the more difficult it was to reach boundary values due to the power of the regulator devices used.

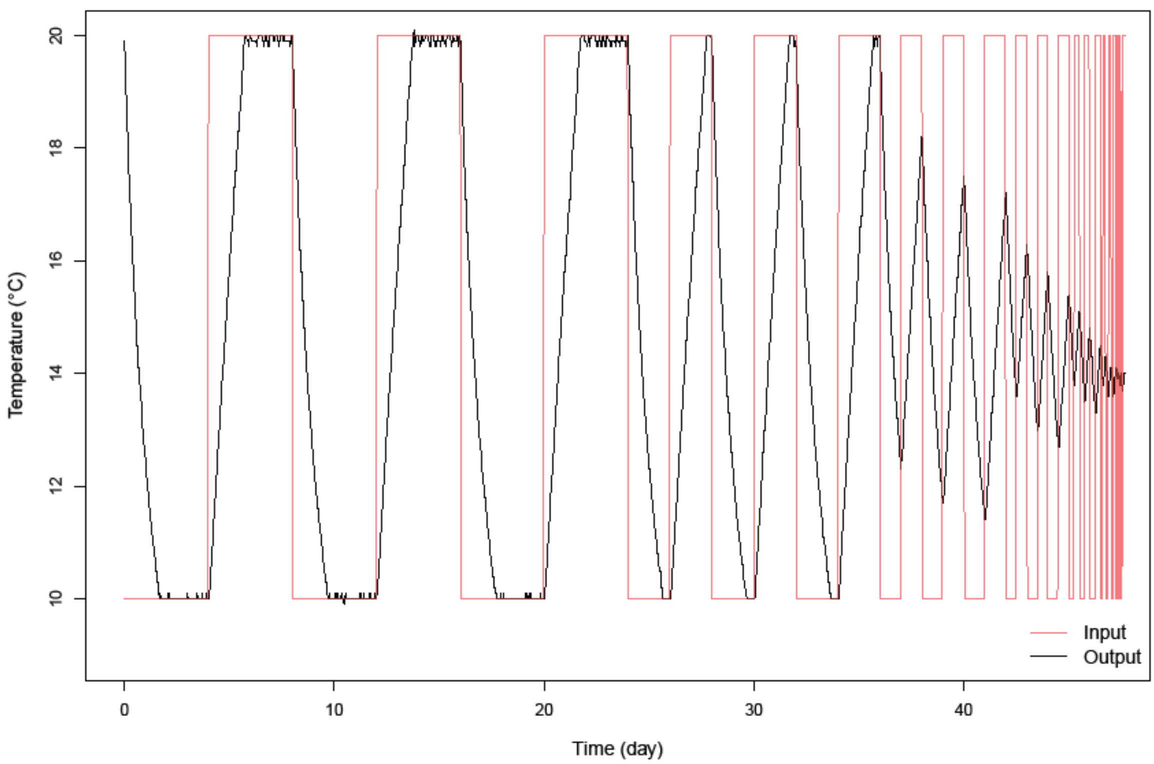

Figure S1. Robustness testing, with the input (red curve) switching from 10°C to 20°C at increasing frequency (every 120, 60, 30, 15, 8, 4 and 2 minutes), and the corresponding output recorded (black curve).

Appendix S2: Materiel used and price

<u>Table S1: List of used materials.</u> Prices reflect those we paid during the experiments, they are subject to change since then. * While the bubbler can be replaced with a pure dioxygen bottle (see *Optoreg* (Ern & Jutfelt, 2024)), this alternative requires additional safety measures due to fire and explosion risks. ** For setups with multiple mesocosms, multiple solenoid valves (negative pathway) can be connected to the nitrogen bottle

Module	Temperature			Dissolved oxygen		
	Item	*Price (€/unit)*	*Quantity*	*Item*	*Price (€/unit)*	*Quantity*
Sensor	Numerical temperature sensor DS18B20 (*Analog Devices, Wilmington, USA*)	12	1 per mesocosm	Optod (*Aqualabo, Champigny-sur-Marne, France*)	1030	1 per mesocosm
Controller	Raspberry Pi 3 Model B V1.2 (*Mouser Electronics, ref:RASPBERRYPI3-MODB-1GB 102110299 3055 RP2040TR7*)	32	1 per case study	Raspberry Pi 3 Model B V1.2 (*Mouser Electronics, ref:RASPBERRYPI3-MODB-1GB 102110299 3055 RP2040TR7*)	32	1
	Power supply 220v AC/ 5v DC 12W (*RS online, ref: 206-4917*)	10	1 per Raspberry Pi	Power supply 220v AC/ 5v DC 12W (*RS online, ref: 206-4917*)	10	1 per Raspberry Pi
	SD card 32 Go (*SanDisk, Milpitas, USA*)	10	1 per Raspberry Pi	SD card 32 Go (*SanDisk, Milpitas, USA*)	10	1 per Raspberry Pi
	Relay card TTL-RELAY16 (*Seeit, Clermont-Ferrand, France*)	33	1 per Raspberry Pi	Relay card TTL-RELAY16 (*Seeit, Clermont-Ferrand, France*)	33	1 per Raspberry Pi
	Interface bridge DS9490R (*Analog Devices, Wilmington, USA*)	43	1 per Raspberry Pi	Arduino Uno (*RS online, ref: 715-4081*)	20	1 per Raspberry Pi
Regulator; positive pathway	Submersible heater EHEIM thermocontrol 25W (*Deizisau, Germany*) [Stochastic time series] // Submersible heater EHEIM thermocontrol 75W (*Deizisau, Germany*) [Stochastic time series incorporating a cyclic pattern]	20	1 per mesocosm [4 heaters for freshwater fishes//5 heaters for macrophytes]	Air pump SUNSUN CT-404 680 L/H* (*Aquastore, ref:CT-404*)	23	1 per mesocosm
Regulator; negative pathway	Aquarium chiller TK500 (*TECO, Ravenna, Italy*)	800	1 per case study	Solenoid valve 24v DC VDW22LA (*SMC, Tokyo, Japan*)	20	1 per mesocosm
	Cold water tank 120 - 114L (*IGLOO, Katy, USA*)	120	1 per case study	Power supply RS PRO 220v AC/ 24v DC 12W (*RS online, ref :175-3306*)	10	1 per solenoid valve
	Copper pipes 2m Ø12mm roller (*Castorama, ref:3506465167557*)	23	1m per mesocosm [2 rollers for freshwater fishes/3 rollers for macrophytes]	Nitrogen source**(*Air liquide, Paris, France)*	Variable	1 per experiment (variable consumption)
	Water pump EHEIM Compact On 300 (*Deizisau, Germany*) // Water pump EHEIM Compact On 1000 (*Deizisau, Germany*)	18 // 35	1 per mesocosm [4 pumps for freshwater fishes/5 pumps for macrophytes]			
	PVC pipes 25m Ø12,5mm roller (*Castorama, ref:5059340349701*)	25	4m per mesocosm [1 roller for freshwater fishes/1 roller for macrophytes]			
Mesocosm	15L glass tanks (35 cm x 20 cm x 25 cm) (*Europrix, ref:01010305*)// 50L plastic tanks (17cm x 78,7cm x 58,6cm) (*Castorama, ref:3663602763192*)	21 // 12	1 per mesocosm [4 glass tanks for freshwater fishes/5 plastic tanks for macrophytes]	43L plastic tank (45cm x 33cm x 28cm) + lid (*Castorama, ref:3663602763154*)	10	1
Total price (€), case study with freshwater fishes (4 mesocosms)			**~1400**	**Total price (€) (1 mesocosm)**		**~1100**
Total price (€), case study with macrophytes (5 mesocosms)			**~1500**			