Disturbance of primary producer communities disrupts the thermal limits of the associated aquatic fauna

J.M. Booth [a,*], F. Giomi [b], D. Daffonchio [c], C.D. McQuaid [a], M. Fusi [d,e]

[a] Coastal Research Group, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa
[b] Via Maniciati, 6, Padova, Italy
[c] King Abdullah University of Science and Technology (KAUST), Biological and Environmental Sciences and Engineering Division (BESE), Red Sea Research Center (RSRC), Thuwal 23955-6900, Saudi Arabia
[d] Centre for Conservation and Restoration Science, School of Applied Sciences, Edinburgh Napier University, Edinburgh, UK
[e] Present address: Joint Nature Conservation Committee, Peterborough PE1 1JY, UK

HIGHLIGHTS

- Primary producers create predictable environmental fluctuation in freshwater habitats
- Cycles of oxygen fluctuation in freshwater channels improve heat tolerance of aquatic fauna
- Anthropogenic removal of primary producers (i.e. dredging) in freshwater channels eliminates patterns of oxygen variability
- Conservation and restoration of aquatic primary producers may offer protection to freshwater animals against climate warming

GRAPHICAL ABSTRACT

Primary producers are responsible for generating oxygen fluctuations in aquatic environments through photosynthetic activity in the day time and respiration during the night time. Aquatic animals have evolved in such dynamic oxygen conditions. Here we show that disruption of ecologically relevant oxygen fluctuation can impair the thermal physiology of aquatic fauna, highlighting the importance of conservation and restoration of aquatic primary producers to protect aquatic ecosystem biodiversity and functioning from global warming.
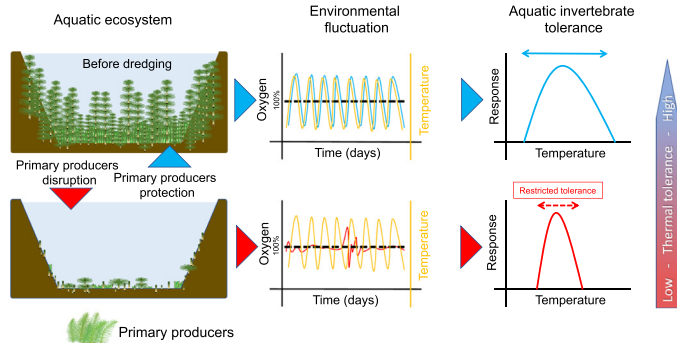
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ABSTRACT

Environmental fluctuation forms a framework of variability within which species have evolved. Environmental fluctuation includes predictability, such as diel cycles of aquatic oxygen fluctuation driven by primary producers. Oxygen availability and fluctuation shape the physiological responses of aquatic animals to warming, so that, in theory, oxygen fluctuation could influence their thermal ecology. We describe annual oxygen variability in agricultural drainage channels and show that disruption of oxygen fluctuation through dredging of plants reduces the thermal tolerance of freshwater animals. We compared the temperature responses of snails, amphipods, leeches and mussels exposed to either natural oxygen fluctuation or constant oxygen in situ under different acclimation periods. Oxygen saturation in channel water ranged from c. 0 % saturation at night to >300 % during the day. Temperature showed normal seasonal variation and was almost synchronous with daily oxygen fluctuation. A dredging event in 2020 dramatically reduced dissolved oxygen variability and the correlation between oxygen and temperature was lost. The tolerance of invertebrates to thermal stress was significantly lower when natural fluctuation in oxygen availability was reduced and decoupled from temperature. This highlights the importance of natural cycles of variability and the need to include finer scale effects, including indirect biological effects, in modelling the ecosystem-level consequences of climate

* Corresponding author at: Coastal Research Group, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa.
E-mail addresses: jen.marie.booth@gmail.com jenny.booth@kaust.edu.sa (J.M. Booth), marco.fusi@kaust.edu.sa (M. Fusi).

change. Furthermore, restoration and management of primary producers in aquatic habitats could be important to improve the thermal protection of aquatic invertebrates and their resistance to environmental variation imposed by climate change.

1. Introduction

Aquatic environments are characterised by natural fluctuations in environmental conditions that occur on multiple scales (Bernhardt et al., 2020). Particularly important is variation in pH, dissolved oxygen and temperature (Booth et al., 2021; Fusi et al., 2021; Vargas et al., 2022). Fluctuations in such abiotic conditions form a framework of variability within which species have evolved (Kroeker et al., 2020) and to which they are adapted, but natural variability normally includes a level of predictability, such as seasonal or diel cycles (Ostrowski et al., 2022). The predictable component in natural fluctuations can include both magnitude and duration, and organisms generally respond to these as cues. For example, photoperiod can be used to anticipate daily temperature variation or to acclimate to seasonal conditions (Huey and Bennett, 1990). In contrast, the persistence of natural populations facing rapid and unpredictable global change largely relies on their phenotypic plasticity (Bitter et al., 2021; Botero et al., 2014). For animals living in temporally and spatially heterogeneous environments, a degree of plasticity is advantageous. The phenotypic plasticity of a population is partly influenced by the level of predictability within the selective environment (Bitter et al., 2021; Botero et al., 2014; Reed et al., 2010; Tufto, 2016), with the costs of plasticity being higher when there is a decline in the predictability of fluctuations so that the optimum phenotype and population phenotype are more frequently mismatched (Reed et al., 2010; Scheiner and Holt, 2012).

Environmental fluctuation and its magnitude and predictability, defined as the temporal autocorrelation of one or more environmental parameters (Bernhardt et al., 2020), is used by many organisms to anticipate favourable conditions for activities such as reproduction and growth (Marshall and Burgess, 2014; Vannini et al., 2008). This reduces uncertainty about future conditions (Donaldson-Matasci et al., 2010) and allows organisms to increase their fitness by matching their phenotype or activity to expected environmental conditions (Reed et al., 2010). Consequently, disruption of the magnitude and predictability of environmental fluctuation, for example the correlation between oxygen levels and the temperature of water, can have variable consequences by altering feedforward mechanisms that allow the organism to adjust their physiology to anticipate a future environmental state (Morash et al., 2018). A classic example of such mechanisms is the avoidance of predation by zooplankton through diel vertical migration, using sunlight as a trigger to anticipate the danger posed by visual predators in the sunlit zone (Iwasa, 1972).

The regular, diel patterns of photosynthesis by primary producers determine highly predictable conditions in aquatic habitats, with photosynthetically-produced oxygen increasing during the day and decreasing during the night (Krumhardt et al., 2020; Wu and Hahn, 2006). Evidence indicates that aquatic fauna living in association with these plant communities benefit from this natural pattern of oxygen fluctuation in terms of their ability to sustain metabolic demands under warming (Blewett et al., 2022; Booth et al., 2021; Giomi et al., 2019).

Given the importance of primary producers as generators of oxygen, we asked whether changes to their biomass could have indirect effects on invertebrate thermal tolerance, testing this possibility with a system subject to human manipulation. Freshwater channels bordering agricultural land are landscape features that form significant freshwater habitats in many countries (Herzon and Helenius, 2008; Keeley et al., 2022). They connect lakes and rivers and represent a valuable biodiversity and conservation feature (Thrupp, 2000; Williams et al., 2003). These channels are colonised by freshwater primary producers, including angiosperms, freshwater algae and photosynthesising microbial biofilms; they support a remarkably diverse invertebrate community (Morris et al., 2016) and provide a rich array of ecosystem services (Sherren et al., 2021; Verdonschot et al.,

2011). These environments are also rapidly altered sporadically and abruptly by anthropogenic activities that can alter natural patterns of environmental change and disrupt the ability of resident animal communities to adapt to different fluctuation regimes (Williams et al., 2003), thus increasing their vulnerability to further change. In agricultural situations, channels are periodically dredged to improve the drainage of cultivated fields. This is a mechanical process that removes aquatic plants from the channels to increase water flow, and, in doing so, disrupts natural patterns of oxygen fluctuation. While oxygen levels may be improved due to the removal of the effects of the respiration and decay of dense plant communities, dredging also disrupts the predictable fluctuation in oxygen they create. This makes such channels a perfect model to investigate the effects of disruptions to natural patterns in dissolved oxygen and the potential consequences for the animal communities associated with aquatic plants.

We characterised the fluctuation of oxygen and temperature in two freshwater agricultural channels recorded over one year and tested the hypothesis that disruption of oxygen fluctuation through dredging will reduce the thermal tolerances of freshwater animals. To achieve this, we exposed representatives of four animal taxa (snails, mussels, leeches, amphipods) to natural oxygen fluctuation and constant oxygen in situ over three acclimation periods (2, 10 and 28 days) and measured their resistance to increasing temperature.

2. Methods

2.1. Environmental monitoring

Monitoring of dissolved oxygen saturation and temperature was carried out in two freshwater channels bordering arable land in eastern England (53.18° N, 0.23° W; Supplementary Fig. 1) between May 2020 and May 2021. Both channels are representative of arable agricultural systems throughout the United Kingdom (UK), with dense beds of the tracheophyte hornwort (*Ceratophyllum* sp.). Two PME miniDOT dissolved oxygen and temperature loggers attached to poles in the centre of the channels were submerged approximately 15 cm deep in the main water flow within the vegetation. The loggers were set to record at 30 min intervals, and data were downloaded periodically. In July 2020, the channels were subjected to dredging, as is typical during this season, to maintain water flow. The data obtained from environmental monitoring were used to inform the experimental treatments and levels of treatment for the animal lethal temperature experiments (e.g. fluctuating vs non-fluctuating oxygen).

2.2. Animal lethal temperature experiments

We selected four of the most abundant animals associated with vegetation in freshwater channel systems in the UK for our study: the snail *Bithynia tentaculata* (Linnaeus 1758), the zebra mussel *Dreissena polymorpha* (Pallas, 1771), the amphipod *Gammarus pulex* (Linnaeus 1758) and the leech *Glossiphonia complanata* (Linnaeus 1758). These animals are found in abundance in hornwort beds at 15–50 cm depth where they are exposed to diel oxygen fluctuation. Within one of the study channels, 18 plastic tanks (35 L) were set up on September 1st September 2021. Nine tanks were perforated with large holes of diameter 20 mm (covered with mesh, diameter 0.3 mm, to prevent animals escaping), allowing the exchange of water subject to oxygen production/respiration by primary producers, and these were assigned to the treatment 'oxygen fluctuation'. Nine tanks were unperforated, with no water exchange allowed, and were assigned to the 'constant oxygen' treatment (Supplementary Fig. 4). For these tanks, water was replaced with fresh environmental water (collected after dawn when 100 % oxygen saturation was reached) every 5 days to prevent the
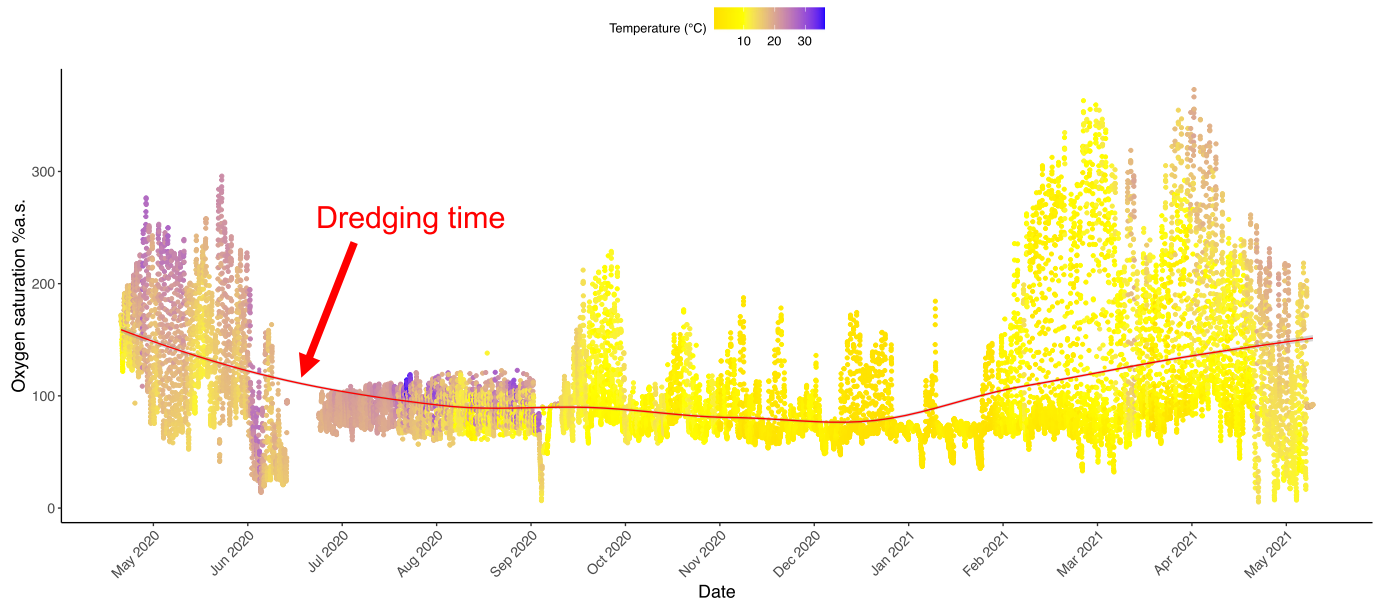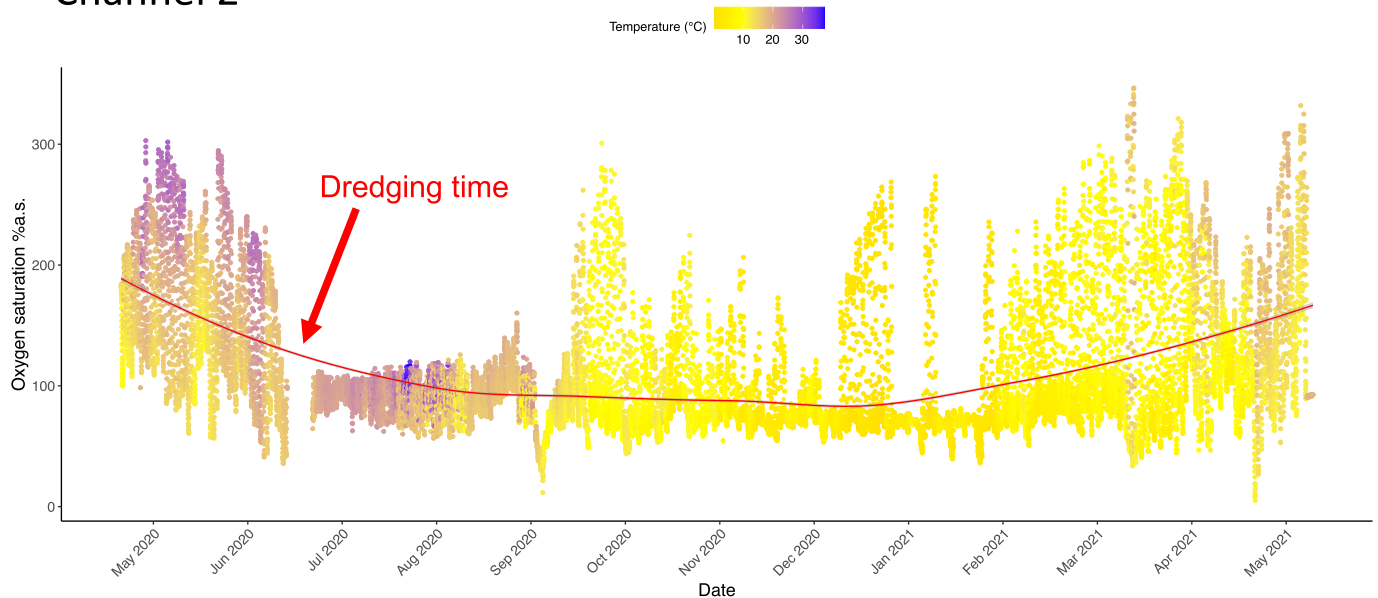
Fig. 1. Dissolved oxygen (% air saturation) in the two channels during the surveyed period. The red line shows daily average oxygen saturation, fitted with a loess regression. Temperature is indicated by the colour of the dots. The red arrows indicate when dredging occurred.

build-up of metabolic waste products and oxygen reduction through animal respiration. For each treatment, we assigned three replicate tanks to one of three acclimation periods: 2 days, 10 days and 28 days. In each replicate, we placed 20 individuals of each of the four study animals (i.e. total n = 60 per treatment/acclimation time for each species). The four species were separated by mesh barriers to reduce predation or competition. Each tank was provided with *Spirulina* algal discs, both whole and ground, every 5 days, for animal nourishment. All the species, except *G. complanata*, feed on algae directly or filter feed (Delong et al., 1993; Gossiaux et al., 1998; Hahn, 2005). Leeches are predators, and while they were not fed, we assume that any impact of diet was consistent across both treatments and had no confounding effect on the experiment. PME miniDOT dissolved oxygen and temperature loggers (ONSET HOBO - UA-002-08) were used to monitor oxygen and temperature during the acclimation period in one

replicate tank for each treatment. In the 'constant oxygen' treatment, oxygen varied between 80 and 110 % and in the 'fluctuating oxygen' treatment, oxygen varied between 0 and 200 % (acclimation environmental data are shown in Supplementary Fig. 4 and the conditions experienced by animals in the natural sites prior to the treatments are shown in Fig. S3).

At the end of each acclimation period (i.e. after 2, 10 or 28 days), animals were extracted from tanks and transported a short distance to the laboratory in tank water. After 3 h acclimation to ensure all animals were healthy and active, they were exposed to a temperature ramp (each replicate formed a different experiment, i.e. 18 temperature ramps were performed). Experimental tanks (13 L) were set up in the laboratory with a PME miniDOT dissolved oxygen and temperature logger, a water pump (BARST) for circulation and a portable immersion heater. For all experiments, to avoid a confounding effect of initial oxygen saturation, all

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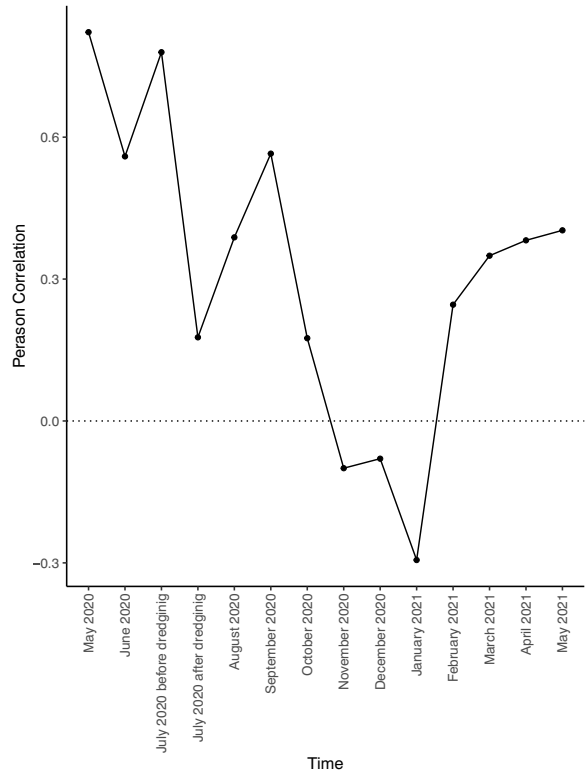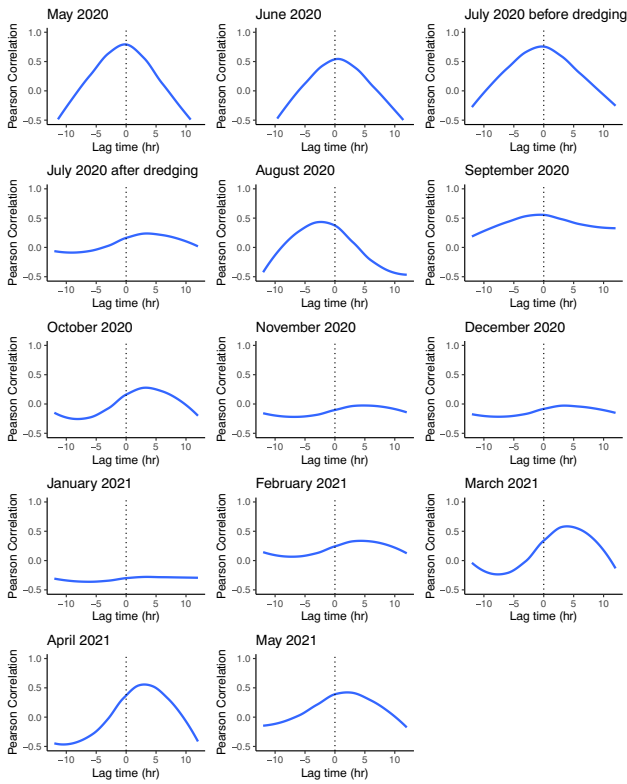
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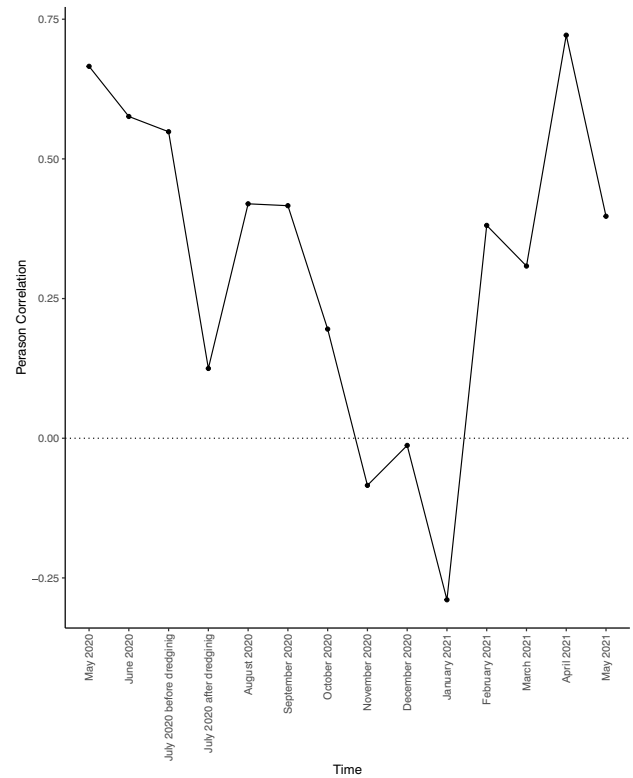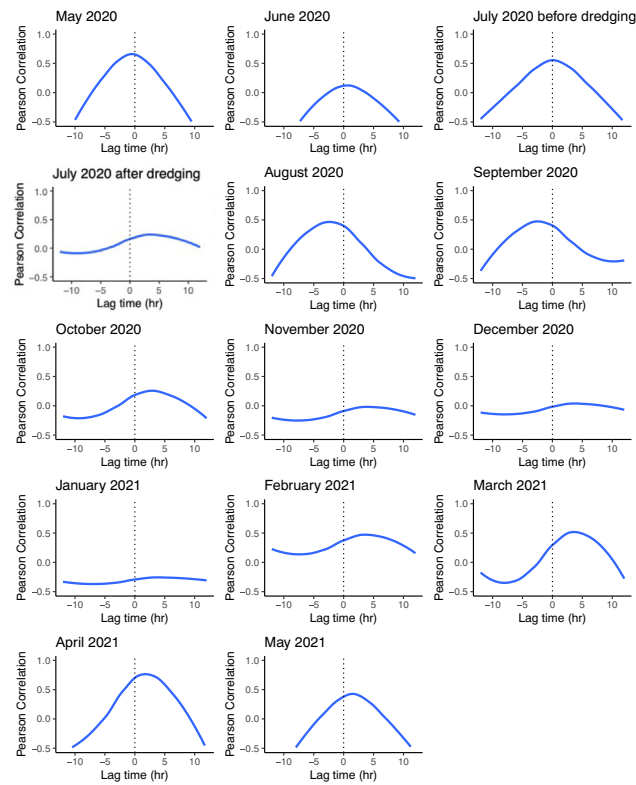
experimental tanks were filled with channel water collected at 100 % oxygen saturation. Animals were transferred to experimental tanks and temperature ramps were started immediately, and warmed at 1 °C per hour initiating at the environmental water temperature (approximately 12–14 °C) up to 42 °C (following Booth et al., 2021), in the experiment with highest lethal temperature. This ramping rate reflects the natural daily warming rate during the warmest part of the year in the study channels. Water temperature was controlled with a temperature controller (Inkbird ITC-308). pH was monitored and oxygen was checked every hour to ensure levels did not drop below 90 % saturation. Mortality was assessed at 0.5 °C intervals (i.e. every 30 min) by assessing animals for movement and attachment to the tank. For leeches and snails that did not remain attached, death was confirmed by gentle prodding (leeches) or checking that the operculum could not be kept closed and there was no response from an external stimulus (snails, Miranda et al., 2010). Amphipods ceased all movement and did not respond to any stimulus when dead (Baldanzi et al., 2015). Mussels were gently tapped to test for the ability to keep the shell valves closed and that there was no response of the exposed mantle tissues to any stimulus (Rajagopal et al., 2003). All animals were examined under a portable stereo microscope. Dead individuals were immediately removed from the experimental tank and the experiments were terminated when all individuals had died (individuals with open valves and non-reactive to a tactile stimulus). Temperature-induced mortality rate and LT50 were measured following Giomi et al. (2016). Animal mortality under conditions of oxygen supersaturation and normoxia was also assessed, with no prior acclimation to fluctuation regimes, in a second experiment. Groups of 10 individuals from the four study species were subjected to the same thermal ramp but under conditions of 100 % oxygen saturation or oxygen supersaturation (160 ± 3 %), achieved with the addition of hornwort and an LED growth lamp (KINGBO, 400–760 nm full spectrum) to promote photosynthesis (light intensity was recorded as 5000 lx +/− 500). To avoid other confounding effects of the growth lamp, the lamp was also used for the 100 % oxygen saturation treatment. Animal mortality was assessed as above.

2.3. Data analysis

All analyses were performed in R version 4.2.1 (R Core Team, 2017). Raw data from the minidot oxygen loggers were downloaded and used as input for the environmental time-series analysis. Data were visualised using the package ggplot2 (Wickham, 2011). To visualise the oxygen and temperature trend in Fig. 1 we used the local polynomial regression (loess). Time series of water temperature and dissolved oxygen concentration were detrended by subtracting a 24 h central moving average to focus on daily rather than long-term variability. Cross-correlations between oxygen and temperature from the detrended time series were calculated using the Pearson correlation coefficient and different time lags using the CCF function in R.

We computed the continuous cross wavelet analysis (R package WaveletComp; Roesch and Schmidbauer, 2018) to identify the dominant periodicities of the covariance of temperature and oxygen time-series data and determine and visually display whether these were regular, periodic oscillations or isolated or intermittent bursts. To compute the continuous wavelet power spectrum (Grinsted et al., 2004) of the temperature and dissolved oxygen time-series, significance levels were assessed against a null hypothesis of red noise. This method allowed us to calculate the wavelet power spectrum, which describes the variability in water temperature occurring at each period or frequency and time point, for each of the time series recorded in both channels analysed. This analysis allowed us to determine the relative contribution of variability at each period or frequency to the total variation observed in each temperature and oxygen time series. Cross wavelet analysis is a technique that was developed in

the 1980s for the simultaneous analysis of two signals, in this case temperature and oxygen, in the frequency and time domains (see for example De Boer and Karemaker, 2019). In simpler terms, this analysis tests for the cyclical periodicity of two variables (environmental parameters in our case) and the correlation of these periodicities. Crosswavelet analysis was performed on oxygen and temperature time series data that were first detrended to eliminate the trend component of the time series (where a trend refers to a change in the mean over time) and to avoid distortion in the detection of the synchronicity of the two variables (see for example Giomi et al., 2019).

We calculated the median lethal temperature for each species, using the function 'dose' in the MASS library (Ripley et al., 2013), for the different groups exposed to 'oxygen fluctuation' and 'constant oxygen' for the three acclimation periods: 2 days, 10 days and 28 days. We ran a generalized linear model with the 'glm' function using a binomial distribution (response variable: 'dead' or 'alive') to fit a curve to temperature and mortality for both treatments for each acclimation period. To test the effect of oxygen saturation (hyperoxia or normoxia) on the freshwater species thermal tolerance (measured as LT50) of the four species, we used the drc package in R (Ritz et al., 2015).

3. Results

3.1. Environmental time-series analysis

Both drainage channels were characterised by the same environmental variability throughout the period analysed (Fig. 1; Supplementary Fig. 2). The time series data revealed high daily oxygen fluctuation throughout the year, ranging from close to 0 % saturation (during the night) and up to >300 % saturation (during the day), though the highest density of oxygen saturation values was around 80–90 % saturation. Temperature followed the expected seasonal variation with the lowest temperatures recorded in winter and reaching >30 °C in the summer (Supplementary Fig. 2). The highest density of temperature readings fell below 10 °C with two density peaks between 17 and 22 °C that occurred during warm periods.

For both channels, the monthly cross-correlation analysis revealed that the relationship between the detrended time series of water temperature and dissolved oxygen concentration was in phase (Fig. 2) in the warmer part of the year (i.e. Spring and Summer), while synchronicity was lost in the colder months. Interestingly, the loss of synchronicity also occurred after the dredging event, removing the correlation between temperature and oxygen variation (Fig. 2). After the dredging event during the early summer in 2020, and in winter, dissolved oxygen variability in the channels was dramatically reduced, though temperature continued to fluctuate as expected (Fig. 1). We therefore used a cross-wavelet analysis to explore whether the cross correlation between oxygen and temperature was altered; the significant correlation between oxygen and temperature, most significant at a 24 h (or daily) resolution, was lost in the period immediately following the dredging (Fig. 3).

3.2. Animal LT50 experiments

At 2 days of acclimation to oxygen treatment, lethal temperatures did not significantly differ between treatments for any species (Supplementary Table 1, Fig. 4). After 10 days of acclimation, a significant effect of oxygen treatment was observed for amphipods (2.1 °C, $GLM_{1, 58}$ = 5.1, P < 0.05), leeches (4.2 °C, $GLM_{1, 58}$ = 6.42, P < 0.05) and mussels (1.5 °C, $GLM_{1, 58}$ = 5.46, P < 0.05). For these three groups, animals exposed to 'oxygen fluctuation' for a period of 10 days had significantly

Fig. 2. Monthly cross-correlations between water temperature and lagged dissolved oxygen concentration, hourly averaged and detrended to analyse the oscillations during the day, show that the two time-series are almost in phase (the Pearson correlation coefficient is highest nearby zero lag). The effect of dredging on the phase of oxygen and temperature is disruptive: in the period after dredging, the two variables lose synchronicity and no significant correlations were detected. This also occurs during the winter when temperature is constantly low and oxygen production is controlled by other factors, i.e. PAR availability.
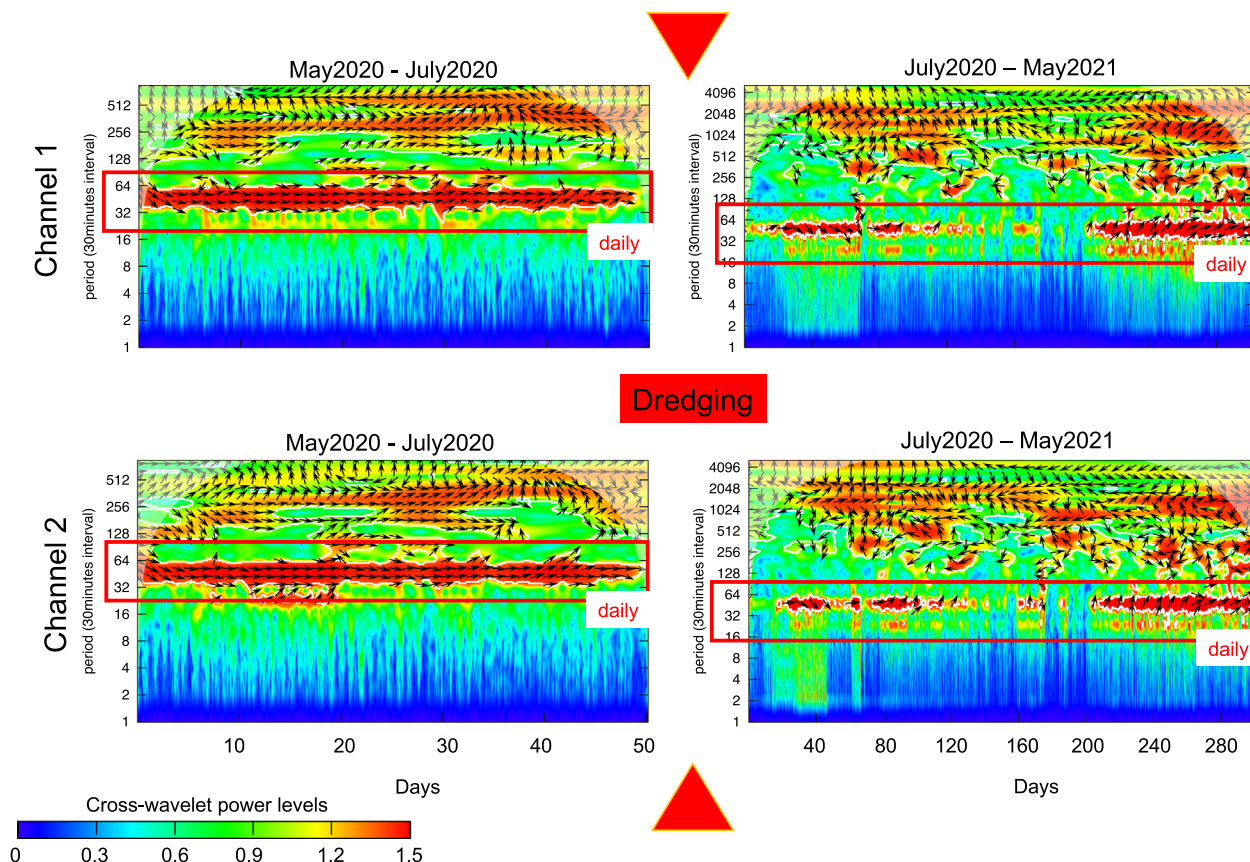
Fig. 3. Cross-wavelet transformation of detrended oxygen and temperature time-series data. The x-axis represents time (in days), while the y-axis represents the periodicity (in half hours). Cross-wavelet power quantifies the relative contribution of variation in water oxygen and temperature at each period to the total variance over time. High power is represented by warm colours and low power is represented by cool colours. Black contours designate regions of significantly high temporal variation compared to a null model (red noise). Values outside of the cone of influence—the bolder colour cone shape—are less reliable due to edge effects. Thick contour indicates the 5 % significance level against red noise. Arrows indicate the time-series relative phase (pointing left: the two time-series are in antiphase; pointing right: in-phase). Red boxes highlight the significant periodicity of co-variation of oxygen and temperature.

higher LT50 values than those exposed to 'constant oxygen' (Supplementary Table 1, Fig. 4). After 28 days of acclimation to 'oxygen fluctuation', all animals groups had significantly higher LT50 values than those exposed to 'constant oxygen' (amphipods: 3.7 °C, $GLM_{1, 58}$ = 6.87, P < 0.01; snails: 0.3 °C, $GLM_{1, 58}$ = 4.24, P < 0.05; mussels: 2.7 °C, $GLM_{1, 58}$ = 11.9, P < 0.001 and leeches: 2.8 °C, $GLM_{1, 58}$ = 182.57, P < 0.0001; Supplementary Table 1, Fig. 4).

All the species showed a significant increase of 1 to 2 °C in LT50 when exposed to conditions of oxygen supersaturation during the thermal ramp (Supplementary Fig. 5).

4. Discussion

In this study, we show that primary producers in freshwater agricultural channels provide an ecosystem service (Chen et al., 2022), by shaping a natural pattern of diel oxygen fluctuation, that in turn affects animal physiology. The removal (i.e. dredging) of these aquatic plants, therefore, disrupts the ecosystem, and the regrowth of the primary producers restores the cycle of daytime oxygen supersaturation and night time oxygen undersaturation.

Freshwater channels bordering agricultural land harbour developed beds of vegetation composed of algae, vascular plants (such as hornworts), phytoplankton and microphytobenthos, which all contribute to diel cycling of oxygen fluctuation. In this study, we confirmed that dissolved oxygen levels in these channels show significant natural fluctuation on a daily scale. During the day, when solar radiation is available and temperature

increases, photosynthesis enriches the water with oxygen, creating conditions of supersaturation while removing carbon dioxide. Conversely, in the night oxygen can become depleted to the point of hypoxia, particularly in warmer periods due to respiration by large standing stocks of primary producers and heterotrophs. The synchronous phase of high oxygenation and high temperature is, therefore, an important feature of freshwater habitats and has been found in tropical and in temperate environments (Andersen et al., 2017; Giomi et al., 2019). Andersen et al. (2017) showed that, in shallow vegetated lakes, submerged vegetation reduced both light and turbulence, promoting stratification of the water column with warm surface and cold bottom water; this, in turn, resulted in daytime oxygen accumulation and carbon dioxide depletion in the surface water due to photosynthesis, while in bottom waters respiration dominated during the day and oxygen was re-introduced through convective mixing at night.

Macrophytes induce considerable changes in water properties, for example, altering hydrodynamics (Desmet et al., 2011), and uncontrolled growth (as is frequent in agricultural areas due to fertiliser run-off) can lead to a dramatic reduction in water flow, therefore reducing the drainage capability of the channels. Intensive management, in the form of regular dredging to remove vegetation and sediment (secondarily also removing associated animals and microbes), is therefore needed. In this study we observed the disrupting effect of dredging on natural patterns of oxygen cycling, which persisted for the subsequent months and the pre-dredging levels of fluctuation were only regained the following spring (Fig. 1). This can be linked to the recruitment of primary producers and to the reduction in the turbidity created by dredging. The loss of synchronicity of oxygen
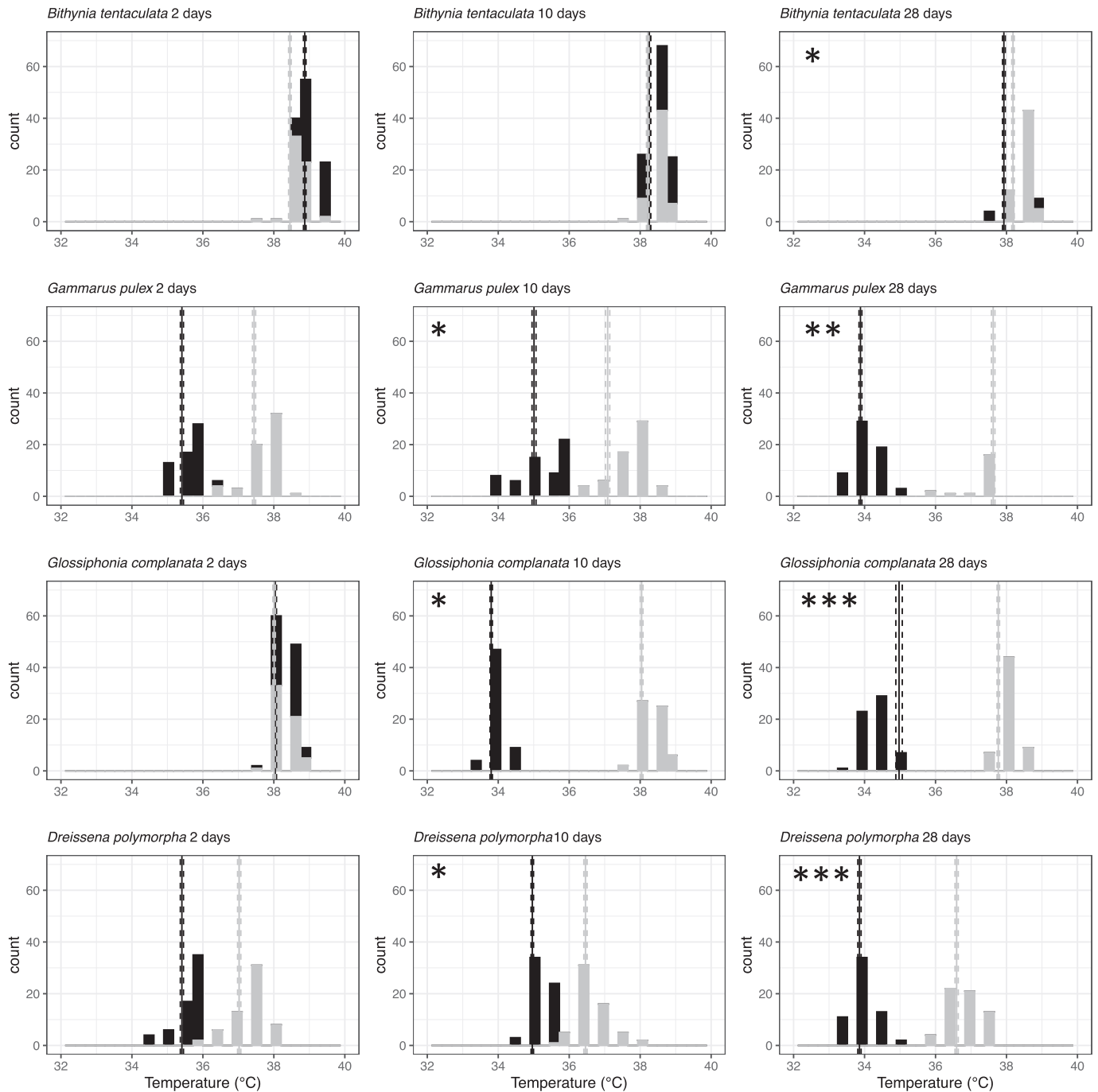
Fig. 4. Mortality density plots of amphipods (*Gammarus pulex*), mussels (*Dreissena polymorpha*), leeches (*Glossiphonia complanata*) and snails (*Bithynia tentaculata*) after (A) 2, (B) 10, and (C) 28 days of acclimation to 'oxygen fluctuation' (blue) and 'constant oxygen' (red). Solid lines indicate LT50 values and dotted lines represent LT50 +/−SE. * represents level of statistical difference between treatments.

and temperature in the period after dredging also occurs during the winter when temperature is constantly low and oxygen production is controlled by other factors, i.e. PAR availability.

The communities living in association with high biomass aquatic plant communities are exposed to diel cyclical fluctuation in oxygen levels and, although the mechanisms are not yet known, recent studies indicate that this can lead to extended thermal tolerance for marine ectotherms, allowing them to sustain increased metabolic demands under warming (Blewett et al., 2022; Booth et al., 2021; Giomi et al., 2019). In particular, marine ectotherms that experience high temperatures during the day have a higher metabolic demand for oxygen; oxygen supersaturation caused by

photosynthesising plants provides the oxygen needed by these animals, allowing them to extend their thermal tolerance during the day (Booth et al., 2021; Giomi et al., 2019). As shown in our current study, like shallow water marine ecosystems, freshwater agricultural channels are highly productive aquatic habitats that represent very dynamic environments, and their associated animal communities experience daily exposure to fluctuating oxygen. Our study showed that that the removal of oxygen fluctuation, through the removal of primary producers, can negatively affect the thermal tolerance of aquatic animals. We found that freshwater amphipods, leeches and mussels maintained in the absence of (or with very limited) oxygen fluctuation for 10 days or longer significantly decreased their
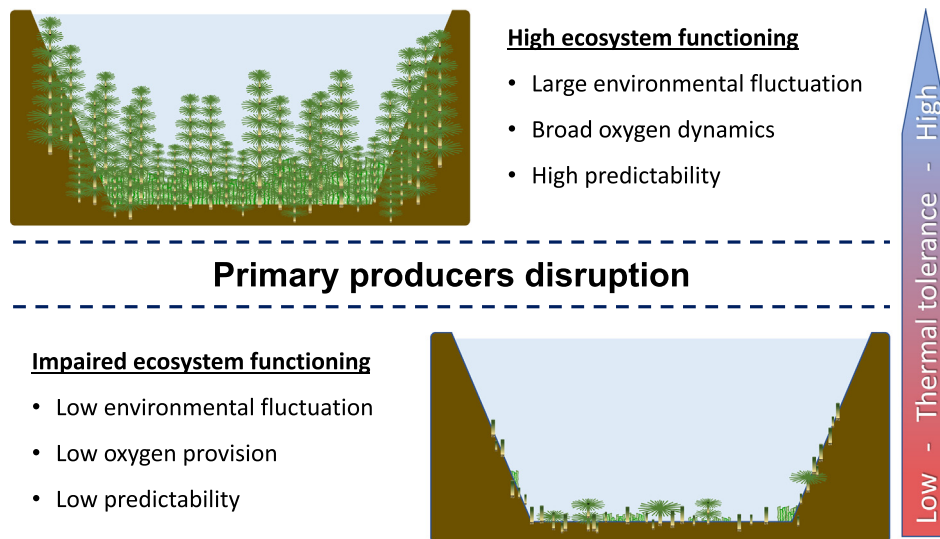
Fig. 5. Conceptual summary of the study. Healthy and productive aquatic vegetation determines continuous and constant oxygen fluctuation, providing animals with daily supersaturation and night time undersaturation during the hottest period of the year. Disruption of these vegetated ecosystems can lead to a loss of oxygen and temperature synchronicity similar to colder periods (i.e. winter).

tolerance of high temperatures. Freshwater snails showed the same response, but this required longer exposure (28 days). The effect of oxygen availability, and in particular of oxygen supersaturation, on heat tolerance in freshwater peracarid crustaceans was examined by Verberk et al. (2018). Although heat tolerance differed among the species studied, the authors found a significant effect of supersaturation on the species' thermal limits measured as lethal temperature, and they found that exposure to oxygen supersaturation increased the thermal tolerance of some species. The increased thermal performance of freshwater ectotherms in response to oxygen supersaturation highlights the role of oxygen availability in shaping the physiological responses of aquatic organisms (Fusi et al., 2021). For example, assessing metabolite profiles of stonefly nymphs under different levels of oxygen availability, Verberk et al. (2013) found thermal tolerance to be higher under conditions of hyperoxia and observed a decrease in the ability of nymphs to survive heat stress under conditions of normoxia, with the onset of metabolic features such as anaerobic metabolism being more pronounced under hypoxia. Certain species are undoubtedly more likely to profit from the benefits of hyperoxia, such as those with a fully aquatic gas exchange system e.g. the freshwater mollusc *Bithynia* (Koopman et al., 2016). Yet, exposure to hypoxia can also be critically involved in the acclimatory response of some animals, as shown in nymphs of the damselfly *Calopteryx virgo* inhabiting freshwater environments. Improved thermal tolerance was not observed under hyperoxia, but instead critical thermal limits were enhanced when nymphs had previously been exposed to hypoxia suggesting that protective heat shock proteins change an organism's capacity to supply oxygen to tissues or alter the oxygen demands of these tissues themselves (Verberk and Calosi, 2012). Gastropods have a high level of well-studied physiological plasticity, which may explain their lack of response to oxygen treatments after ten days of acclimation. For example, the rocky-shore eulittoral-fringe snail (*Echinolittorina malaccana*) is able to depress its metabolism when experiencing prolonged emersion and fluctuating high temperatures (Marshall and Mcquaid, 2010). The removal of natural oxygen fluctuation has also been shown to affect the metabolic performance of the coastal ectotherms the velvet crab (*Necora puber*) and the blue mussel (*Mytilus edulis*) (Booth et al., 2021).

When measuring the thermal tolerance of aquatic species, a ramp method is likely to measure an endpoint higher than the highest temperature recorded in the site where the species lives. For example, when measuring the upper lethal temperature of the amphipod *Talorchestria capensis* in Port Nolloth in South Africa, Baldanzi et al. (2015) recorded a value of 39 °C even though the maximum average temperature in the location is

about 20 °C (i.e. a difference of 19 °C between the environmental temperature and physiological response). Rather than focussing on the absolute value of the endpoint of thermal tolerance, we should interpret these data as the capability of the species to alter its thermal tolerance when exposed to predictable and relevant environmental fluctuation, rather than in the absence of fluctuation (e.g. Booth et al., 2021) or the presence of stochastic fluctuation (e.g. Bitter et al., 2021). The role of exposure to fluctuating environments in inducing change in physiological plasticity has been both theorized (Botero et al., 2014) and demonstrated using fishes as model aquatic species (Morgan et al., 2022). The results obtained are corroborated by the findings in our paper, unveiling the role of environmental fluctuation in shaping the thermal plasticity of aquatic animals. In the particular case of our study, the continuous daily exposure to oxygen fluctuation, including reaching supersaturation levels, can change the physiological response of the study species by triggering physiological processes that modify their performance and resistance. Recently, McArley et al. (2022) demonstrated that exposure to oxygen supersaturation in fishes exerts a protective effect on arterial oxygenation, maintaining a 22 % higher arterial O_2 content post-exhaustive exercise (thereby allowing a higher arterial-venous O_2 content difference) compared to the arterial O_2 partial pressure, O_2 saturation of haemoglobin under conditions of normal oxygen saturation. Our findings also support the hypothesis that a predictable environment can determine the onset of a feedforward response. This allows species to prepare an appropriate response and is defined by Bernhardt et al. (2020, p. 3) as "proactive and allow for living systems to anticipate changes in the environment before they have occurred". Therefore, interference with those factors that determine environmental fluctuations can disrupt species physiological responses, reducing their ability to adapt to environmental changes that are more likely to happen in the context of climate change. This study also highlights that other environmental fluctuations (such as light, pH, and CO2, among others) that can shape the adaptation of aquatic species in a fast-changing world should be investigated.

The importance of fluctuation in shaping animal physiology and plasticity is significant for an understanding of the degree of the plasticity of animal physiological responses to environmental drivers (Huey and Bennett, 1990). The magnitude of diel oxygen variability in these systems exposes animals to hypoxia during the night and oxygen supersaturation (hyperoxia) during the day, shaping the metabolic responses of resident aquatic animals (Giomi et al., 2019). Daily fluctuation in temperature and in oxygen, driven by photosynthesis, results in relatively predictable variability that shapes the adaptation of the associated aquatic species. Bitter

et al. (2021) showed that predictable pH fluctuations enhance the metabolic response of aquatic animals (the Mediterranean mussel, *Mytilus galloprovincialis*), while a decrease in predictability depresses phenotypic plasticity within natural populations, supporting recent theoretical studies (Bonamour et al., 2019; Botero et al., 2014). Similarly, predictable variation of oxygen in aquatic environments has been shown to significantly extend the physiological tolerances of animals to warming (Booth et al., 2021; Giomi et al., 2019; Vargas et al., 2022). For example, exposure to oxygen supersaturation enhances the ability of aquatic ectotherms to maintain an aerobic metabolism even at low oxygen partial pressure, resulting in a significantly lower critical PO_2 (Giomi et al., 2019). This may explain the ability of aquatic organisms to cope with periods of night time hypoxia, when the respiration of primary producers lowers the dissolved oxygen levels in the water (Blewett et al., 2022). The positive effect on animal heat tolerance during exposure to hyperoxia in our experiments confirms hyperoxia to be one of the major drivers in shaping species thermal tolerance and supports the idea that absence of oxygen fluctuation can lead to a reduction of the thermal breadth of the species. However, it has also been demonstrated that repeated exposure to night time oxygen undersaturation, even to the point of hypoxia, can improve the thermal tolerance of aquatic animals, showing that, in general, a fluctuating environment can determine a species' acclimation and modulate oxygen-dependent thermal tolerance (Koopman et al., 2016). It is worth mentioning that different factors other than oxygen availability play a role in ectotherm thermal tolerance, especially when exposure to excessive heat occurs on a chronic or recurrent basis rather than an acute event (Ekström et al., 2016; Magozzi and Calosi, 2015; Rezende et al., 2014; Verberk et al., 2016). However, our case study conforms with a chronic thermal tolerance assay since it implements highly relevant ecological conditions in the field and tests the capacity to sustain a wide tolerance window over a large period of time. Our results are in agreement with the prediction that ample environmental fluctuation drives the thermal tolerance of the aquatic community and further suggest a role of oxygen availability in this mechanism.

Our study shows that hyperoxic conditions can improve the heat tolerance of the study species so that the removal of a period of enhanced oxygen levels during the day can reduce their heat tolerance. Importantly, this disruption happens during the warmest part of the year, enhancing this stress. However, our results show that a period of 28 days was necessary to alter animal response to thermal stress significantly, which means that the period required by the system to recover from dredging could be a time of physiological stress that may ultimately affect species assemblages.

Our data show that the thermal tolerance of freshwater fauna is directly affected by the activities of other species, in this case, oxygen production and consumption by photosynthesising organisms and, indirectly, negatively affected by human disturbance to the system. Environmental fluctuation imposed by aquatic primary producers is an ecosystem service that can protect animals from thermal stress, and potentially other stresses related to climate change. Understanding the effects of current and future warming requires fine-scale assessments of environmental parameters, since broad-scale analyses can mask local patterns (Wethey and Lima, 2012). At this stage, climate change models are unable to capture local processes that may enhance species resistance to the impacts of global warming, therefore downscaling observations and data acquisition should be prioritised. We suggest that protection of primary producers may be a potential solution to offer, in turn, protection to aquatic animals against climate warming (Fig. 5).

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CRediT authorship contribution statement

J.M. Booth: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **F. Giomi:** Conceptualization, Methodology, Writing – review & editing. **D. Daffonchio:** Resources, Writing – review & editing. **C.D. McQuaid:** Resources, Writing – review & editing, Supervision, Funding acquisition. **M. Fusi:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Visualization.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162135.

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