Effects of pollution-induced changes in oxygen conditions scaling up from individuals to ecosystems in a tropical river network

Eriksen, Tor Erik; Jacobsen, Dean; Demars, Benoît O. L.; Brittain, John E.; Søli, Geir; Friberg, Nikolai

Published in:
Science of the Total Environment

DOI:
10.1016/j.scitotenv.2021.151958

Publication date:
2022

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY

Citation for published version (APA):
Eriksen, T. E., Jacobsen, D., Demars, B. O. L., Brittain, J. E., Søli, G., & Friberg, N. (2022). Effects of pollution-induced changes in oxygen conditions scaling up from individuals to ecosystems in a tropical river network. Science of the Total Environment, 814, [151958]. https://doi.org/10.1016/j.scitotenv.2021.151958
Effects of pollution-induced changes in oxygen conditions scaling up from individuals to ecosystems in a tropical river network

Tor Erik Eriksen a,b,⁎, Dean Jacobsen c, Benoît O.L. Demars a, John E. Brittain b,e, Geir Soli b, Nikolai Friberg a,c,d

a Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, 0349 Oslo, Norway
b Natural History Museum, University of Oslo, P O Box 1172 Blindern, 0318 Oslo, Norway
c Freshwater Biological Section, Dept. of Biology, University of Copenhagen, Copenhagen, Denmark
d water@leeds, University of Leeds, School of Geography, Leeds, UK
e Norwegian Water Resources & Energy Directorate (NVE), P O Box 5091 Majorstuen, 0301 Oslo, Norway

HIGHLIGHTS

• O2 regulation capacity (ORC) of tropical aquatic ectotherms was studied in Myanmar.
• River reach diel O2 deficits were investigated using oxygen loggers.
• Eutrophication/organic pollution induced O2 deficits in rivers.
• Closed-chamber studies used to derive ORC for riverine macroinvertebrates (MI)
• Individual ORC scaled up to ecosystem MI composition mirroring O2 deficits.

ABSTRACT

Anthropogenic inputs of nutrients and organic matter are common in tropical lowland rivers while little is known about the pollution-induced changes in oxygen availability and respiratory performance of ectotherms in these high temperature systems. We investigated the effects of agriculture and urban land-use on river water oxygen levels (diel measurements), decomposition rates (Wettex) and macroinvertebrate assemblages (field studies), as well as the oxy-regulatory capacity of eight riverine macroinvertebrate taxa (laboratory study) from a tropical lowland river network in Myanmar. The highest decomposition rates (0.1–5.5 mg Wettex degree day−1) and oxygen stress (≤91% saturation deficits) were found in reaches draining degraded catchments with elevated concentrations of nutrients. All individual macroinvertebrate taxa investigated were to some extent able to regulate their respiration when placed under oxygen stress in the laboratory (regulation value of 0.74–0.89). The oxy-regulation capacity of macroinvertebrate assemblages in the river network were, as predicted, inversely related to diel oxygen stress (maximum deficit; lm, R2 = 0.69), where taxonomic richness and pollution sensitivity (ASPT metric) also declined sharply (lm, R2 ≥ 0.79). Our study shows that eutrophication and organic pollution induce oxygen deficits in tropical rivers but stimulate decomposition rates, which may further deplete oxygen levels. Furthermore, macroinvertebrate oxy-regulatory capacity predicts assemblage composition along gradients in oxygen stress at the ecosystem level. Our findings suggest that tropical lowland river systems could be highly sensitive to pollution by nutrients and organic matter leading to substantial impacts on ectotherm community composition and ecosystem functioning.
1. Introduction

In aquatic ecosystems, oxygen concentration and partial pressure (pO₂) can vary widely through photosynthesis, respiration, temperature and elevation or owing to pollution events (Demars et al., 2011; Jacobsen and Brodersen, 2008; Rostgaard and Jacobsen, 2005). Oxygen is poorly soluble in water and oxygen diffusion is about 8000 times slower in water than in air at 25 °C (Cussler, 1997). Aquatic animals have evolved various strategies to obtain the oxygen needed for aerobic respiration, including uptake across body surfaces, plastrons and air breathing (Maina, 2002). Of those strategies, to obtain the oxygen needed for aerobic respiration, including uptake across body surfaces, plastrons and air breathing (Maina, 2002). Of those strategies, oxy-conformers (i.e., their metabolism track oxygen availability down to a critically low oxygen partial pressure or concentration (pO₂crit), whereas others can to some extent regulate respiration despite diminishing external oxygen availability (oxy-regulators). Aquatic ectotherms therefore show contrasting capabilities to withstand periods of oxygen depletion to maintain their aerobic metabolism. Oxygen availability and macroinvertebrate physiological abilities have been well studied from temperate surface waters (Eriksen, 1963; Nagell, 1973; Nebeker et al., 1996), and to some extent tropical highland rivers (Jacobsen, 2003; Jacobsen and Marin, 2008). However, tropical lowland rivers remain understudied (Connolly et al., 2004).

Tropical biomes are increasingly impacted by land use changes, pollution and global warming (Habell et al., 2019; Newbold et al., 2020; Tordoff et al., 2020), leading to environmental degradation of many river ecosystems and putting further pressure on aquatic communities in need of oxygen for their respiration (Wen et al., 2017). Excessive inputs of nutrients and organic matter to rivers, e.g. from agriculture field and domestic wastewater run-offs, may increase primary production, speed up decompositions rates and lead to oxygen saturation deficits (Hynes, 1966; Rosemond et al., 2015; Saltarelle et al., 2018), most notably at high temperatures, due to higher respiration rates and during darkness due to lack of photosynthesis. Tropical lowland macroinvertebrates are likely more susceptible to pollution and warming events that increase respiration rates and the demand for oxygen, compared to lowland rivers in cooler climates, because the basal metabolic rate of ectotherms increases non-linearly with increasing water temperatures (Dillon et al., 2010). Tropical aquatic macroinvertebrates are therefore especially exposed to oxygen depletion.

We hypothesise that river reaches with high concentrations of nutrients, draining catchments dominated by urban and agricultural land-use, have high decomposition rates and oxygen stress, and are dominated by macroinvertebrates possessing the highest oxygen regulatory capacities. We also expected a loss of taxonomic diversity and oxygen sensitivity in assemblages mediated through physiological constraints of individual taxa. We tested these hypotheses in a tropical lowland river network in Myanmar, representative of other lowland areas of Indo-Burma, with surface waters increasingly impacted by intensified land-use pressures, dominated by deforestation, agriculture and urbanization (Tordoff et al., 2020). We investigated the pollution-induced impacts on diel river oxygen conditions, decompositions rates of an introduced organic assay (Wettex) and macroinvertebrate assemblage (field studies). In the laboratory, we determined the oxygen-regulatory capacity of eight riverine macroinvertebrate taxa common in the study area and derived the oxygen-regulation capacity at the assemblage level through the relative abundances of individual taxa. Finally, we related the assemblage oxygen-regulation capacity to ecosystem (reach) level oxygen stress.

2. Materials and methods

2.1. Field studies

Our study was conducted in the lower part of the Sittaung River basin with the Bago District as our focus (Fig. S1). The Bago District has a population density of ~133 inhabitants per km² with 26.2% living in urban areas (MOIP, 2015). The climate in this region is tropical monsoon (Am following the Köppen climate classification) with distinct wet and dry seasons. The dry season lasts from December to April and the wet season from May to October. December and January are the coldest months and April the warmest, with mean temperatures ranging from 24 °C to 31 °C (Haruyama and Hlaing, 2013). The average annual precipitation was 3185 mm and 2746 mm at Bago City and Zaungu (shown in Fig. S1), respectively (Shrestha and Htut, 2016). There are several pressures acting on river networks in this area with large forested areas being converted to agricultural fields (rice, corn, peanut, sesame, chilli, pigeon pea and vegetables), rubber plantations, roads and other infrastructure at an increasing rate (Eriksen et al., 2021b). Some rivers are also impacted by disturbance from grazing livestock. These impacts lead to higher water temperatures, potentially increased erosion and diffuse pollution loads that influence the aquatic environment negatively through excess sedimentation and nutrients. Most of the population in this region are not connected to a sewer system (~94%), and there is frequent use of water sealed pit latrines (~74%) and ditches with direct discharge to rivers. Traditional pit latrines and buckets are used by ~14% of the population and 10% do not use any toilet (MOIP, 2015). Thus, faecal pollution and other household wastewater enter the river network in populated areas, with the major input coming from the Bago City area and other settlements (Eriksen et al., 2017). There is no heavy industry in this industry and the heavy metal pollution is low (Eriksen et al., 2021b). The sampling sites were at lowland altitudes (<75 m a.s.l.), dominated by sandy substrates (Eriksen et al., 2021b), moderate water current velocities (0.3 ± 0.1 SD m/s) and water depths (20.1 ± 9.8 SD cm; Table 1). In least impacted conditions, the substrate cover had moderate amounts of allochthonous material (tress, twigs and leaves) with some algae, although no macrophytes were present (Eriksen et al., 2021b). Samples were collected from a total of nine lowland rivers and 14 sampling locations, with some sites representing least disturbed and impacted conditions in the same river. Least disturbed sites were designated qualitatively based on no or minimal visual impacts during surveys (well-developed riparian zone, no visible sewage inputs and intact quality of substrates) and having no or low human activities in their catchment, with likely impact on water quality, based on remote sensing by satellite images (see Eriksen et al., 2021b for details).

2.2. Environmental characteristics and degradation of river sites

Land-use at the catchment and reach level (500 m upstream site) was assigned to the three categories 1) forested, 2) cultivated (agriculture/plantations), and 3) converted to villages/urban development, and the assessment of percentage cover of each type for all study sites was undertaken by remote sensing in ArcGIS (version 10.1) using satellite images and open source landcover maps (see Eriksen et al., 2021b for details). From each sampling site, land-use types were quantified using a polygon function in ArcGIS and calculated as the sum of individual land-use type patches catchments and reach. Riparian vegetation was assessed using the MQI index following Rinaldi et al. (2013) by combining quality scores for function and extension of riparian vegetation (category F12 and F13). One water sample was collected when installing oxygen loggers and Wettex in the rivers and was analysed for total phosphorus (totP; NS-EN-ISO-6678:2004), total nitrogen (totN; NS-4743:1993) and alkalinity (alk; NS-EN-ISO-9963:1:1995). Samples for totP and totN were preserved by acidification immediately following collection (1% sulphuric acid, 4 M H₂SO₄) and analysed using accredited methods at the NIVA laboratory in Norway. Water flow velocities (m/s) at the sites were measured using an electromagnetic current meter (OTT MF Pro, Loveland, USA) near the substratum where the oxygen loggers were deployed.

2.3. Oxygen measurements

Diel oxygen concentrations and water temperature were measured through automatic logging for periods of 16–48 h at 13 of the 14 study sites, representing daylight and darkness oxygen conditions. These measurements were conducted during low flow conditions in the period...
November–March (2018–2020). At two of the sites (602T and MYST) oxygen was logged on two occasions, in March 2018 and November 2019, hence a total of 15 logging events. An optical oxygen logger, miniDOT, was used for the analysis. Microbial decomposition rates were measured at eight of the sampling sites to assess ecosystem function–absence at the family levels, except for Oligochaeta. The ASPT and EPTCO metrics have shown promising results in detecting environmental degradation in tropical rivers worldwide (Eriksen et al., 2021a), including the study area (Eriksen et al., 2021b). Most of the studied and identified specimens are kept in the collection of the Natural History Museum, University of Oslo (NHMO; see Supporting information).

### 2.5. Quantifying decomposition rates

Decomposition rates of an introduced organic material assay (Wettex) were measured at eight of the sampling sites to assess ecosystem functioning. The method represents a standardized and inexpensive way to assess decomposition rates in aquatic habitats and will relate to metabolic activity in terms of respiration related to breakdown rates of the Wettex cloth. Wettex® sponge cloths (manufactured by Vileda, Germany) are fully biodegradable, made from 30% cotton and 70% cellulose. Two sites were sampled twice, in 2017 and 2018, and mean decomposition rates for both years were used for the analysis. Microbial decomposition rates were measured by putting four pieces of Wettex (2.5 × 8.5 cm) into fine nylon mesh bags (12 × 7 cm; 250 μm), and coarse mesh bags made from plastic nettings (ca. 1 cm mesh), to infer the role of macroinvertebrates on decomposition rates (mass loss per unit time). From each site, five replicates of both sample types were put into the river, attached to two separate metal chains (~0.5 m) that were anchored to the river substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2). The mesh bags and temperature loggers (HOBO Pendant® Temperature/Light 64K; 10 min. logging intervals) were attached to a metal chain that was anchored horizontally on top of the substratum using tent pegs (Fig. S2).

### Table 1

Environmental characteristics of the sampling sites, catchment land use. Degradation of river sites are indicated by impacted (I) and least disturbed sites (LD). Data type collected are denoted by O = oxygen, M = macroinvertebrates, and W = Wettex. Years denote when oxygen data were collected.

| Site   | Year | Elevation (m) | River width (m) | Water depth (cm) | Water velocity substratum (m/s) | Catchment forested (%) | Catchment cultivated (%) | Catchment urban (%) | Degradation category | Data type collected |
|--------|------|---------------|-----------------|------------------|-------------------------------|------------------------|--------------------------|--------------------|---------------------|---------------------|
| REF1   | 2019 | 44            | 23              | 16               | 0.18                          | 99                     | 1                        | LD                 | OMW                 | O,M,W               |
| IMP2   | 2019 | 17            | 17              | 26               | 0.09                          | 34                     | 64                       | 2                  | I                   | OMW                 |
| IMP3   | 2019 | 45            | 24              | 12               | 0.12                          | 98                     | 2                        | LD                 | OMW                 | O,M,W               |
| MYST   | 2018 | 28            | 20              | 18               | 0.15                          | 64                     | 33                       | 2                  | I                   | OMW                 |
| 602T   | 2018 | 17            | 7               | 20               | 0.28                          | 79                     | 21                       | 1                  | OMW                 | O,M,W               |
| MYST   | 2019 | 17            | 7               | 16               | 0.09                          | 79                     | 21                       | 1                  | OMW                 | O,M,W               |
| 602T   | 2019 | 14            | 14              | 29               | 0.26                          | 71                     | 14                       | 15                 | I                   | OMW                 |
| REF6   | 2020 | 48            | 14              | 41               | 0.43                          | 99                     |                          |                    |                     | LD,OMW              |
| IMP4   | 2020 | 17            | 15              | 16               | 0.34                          | 71                     | 28                       | 1                  | I                   | OMW                 |
| 302T   | 2020 | 34            | 33              | 20               | 0.22                          | 87                     | 13                       |                    |                     | LD                   |
| 303T   | 2020 | 21            | 13              | 49               | 0.32                          | 94                     | 6                        |                    |                     | LD                   |
| 607T   | 2018 | 18            | 5               | 15               | 0.45                          | 62                     | 38                       |                    |                     | LD                   |
| 617T   | 2020 | 11            | 4               | 10               | 0.58                          | 50                     | 50                       |                    |                     | O                   |
| RES1   | 2018 | 72            | 5               | 10               | 0.18                          | 100                    |                          |                    |                     | LD                   |
| 606T   | 2016 | 15            | 3               | 15               | 0.30                          | 1                      | 99                       | 1                  | OMW                 | OMW                 |

* Only one spot measurement of oxygen was taken from this site.
The Wettex dry weight for each sample was measured prior to the studies using an oven (105 °C for 24 h) and cooled to room temperature in a desiccator. Each sample of Wettex (four pieces) had a mean dry weight of 2.26 g (standard deviation ± 0.09). Following collection, the samples were immediately conserved in 99% ethanol to inhibit decomposition. The Wettex was brought back to the laboratory and inspected using a stereoscopic microscope (Leica M205CC). Any invertebrates attached to the Wettex were removed and identified, and the Wettex was then carefully rinsed with water to remove any biofilm. Samples were then dried at 105 °C for 24 h, cooled to room temperature in a desiccator and weighted again. The Wettex was burned at 550 °C for 2 h to ensure removal of all organic material (NS-4764:1980), and weighted again to account for the weight of inorganic particles. Decomposition rates were calculated as the total weight of Wettex lost per sum degree-days for each mesh bag.

2.6. Laboratory respiration study

The respiration studies were conducted in Bago City, Myanmar, using facilities provided by the Bago Forest Department. Macroinvertebrates were collected from four river sites (Bago River and Mazin Chaung). These sampling sites reflected a gradient from low to high eutrophication/organic pollution, with average nutrient concentrations of 9–50 μg TP/L and 235–535 μg TN/L (n = 3), although there were no completely pristine sites sufficiently near our facilities. Our focus was to conduct respiration studies on a sample of common and abundant macroinvertebrate taxa in our study area, representing different functional feeding groups and morphological types (shown in the graphical abstract). We sampled eight taxa, seven insects and oneannelid: Ecnomus sp. (Ecnomidae, Trichoptera; predator and gatherer collector), Barbronia weberi (Blanchard, 1897; Saliidae, Hirudinea; predator). The studies were conducted over eight days by testing the oxygen-regulatory capacity of one taxon daily. Each day, the test organisms were collected from the same sampling site using a hand net and carefully put into water-filled plastic containers that were provided with some substrate (twigs, grass, leaves). The collected animals were brought back to the laboratory within 2 h. In the laboratory, prior to studies, animals were kept in water-filled plastic trays which were saturated with oxygen using an air pump and a diffuser stone. Similar sized animals were used for each of the studies when available to us. Any surplus test organisms stayed in the tray throughout the studies to check for mortality. It took approximately 30 min to set up and start the studies which lasted 2–6 h.

Respiration studies were conducted at 25 °Celsius, according to the “closed chamber” method. We used two sets of glass chambers: small with an average volume of 0.71 mL (standard deviation ± 0.02) and large 2.08 mL (±0.24), depending on the size of test organisms (MicroResp, Unisense, Denmark). The exact water volume of each chamber was calculated as the difference in mass when empty or water filled at 20 °C. Within each chamber, a false bottom was created by enclosing a glass-coated magnet stirrer in a plastic ring and on top a fine mesh netting. Prior to adding the animals, chambers were filled with fully saturated water that was filtered through a 0.45 μm mesh (Merck Milllex-HA) using a syringe. The water used for the chambers was collected from the outlet of a large reservoir (~4 km²) with no known settlements in the catchment (Mazin Dam). Water from the reservoir is mostly used for irrigation of crops during the dry season. Water from the reservoir is mainly used for irrigation of crops during the dry season. Water samples collected from the outlet on two occasions showed low conductivity (20 µS/cm), and low content of calcium (0.8 mg/L), total organic carbon (2.7 mg/L) and TP (9 µg/L). Individual test organisms were then carefully added to each of the chambers. The chambers were closed with glass stoppers with a capillary hole in the middle so that an optical oxygen micro-sensor with point diameter of 500 μm (Oxygen MicroOptode, MicroResp, Unisense, Denmark) could penetrate the respiratory chambers. The narrow liquid-filled capillary hole effectively prevented diffusion of oxygen into and out of the chamber. One chamber filled with water, but no animals inside, was included as a control. However, we did not do this for all the studies because we wanted to have as many test organisms as possible, and because we never observed a change that could be attributed to oxygen consumption. Each glass chamber was individually stirred in a rack with 8 replicates and stirring magnets revolving at 800 rpm. The rack was immersed into a water bath kept at 25 °C (±0.5 °C). Because we did not have the opportunity to use a powered thermo-regulated water bath, the cooling of water was done by adding small ice cubes into the water bath and heating by an aquarium heater (50 W thermocontrol, precision aquarium heater, EHEIM, Germany). Respiration was recorded directly via the Unisense MicroOptode meter connected to a PC with the SensorTrace RATE software (Unisense, Denmark). Calibration of 0 and 100% oxygen saturation was done in the Rate software by readings in air-saturated water (100%) and water made anoxic (0%) by adding sodium dithionite (Na2S2O4), adjusted to air humidity at the time of calibration. As the air pressure and water temperature was not entirely constant, sometimes leading to negative oxygen readings at the end of the studies, a re-calibration was done by Unisense DK following the studies to ensure the best possible comparability between the test organisms. For each taxon, measurements were performed on four to eight individuals, one individual per chamber. The oxygen sensor was adjusted to a fixed position so that the tip protruded 1–2 mm below the capillary hole in the lid of the chamber. The oxygen saturation of the water in each chamber was measured by introducing the oxygen microelectrode and making 15–20 spot measurements. It took a little more than 1 min to do measurements per chamber, i.e., with a setup using eight chambers, it took a little more than 7 min to revisit each chamber for additional measurements. Each study lasted from 2 to 6 h, depending on how quickly the animals consumed the available oxygen. Following the studies, animals were placed individually in tin foils and frozen, brought to the lab in Norway, and dried at 105 °C for 24 h to measure individual dry matter (DM; shown in table S3) weights (SE2-F Microbalance, Sartorius, Germany).

2.7. Analyzing and modeling the respiration curves

The measurements from the individual organisms produced a respiration regression curve based on their respiratory performance, i.e., oxygen consumption was related to diminishing concentration of oxygen. Because metabolic rate (oxygen consumption) varies with body mass (Schmidt-Nielsen, 1984), the measured respiration rates were converted to mass specific respiration rates (MSRR) by dividing the rates of oxygen consumption with the DM of the specimen raised to the 2/3 power based on the assumption that the diffusion of gases through body tissues was controlled by the surface to volume ratio (Dodds et al., 2001). The use of the 2/3 exponent was supported by fitting a power function to the plot of O2 consumption as a function of dry weight based on the initial oxygen consumption rates in the study. Oxygen-regulator capacity (R) was quantified as the integrated sum of standard values (the regression line) across the mass-specific respiration rate (MSRR) divided by same area of a theoretically perfect oxygen-regulator (a horizontal line at maximum uptake), calculated from 0 to 200 mmol/L for all taxa (Lencioni et al., 2008).

2.8. Data exploration and statistical analyses

Data exploration and analyses were done using R version 4.0.2 (R Core Team, 2020). Respiration curves (local regression; LOESS) were drawn using the R package ggplot2 (Wickham, 2009). A principal component analysis (PCA) was performed on the collected environmental variables (water chemistry, land-use, riparian vegetation, sewage/cattle, altitude and water velocity) using the built-in R function prcomp. The data were

T.E. Eriksen et al. Science of the Total Environment 814 (2022) 151958
centred and scaled for the analysis. A principal component regression was subsequently applied to test the response of oxygen stress and Wetex decomposition rates to the principal components. Effects of oxygen stress on macroinvertebrate assemblages were evaluated by means of weighted abundances (WA) of regulation values (R-values derived from the respiration studies), the ASPT and family richness within the orders Ephemeroptera, Plecoptera, Trichoptera, Odonata and Coleoptera (EPTCO). WA of each taxon in kick samples was multiplied by the R-value (WA of R; WAR). If only *Chironomus* was present of those eight taxa (WA = 1), WAR would equal the *Chironomus* R-value. Kick sampling data collected from several years (2016–2018, \( n = 24 \)) was used for this analysis when available (average values), to lower the probability of sampling errors, as ongoing environmental monitoring has indicated much similarity in environmental conditions in this period (Eriksen et al., 2021b; Eriksen et al., 2017). These biological metric responses were investigated using a linear regression model as a function of minimum diel DO saturation (\( n = 14 \)).

### 3. Results

#### 3.1. Environmental characteristics and degradation of river sites

The sampling sites reflected various geological conditions (0.2–1.8 mmol/L alkalinity) and dissolved nutrient concentrations (3–1100 µg T/P/L and 100–5300 µg TN/L; Table 2). Sites located in areas with gleysol soils had generally lower levels of alkalinity compared to nitisol soils (soil types are shown in Fig. S1). The dominant river substrates were sand, with some gravel and silt present at some sites, depending on water velocity and land-use activities. Recorded diel water temperatures from the river sites were on average 24.6–28.1 °C with minimum and maximum levels of 20.6–34.1 °C.

The general trend moving downstream through the river networks was for forested areas to be converted into cultivated areas, human settlements and urban development. River reaches draining areas dominated by urban and agricultural land-use had elevated concentrations of TP and TN, relative to less impacted reaches located upstream, and in the most urbanized areas significant inputs of untreated sewage were observed. Our integrated approach to assess the cumulative degradation was supported by the principal component regression analysis undertaken on the oxygen conditions, decomposition rates (Wetex) and selected environmental variables (shown in Fig. 1). The degradation of riparian zone was also associated with the PCA 1 (shown in Fig. S3) but considered redundant for further analysis.

**Table 2**

Recorded concentrations of total phosphorus (TP) and nitrogen (TN), and alkalinity from the studied sampling sites.

| Site | Year | TP (µg/L) | TN (µg/L) | Alkalinity (pH 4.5 mmol/L) |
|------|------|-----------|-----------|--------------------------|
| REF1 | 2019 | 35        | 220       | 1.8                      |
| IMP2 | 2019 | 55        | 330       | 0.9                      |
| IMP4 | 2019 | 55        | 140       | 2.2                      |
| MYST | 2018 | 37        | 320       | 1.1                      |
| 602T | 2018 | 9         | 425       | 0.2                      |
| 603T | 2019 | 76        | 925       | 0.3                      |
| MYST | 2019 | 5         | 130       | 0.2                      |
| 602T | 2019 | 91        | 860       | 0.2                      |
| REP6 | 2020 | 21        | 370       | 1.2                      |
| IMP4 | 2020 | 23        | 800       | 1                        |
| 302T | 2020 | 13        | 250       | 0.5                      |
| 303T | 2020 | 3         | 340       | 0.5                      |
| 607T | 2020 | 29        | 350       | 0.3                      |
| 617T | 2020 | 34        | 650       | 0.3                      |
| RES1 | 2018 | 36        | 167       | 0.4                      |
| 605T | 2016 | 1100      | 5300      | 1.5                      |

#### 3.2. Diel dissolved oxygen regimes

Land use practices dominated by agriculture/plantations and settlements/urbanization led to volatile oxygen regimes in impacted river reaches with the highest oxygen deficits occurring during darkness (Fig. 2). Water temperatures were lowest in the morning (5–9 a.m.) with shallow rivers heating and cooling the fastest. The highest DO saturations were recorded mid-day, usually between 10:00 and 14:00, with supersaturation (>100%) occurring at some sites (e.g., Fig. 2b). This was the time of day with the highest temperatures and light intensities. The highest DO saturation was recorded in a remote river site, subject to disturbances by grazing cattle, where we observed high densities of epibenthic algae (Res1).

During daylight the saturation reached 115% before dropping to 75% following sunset, showing an oxygen amplitude of 40% within 6 h. The diel pattern was repeated during the next 24 h, likely reflecting algae respiration rates, indicating increased oxygen fluctuations can occur localised in rivers with less intense land use impacts. In some cases, the impacted sites experienced abrupt falls in oxygen saturations (e.g. Fig. 2d, f, h & o) which was not observed from least disturbed sites (Fig. 2a & l). Urban reach development was the land-use type with most impact on minimum diel oxygen concentrations (\( R^2 = 0.81, P < .001; \) Table S4a). Mean DO deficits were higher in darkness (6–92%) compared to daylight (2–78%) with impacted reaches having the highest deficits (Table S1).

#### 3.3. Macroinvertebrate assemblage composition

From the kick sampling data, we recorded a total of 65 families, mostly within the orders Ephemeroptera, Plecoptera and Trichoptera, Coleoptera and Odonata (hereafter EPTCO), Heteroptera, Diptera and Gastropoda. Higher values of EPTCO family richness (scoring 0–17) and ASPT (2.3–6.1) were associated with least disturbed sites whereas impacted sites were dominated by Oligochaeta, Diptera and Gastropoda. The presence of the eight taxa used for the respiration studies at the investigated sites was variable (13–79%; Table S2). The dipteran *Chironomus* and ephemerolopteran *Nigrobaetis* occurred in more than half of the samples, whereas the odonates *P. rubriceps*, *O. sabina* and *L. lineata*, and the trichopterans *Ecnomus* and *A. meridiana* were found in 25–42% of the samples. The hirudinean *Barbronia weberi* was the most infrequent of the eight taxa, only
recorded in 13% of the samples. Chironomus, Nigrobaetis and A. meridiana had the highest abundances of the studied taxa (on average 74–264 individuals per sample).

3.4. Decomposition rates

Wettest decomposition rates were in the range 0.09–1.16 mg degree day\(^{-1}\) for fine mesh bags (1–27% mass lost on average), and 0.12–5.45 mg degree day\(^{-1}\) for coarse (2–67% mass lost; Table S3). A thin layer of biofilm covered the Wettest when it was retrieved. Wettest from coarse mesh bag were usually colonized by macroinvertebrates, typically Chironomidae, Oligochaeta, Simulidae, Hydroptilidae, Baetidae and Caenidae. High numbers of Gastropoda (e.g. Thiaridae and Pachyliidae) were also often found in the coarse bags, and based on the observed decomposition in patches where they were found, they were probably foraging off the Wettest (Fig. S2).

3.5. Macroinvertebrate oxy-regulation capacity

All the organisms tested in the respiration study showed at least some regulatory capacity to oxygen depletion (R-value of 0.74–0.89; Fig. S3). O. sabina (R = 0.89) and Chironomus (R = 0.86) showed high oxy-
regulatory capacity with constant oxygen consumption down to a critical threshold (~30–50 nmol O₂/L) before switching to oxy-conformer respiration (Fig. 3a–b). Ecnomus (R = 0.84) and Nigrobaetis (R = 0.81) had highest respiration rate at intermediate oxygen concentrations (~150 nmol O₂/L), indicating an “oxy-stressor” response, before eventually turning to oxy-conforming (Fig. 3c–d). The respiration curve for B. weberi (R = 0.77) also resembled an oxy-stressor response (Fig. 3e) but individual respiration curves revealed a possible artefact resulting from a rapid oxygen consumption by the largest individual (shown in Fig. S5). Oxygen consumption by P. rubriceps (R = 0.77), A. meridiana (R = 0.77) and L. lineata (R = 0.74) gradually declined in response to oxygen depletion (Fig. 3f–h) and showed the lowest oxy-regulatory capacity of the organisms studied.

3.6. Relationship between environmental degradation, DO and macroinvertebrate assemblages

Oxygen stress, expressed as diel mean and minimum DO saturation deficits, showed negative relationships to principal component axis 1 (hereafter eutrophication impact) by linear regression models (lm; R² = 0.47–0.48, P ≤ .003; Fig. 4a). Oxygen amplitude did not show any significant relationship (lm; R² = 0.15, P = .082). Decomposition rates of Wettex exposed to both micro- and macrobial decomposition, i.e., coarse mesh bags, increased with eutrophication impact (lm; R² = 0.82, P = .001; Fig. 4b), but this was not found for microbial decomposition alone, i.e. fine mesh bags (lm; R² = 0.36, P = .067). Oxy-regulatory capacity

Fig. 3. Mass specific respiration rates (MSRR) in relation to oxygen concentration (nmol/L) for eight macroinvertebrate taxa. Data are from closed chamber respiration studies. The capacity of macroinvertebrate oxy-regulation, R, is indicated in brackets. 95% confidence intervals for the regression curves are shown.
increased in response to oxygen stress expressed as the diel DO minima (lm; $R^2 = 0.69$, $P < 0.001$; Fig. 5a), and notably in urban reaches (lm; $R^2 = 0.75$, $P < 0.001$; Fig. S4b). Biodiversity expressed as family richness of EPTCO (lm; $R^2 = 0.79$, $P < 0.001$) and full assemblage tolerance, expressed as ASPT, increased with oxygen availability (lm; $R^2 = 0.9$, $P < 0.001$; Fig. 5b–c).

4. Discussion

In this study we found that individual macroinvertebrate oxy-regulatory capacity predicts assemblage composition at the ecosystem level in response to external water oxygen concentration. The possession of at least some oxy-regulatory capacity was a common attribute in macroinvertebrates inhabiting the tropical lowland river network, with assemblages at the ecosystem level showing higher capacities in response to pollution-induced oxygen stress, partly through increased decomposition rates in rivers despite low DO concentrations. Although our respiration studies were conducted using relatively few taxa, these were common and abundant with relatively high impact on assemblage oxy-regulatory capacity (WAR).

As the effects of individual stressors associated with nutrient and organic pollution are difficult to disentangle, such as oxygen and ammonium/ammonia (NH$_4^+$/NH$_3^-$), we cannot fully prove a causal link between assemblage composition (WAR) and oxygen stress in field studies. However, strongly supported by our respiration studies, we suggest that oxygen was the primary stressor impacting macroinvertebrate assemblages in the field studies. This contention is supported by Friberg et al. (2010), who found a stronger negative effect of BOD$_5$ (as a proxy for oxygen) on macroinvertebrate taxa than nitrogen in temperate rivers.

4.1. Human activities impact river oxygen condition and decomposition rates

Catchment-scale human activities in rivers are known to impact river oxygen conditions in various ways. Deforestation and removal of riparian vegetation may increase light influx and water temperatures (Moore et al., 2005; Webb et al., 2008), leading to higher primary production (Davies et al., 2008) and respiration rates (Clapcott and Barmuta, 2010). Excessive nutrient inputs can enhance this effect by further speeding up primary production (Davies et al., 2008) and respiration rates (Rosemond et al., 2015), which may lead to undersaturation of oxygen during night because of non-compensated respiration rates from lacking photosynthesis (Mulholland et al., 2005). Our study shows that oxygen deficits in rivers may be chronically high, but sometimes also episodic in periods with
high human activity. Inputs of nutrients and easily degradable organic matter, like sewage, can lead to rapid water deoxygenation induced by micro- and macrobial respiration (Hyne, 1970), and excessive sedimentation (turbidity) may enhance this effect by hindering light influx and reducing benthic primary production. These perturbations were acting in the studied river basin (Eriksen et al., 2021b) and are common to lowland ecosystems of South East Asia in general (Eriksen et al., 2021a). We therefore consider that our results are transferable to other lowland tropical river networks.

Decomposition rates (Wetex) increased in response to nutrient enrichment despite low oxygen concentrations in some reaches. The presence of a biofilm on the Wetex, and the high colonization rates by Chironomus and Gastropoda, indicates that some organisms are well-suited to decompose organic matter even under poor DO conditions. Faster decomposition rates of allochthonous material are expected from moderately enriched waters (Kominoski et al., 2015; Rosemond et al., 2015), as dissolved nutrients stimulate microbial activity (Gulis et al., 2006), although too high nutrient concentrations may also hinder decomposition (Woodward et al., 2012).

Our study shows that tropical lowland rivers are highly sensitive to nutrient enrichment and organic pollution as warm water rivers rapidly become depleted of oxygen while at the same time stimulating ecosystem respiration, creating a positive synergy, that further expands the oxygen deficit. The low solubility of oxygen in warm water river systems, combined with the non-linear increase in the basal metabolic rates of ectotherms (Dillon et al., 2010), renders tropical river ecosystems especially threatened by drivers that increase ecosystem respiration rates, such as warming and pollution, compared to cooler climates. Although the solubility of oxygen declines linearly with increasing water temperatures, the consumption through respiration increases non-linearly, thus creating higher demands than availability for many organisms with limited capability of regulation their oxygen uptake.

4.2. Oxygen stress scaling up from individuals to ecosystems

The link between individual respiratory responses, river reach DO conditions and assemblage composition in the studied river network, shows how pollution-induced oxygen stress scaled up from individuals to the ecosystem level (river reach). Effects of oxygen stress on the macroinvertebrate assemblages were found using functional and compositional attributes, illustrating that major implications on ectotherm communities are expected from warm water ecosystems exposed to eutrophication and organic pollution. As shown in our study, river reaches subject to oxygen stress were dominated by macroinvertebrates possessing high regulation capacities, leading to an assemblage homogenization, because taxa with lowest oxygen-regulatory capacity were partially excluded (see SI for further details).

None of the taxa selected for the respiration study displayed a typical oxy-conformer response, i.e., an oxygen consumption change linearly with oxygen availability. The reason may be that most ectotherms inhabiting tropical lowland rivers require at least some respiration control to withstand higher metabolic rates induced by high water temperatures and low oxygen solubility (Verberk and Bilton, 2013). This may be especially the case in rivers subject to at least some environmental degradation, like in our study, where we were unable to obtain completely pristine sampling sites for the collection of macroinvertebrates. Aquatic ectotherms with low regulation capacities should prefer habitats with low DO fluctuations, i.e. unpolluted waters with low temperature amplitudes, such as forested rivers with shading riparian vegetation. In our data, even in least disturbed states, wide and shallow rivers with well-developed riparian vegetation were subject to high water temperatures amplitudes despite amelioration by the cooler water entering from shaded tributary rivers upstream. This shows that shallow tropical rivers are extreme environments in terms of oxygen regimes and species living on the edge of their distribution ranges may therefore be especially sensitive to disturbances that affect DO conditions.

Responses were evident on metrics measuring organic pollution (ASPT) and biodiversity (EPTCO) with the strongest response recorded for ASPT. The ASPT (Armitage et al., 1983) was initially designed to assess environmental effects of oxygen stress following organic pollution in UK rivers (Hawkes, 1998; Pauley et al., 2014). However, our study from tropical lowland rivers shows that similar responses can also be expected in contrasting ecosystems (Eriksen et al., 2021b), which may explain the worldwide use of ASPT for river bioassessments (Eriksen et al., 2021a). The EPTCO metric likely targets environmental degradation more broadly than ASPT, e.g. by responding to overall habitat degradation, as pollution sensitivity is expected to vary considerably within the orders (e.g. Baptista et al., 2007; Dos Santos et al., 2011), which was shown by the respiration responses in the present study. The loss of biodiversity leading to assemblage homogenization may therefore ultimately threaten ecosystem integrity and the services that they supply (Barnosky et al., 2011; Sanchez-Bayo and Wyckhuys, 2019; Whitehorn et al., 2019).

4.3. Effects of changed oxygen conditions in tropical river networks

Deforestation in Indo-Burma has accelerated from already high rates during the last decades, with effects of global warming already noticeable through elevated temperatures and altered precipitation patterns (Tordoff et al., 2020). The projected future for this region, like several other biodiversity hotspots (Hasell et al., 2019), is that much of the remaining pristine land areas are threatened by environmental degradation, resulting from a combination of high demographic pressures, agricultural land conversion and climate change. At the same time, organic pollution is predicted to increase and represents a major threat to riverine ectotherms (Wen et al., 2017). Aquatic macroinvertebrates inhabiting warm water lowland rivers may be especially exposed in this context as 1) increasing water temperatures decrease oxygen solubility while increasing ectotherm metabolism, 2) removal of forest cover further increases temperatures and turbidity, 3) agriculture and urban development leads to higher inputs of nutrients, sediments and organic pollutants, 4) oxy-regulatory capacity becomes lower when preferred habitats become degraded and unavailable (e.g. Eriksen, 1963), and 5) process rates, including microbial O2 consumption, increase with temperature. To mitigate freshwater oxygen stress in Indo-Burma and elsewhere, it is imperative to protect and restore forested areas, including riparian buffer strips, and to halt the inputs of nutrients and sediments to surface waters. The current land-use practice and human induced perturbations to rivers impacts ecological and biodiversity condition in this area (Eriksen et al., 2021b), and the present study shows that river ectotherm communities may be highly sensitive to pollution-induced oxygen stress.

5. Conclusion

This study gives novel insight into oxygen condition and macroinvertebrate oxy-regulatory capacity in a tropical lowland river network, which are increasingly threatened by human activities worldwide but remain understudied. Respiratory performances on individual taxa in response to oxygen stress (laboratory studies) scaled to the ecosystem level (field studies) where impacted river reaches were dominated by assemblages possessing the highest capacities to regulate their respiration rates. Impacts were also characterised by low biodiversity (EPTCO) and high tolerance of organic pollution (ASPT). This study shows that the current land-use in this part of Myanmar, likely representative of many tropical lowland rivers in the Indo-Burma region, threatens ectotherms communities possessing low oxy-regulatory capacities which may ultimately affect ecosystem services. River oxygen conditions are widely known to impact the aquatic fauna, and we encourage more studies to focus on respiration traits in response to environmental degradation as this is crucial for the survival of many species living in aquatic environments.

CRediT authorship contribution statement

Tor Erik Eriksen: Conceptualization, Investigation, Visualization, Formal analysis, Writing – original draft, Writing – review & editing. Dean Jacobsen: Conceptualization, Investigation, Formal analysis, Writing –
original draft, Writing – review & editing. **Benoit O.L. Demars:** Writing – review & editing. **John E. Brittain:** Writing – original draft, Writing – review & editing. **Geir Selj:** Writing – original draft, Writing – review & editing. **Nikolaif Friberg:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

**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.

**Acknowledgements**

This work was supported by the Royal Norwegian Embassy in Myanmar/Norwegian Ministry of Foreign Affairs and the Norwegian Institute for Water Research (NIVA). The study was a component of a larger collaborative project (The integrated water resources management – institutional building and training) with Department of Forestry (MND), Ministry of Natural Resources and Environmental Conservation (MONREC) of Myanmar and NIVA. We thank Bo Ni, retired director of Watershed Management Division (FD), Zaw Win Myint – the director of Watershed Management Division (FD), Ko Ko Oo (Irrigation and Water Utilization Management Department; IVUWM), Htay Aung (Directorate of Water Resources and Improvement of River Systems; DWR), Toe Aung (FD) and Ingrid Nesheim (NIVA) for providing facilities and personnel to assist our work. A special acknowledgement is given to Eirin Årstein-Eriksen (NIVA), Johnny Hall (NIVA), Mathias Brink Kjeldgaard and the monitoring sampling team in Myanmar for valuable assistance during the studies, including Phyo Wai (IVUWM), Phyo Thet Naing (FD), Phyo Thet (FD), Mya Htet Aung (IVUWM), Thet Myat (FD), Htay Kywe (IVUWM), Zao Myo (FD), Phyo Thet (FD), Swuam Pyaye Aye Aung (FD), Eai Chit Thu Maung (FD) and Mr. Moe (FD).

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151958.

**References**

Armitage, P.D., Moss, D., Wright, J.F., Furse, M.T., 1983. The performance of a new biological water-quality score system based on macroinvertebrates over a wide-range of unpolluted running water sites. Water Res. 17, 333–347. https://doi.org/10.1016/0043-1354(83)90188-4.

Baptista, D.F., Buss, D.F., Egler, M., Giovanelli, A., Silveira, M.P., Nessimian, J.L., 2007. A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest water-quality score system based on macroinvertebrates over a wide-range of unpolluted running water sites (ISO 10870:2012), p. 35. 

Benoît O.L. Demars: Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

Nikolaif Friberg: Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

Kong University Press, Hong Kong.

Jacobson, D., Marin, R., 2008. Biotic Altipiano streams with low richness of macroinvertebrates and large diel fluctuations in temperature and dissolved oxygen. Aquat. Ecol. 42, 643–656. https://doi.org/10.1007/s10686-007-9127-x.

Kominoski, J.S., Rosemond, A.D., Benstead, J.P., Gulis, V., Maerz, J.C., Manning, D.W.P., 2015. Low-to-moderate nitrogen and phosphorus concentrations accelerate microbially driven litter breakdown rates. Ecol. Appl. 25, 856–865. https://doi.org/10.1899/14-11131.1.

Lencioni, V., Bernabo, P., Vanin, S., Di Muro, P., Beltrami, M., 2008. Respiration rate and oxy-regulatory capacity in cold stenothermal chironomids. J. Insect Physiol. 54, 1035–1042. https://doi.org/10.1016/j.jinsphys.2007.07.002.

Maina, J.N., 2002. Structure, function and evolution of the gas exchangers: comparative perspectives. J. Anat. 201, 281–304. https://doi.org/10.1046/j.1469-7580.2002.00099.x.

MOIP - Department of Population MolAp, 2015. Myanmar Population and Housing Census - Main Report Volume 2 - 201 pp. 2015.

Mulholland, P.J., Houser, J.N., Maloney, K.O., 2005. Stream diurnal dissolved oxygen profiles as indicators of in-stream metabolism and disturbance effects: Fort Benning as a case study. Ecol. Indic. 5, 243–252. https://doi.org/10.1016/j.ecolind.2005.03.004.

Nagel, B., 1973. The oxygen consumption of mayfly (Ephemeroptera) and stonefly (plecoptera) larvae at different oxygen concentrations. Hydrobiologia 42, 461–489.

Newbold, T., Oppenheimer, P., Etard, A., Williams, J.J., 2020. Tropical and Mediterranean biodiversity is disproportionately sensitive to land-use and climate change. Nat. Ecol. Evol., 19, 1–13. https://doi.org/10.1038/s41467-020-15109-5.

Newbold, T., Oppenheimer, P., Etard, A., Williams, J.J., 2020. Tropical and Mediterranean biodiversity is disproportionately sensitive to land-use and climate change. Nat. Ecol. Evol., 19, 1–13. https://doi.org/10.1038/s41467-020-15109-5.

Nicholls, R.J., 1996. Water quality - Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters (ISO 10870:2012), p. 36.

NS-4743, 1993. Water analysis - Determination of total nitrogen after oxidation by peroxodisulphate 12 pp.

NS-4764, 1980. Water analysis - Total residue, and total fixed residue in water, sludge and suspended solids. 1980.

NS-EN-ISO-6878, 2004. Water quality - Determination of phosphorus – Ammonium molybdophosphate spectrophotometric method (ISO 6878:2004) 28 pp.

NS-ISO-9963-1, 1995. Water quality - Determination of alkalinity – Part 1: Determination of total and composite alkalinity (ISO 9963:1994) 16 pp.

NS-EN-ISO-10870, 2012. Water quality - Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters (ISO 10870:2012), p. 36.

PSF, 2004. Methodology for the biological monitoring working party (BMWP) score system: derivation of present-only and abundance-related scores from field data. River Res. Appl. 30, 887–904. https://doi.org/10.1002/rra.2686.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
