



Potamodromous fish movements under multiple stressors: Connectivity reduction and oxygen depletion



Paulo Branco^{a,b,*}, José M. Santos^a, Susana Amaral^{a,b}, Filipe Romão^b, António N. Pinheiro^b, Maria T. Ferreira^a

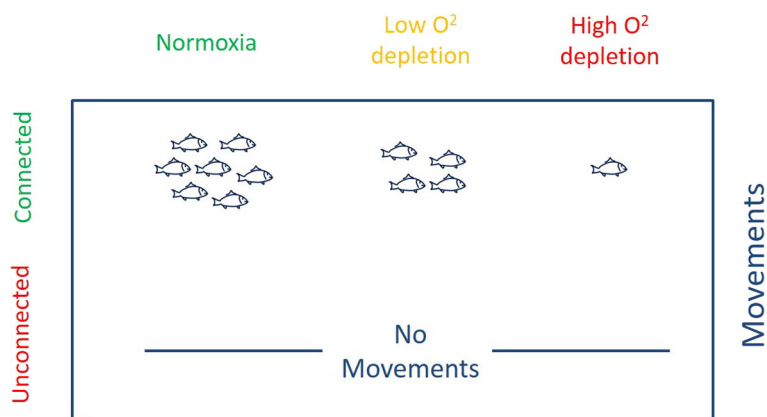
^a CEF – Forest Research Centre, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

^b CERIS – Civil Engineering for Research and Innovation for Sustainability, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

HIGHLIGHTS

- Both stressors and their interactions impact fish movements.
- Lack of connectivity overrode the impact of oxygen depletion.
- During connectivity fish movements decreased with decreasing oxygen concentration.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 24 May 2016

Received in revised form 9 August 2016

Accepted 10 August 2016

Available online xxxx

Keywords:

Multiple stressors
Multifactorial experiments
Potamodromous fish
Water abstraction
Oxygen depletion
Mediterranean systems

ABSTRACT

Rivers are impacted by multiple stressors that can interact to create synergistic, additive or antagonistic effects, but experimental studies on fish encompassing more than one stressor are seldom found. Thus, there is the need to study stressors through multifactorial approaches that analyse the impact of fish exposure to multiple stressors and evaluate fish sensitivity to stressor combinations. Some of the most common impacts to Mediterranean rivers are of two natures: i) water abstraction and ii) diffuse pollution. Therefore, the present study aims at studying the responses of potamodromous fish facing combinations of: 1) a primary stressor (two levels of connectivity reduction due to water scarcity), and 2) a secondary stressor (three levels of oxygen depletion due to increase organic load – of anthropogenic nature). Schools of five wild fish from a cyprinid species (*Luciobarbus bocagei*) were placed in a flume, equipped with see-through sidewalls to allow for behavioural analysis, and subjected to different combinations of the stressors. Results show that at the unconnected level the primary stressor (lack of connectivity) overrode the effect of the secondary stressor (oxygen depletion), but when connectivity existed oxygen depletion caused a reduction of fish movements with decreasing oxygen concentrations. This multifactorial study contributes to improved prediction of fish responses upon actual or projected pressure scenarios.

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* Corresponding author at: CEF – Forest Research Centre, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal.
E-mail address: pjbranco@isa.ulisboa.pt (P. Branco).

1. Introduction

Riverine environments are among the most degraded systems in the world (Sala et al., 2000; Gleick, 2003) and several studies show alterations in fish populations due to human-induced disturbances (Branco et al., 2013a). The Water Framework Directive (WFD, European Commission, 2000) brought innovation to the measurement of surface water quality by using several biological communities as quality indicators, rather than just resorting to chemical parameters (Moss, 2007). To date, WFD has searched for biological indicators of stressors and summed them up in a one-out-all-out principle, neglecting the need for reliable indicators that respond to multiple stressors and provide an integrated assessment of ecosystem health and malfunctioning (Hering et al., 2010). In addition, researchers tend to analyse the impact of individual stressors – changes in the environment that force a response by the biological group of interest (Underwood, 1989) – on biological indicators (Birk et al., 2012), but it is clear that stressors tend to interact, creating multiple and quite complex effects and results (Piggott et al., 2015).

European water resources and ecosystems are impacted by multiple stressors, which affect ecological and chemical quality, water availability and ecosystem functions. Furthermore, increased water demand and climate change are likely to augment the magnitude and number of stressors acting on river ecosystems (Hering et al., 2015), thereby increasing possible interactions. This interaction between different stressors can be manifold: additive when the response is predicted by the sum of the responses to isolated stressors; synergistic when the combined effect is greater than the sum of the effects of isolated stressors; or even antagonistic when effects are smaller than the sum of isolated stressors (Underwood, 1989). Studies focused on multiple stressors seldom appear in freshwater journals (Ormerod et al., 2010), leading to a lack of mechanistic understanding of the interactive effects of stressors, which in turn makes predicting responses to changing environments, risk assessment, management, impact mitigation and ecosystem restoration all harder (Vinebrooke et al., 2004).

Mediterranean rivers have a long history of human settlement and disturbance, exhibiting the highest anthropogenic impacts – e.g. water abstraction, river fragmentation, and hypoxia caused by eutrophication (Gasith and Resh, 1999; Benejam et al., 2010; Mcbryan et al., 2013) – of rivers in any climatic zone (Maceda-Veiga and De Sostoa, 2011). It is expected that these impacts will be exacerbated by future climatic changes, which will increase ecological impacts by reducing connectivity or extending the period during which riverbeds are dry. Alterations in the chemical composition of water will interact with environmental changes and enhance the impact of nutrient loading, deoxygenation, sedimentation and turbidity. In fact, water abstraction and deoxygenation are two of the most important stressors at play in increasingly human-impacted Mediterranean rivers (Picó et al., 2013; Schuhmacher et al., 2016). Dissolved oxygen has a lower concentration in water than in air (Moyle and Cech, 2004), and it has an inverse relationship with water temperature (Kalff, 2000; Ficke et al., 2007). For water-dwelling animals, this leads to an even greater problem, as most are cold-blooded and their metabolic rates increase with water temperature, so a temperature increase will reduce oxygen supply while increasing biological oxygen demand (Kalff, 2000). Rivers receive organic loads from several sources; the biological degradation of this organic matter reduces available dissolved oxygen in the water column. Even though fish have proven to possess a high level of plasticity when coping with hypoxia (Beitinger and Bennett, 2000; Rees et al., 2001; Ford and Beitinger, 2005; Fangue et al., 2006), oxygen depletion has been shown to cause physiological impairment in fish (Behrens and Steffensen, 2007). Cyprinids are the most dominant and abundant group of autochthonous freshwater fish in Mediterranean rivers (Doadrio et al., 2011), contributing to the Mediterranean hotspot of freshwater fish diversity (Myers et al., 2000). Although these fish are known to have a higher tolerance for water deoxygenation than

salmonids (Domenici et al., 2012), by creating isolated patches of water where temperature, metabolic rates of fish and competition increase, connectivity reduction further magnifies the level of stress to which cyprinids are subjected. This is particularly the case of potamodromous species, where movement between habitats is fundamental to life-cycle completion. The isolated impact of these two stressors (water scarcity and oxygen depletion) and their combined impact on freshwater biota thus needs to be ascertained. This requires more nature-like multifactorial experiments where stressors are combined to test the effects of their interaction on the biological response. The purpose of this study was thus to understand the response of a potamodromous fish species to the combination of connectivity reduction (a physical stressor) and oxygen depletion (a chemical stressor), which are both common stressors acting on Mediterranean rivers. To this end we posed several questions: Does connectivity reduction have an impact on fish movements?; Does oxygen reduction have an impact on fish movements?; Is the interaction between connectivity and oxygen reduction significant for fish movements?

2. Materials and methods

Experiments performed in a controlled laboratory environment in which natural conditions can be recreated have been considered preferable to field studies, due to the ability to manipulate the variables of interest while controlling possible confounding factors (Kondratieff and Myrick, 2005). Trials were performed in an experimental 10 m-long by 1 m-wide flume, with a 3% slope (Fig. 1). The sidewalls are composed of glass panels to make it possible to visualise fish behaviour (both upstream and downstream movements) inside the flume. Four evenly spaced polypropylene crosswalls (Fig. 1) were installed to create a water surface disconnectivity for discharges up to $28 \text{ L} \cdot \text{s}^{-1}$. In order to make the conditions similar to those in the field when water moves interstitially, flow discharge was kept constant for both treatments (connected and unconnected) at $28 \text{ L} \cdot \text{s}^{-1}$, while maintaining a sub-superficial hydrological connectivity. Connectivity was assured by increasing water depth within the flume by controlling the tailwater level. This configuration resulted in reduced surface connectivity due to water scarcity, from a higher water level and surface connectivity (achieved by connecting the pools) to a low water level and connectivity fragmentation (disconnection of the pools) – a range which is a characteristic of Mediterranean rivers (Gasith and Resh, 1999). This connectivity stressor was associated with three levels of the secondary stressor oxygen depletion/reduction of dissolved oxygen (DO): a control level (normoxia), a mild depletion (ca. 50% DO), and a severe depletion (ca. 15% DO) (Table 1). In order to provide a framework for the experimental oxygen depletion, the extreme was selected on the basis of the fact that 15% DO (ca. 1.5 mg/L at a temperature of $22 \text{ }^\circ\text{C}$) reflects the value at which DO concentrations ($<2 \text{ mg/L}$) start to become stressful for and sometimes lethal to fish (Rao et al., 2014; Bohlen, 2003) – a situation that can occur as a result of discharges of organic wastes (Seager et al., 2000), which are particularly common in Mediterranean rivers (Lillebø et al., 2007). DO concentrations lower than these have been described as much as 3200 m downstream from sewage outfalls (Birtwell et al., 1983), and have been detected by high-frequency monitoring in peri-urban rivers (Ivanovsky et al., 2016). Oxygen depletion was achieved by adding Sodium sulphite (Park et al., 2014) to the water column within the flume while monitoring oxygen concentration with a HANNA (HI 98193) oxygen probe. This compound has been used in the establishment of low-oxygen and oxygen-deficit conditions in animal research (Crampton, 1998; Peay et al., 2006). Oxygen is bound by the sulphite ion in sodium sulphite, and this makes it possible to control the amount of dissolved oxygen in water (Lewis, 1970). This artificial oxygen reduction was carried out as a proxy for the reduction of dissolved oxygen in water due to the degradation of organic matter inputs to the system. We should note that even though fish were subject to this chemically induced oxygen depletion for a limited time, we cannot fully exclude

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