POTAMODROMOUS FISH RESPONSES TO MULTIPLE STRESSORS: WATER SCARCITY AND OXYGEN DEPLETION

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Rivers are impacted by multiple stressors that affect their ecological and chemical quality, water availability and ecosystem functions. Stressors 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, stressor interactions, and evaluate fish sensitivity to stress combinations. Some of the most common impacts to Mediterranean rivers are of two natures: i) hydromorphological 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 water scarcity and connectivity reduction – naturally occurring in Mediterranean systems), and 2) a secondary stressor (three levels of oxygen depletion due to increase organic load – of anthropogenic nature). For that, schools of five wild fish from a potamodromous 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. The results of this work, and the observed correlations between behaviour and stressor combination, improve our understanding of the effects of the interaction of natural and man-induced stressors on potamodromous fish species, making possible to implement effective management and restoration policies. This work progresses from single variable manipulative experiments to multifactorial experiments, allowing to assist prediction of fish responses upon actual or projected pressure scenarios.

Key-words: Multiple stresses; multifactorial experiments, potamodromous fish, water abstraction, oxygen depletion, Mediterranean systems

1 INTRODUCTION

Mediterranean rivers are characterized by having a harsh environmental setting that will be affected by climate change, prompting complex ecological impacts (reduced connectivity, increased or temporally extended surface of dry river beds) [1]. Chemical stressors interact with this changing environmental setting and increase

the ecological impacts of organic and nutrient loads, deoxygenation, sedimentation and turbidity. Indeed, water abstraction and oxygen depletion are two of the most important stressors imposed by an increasing human activity in Mediterranean rivers. European water resources and ecosystems are impacted by multiple stressors [2] that affect ecological and chemical quality, water availability and ecosystem functions. Furthermore, water demand increase and climate change are likely to augment the magnitude and number of stressors acting upon river ecosystems, potentially increasing possible interactions. The interaction of different stressors can be manifold: additive when the response is predicted by the sum of the responses to isolated stresses; synergistic when the combined effect is greater than the sum of the effects of isolated stresses; or even antagonistic by creating responses smaller than those predicted [3]. Studies focused on multiple stressors are seldom published [4], leading to a lack of mechanistic understanding of stressors interactive effects, which is a barrier for the prediction of responses to changing environments, risk assessment, management, impact mitigation and restoration of ecosystems [5]. There is a pressing need to study stressors through multifactorial approaches that analyse the impact of fish exposure to multiple stressors, stressor interactions, and understand fish sensitivity to stress combinations. This will facilitate an accurate definition of the ecological state of river sites making it possible to implement effective management and restoration policies. The main objective of this work was thus to understand the responses of freshwater fish to multiple stressors encountered in Mediterranean regions.

2 MATERIALS AND METHODS

Experiments were carried out in an experimental flume, 10 m in length and with a slope of 3%. The side walls are composed by glass panels allowing visual observation of fish behaviour within the flume. The flume was “divided” into 5 evenly spaced sections by using 4 perforated polypropylene crosswalls. These crosswalls were designed to create superficial disconnectivity for discharges up to 28 $L.s^{-1}$. This solution allowed recreating Mediterranean (low water level and disconnected “pools”) and temperate river flows (high water level and connected “pools”). This connectivity stressor was associated with three levels of a secondary stressor – oxygen depletion - control (90%), mild depletion (50%) and severe depletion (15%). All experiments were replicated five times. Schools of five wild caught barbel (*Luciobarbus bocagei* – representing potamodromous cyprinids) were acclimated at the center of the flume for 15 min and fish responses under these two stressors were tested for 30 min where fish were let to displace themselves volitionally.

3 RESULTS AND DISCUSSION

Results (Table 1) demonstrate how both treatments and their combination significantly affected movements, including upstream, downstream and total movements. For the unconnected arrangement, only one upstream movement was recorded, during one replicate under the Control situation. Therefore, PerMANOVA [6] post-hoc pair-wise, were not able to identify any differences with respect to oxygen depletion under an unconnected arrangement. However, during the connected arrangement, with water flowing over the crosswalls, there were clear differences in terms of fish behaviour (Figure 1) - fish showed a significant reduction both in upstream and downstream movements with increasing oxygen depletion.

Table 1 – PerMANOVA test results (p-value) (α = 0.05) for upstream, downstream and total number of movements. Connectivity reduction – two levels: Connected and unconnected. Oxygen depletion – three levels: Control (90%), mild depletion (50%) and severe depletion (15%).

Stressor	Upstream	Downstream	Total
Connectivity reduction	<0.001	<0.001	<0.001
Oxygen depletion	<0.001	<0.001	<0.001
Interaction	<0.001	<0.001	<0.001

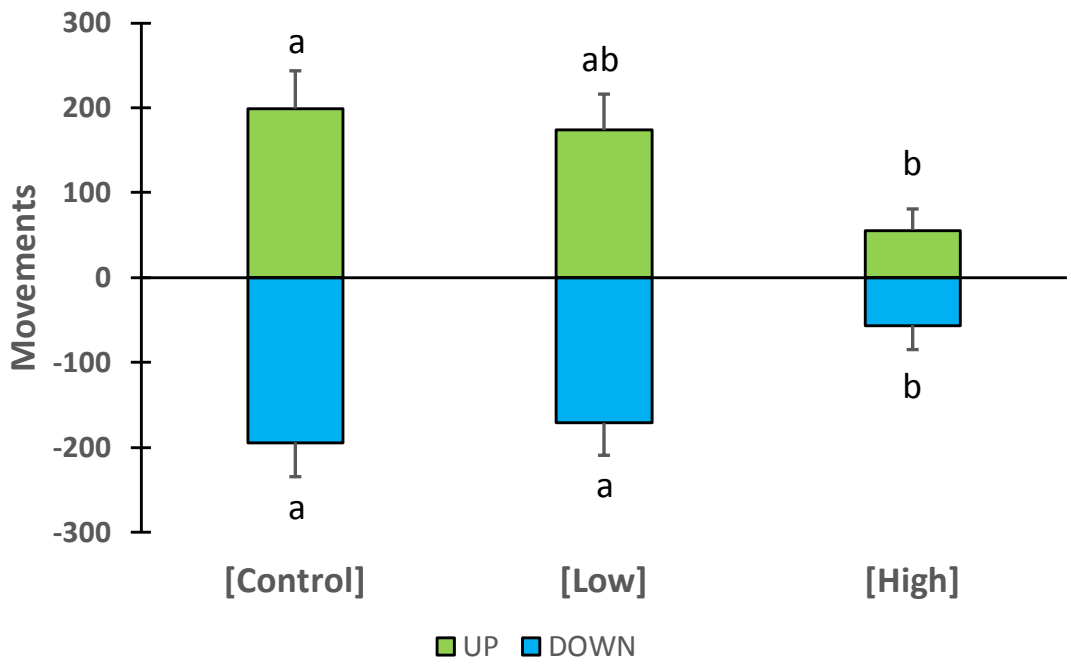

Figure 1. Number of movements of *Luciobarbus bocagei* across the five connected pools and under no oxygen depletion (control), low depletion (50% saturation) and high depletion (15% saturation) conditions. Bars show mean number of movements (of 5 replicates) ± 1 SD. For each direction, conditions that share the same letter are not significantly different on the number of movements according to a pair-wise Kruskal-Wallis ANOVA.

This research shows how the primary stressor, connectivity, clearly limits fish movements regardless of oxygen concentration. Even slight limitations like the one considered in this study, have a movement deletion effect even when movement is "stimulated" by the maintenance of a continuous upstream-downstream flow. During migrations barbel orient themselves against the prevailing current. Somewhat contrary to what was found at a larger scale [7], implying that scale effects might play a role in fish movement responses and that future studies need to account for scale variation. On the other hand, the effect of the secondary stressor, oxygen depletion, on the movement of potamodromous fish is only important when movements are not physically limited by barriers to connectivity. When that happens, a clear decrease in movements with the decrease of oxygen concentration is noteworthy. So, the secondary stressor only plays a major role in fish movement thinning when no connectivity reduction exists. Nevertheless, cumulative impacts can be inferred by the decrease in fish activity within the central flume section, with oxygen reduction, during the unconnected treatment. The number of upstream and downstream movements is almost mirrored and that is possibly due to flume length limitation and to environment exploration. This work progresses from single variable manipulative experiments to multifactorial experiments, approaching what naturally occurs in rivers. Such an experimental approach allows understanding how, at times, variables interact forcing fish behavioural responses to adjust to joint variations of stressors.

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