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Abstract

Heat Hardening in Grey Mullets: Physiological Responses of Juvenile *Chelon labrosus* and *Chelon aurata* Under Simulated Short-Term Marine Heatwaves †

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Abstract

Introduction: Marine heatwaves are increasing in frequency and intensity, posing major challenges for fishes inhabiting shallow coastal ecosystems. Short-term exposure to extreme warming can alter metabolic performance and thermal tolerance, with potential consequences for species persistence and school composition in thermally variable habitats. Understanding the capacity of coastal fishes to withstand acute warming events is therefore essential for predicting ecological responses to climate change. **Objective:** We aimed to determine the effects of simulated marine heatwaves on thermal tolerance and metabolic performance in juvenile grey mullets, Chelon labrosus and Chelon aurata, two abundant sympatric species inhabiting the Ria Formosa lagoon (southern Portugal). **Methodology:** Juvenile mullets acclimated at 17 °C were exposed to simulated heatwave treatments of 23, 27, or 33 °C and sampled either at peak temperature or after 48 h and 1-week recovery at 17 °C. Critical thermal maximum (CTmax, using a 1 °C/min thermal ramp), static oxygen consumption (MO2), and intermittent respirometry parameters were measured. Standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) were derived from intermittent respirometry. A complementary temperature-ramp (>3 h at each temperature step 17, 23, 27 and 33 °C) was performed to evaluate routine metabolic rate and estimate Q10 values across increasing temperatures. Additional plasma and tissue analyses are being conducted to assess energetic substrate mobilization and cellular responses to thermal and oxidative stress. **Results:** CTmax increased significantly with warming in both treatment modes, demonstrating rapid heat hardening in juvenile mullets. Fish exposed to 27 and 33 °C exhibited higher CTmax than control fish, and this elevated tolerance persisted after recovery. *Chelon labrosus* showed slightly higher CTmax values than *C. aurata*. Oxygen consumption increased with temperature, with the strongest responses occurring at 33 °C. SMR increased markedly with warming, particularly in heatwave-exposed fish, while MMR increased mainly at the highest temperature treatment. In contrast, AS showed no clear thermal optimum or decline across treatments. Routine metabolic rate increased non-linearly with temperature in the complementary ramp experiment, with a mean Q10 of 2.28, confirming strong thermal dependence of metabolism. **Conclusions:** Juvenile mullets possess substantial short-term thermal plasticity and can rapidly increase heat tolerance during marine heatwaves but this enhanced tolerance is accompanied by elevated metabolic costs

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under extreme warming, indicating potential energetic trade-offs near upper thermal limits. Differential physiological responses between species may influence school composition and ecological performance across thermal landscapes. Ongoing plasma and tissue analyses will further clarify the energetic and cellular mechanisms underlying thermal and oxidative stress resilience in coastal fishes.

Keywords: eurythermal fish; physiological acclimation; climate resilience; cellular responses

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