Studying behavior and physiological stress in fish: a case study on the effect of artificial light at night on mobility and cortisol excretion and accumulation of thinlip mullet (Chelon ramada)

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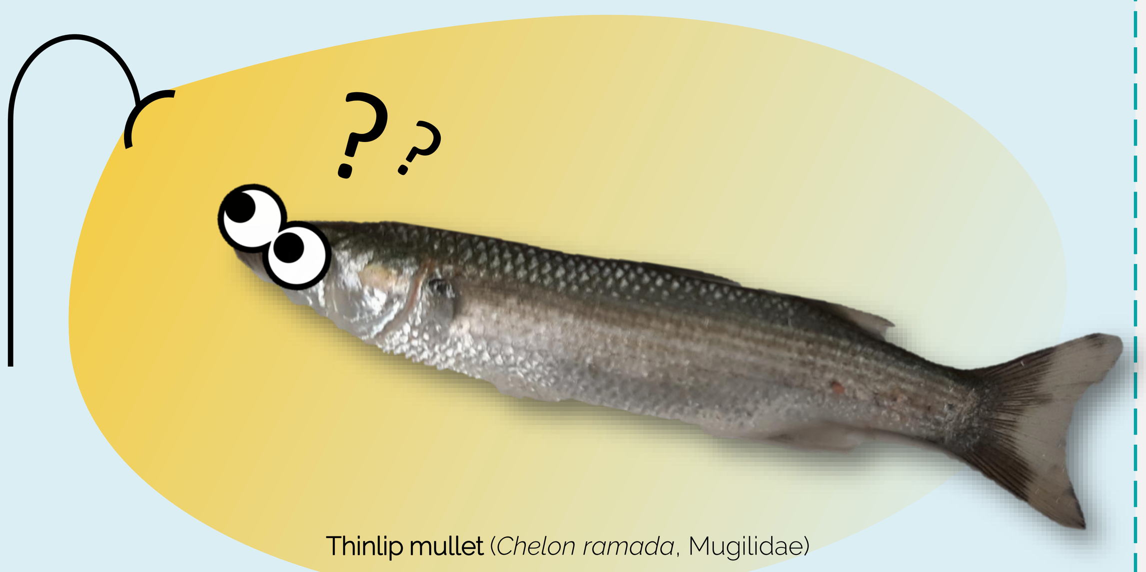
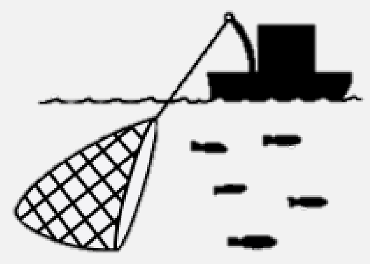
Introduction



Thinlip mullet (*Chelon ramada*, Mugilidae)

Urban ecosystems are submitted to multiple anthropogenic stresses including **artificial light at night** (**ALAN**). ALAN can penetrate into aquatic environments down to 40 m depth depending on water clarity, 3.1 % of the world's marine economic zones impacted down to 10 m (Smyth et al., 2021). However, only **20% of the papers** addressing ALAN's impacts on biodiversity **concern aquatic species** (Bassi et al. 2022). In this work, we used **thinlip mullet** (*Chelon ramada*, Mugilidae) as a biological model. It is a common fish species widely distributed in European urbanized coastal and brackish environments, and is a rare fish representative of the herbivore/detritivore trophic guild. An experiment was set up to investigate the effects of **an alteration of the photoperiod** through ALAN on the **behavior and physiological stress** of thinlip mullet under controlled conditions.

Materials and methods

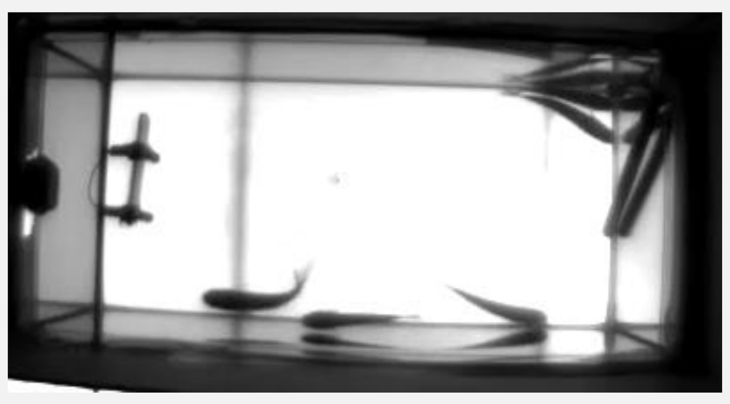


Fishing on 5th April 2023 → **Experiences**: May – June 2023, Repeated 3 times, During 3 days → **Analysis**:

- Cortisol analysis in water and blood
- Behavior analysis

EthoVision XT

Alternate photoperiod (AP) — Continous Photoperiod: direct (dCP), indirect (iCP)

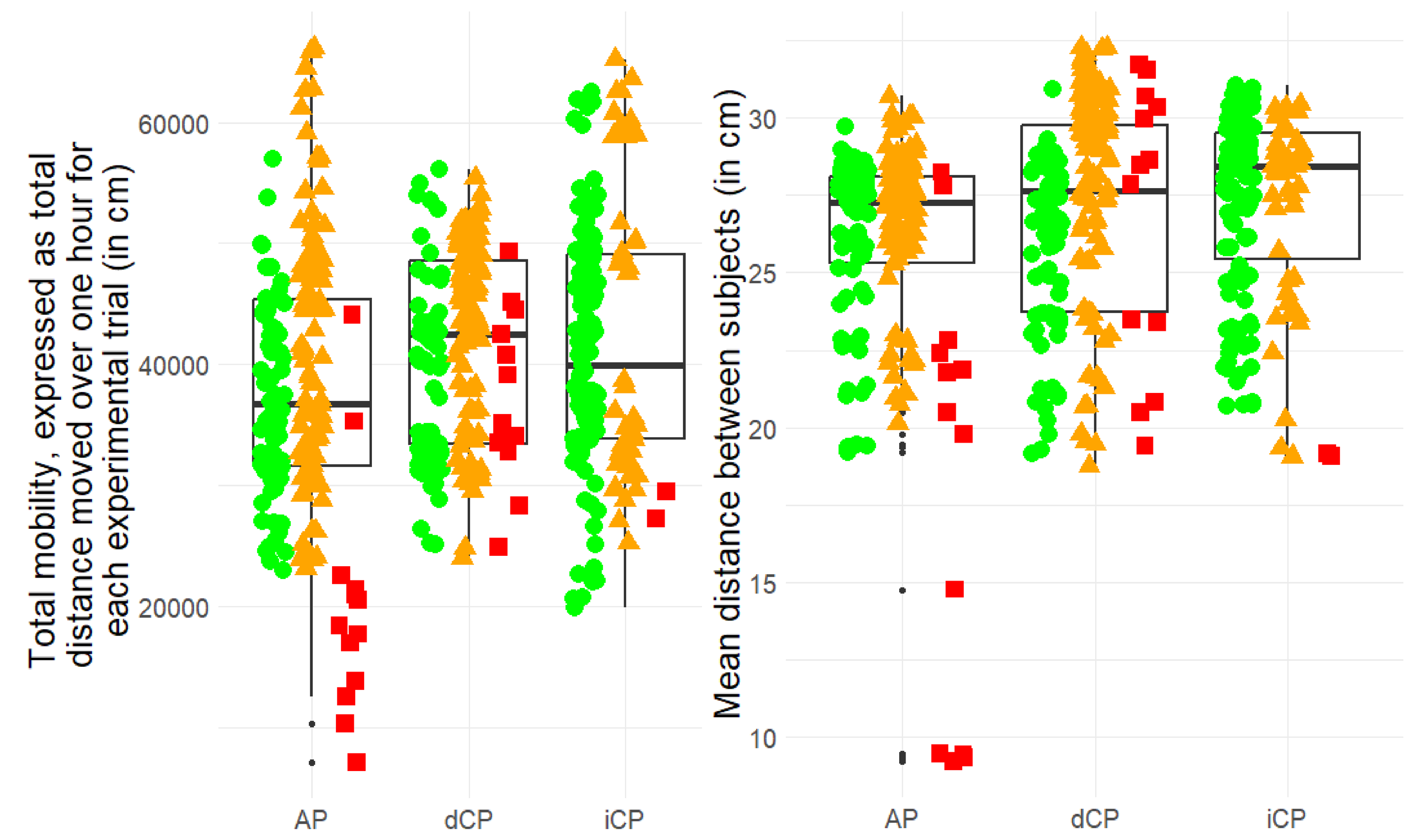


Behavior was recorded continuously over **72 hours** (3 day/night successions) using infrared-equipped cameras in three aquaria (Ctrl=AP, continuous photoperiod with direct light=dCP, continuous photoperiod with indirect light=iCP). Experiences were run **3 times**, using **15 fishes** (5 per aquarium) each time. A total of **38 hours of videos** were interpreted, hours being selected based on a fixed sampling allowing for 3 replicates for each day/night period, Data were stocked and analysed using Ethovison XT 17®, Noldus. Acquisition reliability was assessed using non-detection countings ('subject not found') thresholds (>15%; 15 to 29% and >30%),

Cortisol was analysed in the **water** of each aquarium by sampling 2L in each experimental system every 6 hours (12 samples per trial). Cortisol was quantified from SEP-Pak cartridges extraction (see Ellis et al. 2004). At the end of each trial, **blood** samples were recovered in every fish. Cortisol measurements were expressed as ng g^{-1} biomass of fish.

Results

Behavior



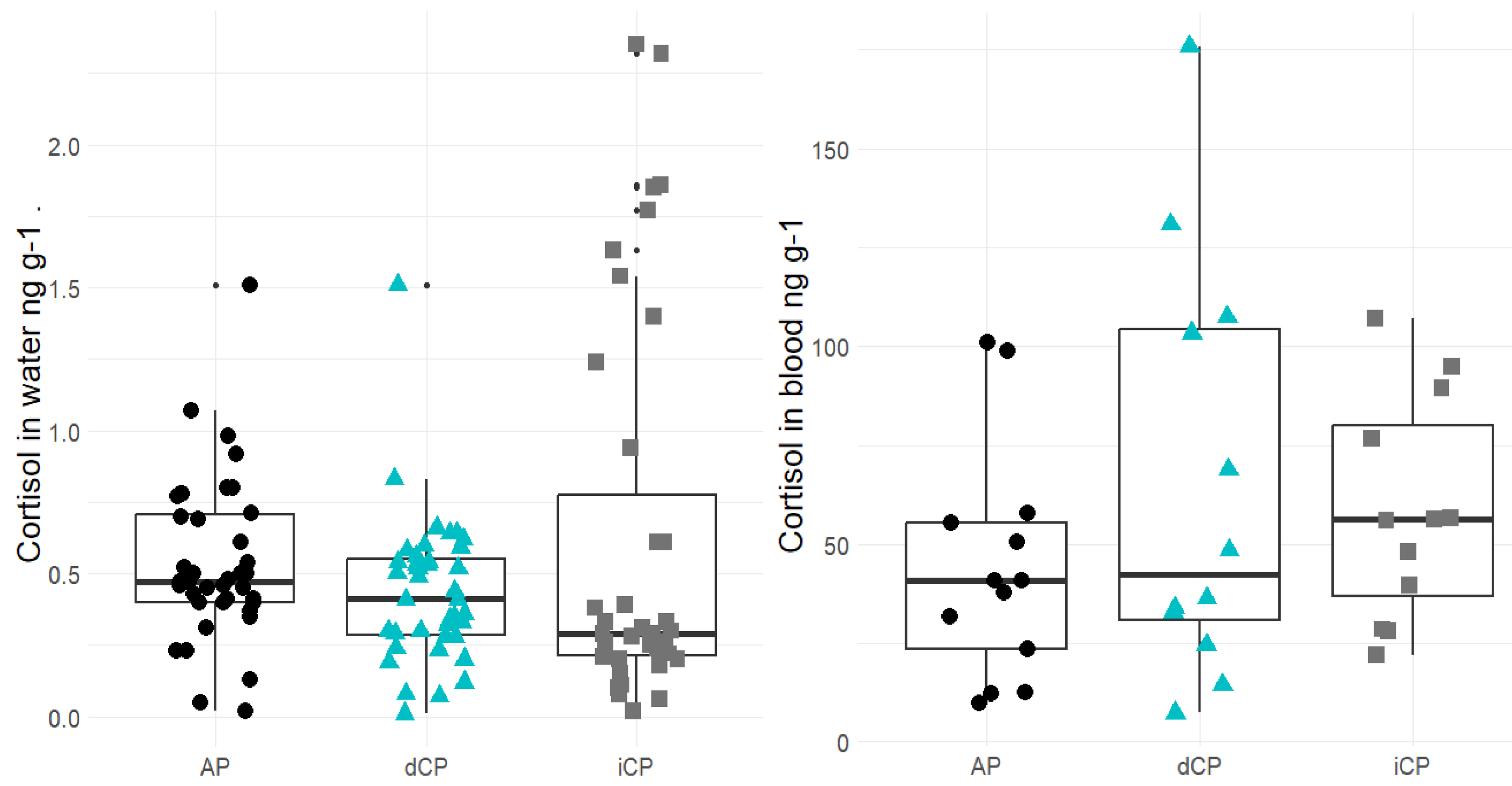
Proportions of undetected individuals by Noldus (= acquired data reliability)

- <15%
- [15-30]%
- >30%

Total distance moved by fishes over the 72h was lower in AP then in dCP/iCP (Kruskall-wallis test, p-value<0,05 ; test post hoc dunn, p-value<0,05).
Fishes were **more distant** one from another in AP than in dCP/iCP (Kruskall-wallis test, p-value<0,05 ; test post hoc dunn, p-value<0,05).

Generalized mixed models with/without aquarium factor.

Cortisol



Condition

- AP
- dCP
- iCP

No significant difference between light conditions (Kruskall-wallis test, p-value>0,05).

Additionnal analyses considering time factor (period during day/night).

Discussion/Conclusion

The behavior of the fish behavior, addressed by the total distance moved and the distance between subjects in the aquaria, varied between normal photoperiod and altered photoperiod conditions. This suggests fish display a behavioral response to ALAN at a short temporal scale (72 hours). As for physiological stress, we detected no difference between treatments in cortisol quantities measured either from the water or the fish blood. These results highlight that behavioral responses are very important to integrate in the study in ecotoxicology; **behavior is a more sensitive parameter**, often overlooked (Bertram et al., 2022).

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