Title: Do aerobic and anaerobic capacities affect the ability to repeatedly escape from predators, and how does personality play a role?

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Abstract

The escape response is the main anti-predator strategy used by fish to escape from predators. It consists of a rapid muscular contraction that quickly thrusts the fish in a direction opposite to that of the predator. Although, this swimming performance involves primarily the activation of white anaerobic muscles, recent evidence suggests that the capacity to aerobically repay an O_2 debt, due to anaerobic activity, can affect the escape response. This link between escape response and aerobic and anaerobic capacity might be especially relevant in the context of escape from multiple predator attacks, i.e. when predators attack in groups. However, this hypothesis has never been tested before. Therefore, this project aims to investigate the relationship between the ability to repeatedly escape from predators and the aerobic scope (AS) and Excess Post-exercise Oxygen Consumption (EPOC). Our results showed that across repeated escape response stimulations, the responsiveness to predator attack simulation declined, probably due to habituation rather than metabolic fatigue, while escape latency remained stable. However, these two variables were not associated with AS or EPOC. While in this report, we only focus on the link between metabolism and behavioural components of repeated escape performance, in the broader project, we also measured how personality ("bold – shy" axis; "anxiety - risk taking" axis) could affect escape response performance. Including this additional analyses is needed to gain a deeper understanding of how physiological and behavioural aspects affect escape response.

Introduction

Escape response performance is a key ecological trait of fishes, crucial for their fitness and survival in nature (Domenici, 2010). Escape response is one of the main antipredator mechanisms. It consists of a fast muscle contraction of the white muscles that quickly propels the fish in a direction opposite to that of the predator (Domenici and Blake 1997). The capacity to escape from predators and to maintain this performance over time drive a strong selective pressures that shape morphology, behavior, and physiology (Domenici and Hale, 2019).

Anaerobic capacity enables rapid escape but incurs an oxygen debt, while aerobic metabolism might support recovery and escape repeatability. Aerobic metabolism, quantified as aerobic scope, represents the capacity to provide O_2 to the tissues for generating a large amount of ATP need to fuel every biological function. While anaerobic capacity is defined as ability to quickly provide ATP to the tissues in absence of O2, but for a limited time frame. Aerobic scope has been used as a performance indicator in fish species (Ern, 2019).

Although escape responses are primarily fueled by anaerobic metabolism, the ability to recover from oxygen debt after repeated escape attempts may depend on aerobic metabolism. To our knowledge, only a previous study investigated the link between escape response and aerobic metabolism (Killen et al., 2015). They found that the latency to escape was negatively linked with time to recovery post exercise (Killen et al., 2015). Furthermore, only few studies investigate both intra- and inter-individual repeatability of escape response, showing that it might vary across species and contexts (Jornod and Roche, 2015; Marras et al., 2011; Nyqvist et al., 2025). While Marras et al. (2011) and Jornod and Roche (2015) found consistent individual performance over time, Nyqvist et al. (2025) reported no individual repeatability. However, very little is currently known about the relationship between aerobic and anaerobic metabolism and the ability to repeatedly escape from predators.

Therefore, this study aims to bridge the gap between escape performance and metabolic capacity. This study was conducted on shiner perch (*Cymatogaster aggregate*), a coastal schooling fish that is subject to high predation pressure. This species serves as a model species for studying escape and metabolic performance due to the abundance, maintenance ease, and measurable swimming behavior. The primary question being explored throughout this study is: Do aerobic and anaerobic capacities affect the ability to repeatedly escape from predators? Hypotheses were generated as follows, Fish with higher aerobic scope and faster recovery (EPOC) will maintain escape responsiveness and performance across repeated stimulations (H_1) and fish with higher anaerobic capacity will show stronger initial escape performance but greater decline due to fatigue across trails (H_2).

Materials and methods

a. Animals

Adult shiner perch, *Cymatogaster aggregate* (*Gibbons, 1854*) were caught by beach seining at Jackson Beach, San Juan Islands, Washington, USA (N 48°31′11″, W 123°0′45″) between July and August 2025.

Fish were transported to Friday Harbor Laboratories and kept in indoor flow-through tanks (92×61×31 cm, 174 l) under natural temperature and photoperiod conditions.

To standardize our measurements, we selected fish within a specific range of body size (N = 54; fork length = 11 cm cm; body mass = 21.21 gr).

Fish were maintained in flow-through seawater so that they were able to feed on plankton present in the water. The day before the experiments, four individuals were randomly selected and fasted for at least 24 h before being used in experiments.

b. Experimental tank for open field and escape response

The experimental setup for the personality and escape response assays consisted of a circular tank (diameter = 107 cm, height = 53 cm) supplied with a flow-through water system. The water level was maintained at 15 cm (volume = 135 l) (Fig. 1). To define the experimental arena, a circular mesh (diameter = 60 cm in diameter) was positioned in the center of the tank. This setup, used to test personality and escape response, will be referred to as the experimental tank from this point on.

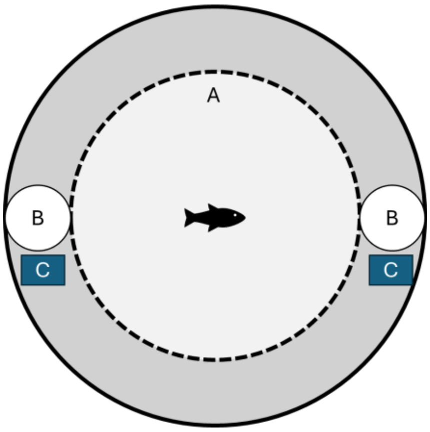

Fig 1. Top view of the experimental tank used for personality and escape response. A) experimental area delimited by a circular mesh, B) Stimulus location, C) 45° mirrors to visualize the impact of the stimulus on the water surface.

c. Experimental protocol

In the current experiment, we measured several physiological and behavioural traits from each individual in the following order (Fig. 2). The details of each measurement are provided in the respective section below. The first day, an individual fish was first transferred to the experimental tank, and left to acclimate for 15 minutes. At the end of the acclimation period, an additional 15 minutes were recorded and then used to assess personality traits. Thereafter, the personality test, the escape response was stimulated as described below. At the end of the escape response test fish were moved back to the holding tank and kept separated in a small mesh enclosure, to rest for 2 hours. After 2 hours resting the fish, were placed into an intermittent flow respirometer to determine standard metabolic rate (SMR). The following day, maximum metabolic rate (MMR), Excess Post-exercise Oxygen Consumption (EPOC) and aerobic scope (AS) were determined.

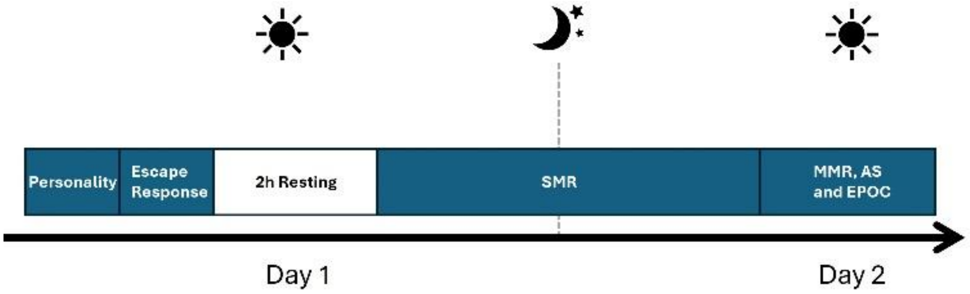

Fig. 2. Diagram showing the trials conducted over the 2 experimental days. Four fish were tested in each trial across the 2 days.

d. Personality

The personality was tested using an Open field test. The fish was placed in experimental tank and let acclimate for 15 minutes. After that, its movement was recorded for 15 minutes using webcam (NexiGo 1080p A07). The personality videos were analysed using the tracking software Trex to obtain the X and Y coordinates of the fish. To estimate the fish personality (bold or shy fish) we measured the following variables: 1) thigmotaxis: percentage of time spent near walls of the arena (1 body length), 2) total distance covered over time, 3) average speed, 4) time spent swimming above 1 body length *s-1.

e. Escape response

The escape response was recorded using Camera Olympus TG-7 at 240fps. After 15 minutes of acclimation and an additional 15 minutes of open field test, the escape response was triggered using mechanical stimulation. To do so, a stimulus (dark cylindrical weight ≈ 220 gr) was released from a height of 145 cm. To avoid visual stimulation before the stimulus impacted the water, the stimulus trajectory was covered by a PVC pipe. The tank was equipped with 2 stimuli, only the closest stimulus to the fish was released to induce the escape response (Fig 1). To assess the ability to perform successive escapes, the fish was stimulated three times at 10± 2 minutes intervals.

The escape response videos were analyzed using Kinovea (v. 2024.1.1.) to obtain the X and Y coordinates of the centre of mass of the fish (CoM) and the head of the fish (HF). We analyzed the following escape response variables: No locomotor variables = 1)Responsiveness, 2) Latency time; Locomotor variables = 3) Cumulative distance (D), 4) Maximum escape speed (U_{max}), 5) maximum acceleration (A_{max}), 6) Turning angle , 7) Turning rate,

f. Metabolic rate

Two hours after the escape response test, fish were placed in intermittent flow respirometry (chamber volume 0.6 l). Oxygen uptake (MO2) of the fish was determined with AquaResp software. The respirometry cycle consisted of 60 s for wait mode and 300 s for measurement and 240 s for flushing mode. The following morning, fish were removed from the respirometry, each individual was manually chased for 3 minutes and air-exposed for 1 minute to estimate the MMR and EPOC. The MMR was calculated as the highest value obtained after the chasing protocol. Aerobic scope was calculated as the difference between MMR and SMR. EPOC was calculated as the area under the $\dot{M}O_2$ curve over the two hours following the manual chasing, representing the energy used for recovery.

g. Statistical analyses

The statistical analyses were conducted in R (v. 4.4.1). The effect of repeated stimulation on the responsiveness of the escape response was tested using a generalised linear mixed model (GLMM) with a binomial distribution, including Trial (stimulation number) as a fixed factor and Fish_ID as a random factor. The relationship between responsiveness and either AS or EPOC was tested using logistic mixed models with Trial as a fixed factor and Fish ID as a random facort. Additionally a Pearson correlations were also calculated within each trial.

The effect of repeated stimulation on escape latency was tested using a LMM with rank-transformed latency as dependent variable, Trial as a fixed factor and Fish ID as a random factor. The relationships of the latency with the aerobic scope, as well as latency and EPOC, were tested using linear mixed models (lmerTest package). Latency to escape was used as the dependent variable. For non-escaping fish, latency was assigned a truncated value of 0.250 s. Aerobic scope (AS) and excess post-exercise oxygen consumption (EPOC) were used as predictors of latency, with stimulus distance included as a covariate. Fish ID was used as random effect to control for repeated measures. Data were rank-transformed when assumptions of normality were not met. Additionally, to examine within-trial relationships, Spearman and Pearson correlations were also calculated between latency and AS or EPOC while controlling for stimulus distance. In this report values are presented as means ± s.e.m and *p Value* < 0.05 was chosen as statistically significant.

Results

a. Responsiveness

The responsiveness declined significantly after the 1rst stimulation ($\chi^2 = 5.76$, $p = 0.016$). The percentage of the fish that responded with an escape response to each stimulation was 81.5% to the first stimulation, 64.8% in the second stimulation and 61.1% in the third stimulation (Fig.3). Responsiveness did not show significant relationship with AS ($p = 0.85$) or EPOC ($p = 0.89$). Within-trial correlations were weak and non-significant for both AS (r = -0.22 and 0.26, all $p > 0.10$) and EPOC (r = −0.12 and 0.14, all $p > 0.40$).

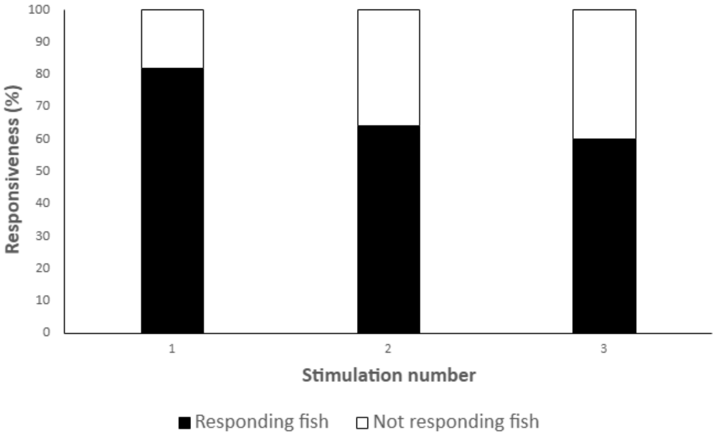

Fig. 3: Responsiveness (%) of the fish over three sequential stimulations.

b. Latency

Latency did not change significantly across trials (LMM, $p = 0.69$), with average latency values were 0.025 ± 0.004 s, 0.027 ± 0.004s, and 0.032 ± 0.008s for Trials 1, 2, and 3, respectively. Neither AS nor EPOC showed a significant association with latency in mixed-effects models ($p = 0.17$ and $p = 0.98$, respectively) (Fig 4). Stimulus distance had a strong positive effect on escape latency in all models ($p < 0.001$), indicating that fish that were closer to the stimulus reacted earlier. Spearman and Pearson correlations performed separately for each of the three trials confirmed the absence of any consistent relationship between metabolic traits and latency ($|\rho| < 0.31$, all $p > 0.14$). Thus, variation in escape responsiveness was explained primarily by distance from the stimulus rather than by aerobic or recovery metabolic capacity.

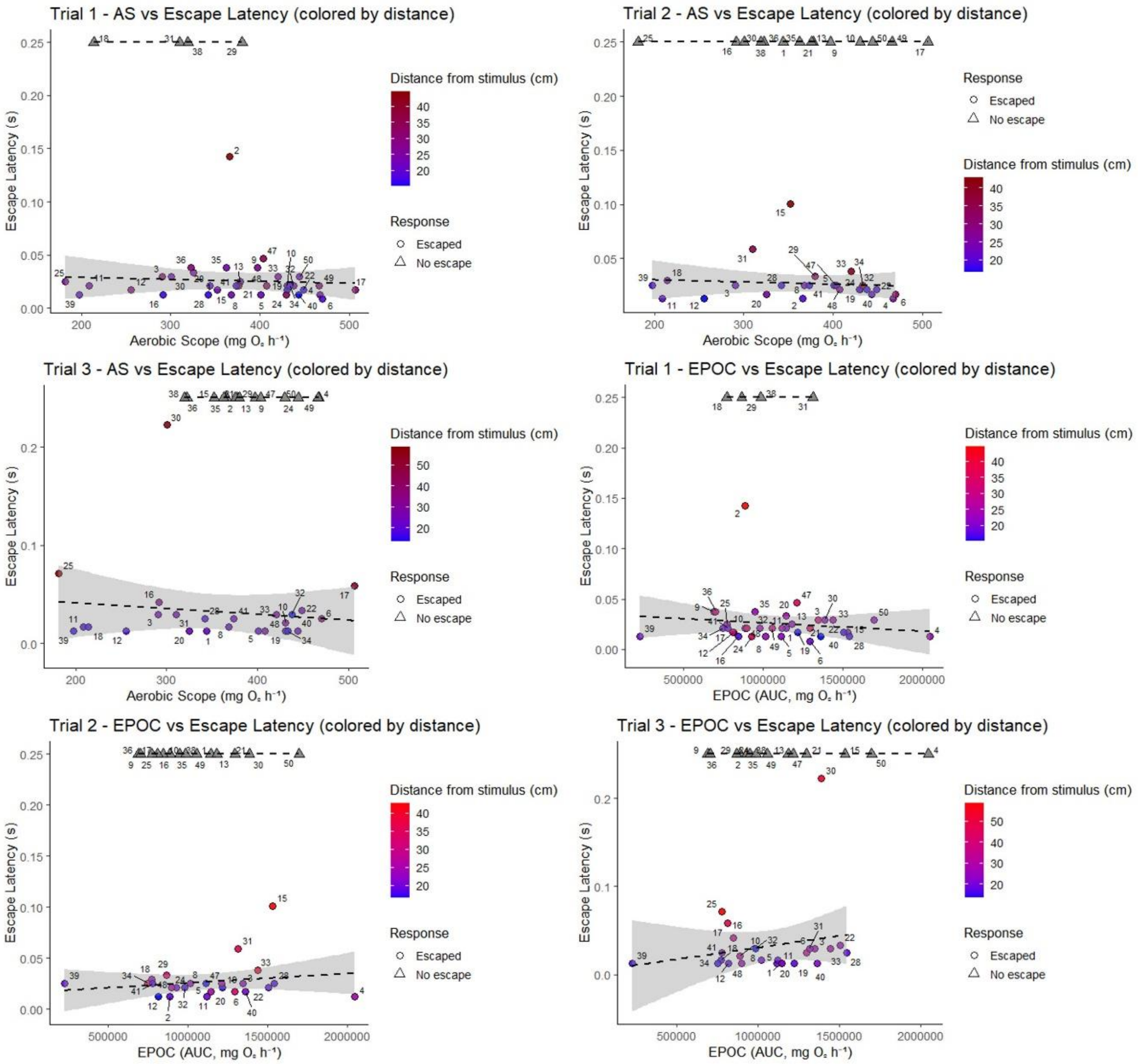

Fig 4: Relationship between response latency to escape and the aerobic scope and EPOC across the three trials. Each chart represents a different trial. Points represent individual fish, with symbols indicating if an escape response occurred or not (Escaped = circles; No escape = triangles) and colours representing the distance of the fish from the stimulus at the time of the stimulation. A max value of 0.250s was given to the fish that did not escaped. Dashed lines is the linear regression with 95% confidence intervals.

Discussion

Across repeated stimulations with an interval of about 10 ±2 minutes, fish showed a strong decline in responsiveness after the first stimulation. This reduction in responsiveness may be explained by habituation effect rather than a trade-off between energy conservation due to the fatigue in performing repeated escapes. Although habituation to recurring stimuli can prevent unnecessary energy expenditure, it may also increase vulnerability to real multiple predator attacks , such as predators attacking in groups. The decline in responsiveness observed here differs from Marras et al. (2011), who found escape performance to be highly repeatable within individuals across repeated stimulations.

Although repeated stimulations resulted in a clear decline in responsiveness, latency did not changes across stimulation. No significant relationship was found between latency and metabolic traits such as aerobic scope or EPOC. These findings are consistent with Killen et al. (2015), who also reported no significant link between metabolic capacity and escape latency. However, Killen et al 2015 found a negative correlation between latency to escape and time to recovery post exercise (Killen et al., 2015), defined as time time for O_2 uptake to return from the MMR to the level of 50% of its total AS.

Limitations and future directions

Although locomotion variables of escape performance were collected, in this report, we did not assess how changes in metabolic capacity relate to locomotor variables. As well, although we recorded personality traits in fish, we did not analyze the link between personality and escape response. In the future, we aim to analyze our data more deeply to examine how aerobic scope, EPOC, and personality traits influence the ability to escape repeatedly from predators. Such integration would provide a more complete understanding of how intrinsic traits shape predator evasion strategies and survival potential in variable environments.

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