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A multivariate analysis using physiology and behavior to characterize robustness in two isogenic lines of rainbow trout exposed to a confinement stress

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HIGHLIGHTS

- Robustness is approached using physiology and behavior measures.
- Data before, during and after a confinement stress are obtained in rainbow trout.
- Sensitivity and resilience are extracted from the temporal patterns.
- Two isogenic lines are characterized using a multivariate analysis.
- The importance of pre-challenge social context was observed.

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ABSTRACT

Robustness is a complex trait difficult to characterize and phenotype. In the present study, two features of robustness in rainbow trout were investigated: sensitivity and resilience to an acute stressor. For that purpose, oxygen consumption, cortisol release, group dispersion and group activity of two isogenic lines of juvenile rainbow trout were followed before and after an environmental challenge. The effect of a 4 h confinement protocol (~140 kg/m^3), which is generally considered as a highly stressful challenge, was investigated. Temporal patterns produced by this experiment were analyzed using multivariate statistics on curve characteristics to describe physiological and behavioral adaptive systems for each isogenic line. The two isogenic lines were found to be highly divergent in their corticosteroid reactivity. However, no correlation between physiological and behavioral sensitivity or resilience was observed. Furthermore, the multivariate analysis results indicated two separate and independent fish group coping strategies, i.e. by favoring either behavioral or physiological responses. In addition, considerable intra-line variabilities were observed, suggesting the importance of micro-environment effects on perturbation sensitivities. In this context, cortisol release rate variability was found to be related to the pre-stress social environment, with a strong correlation between pre-stress aggressiveness and cortisol release rate amplitude. Overall, this approach allowed us to extract important characteristics from dynamic data in physiology and behavior to describe components of robustness in two isogenic lines of rainbow trout.

1. Introduction

Animal breeding programs are evolving, and are now aspiring to new goals. New challenges linked to environmental impact, animal welfare and therefore production sustainability have become important objectives in most breeding programs [16,20,37]. In this context, robustness becomes a key trait for selection. Robustness has been defined by Knap [23] as 'the ability to combine high production potential with resilience to stressors allowing for unproblematic expression of a high production potential in a wide variety of environmental conditions'. However, robustness is a complex trait that is difficult to define and phenotype. The intention to select more robust animals is driven by the desire to obtain animals better able to maintain key life functions despite perturbations [12,23,36]. By characterizing its opposite, vulnerability, many authors have described robustness as a multidimensional concept generally encompassing three main components: exposure, sensitivity and resilience [9,15]. Exposure relates to the degree and duration of a perturbation [1]. Sensitivity refers to the degree to which

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the system is modified or affected by the exposure and resilience to the time taken to recover from the exposure [9].

By conducting an experiment using a controlled exposure to stressor, this study aims at better understanding and characterizing sensitivity and resilience in rainbow trout. The overarching hypothesis of the present study is that repeated measurements of physiological and behavioral traits before, during and after a perturbation of the biological system give a good estimation of its sensitivity and resilience to the stressor and therefore indications on its robustness. Confinement is generally considered as a stressful challenge for fish [3,43,45] but, on the contrary to stressors such as temperature changes, oxygen availability or parasitic infection, confinement is not directly challenging health status. Impact of confinement on several physiological such as osmoregulation, corticosteroid hormones, or behavioral traits like feed intake is now well documented [3,29,46] but, these responses do not help to better face that specific challenge. In that context, strong reactivity to confinement is considered in the present study as a non-robust feature expending energy at the expense of other functions such as growth, immune responses or reproduction [27]. Physiological and behavioral resistance and resilience to a confinement challenge are therefore considered in this study as robustness features. However, such definition cannot be generalized to all stressful situations and, for example in pig or in chicken, strong physiological reactivity was considered to have beneficial effects in specific stressful conditions [34].

In the present study, stress responses to a confinement were analyzed in two isogenic lines of rainbow trout with the aim to compare their robustness components. These lines were previously found to be highly divergent for their ability to grow on a plant-based diet during early-feeding [18]. Both lines show therefore divergent vulnerability for one specific trait in one specific condition. Given this, we decided to characterize these two lines using a different approach by looking at their responses to a perturbation. Therefore, we investigate here if these strains differ in their sensitivity to, and their ability to recover from, a confinement stress. Based on the analysis of the physiological and behavioral response trajectories, associated with a principal component analysis, this study explores the variation in and approaches to characterizing some specific robustness traits in physiology and behavioral sciences.

2. Materials and methods

2.1. Ethical statements

Experiments were conducted within INRA facilities having authorization for animal experimentation (B29-777-02 and B35-238-6). Technical staff and scientists had personal authorizations to conduct animal experimentations in accordance with good animal practice delivered by the DDPP (Service de Protection et de Surveillance Sanitaire des Animaux et des Végétaux). *All experimental procedures used in this study were in respect of the rules edicted by the "National Council for Animal Experimentation" of the French Ministry of Higher Education and Research and the Ministry of Food, Agriculture and Forest. They have been approved by the local "Ethic, Animal Care and Use Committee" provided by the French legislation under the official licence N°7. Agreement number:* R-2012-BS-01.

2.2. Isogenic lines

Two isogenic lines of rainbow trout, *Oncorhynchus mykiss*, were produced by crossing two fully homozygous but different sires (A22 and R23) with two homozygous females both from the same isogenic line (genetically identical females, B57). The resulting individuals were therefore heterozygous A22-B57 and R23-B57 (respectively referred to as A and R in the rest of the text). To avoid any bias due to maternal effects, females spawning the same day were used. The ova were mixed and redistributed in two batches, each being fertilized the same day by one male. Thus, the between-line differences were expected to be genetic only.

Fish of both lines were reared in the same conditions and using the same commercial diet.

Both lines have been previously characterized for their adaptation to a plant-based diet challenge [18].

2.3. Experimental design

The experiment was carried out in two replicates one month apart. Within each replicate, both lines were used.

2.3.1. Acclimation to experimental aquaria

At a mean weight of 1.8 g, 16 fish having similar size of one line were placed in a 1.7 L aquarium for a 2 week acclimation time. For each line, 4 identical aquaria were used. All aquaria were supplied with the same water flow and were visually followed using two cameras placed above. They were also equipped with a small closed external water circuit taking water on one side of the aquaria and bringing it back on the other side. This system allowed, when needed, continuous measures of O_2 concentrations using flow-through oxygen sensors linked to a multi-channel fiber optic oxygen transmitter (oxy-4 mini from PreSens, Precision Sensing GmbH, Regensburg, Germany). The system also allowed water sampling (450 mL) at given time points.

After two days of acclimation, fish ate again and were hand-fed with the same commercial diet as during rearing.

2.3.2. Confinement protocol and sample collection

The experiment consisted of a confinement challenge of 4 hour duration with measurements at 8 time points, before, during and after the challenge (see Fig. 1). The confinement challenge consisted in grouping all fish (mean weight after the acclimation period was 2.1 g) of one aquarium in an immersed net of 0.240 L creating a density of 140 kg/m^3. At each time point, video recording, water sampling and oxygen measures were performed for further analysis as detailed below. At each time point, 10 min of video was recorded, except for the period just after confinement where fish were followed during 30 min. The time point 4, where fish are confined, was not recorded.

2.3.3. Replicate

The second replicate used naïve fish from the same fecundation. Fish weighed 3.8 g at the beginning of the acclimation period. To keep a density similar to the first experiment, only 12 fish were placed per aquarium of 2.3 L. The size of the confinement net was increased in consequence.

2.3.4. Fish sacrifice for invasive measures

In parallel to the first replicate, 8 aquaria per line were added to the experiment enabling us to obtain invasive measures at each time point shown in Fig. 1 (one aquarium per line and per time point). These aquaria were treated identically to the other aquaria, but were not equipped with a camera nor a closed external water circuit. At a given time point, all fish from the aquarium were caught and killed with 2-phenoxyethanol and frozen at −20 °C.

2.4. Sample analysis

2.4.1. Group behavior measurements

Two group behavior indexes were calculated from the video using the method developed by Sadoul et al. [47]. These group behavior indexes translate group dispersion of the shoal in the aquarium and group swimming activity. The method gives a value for both indexes every half second of the video. We summarized these data by calculating the mean for each index and aquarium per 10 min.