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## Testing fat content or feeding behaviour as indirect selection criteria for feed efficiency using trout isogenic lines

S. Pouil<sup>1\*</sup>, T. Kernéis<sup>2</sup>, E. Quillet<sup>1</sup>, D. Lallias<sup>1</sup>, L. Labbé<sup>2</sup>, F. Phocas<sup>1</sup>, and M. Dupont-Nivet<sup>1</sup>

<sup>1</sup> Université Paris-Saclay, INRAE, AgroParisTech, GABI, 38350, Jouy-en-Josas, France; <sup>2</sup> INRAE, UE 0937 PEIMA (Pisciculture Expérimentale INRAE des Monts d'Arrée), 29450, Sizun, France; \*[simon.pouil@inrae.fr](mailto:simon.pouil@inrae.fr)

### Abstract

Phenotyping individual feed intake through direct measurements remains complex in fish, and one way to overcome this challenge is to find indirect criteria for estimating feed efficiency. This study investigated the correlations between fish residual feed intake (RFI) and fat content or the number of feed demands in ten isogenic lines of rainbow trout. We found positive correlations between RFI and fat content indicating that fatter fish tend to be less feed efficient. However, the association between RFI and fat content was not constant over time and was driven mostly by one replicate from one line. We also found positive correlations between RFI and the total number of feed demands. Our findings highlight the interest of using fat content in selective breeding programs to improve feed efficiency and highlighted the interest in exploring the relationship between feed efficiency and feeding behaviour in fish.

### Introduction

Improving feed efficiency (FE) in fish can be achieved through various approaches, including husbandry, nutrition, and genetics. FE is one of the main traits of interest for fish breeding programs (Chavanne et al., 2016). However, unless infrastructure is available for separately rear of families as previously done for Atlantic salmon in Norway, selection for FE remains challenging, as it requires precise recording of individual feed intake (FI). Several approaches can be used for phenotyping individual FI in fish, including video-recording during feeding, the use of radio-opaque feed, and the counting of uneaten pellets in individual rearing. Nevertheless, these methods have major drawbacks as reviewed by de Verdal et al. (2018).

On an experimental scale, one tool to overcome the drawbacks of typical FI assessment methods is using isogenic lines (Quillet et al., 2007), within which all individuals are genetically identical. Because the genetic variability within an isogenic line is zero, fish belonging to the same line may be reared in the same tank allowing a precise assessment of FI of their shared genotype under conditions close to production systems (Knap and Kause, 2018). In addition, combining several isogenic lines gives access to the between-line genetic variability of FE (Grima et al., 2008).

Many indirect criteria for estimating FE have been studied. Body weight loss and gain during feed deprivation (FD) and subsequent compensatory growth (CG) periods and fat content have been suggested as indirect estimators of FE (de Verdal et al., 2018). Feed efficiency can also be linked to feeding behaviour. For example, Martins et al. (2006) have shown that African catfish with better FE spend less time eating. In Nile tilapia, Martins et al. (2011) found that FE was negatively correlated with feeding latency and positively correlated with feeding time and the number of feeding acts.

The objectives of the present study were to investigate the links between residual feed intake (RFI), fat content and feeding behaviour.

### Materials & Methods

**Heterozygous isogenic lines.** Parents were derived from the INRAE homozygous isogenic lines previously established after two generations of gynogenesis (Quillet et al., 2007). Ten heterozygous isogenic lines were produced by mating females from a single maternal homozygous isogenic line (named B57) with sex-reversed XX males from ten other homozygous isogenic lines (A02, A03, A22, A36, AB1, AP2, B45, B61, N38, R25) to produce genetically identical heterozygous (h) individuals exhibiting performances across the range of the population from which the isogenic lines are derived. Only one maternal line was used to avoid maternal effects which could bias the results.

**Successive experimental phases.** The experiment reported here started at 137 days post fertilization (dpf). Feed intake was measured three times: from 265 dpf to 297 dpf (fi1) then from 439 to 469 dpf (fi2) and from 522 to 547 dpf (fi3). The first period (fi1) of FI assessment was performed under normal growth conditions, while the two others (fi2, fi3) followed FD periods. From 137 to 549 dpf, each line was reared separately into 1.8 m<sup>3</sup> outdoor tanks, with three triplicates per line, supplied with river water (11.3-16.0°C). In order to keep a density below 50 kg m<sup>-3</sup>, regular random eliminations were performed, and 100 fish per replicate remained at the end of the experiment. Commercial pellets were distributed by self-feeders (Imetronic®, France) throughout the experiment.

**Weight and residual feed intake.** Body weight (BW) was measured regularly throughout the experiment (n ≈ 100 fish for each of three replicates). A way to measure FE is to estimate the residual feed intake (RFI) using linear regression analysis:

$$RFI = FI - (\beta_0 + \beta_1 \times BW^{0.8} + \beta_2 \times BWG) \quad (1)$$

With FI being the observed feed intake and  $(\beta_0 + \beta_1 \times BW^{0.8} + \beta_2 \times BWG)$  being the predicted feed intake based on a linear regression of the observed FI on metabolic body weight ( $BW^{0.8}$ ) and body weight gain (BWG).

**Fat content.** The total fat content in muscle was recorded three times during the experiment (at 471, 520, and 549 dpf, i.e. measurements made at the end of fi2, right before fi3 and at the end of fi3, respectively; n ≈ 100 fish per replicate) using a Fish Torry Fat-meter® (Dourin et al., 1998).

**Feed demand.** Self-feeders were used continuously throughout the experiment to characterize feeding behaviour for the ten isogenic lines by recording feed demands (i.e., rewarded, unrewarded, and both of them). This feeding technique is based on the learning ability of fish, and feed is delivered depending on their demand. In a self-feeding system, fish are assumed to precisely control their feeding by activating a "trigger" sensor. All the self-feeders were connected to a computer system recording the feed demands.

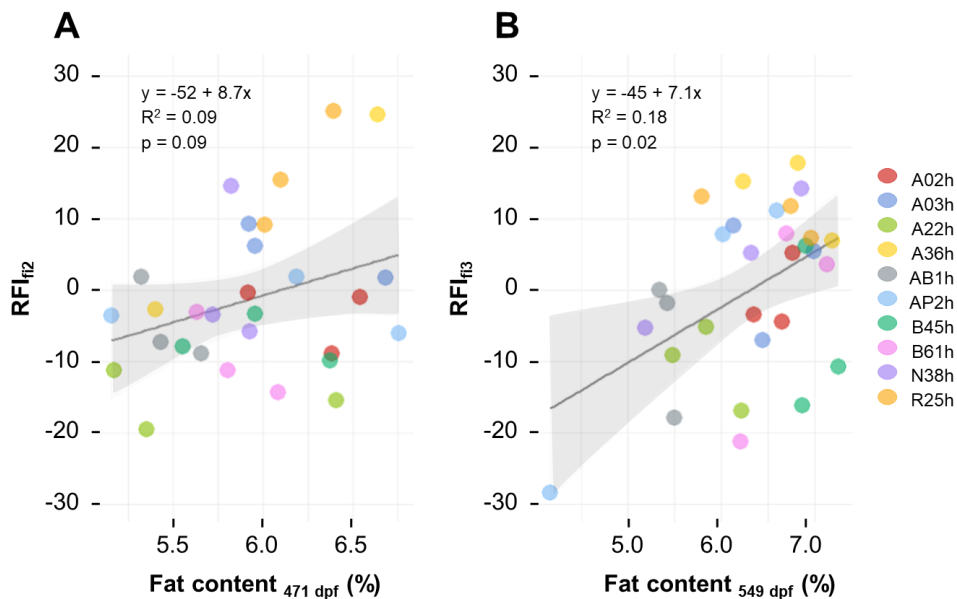
## Results

The correlations between RFI derived from the first measurement under normal feeding regime (fi1) and the two others measures following a FD period (fi2 and fi3) were low ( $r \leq 0.25$ ,  $p \geq 0.18$ ). However, the correlation between the last two RFI measured after FD, i.e. fi2 and fi3, was significant ( $r = 0.58$ ,  $p < 0.01$ ).

Muscle fat content was comparable across the lines at 471 dpf ( $5.9 \pm 0.8\%$ ), but a significant line effect was observed at 520 and 549 dpf. On average, fish lost between 20% (B61h) and 58% (N38h) of muscle fat during the FD period while they gained from 31% (AB1h) to 150% (N38h) of their muscle fat during the re-feeding period.

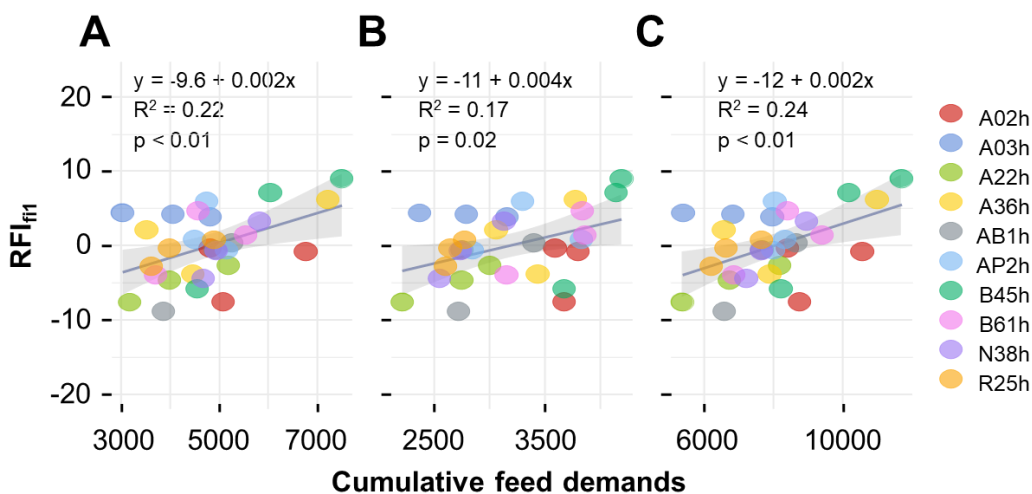
We found a positive correlation between RFI calculated from the two FI measurements following FD periods (fi2 and fi3) and the fat content measured at the end of the FI

measurements (at 471 and 549 dpf). This correlation was only significant for the fi3 measurement period but mostly driven by one replicate belonging to AP2h (Figure 1). Overall, we found that RFI increased when fat content increased.



**Figure 1. Relationship between fat content in muscle measured at 471 and 549 dpf, i.e. right after the two last feed intake periods: from 439 to 469 dpf (fi2) and from 522 to 547 dpf and the RFI calculated for the same periods: (A) fi2 and (B) fi3. Dots sharing the same colour are replicates of the same isogenic line. Grey shading areas indicate the 95% confidence intervals.**

The daily profile of feeding behaviour differed significantly between lines only during the two last FI measurement periods (fi2 and fi3). Interestingly, we found significant correlations between RFI calculated from the first FI measurement period (fi1) and feeding behaviour, assessed as the total number of feed demands over the same period ( $r = 0.42-0.49$ ,  $p \leq 0.02$ ; Figure 2). Such correlations were not found for the other FI measurement periods.



**Figure 2. Relationship between the daily feed demands: (A) unrewarded, (B) rewarded and (C) total and the RFI calculated during the first feed intake period (from d 265 to 297 dpf; fi1). Dots sharing the same colour are replicates of a same isogenic line. Grey shading areas indicate the 95% confidence intervals.**

## Discussion

Previous findings highlighted the relationship between fat content and feed efficiency in rainbow trout and other salmonid species. In rainbow trout, divergent selection for muscle fat content has been carried out (Quillet et al., 2005). After four generations of selection, fish from the lean-muscle selected line had lower body and muscle lipid contents, and their feed conversion ratio (FCR) was improved by about 6-9% compared to the fat-muscle selected line (Kamalam et al., 2012). Kause et al. (2016) have shown that rainbow trout with a low body fat content and low muscle fat content were more efficient in feed and protein utilization, highlighting that controlling fat deposition in fish breeding programs could improve FE and reduce nutrient waste. Our results, from isogenic lines, suggest that such conclusions need to be qualified.

In brown trout, Mambrini et al. (2004) found that selection for growth led to changes in feeding activity as assessed using self-feeders, but differences between control and selected lines were significant for only three out of the five periods investigated. We obtained similar results for rainbow trout isogenic lines. RFI was positively correlated with the number of feed demands at a routine feeding regime (i.e., without any FD), but not after FD periods. In the regular feeding regime, the most feed-efficient isogenic lines (lower RFI) were the ones that requested less feed via the self-feeders. Such findings are in accordance with Martins et al. (2006, 2011), who found that, in African catfish or in Nile tilapia, the most feed efficient fish were the less active for eating.

To conclude, while we did not find clear evidence about the relevance of using fat content as an indirect selection criterion for FE in breeding programs, our results showed the interest in exploring other indirect selection criteria, particularly linked to feeding behaviour.

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