



Biopsy sampling of red muscle does not affect physiological performances in rainbow trout

Morgane Pengam^a, Asrar Lehodey^a, Patrick Calvès^a, Jean-Baptiste Quéméneur^b,
Karine Salin^b, Bernard Simon^a, Aline Amérand^{a,*}

^a Univ Brest, Laboratoire ORPHY EA 4324, Brest F-29200, France

^b Univ Brest, CNRS, IRD, Ifremer, LEMAR, Plouzané F-29280, France

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ABSTRACT

Muscular biopsy is a non-lethal muscle sampling technique allowing for the fish to be returned to its natural environment or its tank after sampling. This technique offers the opportunity for the scientific community and fish farmers to carry out assays on very small muscle samples (between 1 and 40 mg) such as heavy metal, trace elements, lipid composition or muscle energetic metabolism to evaluate, for instance, the health of the fish. The aim of the present study was to determine if a red muscle biopsy affects rainbow trout (*Oncorhynchus mykiss*) survival and their physiological performances (swimming and hypoxia resistances). Each group, fish that had a biopsy (n = 30) or fish that did not (n = 30), was subsequently tested for either a hypoxia resistance test (HRT) or a swimming resistance test (SRT). HRT and SRT were conducted 7- and 10-days post-surgery (dps), respectively. Biopsy had no effect on hypoxia resistance and on swimming parameters (sustained and critical swimming speeds, tail beat frequency, routine and maximal oxygen consumptions). Even if no significant effect was observed between control and biopsy groups on morphometric parameters (body weight variation and condition factors), all the trout lost weight which can be explained by a post-surgery trauma such as human manipulation stress or a local inflammation. More specifically, body weight variation was significantly more important in the 7-dps group compared to the 10-dps group which had the opportunity to eat three more days compared to the 7-dps group. Corroborated with a principal component analysis, we showed that a red muscle biopsy is a good approach as it had no effect on whole-animal performance 7- and 10-dps and it had no effect their survival.

1. Introduction

For several years, fish muscular biopsy has interested the scientific community and fish farmers. This technique enables to perform a non-lethal muscle sampling, while allowing the fish to be returned in its natural environment or in its experimental tank after the biopsy procedure (Crawford et al., 1977; Henderson et al., 2016; Knight et al., 2019). Several publications have demonstrated a wide range of assays that can be performed on small muscle samples (between 1 and 40 mg) such as quantifications of heavy metal (Cyr et al., 2023), trace elements (Stahl et al., 2021), lipid composition (Péron et al., 2023) or mRNA (Pengam et al., 2021, 2020). Also, recent studies evaluated the red muscle energetic metabolism through mitochondrial oxygen consumption from 5 mg muscle biopsy (Quéméneur et al., 2022; Thorat et al., 2024). Biopsy could be relevant in the aquaculture sector to evaluate the quality of fish flesh destined for human consumption through detecting

bacterial or fungal infections. Muscle biopsy can therefore be used to carry out many analyses and answer scientific and economic questions, but studies on the biopsy effects on fish physiological performances and on their survival are still very scarce.

We propose to investigate the effects of this type of biopsy on rainbow trout (*Oncorhynchus mykiss*). Thus, this study focused on the biopsy effects on the swimming performance, the whole oxygen consumption, the ability to resist water oxygen content variations and morphometric parameters.

The red muscle of fish is involved in swimming whatever its type or its intensity (Tudorache et al., 2013). Thus, we explored the short-term effects of a red muscle biopsy (one-week post-biopsy) on swimming performances evaluated through the maximal sustained swimming speed (U_{ms}), the critical swimming speed (U_{crit}) and tail beat frequency. Fish survival depends on the energy production capacity for their physiological needs. So, routine and maximal whole organism oxygen

* Correspondence to: Université de Brest, UFR Sciences et Techniques, ORPHY – EA 4324, 6 avenue Victor Le Gorgeu, Brest 29238, France.

E-mail address: aline.amerand@univ-brest.fr (A. Amérand).

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consumptions were explored. Fish metabolism is divided into an incompressible standard metabolism to maintain the physiological functions essential to life and a variable routine metabolism depending on the activity (Chabot et al., 2016). According to research, biopsy can cause a metabolic disorder (Basu and Shukla, 2013) inducing standard and/or routine metabolism changes. Indeed, immunity responses and factors involved in tissular healing after biopsy (which be compared to an injury) can induce an increase in energetic cost (Ingerslev et al., 2010), which means energy may no longer be available for growth, digestion or physical performance for example. Adaptation to changing environmental condition requires uncompromising energy expenditure if these variations become extreme. In nature, in pisciculture or in experimental tanks, fish could be confronted to a hypoxia shock resistance in their environment due to an increase in temperature (Strowbridge et al., 2021), an accidental pollution (Gomez Isaza et al., 2021), tank size or water supply problem (Oldham et al., 2018; Zhan et al., 2023) for example. In the present study, we have also explored the red muscle biopsy effect on hypoxia resistance on rainbow trout.

2. Materials and methods

All experimental procedures were designed in accordance with animal experimental authorization (APAFIS#30672–2021032521561454 v4). The three “Rs” (replace, reduce and refine) were respected during stabling and experiments.

2.1. Animals

Sixty juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from a local fish farm (EARL pisciculture de Lescoat, Lesneven, France). At their arrival at the laboratory, fish were acclimated in 400 L polyethylene tanks at least 10 days before the experiments. Tanks were filled with continuously renewed and aerated tap water at 15°C with a natural photoperiod (15 h : 9 h light:dark). Trout were fed to satiety 5 days a week with commercial pellets (trout granules, Skretting, France).

Seven or ten days before starting experimental hypoxia or swimming resistance tests respectively, trout were anesthetized allowing morphometric measurements, identification and biopsy procedures. During surgical procedure, the trout randomly fished were placed on a surgical table in a wet cloth with a tricaine MS-222 (80 mg.L⁻¹) constant and sufficient flow to the mouth to allow discharge through gills. For all trout ($n = 60$), an identification tag (Glass tag 1.4 × 12 mm, Biolog-id, Bernay, France) was subcutaneous implanted between the head and the dorsal fin. On 30 random trout, a skin incision (length < 5 mm; depth < 2 mm) was made on the left side with a scalpel between the dorsal and caudal fins just below the lateral line and a sample of red muscle (< 5 mg) was collected using a biopsy punch (2 mm LCH-PUK-20, Kai Medical, Solingen, Germany) (Fig. 1). Scalpel incision was disinfected

using chlorhexidine and filled with powdered bandage (ORAHESIVE, ConvaTec®, Deeside, UK) (Quéméneur et al., 2022). The body mass (BM: 174.7 ± 3.0 g, $n = 60$) and the body length (BL: 24.5 ± 0.1 cm, $n = 60$) were then measured for each animal that was subsequently placed in a recovery tank. Finally, trout that had recovered from anesthesia were placed in a specific tank: control ($n = 30$) or biopsy ($n = 30$). All trout survived to biopsy.

At the beginning and at the end of the experiment, condition factor (CF) was calculated for each fish using the following equation: $CF = \frac{BM \times 100}{BL^3}$ where BM is expressed in g and BL in cm (Fulton, 1902).

Then, the trout were randomly divided into four group ($n = 15$ for each group; Fig. 2): “control – hypoxia resistance test (HRT)”; “biopsy – HRT”; “control – swimming resistance test (SRT)” and “biopsy – SRT”. Hypoxia (HRT) and swimming (SRT) resistance tests are described below.

Based on Quéméneur et al. (2022), biopsy had no effect on infection and inflammation responses 3-, 14- and 28-days post-biopsy (dps) in sea bass. Thus, a period of more than 3 days (here, at least 7 days) was maintained between anesthesia and experimental procedures. We felt that this period of at least seven days was necessary in the context of this initial study in order to observe the healing process, which proved to be satisfactory.

2.2. Experimental protocols

2.2.1. Hypoxia resistance test (HRT)

Seven dps, the oxygen tension at loss of equilibrium (P_{LOE}) was determined on 30 fish (Control $n = 15$; Biopsy $n = 15$). The fish were fasted for 24 h before the beginning of the experiment. The HRT was carried out in two stages to have 15 animals maximum per test (control and biopsy individuals mixed). One day before experimentation, the trout were placed in a circular tank (diameter: 128 cm; height: 75 cm) containing 200 L of water. During HRT, the tank was covered by a tarp to limit light and gas exchange between water and air. The HRT consisted of a fast decrease in water oxygenation (from nearly 100–30 % air saturation in 1 h), followed by a gradual decrease (5 % air saturation per hour) until the experiment ended. Water oxygenation was controlled and homogenized by bubbling nitrogen with a porous stone placed in a pump inside the tank. When a loss of a fish equilibrium was observed, the P_{LOE} of the fish was considered as reached and the corresponding time and oxygenation rate were recorded (Roze et al., 2013). Then, the trout was immediately fished and killed by cerebral concussion. The experiment finished when all the fish in the tank had reached their P_{LOE} . The body mass (BM: 157.0 ± 4.2 g, $n = 30$) and body length (BL: 24.7 ± 0.2 cm, $n = 30$) were measured for each animal allowing to measure the final condition factor.

2.2.2. Swimming resistance test (SRT)

Ten dps, the swimming performance was evaluated on 30 fish (Control $n = 15$; Biopsy $n = 15$) in a confined Brett-type swimming tunnel. Before the beginning of SRT, fish fasted for 24 h and each fish was placed in the swimming tunnel at least for 1 h with a water velocity of 0.05 m.s⁻¹ to acclimate its new condition. Then, the water velocity was increased by 0.10 m.s⁻¹ every 10 min until exhaustion, in water maintained at 15 ± 0.5°C.

2.2.2.1. Maximal sustained swimming speed (U_{ms}). Maximal sustained swimming speed (U_{ms}) which corresponds to the borderline between aerobic and anaerobic speeds (Kraskura et al., 2024), was determined checking trout bursts. When three bursts were observed in less than a minute, U_{ms} was considered as reached. Swimming test performance was continued until reaching critical swimming speed (U_{crit} , see below). U_{ms} was calculated for each animal using this equation inspired by Brett's equation: $U_{ms} = V_{n-1} + V_i \times \frac{V_n}{T_i}$, where V_{n-1} is the velocity of the last completed swimming speed before the three bursts in less than a

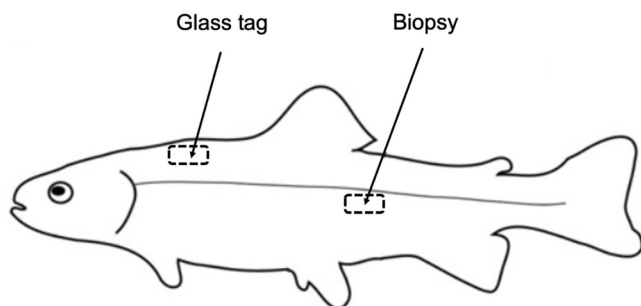


Fig. 1. Illustration of identification tag and biopsy localizations on the fish. On surgery fish, an identification glass tag was implanted ($n = 60$) and after a skin incision on the left side, a red muscle sample (< 5 mg) was collected ($n = 30$) using a biopsy punch. Illustration modified from Ingerslev et al. (2010).

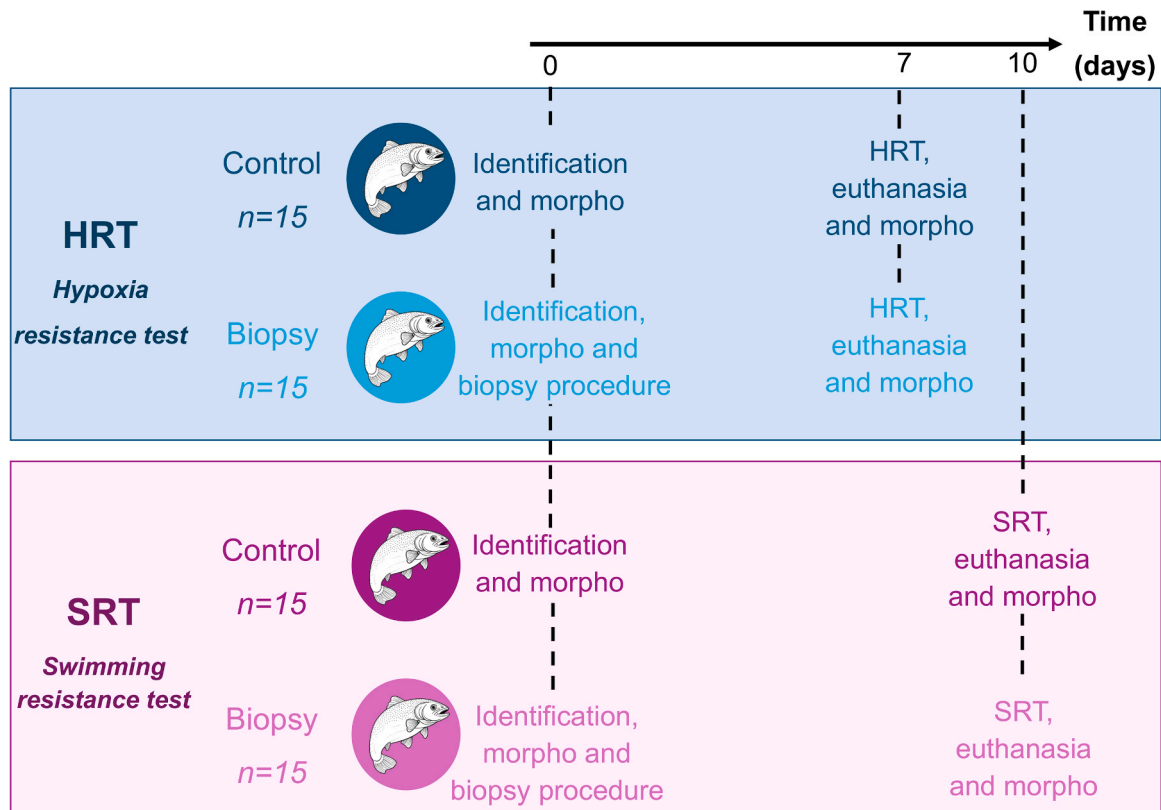


Fig. 2. Experimental protocol design. At $t = 0$, all trout ($n = 60$) were identified, morphometric measurements (body length and weight) were conducted, and 30 trout were randomly biopsied. Seven days post-biopsy, 30 trout ($n = 15$ Control; $n = 15$ Biopsied) performed a hypoxia resistance test. Ten days post-biopsy, 30 trout ($n = 15$ Control; $n = 15$ Biopsied) performed a swimming resistance test. Morpho: morphometric measurements.

minute ($\text{m}\cdot\text{s}^{-1}$); V_i is the velocity increment ($0.1 \text{ m}\cdot\text{s}^{-1}$); t_n is the duration of the last step after three bursts in less than a minute (min) and T_i is the duration of each step (10 min).

2.2.2.2. Critical swimming speed (U_{crit}). U_{crit} was reached when the fish stopped swimming after three manual stimulations with a rod. Then, the trout were fished and killed by cerebral concussion. U_{crit} was measured according to Brett (1964) and Farrell (2008) and calculated for each animal using Brett's equation: $U_{crit} = V_{n-1} + V_i \times \frac{t_n}{T_i}$, where V_{n-1} is the velocity of the last completed swimming speed ($\text{m}\cdot\text{s}^{-1}$); V_i is the velocity increment ($0.1 \text{ m}\cdot\text{s}^{-1}$); t_n is the duration of the last step (min) and T_i is the duration of each step (10 min).

The body mass (BM: $171.2 \pm 3.9 \text{ g}$, $n = 30$) and body length (BL: $24.8 \pm 0.2 \text{ cm}$, $n = 30$) were measured for each animal allowing to express U_{ms} and U_{crit} in $\text{BL}\cdot\text{s}^{-1}$ and to measure the final condition factor.

2.2.2.3. Tail beats. Three short videos (15 s) were taken during the 0.35 and $0.45 \text{ m}\cdot\text{s}^{-1}$ steps to determine the number of tail beats of each fish.

2.2.2.4. Routine and maximal oxygen consumptions ($\dot{M}O_2$). Throughout the SRT, the trout oxygen consumption ($\dot{M}O_2$) was measured with an oxygen meter (Witrox 1, Loligo®Systems) in order to determine routine and maximal $\dot{M}O_2$. Every step, water was reoxygenated for 1 min. Routine $\dot{M}O_2$ ($\dot{M}O_{2 \text{ rout}}$), allowing to obtain the routine metabolic rate (RMR), was determined by selecting a data range of 20 min during acclimatation stage in the swimming tunnel. Maximal $\dot{M}O_2$ ($\dot{M}O_{2 \text{ max}}$), allowing to obtain maximal metabolic rate (MMR), corresponds to the peak of trout oxygen consumption during SRT.

2.3. Statistical analysis

All the results were expressed as means \pm standard error of means (SEM). The statistics were performed using RStudio software (©2024 by Posit Software, PBC). The normality of distribution was tested using the Shapiro-Wilk test. The homogeneity of population was tested by Bartlett test (when the normality of distribution was verified) or by Levene test (when the normality of distribution was not verified). Adapted tests (t test or Mann-Whitney) were then performed to evaluate the effect of the biopsy (comparison of means between control and biopsy groups). To compared initial to final condition factors a paired t test or a Wilcoxon test was performed. The significance threshold was set at $p < 0.05$.

The principal component analysis (PCA) was performed for HRT and SRT groups on all explored data using the *FactoMineR* package.

3. Results

3.1. Oxygen tension at loss of equilibrium (P_{LOE})

Biopsy had no significant effect on the oxygen tension at loss of equilibrium (P_{LOE}) of the trout (Fig. 3).

3.2. Swimming data

The data summarized in Table 1 indicated that maximal sustained and critical swimming speeds did not differ between control and biopsied fish. Moreover, no biopsy effect was observed on the number of tail beats measured during 15 s at 0.35 and $0.45 \text{ m}\cdot\text{s}^{-1}$. Routine and maximal $\dot{M}O_2$ were also not significantly different between control and biopsy groups.

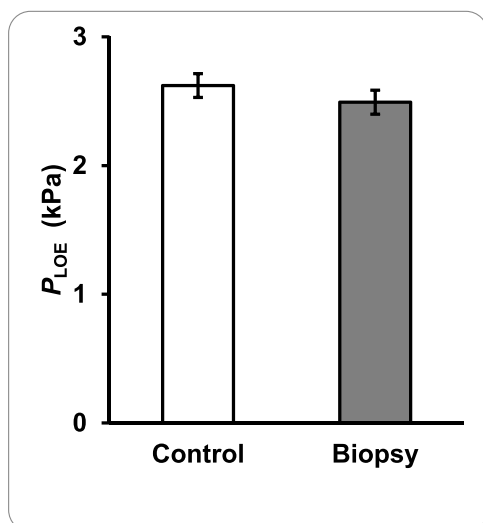


Fig. 3. Oxygen tension at loss of equilibrium (P_{LOE}) of rainbow trout. Results are means \pm SEM. Control: $n = 15$; Biopsy: $n = 15$.

Table 1
Swimming speeds, tail beats and oxygen consumption ($\dot{M}O_2$) of rainbow trout.

	Control $n = 15$	Biopsy $n = 15$	p-value	
Maximal sustained swimming speed (BL. s^{-1})	2.06 ± 0.14	2.22 ± 0.13	$p = 0.42$	
Critical swimming speed (BL. s^{-1})	2.96 ± 0.14	2.96 ± 0.11	$p = 0.93$	
Number of tail beats for 15 s	At 0.35 m. s^{-1}	42.49 ± 1.10	41.45 ± 1.13	$p = 0.51$
	At 0.45 m. s^{-1}	44.71 ± 0.50	44.26 ± 0.86	$p = 0.79$
Oxygen consumption ($mmol.h^{-1}.kg^{-1}$)	Routine	5.03 ± 0.79	4.81 ± 0.70	$p = 0.84$
	Maximal	17.02 ± 1.01	16.66 ± 1.31	$p = 0.59$

Results are means \pm SEM. For oxygen consumption data, $n = 14$ for control and biopsy groups. Italic font for p-value signified a used of Mann-Whitney test.

3.3. Morphological data

3.3.1. Weight variation

In both 7- and 10-dps groups, weight variation did not differ significantly between control and biopsied trout (Table 2). For control and biopsy groups, significant differences were shown between 7- and 10-dps.

3.3.2. Condition factors

Here, the post-surgery period is considered, not the tests (SRT and HRT) performed at the end of this time. In both experimental groups (7- and 10-dps), biopsy had no significant effect on initial and final

Table 2
Weight variation 7- and 10-days post-surgery in rainbow trout.

	Control $n = 15$	Biopsy $n = 15$	p-value	
Weight variation (g)	7-dps	-7.77 \pm 0.65	-7.47 \pm 0.58	$p = 0.74$
	10-dps	-5.04 \pm 1.07	-4.71 \pm 1.57	$p = 0.87$
p-value	$p = 0.04$	$p = 0.01$		

Results are means \pm SEM. Weight variations correspond to the weight difference between the initial weighing (upon biopsy) and the final weighing (upon animal killing). dps: days post-biopsy. Italic font for p-value signified a used of Mann-Whitney test.

condition factors (Table 3). For each group, initial condition factor was significantly different from final condition factor.

3.4. Principal component analysis

The principal component analysis (PCA) showed that control and biopsied distributions of SRT and HRT groups were, respectively, partially or entirely superimposed indicating that biopsy had no significant effect on explored data.

4. Discussion

Although muscular biopsy pertinence has already shown to respond to ecological or economical questions, the present study is the first to explore in the same paper the effects of a red muscle biopsy on the swimming performance, whole organism oxygen consumption, hypoxia resistance and morphometric parameters in rainbow trout.

Red muscle is involved in several swim types like sustained or critical swimming (Tudorache et al., 2013). In their environment, the sustained aerobic swimming is used by fish for steady swimming against low flow, for daily behavior or foraging. When the fish is at borderline aerobic and anaerobic speeds, bursts could be observed (Kraskura et al., 2024) and this speed is considered as the maximal sustained swimming speed (U_{ms}) which corresponds to the maximal aerobic performance (Marras et al., 2010). Critical swimming speed (U_{crit}) could be carried out in laboratory by swimming the fish to exhaustion and the U_{crit} test permits to examine maximum swimming energetic capacity such as oxygen consumption rates (Kraskura et al., 2024). Even if red muscle is involved in both swims, no significant difference was observed neither in U_{ms} nor U_{crit} between control and biopsy groups (Table 1). These results indicate that a lesion of this muscle through biopsy had no or no lasting effects after 10 days post-surgery (dps) on rainbow trout swimming performance. It is known that a linear relation exists between swimming speed and frequency of tail beats in fish (Bainbridge, 1958; Gay, 2013). However, it cannot be ruled out that biopsied trout had a swim performance similarly to control fish through an increase of the tail beats frequency to compensate for a decrease in propulsion force with each beat (not determined). In this study, the number of tail beats measured for 15 sec during swimming resistance test significantly increased by 5 % between 0.35 and 0.45 $m.s^{-1}$ water velocity whatever experimental group. However, this frequency was not significantly affected by biopsy at both 0.35 and 0.45 $m.s^{-1}$ water velocity which leads to the hypothesis that fish propulsion force and so, mechanics properties was not modified by a red muscle biopsy (Table 1).

Resistance tests took place 7- (for HRT) and 10-dps (for SRT). In both 7- and 10-dps groups, weight variation was not significantly different between control and biopsy groups (Table 3). This observation is supported by Schmidt et al. (2016) which did cuts on rainbow trout muscle, more numerous (four) and larger (cylinder: 6 \times 3 mm) than in our case and they observed no significant growth difference 7- and 14-dps between control and biopsied fish. However, in our study, all the trout lost weight after surgery. More precisely, we observed that 7-dps fish had a more important and significative negative weight variation compared to those in the 10-dps group (Table 2). A study conducted on tilapia (*Oreochromis aureus*) indicated that all anaesthetized fish lost weight during the first 10-dps and then, progressively regained weight (Thoreau and Baras, 1997). We hypothesized that our experimental fish lost weight during the 7-dps and progressively regained weight. 10-dps trout had the opportunity to eat and assimilate more nutrients compared to 7-dps trout (three more days). Moreover, for all groups, the red muscle biopsy had no effect on initial and final condition factors (control and biopsy compared; Table 3). However, initial and final condition factors were significantly different in both 7- and 10-dps groups which can be explained by the weight loss after surgery. This weight loss could be due to stress caused by human manipulations, surgery or localized inflammatory response caused by biopsy. Having a longer resting period

Table 3
Initial and final condition factors 7- and 10-days post-surgery in rainbow trout.

Condition factor		7-dps			10-dps		
		Control <i>n</i> = 15	Biopsy <i>n</i> = 15	<i>p</i> -value	Control <i>n</i> = 15	Biopsy <i>n</i> = 15	<i>p</i> -value
Initial	Initial	1.13 ± 0.02	1.18 ± 0.02	<i>p</i> = 0.12	1.20 ± 0.02	1.21 ± 0.02	<i>p</i> = 0.93
	Final	1.03 ± 0.01	1.06 ± 0.02	<i>p</i> = 0.21	1.11 ± 0.02	1.13 ± 0.03	<i>p</i> = 0.57
	<i>p</i> -value	<i>p</i> < 0.001	<i>p</i> < 0.001		<i>p</i> < 0.001	<i>p</i> < 0.001	

Results are means ± SEM.

Condition factor = $\frac{BM \times 100}{BL^3}$ with body mass (BM) in g and body length (BL) in cm.

Initial: condition factor measured during surgery.

Final: condition factor measured at the end of the HRT or SRT.

dps: days post-surgery.

Italic font for *p*-value signified a used of Mann-Whitney or Wilcoxon test.

between surgery and experimental procedures (more than 10 days) could allow fish to gain weight. Schmidt et al. (2016) explored rainbow trout biopsy effects on gene expression involved in inflammatory responses in a white muscle sample collected near biopsy cut. Interleukine (IL)-1β mRNA rate was significantly higher in biopsied than in control group until 100 days post-biopsy even if the difference was less pronounced after 38 days. Quéméneur et al. (2022) showed no systemic infection and inflammation 3-, 14- and 28-days post-biopsy respectively explored by plasmatic lysozymes rate and IL-1 and tumor necrosis factor mRNA levels in sea bass kidney. With these two studies, we supposed that biopsy could induce local inflammatory responses but did not affect the whole animal, limiting the food intake of fish at first, as they focused their energy expenditure on healing. $\dot{M}O_2$ rout and $\dot{M}O_2$ max which correspond respectively to routine (RMR) and maximal metabolic rate (MMR) could vary due to inflammatory and immunity responses caused by biopsy for example (Ingerslev et al., 2010). RMR and MMR were not affected by muscle biopsy in rainbow trout (Table 1). Thoral et al. (2024) also showed that a red muscle biopsy had no effect on goldfish metabolic rates. In their natural or captive environment, fish swim and eat simultaneously. After a meal routine, $\dot{M}O_2$ of juvenile rainbow trout increased to 60–80 % of the $\dot{M}O_2$ max (Alsop and Wood, 1997). To assess swimming energetic cost, our study was performed on rainbow trout fasted for 24 h. If we measured the oxygen consumption at 7-dps (and

not at 10-dps), we may be able to show a biopsy effect on these two metabolic rates. Nevertheless, with a provoked environmental acute 7-dps constraint such as hypoxia, biopsy had no significant effect on the oxygen tension at loss of equilibrium (P_{LOE}) which represents one of the most relevant indicators for hypoxia tolerance in fish (Ekström et al., 2021; Zhang et al., 2025). This result indicated that biopsy did not alter the resistance to hypoxia of the fish (Fig. 3).

To conclude, the red muscle biopsy method presented in this paper had no effect on rainbow trout physiological parameters such as swimming performance and hypoxia resistance and on their survival. Indeed, as indicated by PCA, biopsy did not affect the studied parameters taken together (Fig. 4), control and biopsied groups behaved almost identically. All obtained results confirmed that it is possible to do longitudinal studies in rainbow trout. Thus, experimental fish could become their own control group reducing considerably the number of animals used for this type of studies, in accordance with the three “Rs”. To avoid affecting the health and survival of the fish, red muscle biopsy must be used to sample small tissue pieces because this muscle represents only 5 % of the total mass of skeletal muscle (Stickland, 1983). A histological exploration would be relevant to determine whether the biopsy had a detrimental effect on the neighbouring muscle. Through the weight aftercare, our study showed that more than 10 days between surgery and experimental procedures are required to dissipate stress caused by

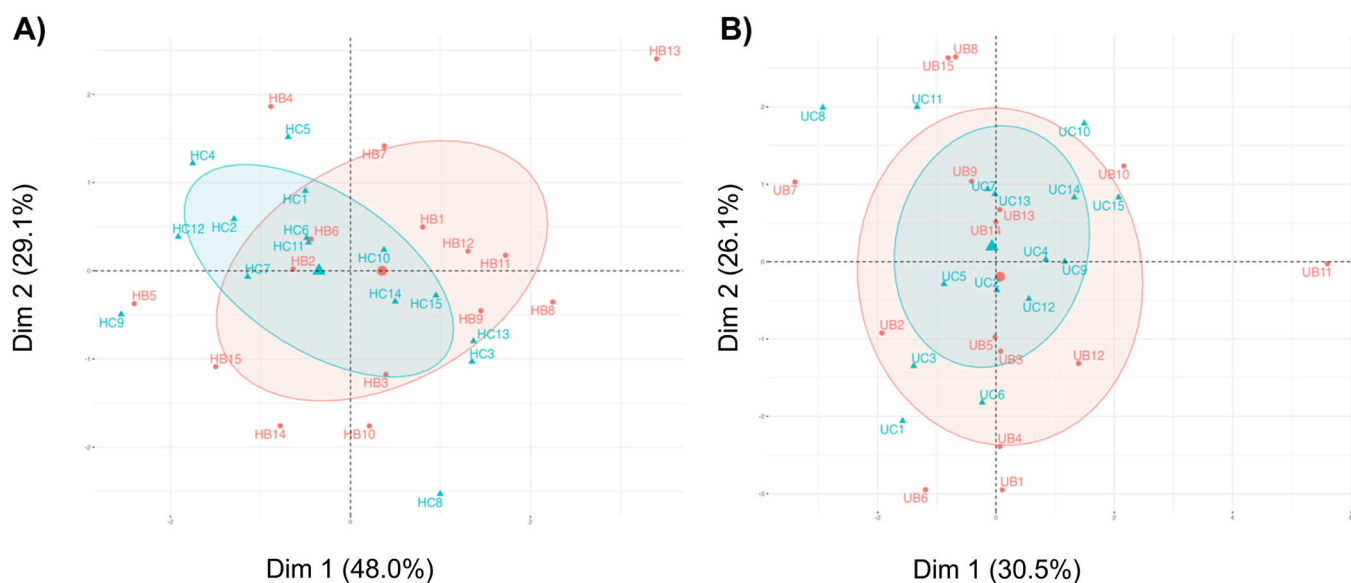


Fig. 4. Principal component analysis (PCA) performed using all data explored in (A) HRT (H) group (P_{crit} , weight variation, initial and final CF) and (B) SRT (U) group (U_{crit} , U_{ms} , number of tail beats, standard and maximal oxygen consumption, weight variation, initial and final CF). Control (C) and biopsied (B) trout were indicated in blue and in red, respectively. CF: condition factor; HRT: hypoxia resistance test; SRT: swimming resistance test.

surgery and human manipulation. Wild fish are more exposed to stress factors than farm-raised or experimental fish. May it be possible to release fish into wild after a muscular biopsy? In this sense, it would be relevant to examine shorter term biopsy effects in trout (1- or 3-dps for example).

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CRediT authorship contribution statement

Amerand Aline: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Asrar Lehodey:** Writing – review & editing, Visualization, Resources, Investigation. **Morgane Pengam:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bernard Simon:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Karine Salin:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Jean-Baptiste Quéménéur:** Writing – review & editing, Resources, Methodology. **Patrick Calvès:** Writing – review & editing, Resources, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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