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Selection for stress responsiveness and slaughter stress affect flesh quality

in pan-size rainbow trout, *Oncorhynchus mykiss*

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Abstract

The control of slaughter stress is of importance with regard to both fish welfare and flesh quality. Muscle characteristics and instrumentally measured quality parameters were determined in rainbow trout lines selected for high-responsiveness (HR) or low-responsiveness (LR) of plasma cortisol to an acute confinement stressor. Measurements were made in both unstressed and stressed fish (a 15 min period of confinement before slaughter) from both lines. Compared to LR fish, HR fish were smaller, had a slightly higher condition factor, lower fat-meter-values, and higher carcass yield. No difference between the lines was observed for muscle pH, either at slaughter or at 72 h *post-mortem* (pm). Fillets from HR fish had a lower muscle dry matter content and had higher lightness (L*) value for raw fillet. Fillet redness (a*) was lower for fish from the HR line for both raw fillet at slaughter and 72 h pm, and for cooked fillets. Fillet firmness was higher for fish from the HR line for raw fillet, but lower after cooking. Both white and red muscle fibers of HR fish were smaller than those in LR fish and HR fish had a thicker red muscle than LR fish. Imposition of an acute confinement stressor before slaughter induced a differential plasma cortisol response in the HR and LR fish. Pre-slaughter stress also lowered muscle initial pH, lowered red muscle mean diameter, and reduced raw fillet mechanical resistance, but increased cooked fillet firmness and had no effect on fillet color. Almost no interaction between selection line and pre-slaughter stress effects was observed showing that slaughter stress had similar consequences in both lines. Overall, the HR/LR trout model gave new insights in the comprehension of trout flesh quality and showed that the level of plasma cortisol response did not affect the impact of slaughter stress on fillet quality.

Highlights

Selection for stress response affects muscle features and flesh quality in rainbow trout

This work confirms some effects of slaughter stress on trout flesh quality

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The effect of slaughter stress on flesh quality was similar in high-responsive and low-responsive stressed fish.

Keywords

Flesh quality, stress, muscle fiber, texture, cortisol.

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1. Introduction

The stress response is an adaptive mechanism in animals that are disturbed or threatened by biological, physical or chemical stressors (Ashley, 2007). In fish farming, fish are subjected both to acute stressors, such as handling, and to chronic stressors including changes in their environment (temperature, water quality, salinity), interactions with other fish, and prolonged physical stressors (e.g. transport, crowding) (Pickering, 1992; Bonga, 1997). In fish, cortisol is the principal corticosteroid released following activation of hypothalamic-pituitary-interrenal axis (Pickering, 1992) and cortisol is widely used as an indicator of stress. A maladaptive stress response impairs feeding and growth, adversely affects immunocompetence, and interferes with reproductive processes (Pickering, 1992; Bonga, 1997; Portz et al., 2006). Selective breeding, to reduce the responsiveness of fish to stressors, has been investigated as a possible strategy to overcome some of the undesirable outcomes associated with aquacultural stressors (Pottinger and Carrick, 1999) .

In rainbow trout (*Oncorhynchus mykiss*), the magnitude of cortisol response was shown to be a trait with a moderately high heritability ($h^2 \approx 0.41$) and families with divergent high (HR) or low (LR) responsiveness to a standardized stressor were constituted (Pottinger and Carrick, 1999).

Selection for stress responsiveness was found to affect growth (Fevolden et al., 2002; Pottinger, 2006; Trenzado et al., 2006), feed efficiency (Overli et al., 2006) and behaviour (Pottinger and Carrick, 2001; Overli et al., 2002; Schjolden et al., 2005). However, the impact of selection for stress response on muscle characteristics and fillet quality has not yet been investigated.

As previously mentioned, chronic stress can reduce growth performance of rainbow trout (Ashley, 2007) and may consequently therefore affect muscle characteristics. It is still unclear whether chronic stress affects muscle growth (Galt et al., 2014) and no consequences of

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chronic stress on flesh quality have been reported. Conversely, the adverse effects on flesh quality of acute stress before slaughter are well documented in most species. Pre-slaughter stress, caused by crowding and handling, often leads to an intense muscle activity associated with an accelerated entry into rigor mortis, nucleotide breakdown, and muscle pH fall (Sigholt et al., 1997; Skjervold et al., 1999; Thomas et al., 1999; Robb et al., 2000; Roth et al., 2002; Wills and Proctor, 2004). Some consequences of pre-slaughter stress on organoleptic properties of the fillet have been reported in various species (Poli et al., 2005). A negative impact on fillet color and a softer texture (Faergemand et al., 1995; Sigholt et al., 1997; Gomez-Guillén et al., 2000; Robb et al., 2000; Roth et al., 2002; Kiessling et al., 2004; Wills and Proctor, 2004; Lefevre et al., 2008) are the main effects that have been observed. More recently these effects have been linked with muscle proteome changes in Atlantic salmon, sea bream and sea bass (Veiseth-Kent et al., 2010; Addis et al., 2012; Silva et al., 2012) showing that both contractile proteins and sarcoplasmic proteins are impacted by slaughter stress. Moreover, slaughter stress was also shown to promote cathepsins expression and activity with consequences for muscle structural integrity (Bahuaud et al., 2010).

Although a genetic influence on the consequences of slaughter stress for flesh quality in fish has not yet been demonstrated, it can be assumed to be present by analogy with findings in terrestrial animals (Terlouw et al., 2008).

The two main objectives of this study were 1) to examine (using HR and LR rainbow trout families) whether genetic selection on stress response had any impact on muscle characteristics and flesh quality and 2) to determine whether the effects of slaughter stress had similar effects on the quality of fillet in fish selected as HR or LR.

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2. Material and methods

2.1. Fish and rearing conditions

The selection procedure and the effect of the breeding program on the magnitude of the cortisol response to a stressor has been described elsewhere (Pottinger and Carrick, 1999, 2001; Øverli et al., 2005). In brief, the HR and LR rainbow trout lines were initiated in 1996 at the Windermere facility of the Centre for Ecology & Hydrology (Cumbria, U.K.) by repeated measurement of the cortisol response to a standardised stressor (3h confinement in 50 L water in groups of 6-7 individuals once monthly) in individually tagged (passive integrated transponder; PIT) 2-year-old rainbow trout (pedigree unknown) kept in groups of 25 in 1500 L holding tanks. Individuals were ranked according to their mean stress-induced plasma cortisol concentration across five episodes of confinement. The four fish in each tank exhibiting the highest response (HR) and the four exhibiting the lowest response (LR) were used to produce the F1 generation which consisted of 15 HR and 14 LR families each resulting from a unique male-female cross. These progeny were tested, using the standardised confinement stressor, on ten occasions between 1997 and 1999 and the six LR families with the lowest mean cortisol response during this period and the six HR families with the highest mean cortisol response were identified and used for further work. The F2 generation (2000) was created from unique crosses conducted within the two most divergent families of F1 fish following the testing of individuals within those families during 1999. The two most divergent families within the F2 generation were used to create F3 lines (2003) and the F4 lines were generated from pooled gamete crosses of randomly selected F3 parents (2006). The F4 fish were tested for stress responsiveness in 2006 and 2007 using the standardised confinement stressor and found to exhibit a divergence in their cortisol stress response that was consistent with that of earlier generations.

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Fifty, 18 months old, F4 HR and LR rainbow trout were transferred to each of eight (4 x HR and 4 x LR) experimental tanks (circular, 1.8 m diameter, 1500 l, glassfibre tanks) in September, one month before the sampling date. The fish were taken randomly from holding tanks, of identical specification to the experimental tanks, within which the F4 HR and LR lines were separately maintained. Each tank was supplied with untreated lake water at a flow of 25 l/min and was exposed to a natural photoperiod and ambient temperature. Fish were fed five times per week at 1.5% body weight with a commercial diet (Skretting Standard Expanded 40, without added pigments).

The experimental work was carried out under Home Office licence in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.2. Slaughter procedure

Fish were fasted for 48 h before slaughtering. Fish from the HR and LR lines (n = 40 per line; 10 fish in each of four tanks) were subjected (stress: S) or not (no stress: NS) to an acute stressor comprising 15 min confinement immediately before slaughter. In each tank, unstressed (NS) fish (n = 5 per tank) were netted rapidly and anesthetized in 2-phenoxyethanol (1:2000). Then the water level in the tank was lowered rapidly until the dorsal fins of the fish were exposed to air and after 15 min the stressed (S) fish were netted (n = 5 per tank) and anesthetized as for the NS fish. The experimental design comprised four groups, HR-NS, HR-S, LR-NS, and LR-S, each of 20 trout (n = 5 per treatment x 4 replicates).

However, sexually mature fish (gonad weight > 1% of body weight), which were not detected visually, were finally excluded from the analysis, so the actual number of fish employed in the study was ≥ 16 per treatment.

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2.3. Measurements and sampling at slaughter

The fish traits measurements were indexed according to the Animal Trait Ontology for Livestock (ATOL: http://www.atol-ontology.com/index.php/en/les-ontologies-en/visualisation-en (Golik et al., 2012)).

A blood sample was taken immediately after sedation for plasma cortisol (ATOL:0002287) determination, and then fish were bled by gill arch section. Blood samples were centrifuged (3000g, 1 min) and separated plasma was stored frozen at 20°C until assay (Pottinger and Carrick, 2001) which was conducted within two weeks.

On whole fish, muscle lipid content (ATOL:0001663) was measured using the Torry Fish Fat Meter® (Distell Industries Ltd, Scotland). Fish were wiped with paper tissue to remove excess water and mucus. The instrument was firmly applied on dorsal musculature, parallel to the lateral line, between the head and the dorsal fin of both sides of the fish (Douirin et al., 1998). The fat-meter value is the mean of these two measurements.

Individual body weight (BW, ATOL:0000351) and standard length (L, ATOL:0001658) were measured and the condition factor (ATOL:0001653) was calculated as $K = BW/L^3$.

Fish were eviscerated and carcass, viscera, liver, and gonad were weighed. Yields were calculated as: carcass yield = carcass weight/BW (ATOL:0000548), Viscera-Somatic Index VSI = viscera weight/BW (ATOL:0002259), Hepato-Somatic Index HSI = liver weight/BW (ATOL:0001121), and gonado-somatic index GSI = gonad weight/BW (ATOL:0001799).

After filleting, fillet color (ATOL:0001017) was assessed using a portable Minolta Chromameter CR-200 (Minolta, France) equipped with light source C and a 2° observer angle, calibrated to a white standard. For each fillet, two measurements were conducted on the interior part of fillet, one anterior to the dorsal fin and the other anterior to the anal fin. The mean of these two measurements values was considered. Data were expressed in L*, a*, b* system, representing lightness, redness, and yellowness, respectively, as recommended by

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CIE (CIE, 1976).

The muscle initial pH (ATOL:0001684) was measured, in the front part of the fillet, within 30 min and 1 hour *post-mortem* using a Hanna HI 9025C pH meter (Hanna Instruments Srl, Italy) equipped with a Hanna FC200 penetration electrode (Hanna Instruments Srl, Italy). Fillet temperature (ATOL:0000067) was measured simultaneously to correct pH values.

Fillets were packed in plastic bags within 1 hour after fish death, and stored in ice until further analysis at 72 h *post-mortem*.

2.4. Muscle histological analysis

Deep white muscle and superficial red muscle samples were taken, within one hour after fish death, just beneath the dorsal fin, fixed in Carnoy fixative (absolute ethanol, chloroform, acetic acid, 6:3:1) for 7 days at 4°C, dehydrated in alcohol and alcohol/butanol, embedded in paraffin, and 10 μm sections were then cut and stained with Sirius Red and Fast Green 0.1% in saturated picric acid (Lopez-De Leon and Rojkind, 1985). Three or four microscopic fields were digitized for red and white muscle respectively. Only areas presenting fibers with transversal section, were digitized for each histological section. Transverse sectional areas of individual white and red muscle fibers (600-1000 fibers per fish for white muscle and 200-500 fibers for red muscle) were measured using Visilog 6.5 for Windows. As histological treatments including paraffin embedding lead to muscle fiber shrinkage, individual muscle fiber area was multiply by a shrinkage correction (SC) factor calculated as follows: SC = (total image area ‑ connective tissue area) / (fiber total area)). Muscle fiber diameters (D) (ATOL:0000458) were then calculated using the formula $D = 2\sqrt{(area/\pi)}$ under the assumption that individual fiber cross-sections were circular. Fiber density (number of fibers/surface unit) was calculated (ATOL:0001691), and the amount of white muscle fibers with a diameter lower than 20 μm and higher than 90 μm was calculated

The maximum thickness of red muscle was measured and the number of muscle fibers at this level was counted. The figure illustrating the thickness of red muscle (Figure 2) was created using ScientiFig (Aigouy and Mirouse, 2013).

2.5. Physical measurements of fillet quality parameters at 3 days (raw) and 6 days (cooked) *post-mortem*

At 3 days *post-mortem*, physical measurements of quality parameters were done on one raw fillet, whereas the second fillet was cooked. Pieces of fillets without skin were cooked for 1 to 2 min, depending on the sample weight, in a domestic microwave oven (Samsung M192DN) at 450 W in a closed bowl to reach a core temperature of 65-70°C. Fillets were then cooled to room temperature, packed in plastic bags and stored at 4°C in a cold room until further analysis. Physical measurements of quality parameters for cooked fillet were assessed at 6 days *post-mortem* except for color which was evaluated immediately after cooking and cooling.

Fillet color was measured as previously described at slaughter.

Ultimate pH (ATOL:0001684) was measured with 5 g muscle, sampled in the anterior part of the fillet, homogenized in four volumes of distilled water.

Dry matter content (ATOL:0000101) was determined by drying about 5 g of minced fillet (anterior part) for 40 h in an oven at 105°C.

The mechanical strength (ATOL:0001649) of the middle part of the fillet (64 mm length beneath the dorsal fin) was analyzed using a Kramer shear cell mounted on a static load cell of 2 kN (INSTRON 5544, INSTRON Ltd., England). The maximum force, force at low (20%) and high (80%) deformation, and the work until the maximum force were calculated. All these parameters were divided by the weight of the sample (Szczesniak et al., 1970).

2.6. Statistical analysis

All results are expressed as mean ± standard deviation. A two-way MANOVA was used to test the effects of line (HR vs LR) and stress before slaughter (NS vs S). As fish mean body weight was different between HR and LR groups, Pearson correlation coefficient with body weight was calculated for all parameters. When this correlation was significant (p<0.05), a two-ways MANCOVA, with body weight as a covariate, was used to test the effects of line (HR vs LR) and stress before slaughter (NS vs S). The level of significance was set at p<0.05. Significant differences between means were determined using Newman-Keuls test. The Pearson correlation coefficient was also calculated to analyze the significance of linear relationships between variables. All these analyses were carried out using Statistica for Windows (version 5.1). The number of fish measured for each parameter is specified in tables.

3. Results

3.1. Fish characteristics at slaughter

Plasma cortisol levels for each line pre- and post-stress are shown in Figure 1. Cortisol levels in unstressed fish (NS) sampled immediately after sedation were not different between the HR and LR lines. In both lines the imposition of an acute confinement stressor (S) significantly increased plasma cortisol concentrations before slaughter and here a significant statistical interaction between selection lines and stress effect was apparent (p<0.001) with the stress-induced increase in plasma cortisol being 74% higher in HR-S fish than for LR-S fish.

Morphometric parameters, adiposity and process yields are summarized in Table 1 for each experimental group. Body weight were greater in fish from the LR line than those from the HR line (p<0.001). In addition, the condition factor K was slightly higher in the HR fish suggesting a more compact morphology for fish from this line. Fish from the LR line gave higher fat-meter values than those from the HR group suggesting higher muscle adiposity for

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fish from LR line. HR fish had a higher carcass yield (89.7%) than LR fish (88.7%) and accordingly a lower viscero-somatic index (VSI; p<0.001). HR fish gave slightly higher hepato-somatic index (HSI) than LR fish (1.7% vs 1.6% respectively, p<0.05). Gonadosomatic indices (GSI) were low as expected, since only immature fish were kept for quality assessment. Nonetheless, significantly higher GSI values were measured for HR fish compared to LR fish (0.14% vs 0.09%, respectively, p<0.05). No differences between NS and S fish in both lines were detected for any of these fish characteristics and no interactions between the effects of selection and slaughter stress were observed.

3.2. Muscle pH and dry matter content

The muscle pH and dry matter content data are summarized in Table 2. In unstressed fish, there was no difference between HR and LR lines in muscle pH at slaughter, 72 h pm, or for cooked fillets. In fish from both HR and LR lines slaughter stress significantly lowered muscle pH (a mean difference of -0.31 pH units) as measured within two hours after death (p<0.001). No effect of slaughter stress was observed on muscle pH measured at 72 h pm or on cooked fillet, and no interaction between selection lines and stress was observed. The *post-mortem* decrease in muscle pH was lower for stressed fish compared to unstressed fish (p<0.001). Dry matter content of LR fish was higher than those of HR fish in both raw and cooked fillet (mean differences of +1.1% and +1.2% respectively, p≤0.001). No effect of slaughter stress or interaction between selection line and stress was evident for fillet dry matter content.

3.3. Fillet color

The color of fillets was evaluated on raw fillet within 2 hours after slaughter and at 3 days *post-mortem* (pm), and on cooked fillet. Data obtained at slaughter are presented in Table 3, and similar results were measured at 3 days pm. Raw fillets from HR and LR fish exhibited differences in all color parameters: lightness (L*), redness (a*) and yellowness (b*). However

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when body weight was considered as a covariate in the analysis of variance, some of those differences were no longer significant. The mean L* of raw fillets from LR fish was lower than that of HR (44.7 vs 46.5, $p < 0.001$ at slaughter time), whereas a* and b* tended to be higher in LR fish than HR fish. For cooked fillets, differences between lines for L* were less pronounced but higher values of a* and b* were still observed for fillets from LR fish compared to HR ($p < 0.05$). Slaughter stress had no influence on fillet color in either raw or cooked fillets. Some interactions between selection line and stress effects were observed, especially when body weight was considered as a covariate. For example, yellowness of cooked fillet was higher for LR fish only for unstressed fish, and higher values for stressed fish were measured only in HR line.

3.4. Mechanical resistance of raw and cooked fillets

The data for fillet mechanical resistance is summarized in Table 4. For raw fillets, the mechanical resistance of HR fish was higher than that of LR fish whatever the parameter considered ($p < 0.01$ +19.8% for specific resistance parameter for example). A significant decrease in mechanical resistance due to slaughter stress was measured for specific resistance (-12.5%) and force at 80% of deformation ($p < 0.05$ and $p < 0.001$, respectively). We noted that the effect of selection line observed for resistance at low (20%) deformation tended to be due to NS fish (interaction $p = 0.09$). Moreover, for mechanical resistance at high (80%) deformation, the effect of stress tended to be due to HR fish (interaction $p = 0.09$).

For cooked fillet, opposite results were observed for both specific resistance and work. Higher values were obtained for fillets from LR fish compared to HR ones, and higher values were obtained for stressed fish compared to non-stressed fish. No interaction between selection line and stress effect was observed.

3.5. Muscle histological analysis

The muscle histology data are presented in Table 5. White muscle fibers in HR fish had smaller mean diameter than those in LR fish (39.7 ± 3.3 vs 42.2 ± 3.0, respectively), and HR fish had more small fibers (Ø<20 μm), fewer large fibers (>90 μm), and, as a consequence a higher density of fibers (508 ± 68 fibers/mm^2 vs 456 ± 59 fibers/mm^2, respectively). However, LR fish were bigger, and when fish weight was taken into account in the statistical analysis, the line effect was no longer significant except that the amount of large fibers remained lower for HR fish. Stressed fish had a higher white muscle fiber mean diameter ($p<0.05$), a lower white muscle fiber density ($p<0.01$), and more large fibers ($p<0.05$).

With regard to red muscle, LR fish had a larger mean fiber diameter (30.0 ± 3.0 μm) than HR fish (26.3 ± 3.0 μm; $p\leq0.001$). Accordingly, HR fish had an overall higher fiber density (3167 ± 585 fibers/mm^2) than LR fish (2629 ± 501 fibers/mm^2; $p<0.05$). Stressed fish had a lower mean red muscle fiber diameter than the control fish ($p<0.05$) but comparison of the means showed that the difference was only significant for HR fish. In addition, the maximal thickness of red muscle of all fish was also analyzed. Representative histological images obtained from HR and LR fish are presented in Figure 2. HR fish had a wider red muscle than LR fish both in absolute (+17%) and relative (+20%) terms as HR fish were smaller. Calculation of the average number of fibers through thickness showed that HR fish had more red fibers along the red muscle thickness than LR fish (88 ± 19 vs 64 ± 13, $p<0.001$).

No interaction between selection lines and stress was observed for muscle histological parameters.

3.6. Correlation analysis

Tables 6 and 7 summarize the correlation between instrumentally measured quality variables and other measured parameters. Correlations with plasma cortisol concentrations were only calculated for stressed fish. Significant correlations highlighted differences between HR and

LR fish. For instance, the plasma cortisol response to acute stress was negatively correlated to muscle dry matter content (r=-0.61, p<0.001) as LR fish gave higher values of muscle DM content. Similarly, correlations with fillet color or mechanical résistance parameters were consistent with differences measured between selection lines. As fish from the two lines had significant differences in body weight, correlation of quality parameters with body weight may also be confounded by some effects of selection/line. Surprisingly, fillet lightness (L*) measured at slaughter was negatively correlated with fish adiposity (fat-meter[®] value and muscle DM content) whereas fillet yellowness (b*) was positively correlated to fatness (+0.49<r<+0.69, depending on fatness parameter and time of color measurement, p<0.001). Muscle fiber density was negatively correlated to muscle dry matter content and to fillet yellowness. Mechanical resistance parameters or raw fillet were negatively correlated to fat-meter[®] value (r=-0.39, p<0.05 for specific resistance) and more significantly to muscle dry matter content (r=-0.48, p<0.001 for specific resistance). Interestingly, mechanical resistance of raw flesh was positively correlated to muscle fiber density, especially for white muscle (r=+0.57, p<0.001 for specific resistance). Specific resistance of raw fillet was also positively correlated to both initial and ultimate muscle pH (r=+0.34 and r=+0.37, respectively, p<0.01). Correlations measured for mechanical resistance of cooked fillet were globally the opposite of those observed for raw fillet: positive correlation with muscle dry matter content and negative correlation to muscle pH. Nevertheless no correlation was observed between instrumental texture measurement of cooked fillets and muscle fiber size/density.

4. Discussion

The aim of this study was to assess the effects of selection for divergent responsiveness to a stressor on muscle characteristics and flesh quality in rainbow trout and to determine whether a pre-slaughter stress differently affects the flesh quality of the two selected lines.

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4.1. Effects of line

Fish from the two lines were genetically selected as HR or LR on their plasma cortisol response to an acute confinement stressor (Pottinger and Carrick, 1999). Accordingly, stress-induced plasma cortisol levels in HR fish were 74% higher than those of LR fish whereas unstressed fish of the two lines had similar cortisol levels. Our results are consistent with those already obtained with these lines (Pottinger and Carrick, 1999; Trenzado et al., 2003).

In our study, LR fish had a higher body weight and length than HR fish. The better growth performance of LR fish has already been observed in previous studies (Fevolden et al., 2002; Trenzado et al., 2006) and LR fish have been reported to show a better feed intake and feed efficiency than HR animals (Overli et al., 2006; Trenzado et al., 2006). HR fish had slightly higher condition factor than LR fish suggesting a more "compact" morphology. This observation has not been reported in previous studies comparing HR and LR lines. This difference in morphology seems to be independent of overall growth since LR fish present a higher body weight but a lower condition factor whereas bigger fish usually present higher condition factor (Haffray et al., 2013). However, imposing divergent selection on only one trait can induce correlated responses in other characters. A similar change in trout morphology was, for example, obtained after divergent selection on muscle fat content (Quillet et al., 2007), and condition factor generally exhibits a higher heritability than body weight in rainbow trout (Haffray et al., 2013).

Higher fat-meter value, viscero-somatic index, and muscle dry matter content suggested that LR fish had higher adiposity than HR fish and this difference seemed to be present across all the lipid storage sites. Although this observation appears consistent with the known lipolytic action of cortisol (Sheridan, 1994; Bonga, 1997) basal plasma cortisol content did not differ between the two selected lines. So in non-stressed conditions, which presumably comprise most of the fish's lifespan, HR fish and LR fish had similar levels of circulating cortisol.

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Nevertheless aquacultured fish are regularly submitted to acute stressors due to unavoidable aspects of the intensive rearing regime and this may result in sufficiently frequent activation of the stress axis to exert an effect on lipid disposition. It should also be noted that the HR fish were smaller and it is well established that fat content increases with size and growth rate of fish (Fauconneau et al., 1995).

Muscle fiber size differed between the HR and LR lines. HR fish had smaller muscle fibers and consequently a higher fiber density in both white and red muscles. This result was consistent with the difference in size between HR and LR fish with HR fish being smaller than LR fish. The difference in white muscle characteristics between HR and LR fish was eliminated when body weight differences between the lines were taken into consideration. The increase in muscle fiber size with fish body size is largely documented and it is well known that the hypertrophy of muscle fibers is associated with overall growth (Johnston, 1999). However, for red muscle the difference between the two lines could not be totally explained by fish size, as a significant difference persisted even when body weight was included as a co-variable. Moreover HR fish had a thicker red muscle, so HR fish had more and smaller red muscle fibers than LR fish. This higher development of red muscle could be related to differences in swimming activity between the two lines, as red muscle is used in routine swimming (Davison, 1997). However, it has been reported that LR fish exhibit a greater degree of locomotor activity than HR fish under routine conditions (Schjolden et al., 2006). Moreover, we could hypothesize that a difference in muscle activity between the two lines would also have favored hypertrophy of white muscle fibers, as previously described (Bugeon et al., 2003), and no difference between lines was observed for white muscle. So the differential red muscle development between the two lines may be an incidental response to the selection process.

Surprisingly, flesh color differed between the two selected lines. The trout of this study did

not received a pigment-supplemented diet which explains the low values measured for color parameters, especially for redness (a*) values lower than zero. Fillets from HR fish had lower values of yellowness (b*) than fillets from LR fish. In addition to the astaxanthin pigment added in commercial food for salmonids, flesh color of fish can also be affected by other carotenoid pigments such as lutein (yellow pigment) as has been shown with catfish (Li et al., 2007). We can suppose that such a pigment was present in raw materials of the commercial diet so, the difference in fillet yellowness measured between the two lines, suggest a physiological difference in yellow pigment assimilation as a correlated response to the divergent stress genetic selection. Indeed, family differences in flesh carotenoid pigment content have long been observed for rainbow trout (Torrissen and Naevdal, 1984) and a genetic determinism of fillet color has been confirmed (Kause et al., 2008). Moreover fillets from HR fish were also lighter (L*>) than those from LR fish. Higher lightness values are often associated with higher fat content (Marty-Mahe et al., 2004). This is not the case in our study as the leaner HR fish gave the higher fillet lightness values. Fillet lightness is also associated with muscle structure, especially fiber size. Muscle fibers with larger diameter scatter more light resulting in a paler flesh (Johnston et al., 2000) which could explain the lower values of fillet lightness measured for LR fish, which had bigger muscle fibers. Indeed, a positive correlation was observed between fillet lightness measured at slaughter and white muscle fiber density ($r=0.35$, $p<0.01$).

Mechanical resistance measurements showed that raw fillets from HR fish were firmer than LR ones. This result can be related to muscle organization, especially muscle fiber size. HR fish had smaller white muscle fibers and a higher fiber density than white muscle of LR fish, and fiber density of white muscle fibers was positively correlated to mechanical resistance of raw flesh ($r=0.57$, $p<0.001$). This suggests that muscle structure can partially explain the variability of the raw flesh texture. Such a correlation has already been found in rainbow trout

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(Lefevre et al., 2008; Lefevre et al., 2015), in brown trout (Bugeon et al., 2003) and in Atlantic salmon (Johnston et al., 2000). Furthermore, HR fish had a lower adiposity than LR fish and this difference in lipid content may have contributed to the softer flesh in LR fish compared to HR. Indeed, lipid content has clearly been shown to decrease fillet mechanical resistance in rainbow trout (Johansson et al., 2000; Lefevre et al., 2015), and in the present study a negative correlation was observed between adiposity parameters and mechanical resistance of fillet. Surprisingly, the opposite result was obtained after cooking, with fillets from LR fish being more resistant than those of HR fish and negative correlations being observed between raw and cooked mechanical resistance parameters. This difference between the two lines cannot readily be explained with the parameters measured in this study and suggests that fillets from HR and LR may differ in their collagen content or related properties. Indeed, a higher collagen content leads to a firm raw flesh but is associated with a soft and elastic cooked fish meat (Sato et al., 1986). So fillets from HR fish may have a higher collagen content which could contribute to their raw firmness and softening after cooking. This hypothesis is supported by the work of Johansen et al. (Johansen et al., 2011) who reported that HR fish have higher cardio-somatic index, associated with elevated collagen (Col1a2) expression and deposition in heart tissue. Such a difference remains to be confirmed in skeletal muscle.

4.2. Effects of stress at slaughter

Classical effects of pre-slaughter stress were observed in the present study. Stress induced a significant increase in plasma cortisol concentration, lowered muscle pH measured immediately after slaughter, decreased red muscle fiber size, and lowered mechanical resistance of raw fillets. The plasma cortisol levels in unstressed fish corresponded to rested fish whereas plasma cortisol levels in stressed fish corresponded to acutely stressed trout

(Lefevre et al., 2008). So our surrogate slaughter procedure succeeded in producing unstressed (NS) and stressed fish (S).

A decrease in muscle pH due to stress is a consequences of the production of lactic acid by glycolysis (Thomas et al., 1999) induced by muscle activity in the stressed fish. However, the decline in pH that we measured was limited compared to those observed during a stressful slaughter method (Thomas et al., 1999; Lefevre et al., 2008). Visual observation of fish during the stress challenge employed in this study revealed that fish exhibited limited locomotory activity which is consistent with the limited pH drop seen following the stressful challenge. No difference between NS and S fish was seen in the ultimate pH measured at 72 h post-mortem. Due to the relative levels of initial pH values, the post-mortem decrease in muscle pH was higher for NS fish compared to S fish. However, the post-mortem pH decline was nonetheless detectable for S fish, in contrast to the situation sometimes observed after slaughter stress (Lefevre et al., 2008), confirming that the present stress challenge was not exhausting for the fish.

Stressed fish had a smaller mean red muscle fiber diameter than unstressed fish, and this was most apparent in the HR group. A similar result was previously observed with stressed rainbow trout of a similar size (Lefevre et al., 2008). This may be related to increased muscle metabolism in relation to the increase in muscle activity during the confinement stressor. In red muscle, glycogen is not the main fuel for muscle cell activity but lipid catabolism could be responsible for the decrease in fiber size. This would be in accordance with a study showing that slaughter stress affects the concentration of lipid oxygenated products in rainbow trout (Secci et al., 2016). However, the few other studies that have reported any effect of slaughter stress on muscle composition showed no effect of killing procedure on muscle lipid content in Atlantic salmon (Gomez-Guillén et al., 2000; Kiessling et al., 2004). Moreover, we observed this effect mainly in the HR group, in which fish seemed to be

particularly lean. Another hypothesis could be the mobilization, during the confinement period, of protein reserves. This mechanism is supported by several studies reporting that slaughter stress affects muscle structural proteins, especially those in the extracellular matrix (Jerrett et al., 1996; Sato et al., 2002). A recent study reporting an increase in gene expression and enzyme activity of cathepsins in stressed muscle salmon corroborate this hypothesis (Bahuaud et al., 2010).

Killing procedure has often been shown to affect fillet color in salmonids (Kiessling et al., 2004; Roth et al., 2006; Lefevre et al., 2008). This effect could be related to muscle activity before death as a similar effect can be obtained after electrical stimulation (Robb et al., 2000). The color of fillet was not altered by pre-slaughter (confinement) stress in the present study. This may be related to limited muscle activity during the confinement period, as already discussed with respect to pH values. Moreover, it should be noted that in the present study, fillets were not pigmented which differed from many studies with salmonids.

In stressed fish the raw fillet exhibited a lower mechanical resistance than that of unstressed fish, an observation that is consistent with previous studies on various species including salmonids (Faergemand et al., 1995; Sigholt et al., 1997; Roth et al., 2002; Kiessling et al., 2004; Merkin et al., 2014), cod (Stien et al., 2005; Kristoffersen et al., 2006), eel (Morzel and van de Vis, 2003) and turbot (Morzel et al., 2003). An opposite effect of slaughter stress on fillet texture was reported in salmon subjected to crowding where an increased firmness of flesh was observed but after long-term exhaustive exercise (24hrs) (Skjervold et al., 2001). Slaughter stress accelerates post-mortem muscle metabolism (Morzel and van de Vis, 2003) and induces a softening of flesh by an accelerated proteolysis of myofibrils and connective tissue (Jerrett et al., 1996; Roth et al., 2002; Kiessling et al., 2004; Morzel et al., 2006) and an increase in lipid oxidation (Giuffrida et al., 2007). After exposure to slaughter stress, a relationship between muscle degradation and cathepsin gene expression and activities was

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observed in Atlantic salmon (Bahuaud et al., 2010). Surprisingly, cooking the fillets produced opposite results with the flesh of stressed fish being more resistant than that of unstressed individuals. As previously mentioned, such a result is difficult to explain on the basis of what is actually known about the factors that determine fish flesh texture.

4.3. Differential consequences of slaughter stress between lines

A primary aim of the present study was to determine whether the impact of slaughter stress on flesh quality varied between HR and LR fish, divergently selected for their cortisol response to stress. Surprisingly, except for cortisol concentration after confinement stress, we observed no interaction between selection line and stress effects. This suggests that the consequences of slaughter stress on flesh quality were not affected by the amplitude of the cortisol response to confinement stress. So fish with a limited cortisol response displayed similar negative impact of slaughter stress on their flesh quality as fish with a high cortisol response. This result can lead to two hypotheses: 1) there was no relationship between fish cortisol response and consequences of stress on fillet quality, and the measured change in fish quality after stressful slaughter conditions is primarily the result of acute locomotory activity; or 2) The threshold for an effect of stress on muscle metabolic or biochemical traits is below that reached by the LR fish and additional elevation of blood cortisol levels beyond this threshold level have no further impact upon flesh quality.

Overall, we can conclude that the present study suggests that selection for stress responsiveness has no impact on the effects of slaughter stress on fillet quality.

4.4. Relationship between flesh quality parameters

As many differences were measured between HR and LR fish, correlation analysis often corroborated various values of measured parameters between those two groups. Nevertheless, the main aim of the present study was to clarify the relationship between the level of stress-induced plasma cortisol and consequences of stress on fillet quality parameters. Interestingly,

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the plasma cortisol level of stressed fish correlated with most of the measured quality parameters, except muscle pH as discussed above.

Cortisol level was negatively correlated with muscle dry matter content which was consistent with the lipolytic action of cortisol (Sheridan, 1994; Bonga, 1997; Weil et al., 2013). Cortisol was positively correlated to fillet lightness (L*) measured at slaughter. This was surprising as, when unstressed and stressed fish are compared, slaughter stress usually induced a darker fillet (Kiessling et al., 2004; Roth et al., 2006; Lefevre et al., 2008). Nevertheless, we observed that fillet L* was also correlated to muscle pH. So this result suggest that the fillet darkening effect, often measured after stressful slaughtering conditions, may be mediated by the decrease in muscle pH. In our study, the pH decrease was not related to the level of stress, as was the case for fillet lightness. The magnitude of the cortisol response was also positively correlated with raw fillet mechanical resistance and negatively correlated to cooked fillet mechanical resistance. This was unexpected as, when comparing unstressed and stressed fish, slaughter stress generally decreases fillet firmness in fish. An individual correlation was indeed measured with pork meat (Choi et al., 2012) and we expected that the genetic model of HR-LR trout would be an ideal model to observe such a relationship. A weak, but significant, correlation was measured for mechanical resistance of cooked fillet, but not for raw fillet.

In addition to the consequences of stress on flesh quality, this study provides a data set to explore correlations between quality parameters. Surprisingly, fatness criteria were negatively correlated to fillet lightness. An opposite relationship is generally observed (Lefevre et al., 2015) maybe due to a greater myosepta area in fattier fish (Marty-Mahe et al., 2004). This result may be biased by the substantial difference between the two lines in both adiposity and fillet lightness, and the fact that, contrary to most previous work, we measured unpigmented fillets. The present model of HR/LR trout did not appear to be a relevant model to explore the factors determining fillet color. In contrast, the relationship between fatness criteria and

mechanical resistance was consistent at least for raw fillets. Fat-meter value and muscle dry matter content were negatively correlated to raw fillet mechanical resistance. Indeed higher muscle lipid content was shown to decrease firmness in rainbow trout (Lefevre et al., 2015). Moreover, mechanical resistance of the raw fillet was positively correlated to muscle fiber density, especially in white muscle ($r = 0.57$, $p<0.001$ for specific resistance). Such a result is classically observed (Lefevre et al., 2008; Lefevre et al., 2015) and the present study confirmed a relationship between the muscle fiber size and the raw flesh firmness in pan-sized rainbow trout. So, the higher HR raw fillet mechanical resistance may be due both to lower lipid content and smaller fiber size.

One of the aims of the present study was to explore the HR/LR trout model and obtain new insights into the effects of slaughter stress on fish flesh quality. This biological model was, in the final analysis, quite complex. Other phenotypic traits associated with the response to genetic selection for stress responsiveness, such as growth rate, fish shape or muscle pH, may influence flesh quality. As a result, it was difficult to discriminate effects of differential intensity in stress responsiveness between HR and LR fish.

5. Conclusion

The present study was designed to assess the effects of genetic selection on the cortisol stress response on muscle characteristics and flesh quality in rainbow trout, and to assess whether slaughter stress has dissimilar effects on the flesh quality of the two selected lines. The selected HR and LR lines exhibited differences in many characteristics including growth, adiposity, fillet color and mechanical resistance, and red and white muscle fiber size. Slaughter stress affected some fillet quality parameters, but to a lesser extent than genetic selection did, and in a similar way for both the high responding and low responding lines. These findings indicate that the intensity of cortisol response due to stress before slaughter was not an indicator of flesh quality. This work also showed that selection of low stress-

responding fish was not only inefficient in terms of reducing the impact of slaughter stress on fillet quality, but genetic selection may generate unfavorable correlated responses such as fattier and softer fish.

6. Acknowledgements

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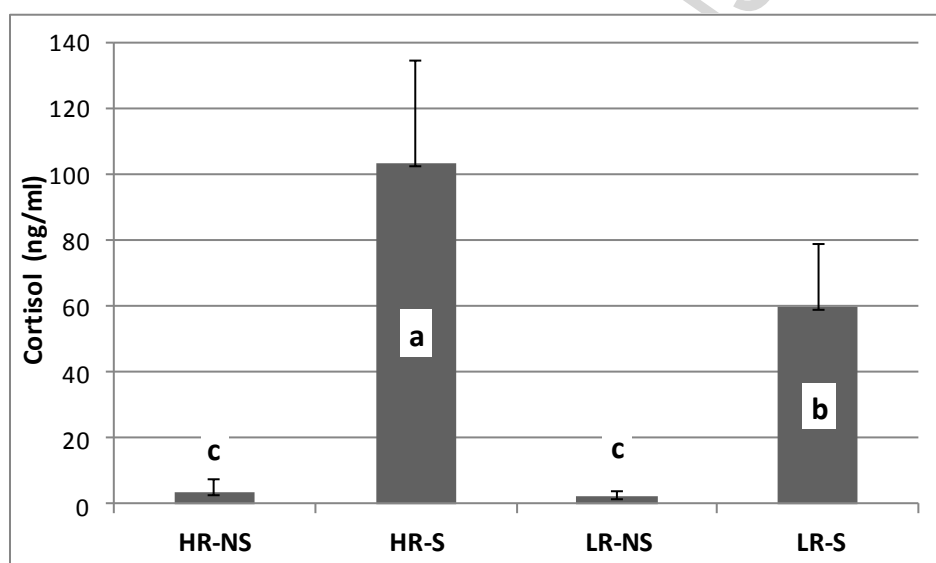**Figure 1 :** Plasma cortisol content in high responsive (HR) and low responsive (LR) rainbow trout submitted (S) or not (NS) to an acute stress before slaughter. Mean ± standard deviation, n ≥16; bars with different letter are significantly different (p<0.001).
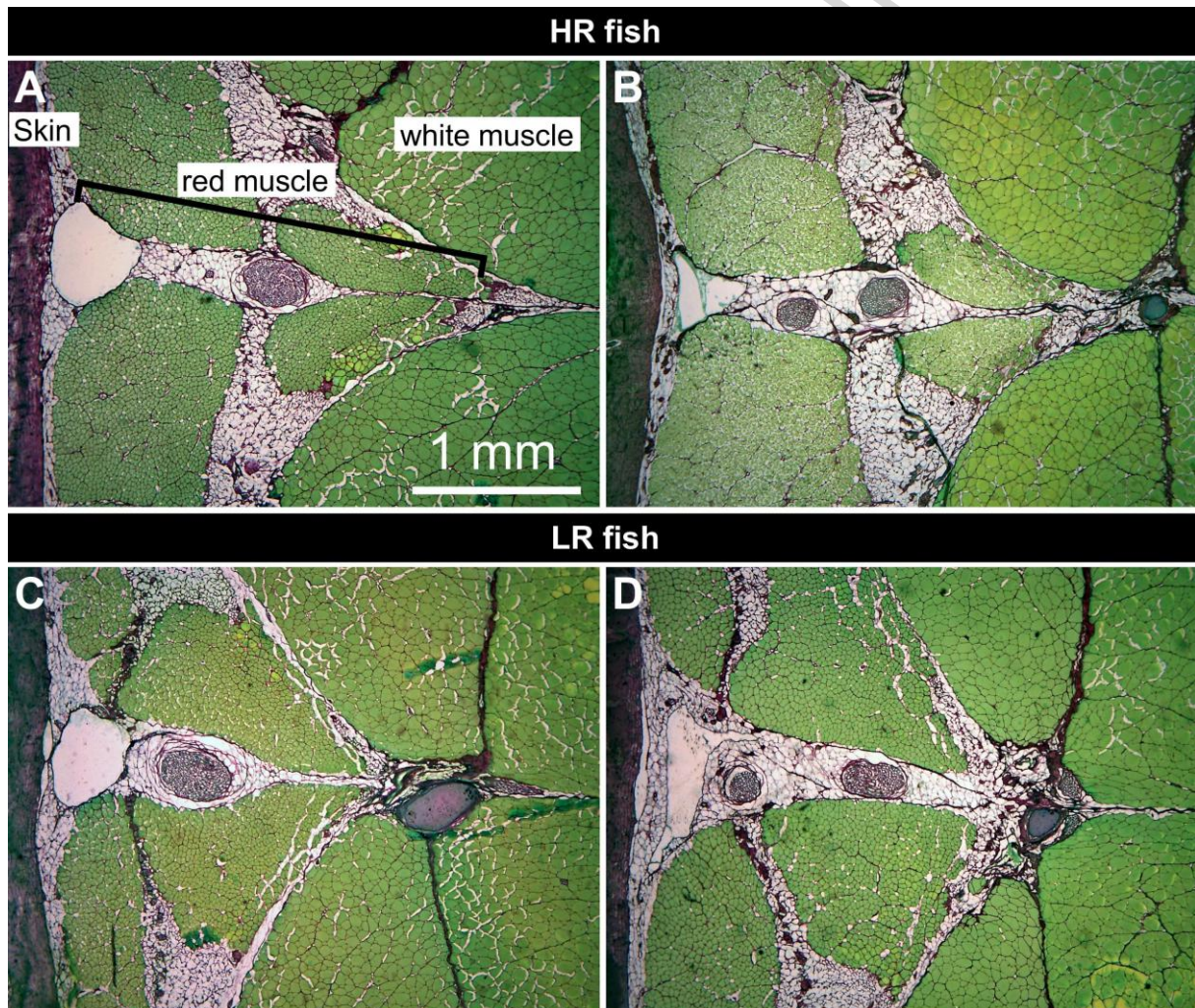
Figure 2 : Illustration of red muscle thickness measured on histological samples stained with

Sirius Red and Fast Green for HR fish (A, B) and LR fish (C, D).

Table 1 : Effects of divergent selection for stress responsiveness and slaughter stress on fish morphometric parameters, adiposity and process yields. Mean ± standard deviation, n ≥ 16.

line :	HR		LR		MANOVA Line x Stress[1]				MANCOVA Line x Stress W cov[2]		
stress :	NS	S	NS	S	Line effect p =	Stress effect p =	Line x Stress p =	Cor W (p)	Line effect p =	Stress effect p =	Line x Stress p =
Weight (g)	242±47[b]	225±31[b]	271±45[a]	279±43[a]	**<0.001**	0.683	0.225	-	-	-	-
K (=W/FL[3])	1.16±0.06[a]	1.16±0.03[a]	1.13±0.08[a]	1.14±0.05[a]	**0.038**	0.935	0.716	0.12 (0.318)	-	-	-
Fat-meter value	2.2±0.7[b]	2.5±0.7[b]	3.3±0.8[a]	3.4±0.8[a]	**<0.001**	0.320	0.415	**0.39 (0.001)**	**<0.001**	0.273	0.281
Carc. yield (%)	89.8±1.2[a]	89.6±0.1[a]	88.3±1.4[b]	89.1±1.2[ab]	**<0.001**	0.322	0.114	**-0.24 (0.040)**	**0.010**	0.347	0.089
VSI (%)	7.4±1.0[b]	7.6±0.7[b]	9.1±1.4[a]	8.4±1.1[a]	**<0.001**	0.342	0.120	**0.38 (0.001)**	**0.001**	0.384	0.063
HSI (%)	1.7±0.2[a]	1.7±0.2[a]	1.6±0.2[a]	1.5±0.2[a]	**0.018**	0.546	0.390	-0.22 (0.062)	-	-	-
GSI (%)	0.16±0.19[a]	0.12±0.07[ab]	0.11±0.06[ab]	0.06±0.05[b]	**0.029**	0.098	0.881	0.02 (0.872)	-	-	-

(1) : Simple Multifactorial ANalysis Of VAriance; (2) : Multifactorial ANalysis Of Variance with body weight as a covariable; HR : High Responsive; LR : Low Responsive; NS : Not stressed; S : Stressed; p : probability; Cor W : Correlation coefficient with body weight; SL : Standard Length; K : condition factor; W : Body Weight; FL : Fork Length; Carc. : Carcass; VSI : Viscero-Somatic Index; HSI : Hepato-Somatic Index; GSI : Gonado-Somatic Index; Values with different letter are significantly different (p<0.05).

Table 2 : Effects of divergent selection for stress responsiveness and slaughter stress on muscle pH and dry matter content. Mean ± standard deviation, n ≥ 16.

line :	HR		LR		MANOVA Line x Stress[1]				MANCOVA Line x Stress W cov[2]		
stress :	NS	S	NS	S	Line effect p =	Stress effect p =	Line x Stress p =	Cor W (p)	Line effect p =	Stress effect p =	Line x Stress p =
at slaughter											
pHi	7.00±0.16[a]	6.77±0.13[b]	7.01±0.19[a]	6.63±0.19[c]	0.094	**<0.001**	0.070	**-0.25 (0.039)**	0.643	**<0.001**	0.133
at 72 h post-mortem											
pHu	6.42±0.06	6.40±0.06	6.44±0.07	6.43±0.11	0.208	0.656	0.877	-0.03 (0.832)	-	-	-
DMC (%)	22.4±0.5[b]	22.5±0.5[b]	23.7±1.1[a]	23.4±0.8[a]	**<0.001**	0.490	0.379	**0.54 (<0.001)**	**<0.001**	0.569	0.143
cooked fillet											
pHc	6.68±0.04	6.66±0.04	6.67±0.05	6.69±0.09	0.290	0.914	0.129	-0.02 (0.901)	-	-	-
DMC (%)	25.9±0.6[b]	25.9±0.9[b]	27.1±1.5[a]	27.3±1.0[a]	**<0.001**	0.740	0.786	**0.42 (<0.001)**	**0.001**	0.656	0.969
post-mortem evolution											
Delta pH	-0.58±0.19[c]	-0.37±0.16[b]	-0.57±0.18[c]	-0.20±0.17[a]	**0.030**	**<0.001**	0.069	**0.23 (0.049)**	0.256	**<0.001**	0.118

(1) : Simple Multifactorial ANalysis Of VAriance; (2) : Multifactorial ANalysis Of Variance with body weight as a covariable; HR : High Responsive; LR : Low Responsive; NS : Not stressed; S : Stressed; p : probability; Cor W : Correlation coefficient with body weight; pHi : initial pH (<2 h post-mortem); pHu : ultimate pH (72 h post-mortem), pHc : pH of cooked fillet; Delta pH = pHu-pHi; DMC : Dry Matter Content; Values with different letter are significantly different (p<0.05).

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Table 3 : Effects of divergent selection for stress responsiveness and slaughter stress on fillet color measurements. Mean ± standard deviation, n

≥ 16

line :	HR		LR		MANOVA Line x Stress[1]				MANCOVA Line x Stress W cov[2]		
stress :	NS	S	NS	S	Line effect p =	Stress effect p =	Line x Stress p =	Cor W (p)	Line effect p =	Stress effect p =	Line x Stress p =
at slaughter											
L*	46.8±1.8[a]	46.3±1.2[a]	45.0±1.2[b]	44.4±1.6[b]	**<0.001**	0.118	0.803	**-0.47 (<0.001)**	**<0.001**	0.077	0.882
a*	-1.0±0.7[c]	-0.9±0.5[bc]	-0.4±0.7[a]	-0.3±0.7[ab]	**<0.001**	0.467	0.651	-0.08 (0.499)	-	-	-
b*	7.24±2.3[b]	7.6±1.5[b]	9.8±2.4[a]	8.6±2.6[ab]	**0.001**	0.449	0.166	**0.63 (<0.001)**	0.321	0.525	**0.011**
Cooked fillet											
L*	79.2±2.1[a]	78.8±2.0[a]	77.7±2.5[a]	79.1±3.2[a]	0.358	0.419	0.126	**0.35 (0.002)**	**0.007**	0.268	0.279
a*	-2.9±0.9[b]	-2.7±0.9[b]	-1.7±0.8[a]	-2.0±0.9[a]	**<0.001**	0.773	0.142	**0.58 (<0.001)**	**0.013**	0.927	**0.019**
b*	17.4±3.4[c]	20.6±2.6[b]	23.7±4.9[a]	22.4±4.6[ab]	**<0.001**	0.393	**0.034**	**0.59 (<0.001)**	**0.031**	0.202	**0.001**

(1) : Simple Multifactorial ANalysis Of VAriance; (2) : Multifactorial ANalysis Of Variance with body weight as a covariable; HR : High Responsive; LR : Low Responsive; NS : Not stressed; S : Stressed; p : probability; Cor W : Correlation coefficient with body weight; L* : lightness, a* : redness; b* : yellowness; Values with different letter are significantly different (p<0.05).

Table 4 : Effects of divergent selection for stress responsiveness and slaughter stress on fillet mechanical resistance. Mean ± standard deviation,

n ≥ 16.

line :	HR		LR		MANOVA Line x Stress[1]				MANCOVA Line x Stress W cov[2]		
stress :	NS	S	NS	S	Line effect p =	Stress effect p =	Line x Stress p =	Cor W (p)	Line effect p =	Stress effect p =	Line x Stress p =
raw flesh											
Fmax/w (N.g^{-1})	20,2±3,2^a	19,3±2,0^{ab}	18,1±2,1^b	16,3±2,0^c	**<0.001**	**0.022**	0.424	**-0.49 (<0.001)**	**0.005**	**0.010**	0.712
F20%/w (N.g^{-1})	0,61±0,05^a	0,58±0,08^{ab}	0,54±0,06^b	0,56±0,06^b	**0.005**	0.639	0.085	0.04 (0.754)	-	-	-
F80%/w (N.g^{-1})	9,6±0,2^a	8,5±0,6^b	8,0±0,8^{bc}	7,6±0,9^c	**<0.001**	**<0.001**	0.086	-0.03 (0.776)	-	-	-
cooked flesh											
Fmax/w (N.g^{-1})	17,3±2,6^b	19,5±2,3^{ab}	20,4±2,7^a	22,0±5,4^a	**0.001**	**0.027**	0.738	0.10 (0.393)	-	-	-
F20%/w (N.g^{-1})	0,52±0,07^{ab}	0,58±0,12^a	0,53±0,09^b	0,52±0,06^{ab}	0.321	0.156	0.116	**-0.57 (<0.001)**	0.158	0.153	0.288
F80%/w (N.g^{-1})	6,5±0,8^a	6,9±1,0^a	6,9±0,8^a	6,9±1,0^a	0.477	0.391	0.357	**-0.31 (0.009)**	**0.034**	0.450	0.622

(1) : Simple Multifactorial ANalysis Of VAriance; (2) : Multifactorial ANalysis Of Variance with body weight as a covariable; HR : High Responsive; LR : Low Responsive; NS : Not stressed; S : Stressed; p : probability; Cor W : Correlation coefficient with body weight; Fmax/w = maximal force / sample weight; F20%/w = Force at 20% deformation / sample weight;: F80%/w = Force at 80% deformation / sample weight; W/w = Work until Fmax / sample weight; Values with different letter are significantly different (p<0.05).

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Table 5 : Effects of divergent selection for stress responsiveness and slaughter stress on muscle histologically-measured parameters. Mean ± standard deviation, n ≥ 16 for white muscle, n ≥ 11 for red muscle.

line :	HR		LR		MANOVA Line x Stress[1]				MANCOVA Line x Stress W cov[2]		
stress :	NS	S	NS	S	Line effect p =	Stress effect p =	Line x Stress p =	Cor W (p)	Line effect p =	Stress effect p =	Line x Stress p =
White muscle fiber size											
Dens. (fib/mm²)	531±66[a]	485±63[b]	469±62[b]	442±53[b]	**<0.001**	**0.015**	0.522	**-0.54 (<0.001)**	0.113	**0.003**	0.164
M D (µm)	38.6±2.4[b]	40.9±3.7[a]	42.1±2.7[a]	42.2±3.3[a]	**0.001**	0.107	0.120	**0.54 (<0.001)**	0.196	**0.038**	**0.015**
% small fibers (< 20 µm)	20.7±3.7[a]	18.1±4.4[ab]	16.3±3.3[b]	17.5±4.8[b]	**0.013**	0.515	0.054	**-0.38 (0.001)**	0.275	0.406	**0.016**
% large fibers (> 90 µm)	2.2±1.7[c]	3.8±2.9[b]	5.0±3.0[ab]	5.8±2.1[a]	**<0.001**	0.060	0.481	**0.53 (<0.001)**	**0.036**	**0.022**	0.167
Red muscle fiber size											
Dens. (fib/mm²)	2955±633[b]	3398±448[a]	2584±439[b]	2673±565[b]	**<0.001**	0.060	0.207	**-0.49 (<0.001)**	**0.014**	0.071	0.425
M D (µm)	27.6±3.3[b]	24.9±2.0[c]	30.5±2.5[a]	29.5±3.4[ab]	**<0.001**	**0.020**	0.253	**0.49 (<0.001)**	**0.001**	**0.023**	0.485
Red muscle thickness											
Absolute value (mm)	2.4±0.5[a]	2.2±0.5[ab]	1.9±0.4[b]	1.9±0.4[b]	**0.001**	0.522	0.149	0.14 (0.293)	-	-	-
Relative value (% of body thickness)	15.5±3.0[a]	14.9±2.9[a]	11.8±2.2[b]	12.5±2.3[b]	**<0.001**	0.908	0.362	-0.16 (0.218)	-	-	-
Number of fibers	88±20[a]	87±19[a]	61±12[b]	67±14[b]	**<0.001**	0.587	0.410	-0.10 (0.465)	-	-	-

(1) : Simple Multifactorial ANalysis Of VAriance; (2) : Multifactorial ANalysis Of Variance with body weight as a covariable; HR : High Responsive; LR : Low Responsive; NS : Not stressed; S : Stressed; p : probability; Cor W : Correlation coefficient with body weight; Dens. : fiber density; MD : mean diameter; Values with different letter are significantly different (p<0.05).

Table 6 : Correlation within instrumentally measured quality variables, and between quality variables and others measured parameters, n=71 except for

L*r, a*r, and b*r : n=70, for FDens RM : n=61, and for Cort : n=35.

	BW	K	Fat	FDens WM	FDens RM	Cort$	pHi	pHu	Del pH	pHc	dmr	dmc	L*s	a*s	b*s	L*r	a*r	b*r	L*c	a*c
Cort$	-0.45 **	0.20	-0.28	0.18	0.51 **	-														
pHi	-0.25 *	-0.04	-0.29 *	0.39 **	0.16	0.25	-													
pHu	-0.03	-0.08	0.15	0.13	0.23	0.09	0.12	-												
Del pH	0.23 *	0.02	0.33 **	-0.34 **	-0.08	-0.19	-0.95 ***	0.21	-											
pHc	-0.01	-0.11	0.10	0.21	0.14	-0.12	0.13	0.68 ***	0.09	-										
dmr	0.54 ***	-0.03	0.53 ***	-0.44 ***	-0.43 **	-0.61 ***	-0.17	-0.26 *	0.08	-0.27 *	-									
dmc	0.42 ***	-0.10	0.60 ***	-0.34 **	-0.30 *	-0.51 **	-0.29 *	-0.31 **	0.18	-0.16	0.77 ***	-								
L*s	-0.47 ***	-0.01	-0.37 **	0.35 **	0.41 **	0.45 **	0.39 **	-0.01	-0.39 **	-0.15	-0.46 ***	-0.42 ***	-							
a*s	-0.08	0.03	0.13	-0.04	-0.20	-0.36 *	0.01	0.00	-0.00	0.11	0.18	0.23 *	-0.53	-						
b*s	0.63 ***	-0.08	0.49 ***	-0.42 ***	-0.48 ***	-0.34 *	-0.14	-0.20	0.07	-0.25 *	0.67 ***	0.56 ***	-0.33	-0.20	-					
L*r	-0.43 ***	0.05	-0.35 **	0.08	0.16	0.17	-0.07	-0.30 *	-0.03	-0.31 *	-0.18	-0.20	0.38 **	-0.01	-0.37 **	-				
a*r	-0.00	-0.08	0.29 *	0.07	-0.04	-0.22	0.17	0.32 **	-0.06	0.22	0.14	0.20	-0.34	0.65 ***	-0.08	-0.50 ***	-			
b*r	0.60 ***	-0.08	0.50 ***	-0.48 ***	-0.45 ***	-0.31	-0.23	-0.20	0.17	-0.27 *	0.69 ***	0.51 ***	-0.36	-0.18	0.93 ***	-0.26 *	-0.14	-		
L*c	0.36 **	0.20	-0.12	-0.25 *	-0.19	-0.30	-0.17	-0.40 **	0.03	-0.38 **	0.20	0.08	-0.09	-0.27 *	0.25 *	-0.02	-0.26 *	0.23	-	
a*c	0.58 ***	-0.09	-0.45 ***	-0.39 **	-0.47 ***	-0.31	-0.14	0.00	0.14	-0.09	0.47 ***	0.43 ***	-0.42	0.23 *	0.57 ***	-0.45 ***	0.42 ***	0.51 ***	0.16	-
b*c	0.59 ***	-0.05	0.52 ***	-0.51 ***	-0.37 **	-0.31	-0.27 *	-0.15	0.21	-0.25 *	0.65 ***	0.48 ***	-0.30	-0.18	0.83 ***	-0.31 *	-0.11	0.87 ***	0.18	0.53 ***

BW: body weight; K: condition factor; Fat: Fat-meter value; FDens: Fiber density; WM: White Muscle; RM : Red Muscle; Cort : Plasma cortisol content; pHi: initial pH; pHu: ultimate pH; DelpH: Delta pH; pHc: cooked pH; dmr: raw muscle dry matter content; dmc: cooked muscle dry matter content; L*s, a*s, b*s: lightness, redness, yellowness at slaughter; L*r, a*r, b*r: lightness, redness, yellowness for raw flesh at 72 h *post-mortem*; L*c, a*c, b*c: lightness, redness, yellowness for cooked flesh; 0"*" : Not Significant; *: p<0.05, **: p<0.01, ***: p<0.001.
$: Correlations with plasma cortisol concerns only stressed (S) fish.

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Table 7 : Correlation within fillet mechanical resistance parameters, and between mechanical resistance parameters and others measured parameters,

n=71 except for L*r, a*r, and b*r : n=70, for FDens RM : n=61, and for Cort : n=35.

	BW	K	Fat	FDens WM	FDens RM	Cort$	pHi	pHu	Del pH	pHc	dmr	dmc	MF/wr	F20/wr	F80/wr	W/wr	MF/wc	F20/wc	F80/wc
MF/wr	-0.49 ***	0.19	-0.39 **	0.57 ***	0.43 **	0.45 **	0.34 **	0.37 **	-0.23	0.25 *	-0.48 ***	-0.51 ***	-						
F20/wr	0.04	0.26 *	-0.18	0.12	0.17	0.21	0.05	-0.07	-0.07	0.04	-0.16	-0.15	0.14	-					
F80/wr	-0.03	0.32 **	-0.32 **	0.42 ***	0.21	0.51 **	0.31 **	0.24 *	-0.22	0.37 **	-0.38 **	-0.38 **	0.63 ***	0.38 **	-				
W/wr	-0.03	0.27 *	-0.28 *	0.34 **	0.30 *	0.35 *	0.14	0.37 **	-0.02	0.38 **	-0.33 **	-0.38 **	0.76 ***	0.22	0.71 ***	-			
MF/wc	0.10	-0.18	0.20	-0.17	-0.19	-0.38 *	-0.35 **	-0.37 **	0.22	-0.25 *	0.32 **	0.38 **	-0.25 *	-0.11	-0.46 ***	-0.30 *	-		
F20/wc	-0.57 ***	-0.22	-0.21	0.29 *	0.24	0.11	0.04	-0.06	-0.06	-0.04	-0.32 **	-0.28 *	0.26 *	-0.06	-0.07	0.03	0.16	-	
F80/wc	-0.31 **	-0.03	-0.02	0.07	0.04	-0.27	0.03	-0.24 *	-0.11	-0.09	0.14	0.15	-0.05	-0.12	-0.17	-0.09	0.53 ***	0.58 ***	-
W/wc	0.29 *	-0.14	0.23	-0.26 *	-0.28 *	-0.44 **	-0.49 ***	-0.37 **	0.36 **	-0.14	0.36 **	0.49 ***	-0.26 *	--0.09	-0.45 ***	-0.25 *	0.86 ***	-0.02	0.34 **

BW: body weight; K: condition factor; Fat: Fat-meter value; FDens: Fiber density; WM: White Muscle; RM : Red Muscle; Cort : Plasma cortisol content; pHi: initial pH; pHu: ultimate pH; DelpH: Delta pH; pHc: cooked pH; dmr: raw muscle dry matter content; dmc: cooked muscle dry matter content; MF/wr: Max Force/sample weight, F20/wr: F20%/sample weight, F80/wr: F80%/sample weight, W/wr: Work/sample weight for raw flesh; MF/wc: Max Force/sample weight, F20/wc: F20%/sample weight, F80/wc: F80%/sample weight, W/wc: Work/sample weight for cooked flesh.; 0"*" : Not Significant; *: p<0.05, **: p<0.01, ***: p<0.001.

$: Correlations with plasma cortisol concerns only stressed (S) fish.

Selection for stress responsiveness and slaughter stress affect flesh quality in pan-size rainbow trout, *Oncorhynchus mykiss*

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Statement of Relevance

Stress in fish is a permanent concern in aquaculture, and the stress associated with slaughter needs to be minimized in order to preserve flesh quality. The present work shows that similar adverse effects of slaughter stress on flesh quality are seen in rainbow trout from both low stress-responding and high responding lines. Genetic selection for low stress responsiveness does not appear to offer benefits to manage slaughter-stress consequences on flesh quality.

Statement of Relevance – Short version < 60 characters

This study gives new insights on slaughter stress effects.