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Original article

Impact of production technology on morphological lesions in the gills of commercial rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792)

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Abstract

The most popular rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) production technologies include both an extensive method with the flow through system (FTS) and an intensive method with the recirculating aquaculture system (RAS). Their impact on the fish was evaluated with a morphological assessment of the gills, as these are organs susceptible to environmental changes. Trout of 350 – 500 g body mass were caught for trial in spring and autumn, with 36 fish originating from 3 fish farms with the FTS system and an equal number from 3 RAS fish farms (n = 6). The fish were macroscopically examined and the gills were investigated microscopically (haematoxylin and eosin staining). Hypertrophy and hyperplasia were most commonly detected, amounting to 89% of all structural abnormalities. These lesions were slightly more common in the FTS, especially in autumn, whereas the changes to the blood vessels in the gills were observed more frequently in the rainbow trout from the RAS system than in the fish obtained from the FTS technology (the difference was statistically significant). The morphological lesions in the mucous cells of the gills were detected at a comparable severity regardless of the technology or production season. The predominantly low values of the histopathologic indices, which described the microscopic lesions in the gills of fish from the FTS and RAS systems, showed that the examined organ was most often free of lesions or demonstrated only minor morphological damage regardless of the production technology.

Key words: gills, rainbow trout, morphological lesions, fish production technology

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Introduction

Presently, apart from the extensive flow through system (FTS) used in rainbow trout production, intensive rearing is more commonly implemented with the recirculating aquaculture system (RAS). It is thus necessary to investigate the impact of these rainbow trout production technologies on the fish organism, including the morphology of gills, using a histopathological evaluation to explore the health of fish xposed to a variety of factors both under laboratory (Cerquiera and Fernandes 2002, Mazon et al. 2002, Oliveira-Ribeiro et al. 2002, Thophon et al. 2003) and environmental (Teh et al. 1997, Stentiford et al. 2003, Flores-Lopez et al. 2005) conditions. If used for such a purpose, pathomorphology is easier and faster than functional changes and demonstrates abnormalities earlier than the latter (Hinton and Lauren 1990, Strzyżewska et al. 2016). Many authors favour histopathology as the method of choice for monitoring the impact of the environment on the reared fish (Poleksic et al. 1999, Poleksic et al. 2002).

The gills in fish, due to their extensive surface and the fact they are a target for numerous water-borne pathogens, demonstrate susceptibility to environmental changes (Poleksic and Mitrovic--Tutundzic 1994, Cerquiera and Fernandes 2002, Mazon et al. 2002, Fanta et al. 2003, Fernandes and Mazon 2003, Mir and Channa 2009, Movahedinia et al. 2009) and react to minor physical or chemical changes in the environment (Cerquiera and Fernandes 2002, Strzyżewska et al. 2016). Damage to the structure of gills may result both from a generalized stress response of the whole body and the local effect of pathogens (Osman et al. 2009, Farell et al. 2010).

The aim of this paper is to assess the impact of the FTS and RAS systems on the morphological pattern of the gills in rainbow trout.

Materials and Methods

The studies were carried out on six fish farms that used the same feed ration but with different rainbow trout production technologies: FTS on three farms (1 – 3) and RAS on the other three (4 – 6) – n = 6/farm. Six rainbow trout (of 350 – 500 g each were collected from each farm in spring and autumn. The fish were sedated with 2-phenoxyethanol at a dose of 0.6 – 1.0 mL/dm^3 of water and were weighed and examined macroscopically. The gill specimens were sampled from the second gill arch located on the right side of the trout's head (Movahedinia et al. 2009) within 1 to 2 minutes after a given fish was sacrificed. The samples were fixed in 7% buffered formalin for 48 h and then decalcified with an EDTA solution for 3 – 7 days (Speare and Fergusson 1989, Flores-Lopes and Thomaz 2011). The sections were then impregnated in fluids (Leica TP1020, Germany) and embedded in paraffin blocks (Leica EG1150H, Germany). Two blocks were prepared for each fish, of which two 5 µm sections were cut perpendicularly to the long axis of the gill filaments (rotational microtome, Leica RM, Germany). The microtome sections (2 from each block) were stained with haematoxylin and eosin (Leica AutostainexXL, Germany) (Bancroft and Gamble 2008). The mounts were microscopically investigated (500 secondary lamellae for each mount) under a Nikon Eclipse 80i optical microscope equipped with a Nikon PS – Fi1 digital camera (Eclipse 80i; Nikon, Japan) using an NIS-Elements BR 2.30 programme (Nikon, Japan). Each mount was analysed by two independent histopathologists to minimize statistical bias.

The morphological lesions were categorized into 4 groups (G1 – G4) by their type and location (Table 1). Among them, lesions associated with the functions of the organ and related to the tissue damage were distinguished and classified by severity: 1st stage lesions – not affecting the regular functions of the tissue; 2nd stage lesions – more advanced and impacting the functions of the tissue, and 3rd stage lesions – very severe and causing irreversible damage to the organ. The groups were analysed semi-quantitatively by calculating the histopathological index (HAI) as modified by Poleksic and Mitrovic-Tutundzic (1994). The HAI value was calculated for each fish with the formula: HAI = 1 x SI + 10 x SII + 100 x SIII, where SI, SII, and SIII correspond to the number of lesions found for the stages 1 – 3. HAI values between 1 and 10 indicated morphological lesions without any impact on the regular functions of the gills; between 11 and 20 – minor damage to the gills; between 21 and 50 – moderate damage and from 51 to 100 – extensive and irreversible damage to the organ (Poleksic and Mitrovic-Tutundzic 1994). Moreover, each lesion was assigned a numerical score from 0 to 6 describing its extent, where: 0 meant no lesions; 2 – minor lesions; 4 – moderate lesions and 6 – extensive (diffuse) lesions (Bernet et al. 1999). Analysis using a Statistica 10 StatSoft package served to determine the location and variation measures for the examined variables within the investigated groups. Statistical differences between FTS and RAS were calculated using the Mann-Witney U test with Bonferroni's adjustment (StatSoft, Inc. 2013).

Table 1. Classification of morphological lesions included in evaluation of rainbow trout gills.

Denomination of lesions	Stage
G1: Hypertrophy and hyperplasia of gill epithelia	
1. Hyperplasia of gill filament epithelium	I
2. Hyperplasia of lamellar epithelia	I
3. Decrease of inter-lamellar space	I
4. Epithelial lifting of gill filament epithelium	I
5. Epithelial lifting of lamellae	I
6. Intercellular oedema	I
7. Incomplete fusion of several lamellae	I
8. Complete fusion of several lamellae	I
9. Complete fusion of all the lamellae	II
10. Rupture and peeling of gill filament epithelium	II
11. Rupture of the lamellar epithelium	II
G2: Lesions in mucous and chloride cells	
1. Hypertrophy and hyperplasia of mucous cells	I
2. Empty mucous cells or their disappearance	I
3. Hypertrophy and hyperplasia of chloride cells	I
G3: Blood vessel lesions	
1. Lamellar telangiectasia	I
2. Filament blood vessel enlargement	I
3. Haemorrhages with rupture of epithelium	II
4. Aneurisms	II
G4: Terminal stages	
1. Fibrosis	III
2. Necrosis	III

Results

It was found that the gross pattern of rainbow trout was regular in most cases. Abnormalities were rarely observed and no pathognomonic lesions indicating disease processes were detected. The skin and scales were integral and continuous with only sporadic defects that were slightly more common in the recirculating aquaculture system (RAS) fish. Macroscopically in the gills, hyperaemia was observed in 4 fish and ecchymosis in 3 individuals. These circulatory disturbances were most often reported in autumn in the RAS fish.

The majority of rainbow trout demonstrated the regular microscopic pattern of the gills. Morphological lesions in the gills detected microscopically were observed both in the fish from the flow through system (FTS) (Fig. 1A, B) and RAS (Fig. 1C, D) technologies. Structural abnormalities classified into the G1 group, namely, of hypertrophy and hyperplasia types, constituted 87.75% of all microscopic morphological lesions (Fig. 2). They were more frequently observed in the FTS fish in autumn. Within this group of lesions, hyperplasia of the lamellar epithelium (26%) and epithelial lifting of the lamellae (25%) predominated (Fig. 3). Hyperplasia of the gill filament epithelium (19%) was relatively common (Fig. 1B, C). The other lesions depicted in Figure 3 constituted from 2% to 13% of all structural abnormalities.

Among the G2 type lesions, hypertrophy and hyperplasia of the mucous cells and chloride cells were occasionally recorded (Fig. 1C, D, Fig. 2).

The morphological lesions categorized into the G3 group, namely, those affecting the blood vessels, occurred in 7.5% of the recorded lesions. Aneurysms (Fig. 1B) and telangiectasia (Fig. 1D) were reported with haemorrhages and rupture of the epithelium being relatively less common (Fig. 1A, C).

The terminal morphological lesions (G4) were sporadic. They did not carry any statistical value and translate into the investigated population.

For the rainbow trout caught in spring on the FTS fish farms (denoted with the numbers 1 – 3), 44% of the HAI values were within the 11 – 20 range (minor morphological damage to the gills) and 33% fell into the 0 – 10 range (morphological lesions without any impact on the regular function of the organ). For the same period, but for the fish from the RAS farms (No. 4 – 6), it was calculated that 50% of the HAI values were between 0 and 10 while 38% fell within the 11 – 20 range. In the autumn season in the fish from the FTS technology, the HAI values from 0 to 10 were predominant (67%) with the other group constituting approximately 16% each. The

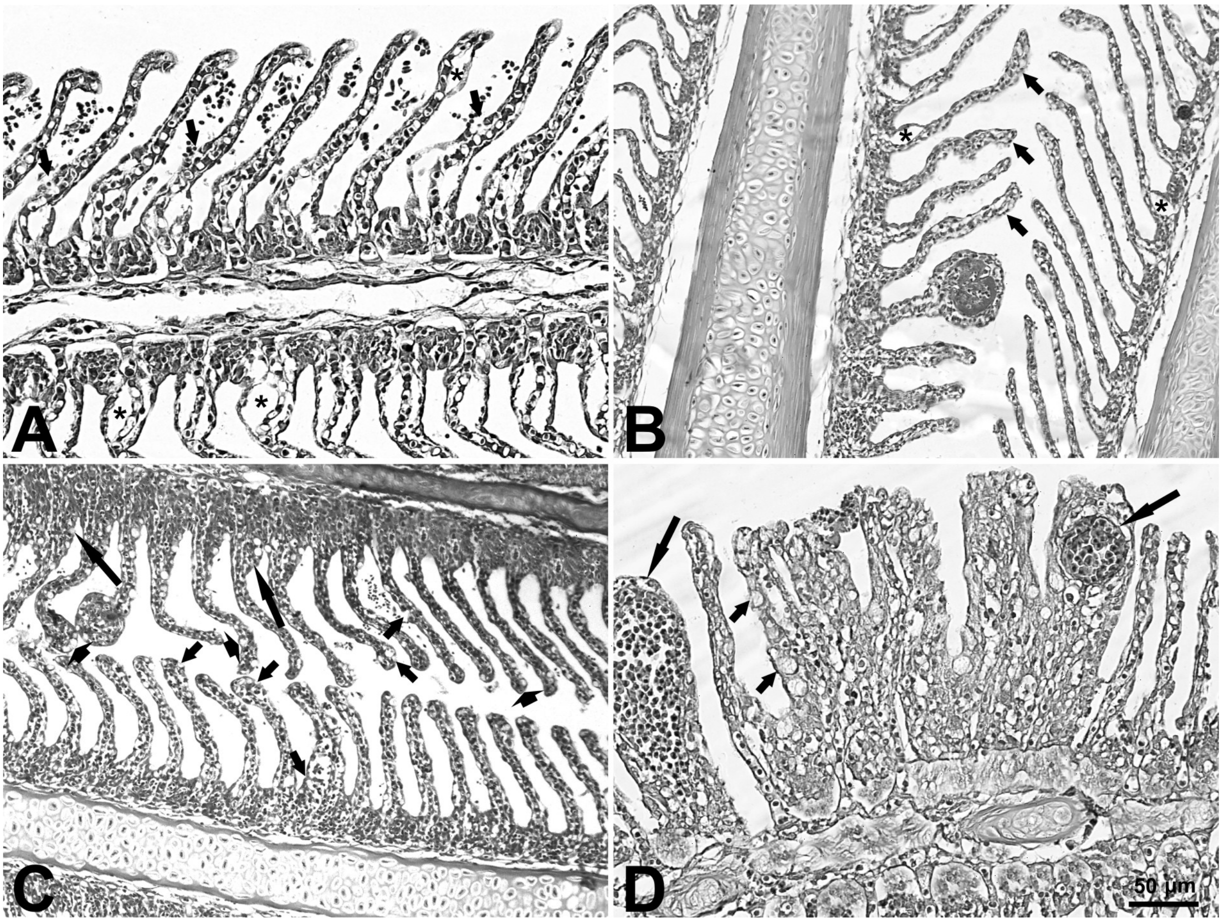

Fig. 1. Morphological lesions in the gills of rainbow trout from extensive (A, B) – FTS and intensive (C, D), RAS, technologies: A. The gills of trout caught in autumn: epithelial lifting (asterisks), haemorrhages with rupture of the epithelium (arrows). B. The gills of trout caught in spring: an aneurysm forming on the left, local epithelial lifting (asterisks), hyperplasia of the gill epithelium (arrows) and on the right most of the lamellae are regular. C. The gills of trout caught in autumn: incomplete fusion of the lamellae (long arrows), hyperplasia of the gill epithelium (short arrows) with clubbing of the lamellae, hypertrophy of the mucous cells (arrow heads), small haemorrhages with rupture of the epithelium. D. The gills of trout caught in spring: telangiectasia (long arrows), lamellar fusion, hypertrophy and hyperplasia of the mucous cells (short arrows). HE staining, magnification as on the scale located on the D pattern.

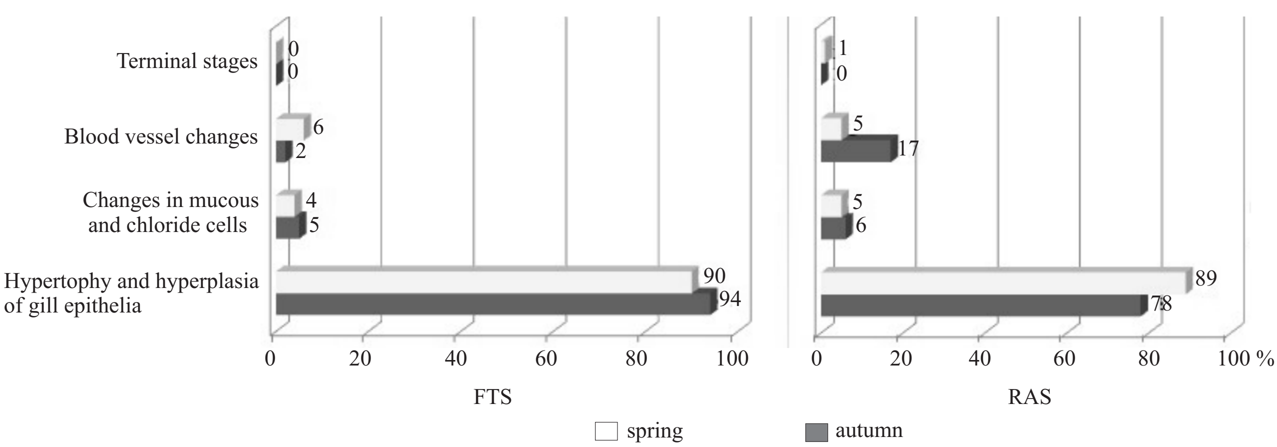

Fig. 2. Percentage compilation of the morphological lesions in the gills of rainbow trout from extensive (FTS) and intensive (RAS) production technologies and collected for trials in spring and autumn, taking into account histopathological index as modifiied by Poleksic and Mitrovic-Tutundzic (1994) to account for Bernet's et al. (1999) numerical score.

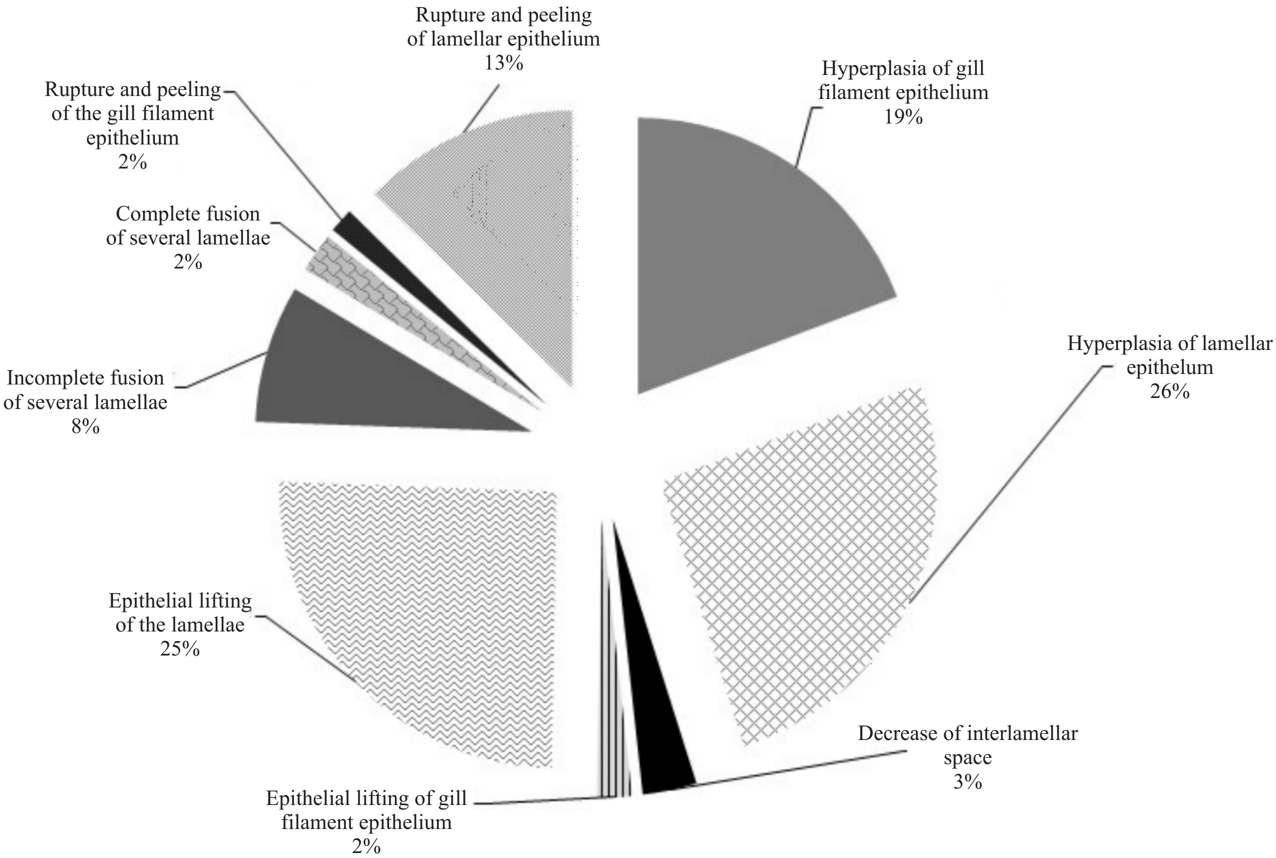

Fig. 3. Percentage compilation of the hypertrophy and hyperplasia type lesions in the gill epithelium rainbow trout from extensive (FTS) and intensive (RAS) production technologies caught for trials in spring and autumn, taking into account histopathological index as modified by Poleksic and Mitrovic-Tutundzic (1994) to account for Bernet's et al. (1999) numerical score.

HAI values for the RAS trout were 11 – 20 in 67% and 0 – 10 in 33%.

For the fish caught on the FTS and RAS fish farms, the HAI values in the 50 – 100 range were not recorded and the 21 – 50 values ranged from 12% to 22%.

The statistical analysis of the prevalence of morphological lesions in the gills indicates that the dispersion of extreme values is larger for the RAS technology in spring and for the FTS technology in autumn. Higher scores were shown for the FTS technology versus RAS in autumn and these differences were statistically significant (Fig. 4, Table 2).

Discussion

The most commonly observed morphological lesions in the gills of rainbow trout from FTS and RAS production technologies included: hyperplasia of the lamellar epithelium and epithelial lifting and separation from the basal membrane of the gill lamellae. The same lesions have been reported in many fish species (Thophon et al. 2003, Monteiro et al. 2008, Bais and Lokhande 2012) as a result of numerous irritating factors (Movahedinia et al. 2012). In addition, it has been found that epithelial lifting is the primary response of the gills to various contaminants.

Our previous studies demonstrated partial adhesions of the gill lamellae, which was a consequence of severe and advanced lesions, namely hypertrophy and hyperplasia of the gill epithelium. Both hypertrophy and hyperplasia as well as epithelial lifting and partial fusion of the secondary lamellae are examples of defensive protective mechanisms resulting in an increase in the distance between the external environment and the blood and leading to a barrier against contaminant and toxin penetration being constructed (Poleksic and Mitrovic-Tutundzic 1994, Poleksic et al. 1999, Singhadach et al. 2009).

In the present study, the morphological lesions in the gills of trout categorized as the G1 group were more common in FTS technology and were independent of the season. This may indicate that in extensive production technology the trout are exposed to a greater number of environmental pathogens which cause relatively mild morphological lesions.

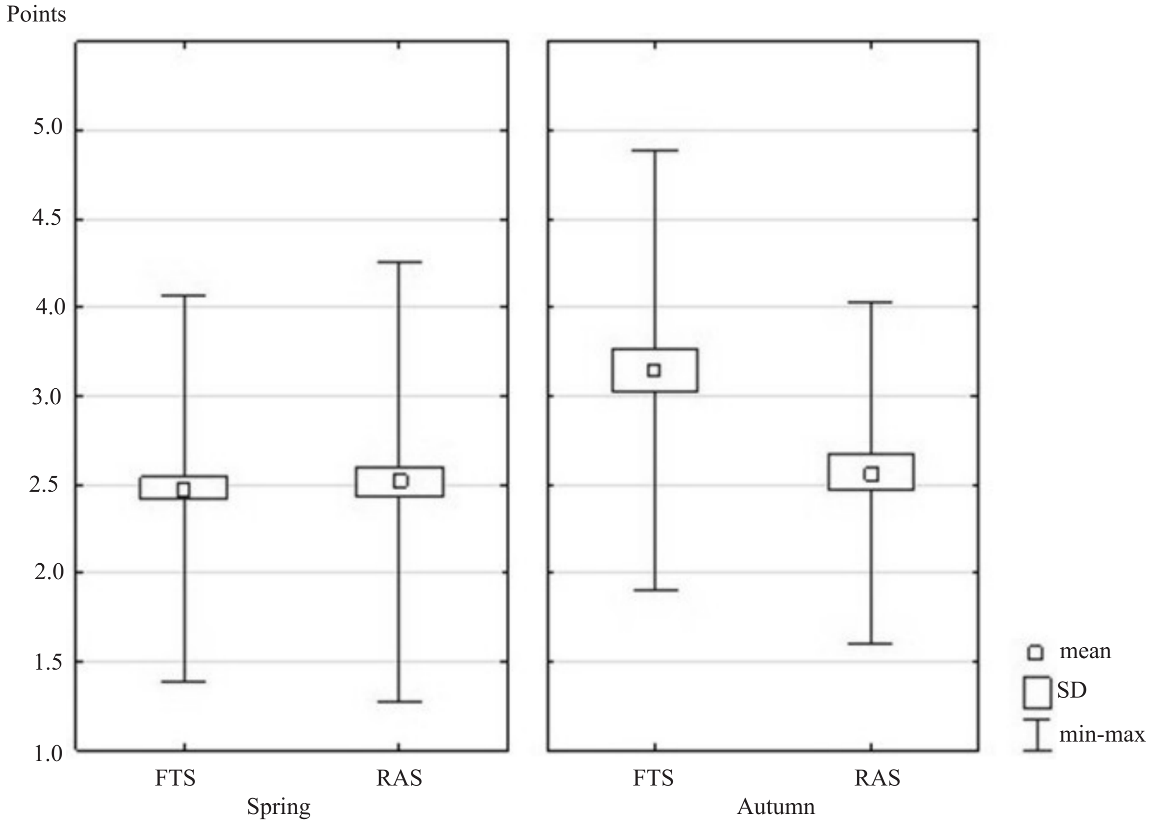

Fig. 4. Dispersion of point values for rainbow trout caught using extensive (FTS) and intensive (RAS) production technologies in spring and autumn.

Table 2. Statistical presentation of average point values for morphological lesions in gills of rainbow trout using extensive (FTS) and intensive (RAS) technologies.

Lesion	Status	Production technologies	Statistical differences
G1	SI	FTS 2.61+1.28 RAS 12.03+8.5	**
	SII	FTS 2.93+0.93 RAS 2.48+1.51	
G2	SI	FTS 2.56+1.09 RAS 1.94+0.85	*
G3	SI	FTS 1.40+0.55 RAS 2.67+1.21	*
	SII	FTS 1.21+0.42 RAS 1.80+0.77	

* statistical difference between means significant at $p \leq 0.05$, **statistical difference between means significant at $p \leq 0.01$, G1 – hypertrophy and hyperplasia of gill epithelia, G2 – lesions in mucous and chloride cells, G3 – blood vessel lesions, SI – stage 1 lesions, which do not disturb regular functioning of tissue, SII – stage 2 lesions, more advanced and impacting functions.

Excessive mucous production and hyperplasia and hypertrophy of the mucous cells in the gills are a response to the impact of numerous external irritant factors (Lease et al. 2003, Svobodova et al. 2005), a reduction in the oxygen content (Fernandes and Mazon 2003) or to an increase in nitrogen metabolic wastes in water (Capkin et al. 2009). The detailed morphological lesions were reported at a similar severity regardless of the production technology or the season.

The blood vessel lesions in the gills were detected in 6% of the examined samples and were more common in the RAS rainbow trout than in the fish from the FTS technology (the difference is significant). Of these, telangiectasia and aneurysms were most common. The expansion of vascular spaces in the gill lamellae is associated with increased heart rate and blood pressure. This results in enhanced blood flow through the gills and, consequently, leads to a reduction in the gill vascular resistance. Blood vessel lesions may result from mechanical injuries (during catching, prolonged transport or intensified culture). These lesions were more frequently observed in our previous studies probably for these reasons.

The trout cultures in the FTS and RAS technologies were in a good health condition as evidenced by the low number of recorded gross lesions and low values of the histopathological indices that describe the microscopic lesions in the rainbow trout gills. When comparing the HAI values for the trout cultured in both technologies, slightly higher values of this index were evident for the FTS fish, regardless of the production season. This may suggest that fish cultured in this system are exposed to a higher number of environmental factors.

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