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Prevalence and severity of cardiac abnormalities and arteriosclerosis in farmed rainbow trout (*Oncorhynchus mykiss*)

J. Brijs[a,*], P. Hjelmstedt[a,b], C. Berg[a], I.B. Johansen[c], H. Sundh[b,d], J.A.C. Roques[b,d], A. Ekström[d], E. Sandblom[d], K. Sundell[b,d], C. Olsson[d], M. Axelsson[d], A. Gräns[a,b]

[a] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden
[b] Swedish Mariculture Research Center, SWEMARC, Faculty of Science, University of Gothenburg, Gothenburg, Sweden
[c] Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway
[d] Department of Biological and Environmental Sciences, Faculty of Science, University of Gothenburg, Gothenburg, Sweden

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ABSTRACT

Cardiovascular disease may pose a major threat to the health and welfare of farmed fish. By investigating a range of established cardiovascular disease indicators, we aimed to determine the prevalence, severity and consequences of this affliction in farmed rainbow trout (*Oncorhynchus mykiss*) from an open cage farm in the Baltic Sea, an open cage farm in a freshwater lake, and a land-based recirculating aquaculture system. We also aimed to identify environmental, anthropogenic and physiological factors contributing towards the development of the disease. The majority of trout possessed enlarged hearts with rounded ventricles (mean height:width ratios of 1.0–1.1 *c.f.* ~1.3 in wild fish) and a high degree of vessel misalignment (mean angles between the longitudinal ventricular axis and the axis of the bulbus arteriosus of 28–31 °**c.f.** ~23° in wild fish). The prevalence and severity of coronary arteriosclerosis was also high, as 92–100% of fish from the different aquaculture facilities exhibited coronary lesions. Mean lesion incidence and severity indices were 67–95% and 3.1–3.9, respectively, which resulted in mean coronary arterial blockages of 19–32%. To evaluate the functional significance of these findings, we modelled the effects of arterial blockages on coronary blood flow and experimentally tested the effects of coronary occlusion in a sub-sample of fish. The observed coronary blockages were estimated to reduce coronary blood flow by 34–54% while experimental coronary occlusion adversely affected the electrocardiogram of trout. Across a range of environmental (water current, predation), anthropogenic (boat traffic intensity, hatchery of origin, brand of feed pellets) and physiological factors (condition factor, haematological and plasma indices), the hatchery of origin was the main factor contributing towards the observed variation in the development of cardiovascular disease. Therefore, further research on the effects of selective breeding programs and rearing strategies on the development of cardiovascular disease is needed to improve the welfare and health of farmed fish.

1. Introduction

Cardiovascular disease is a class of diseases that involves the heart and/or blood vessels, and is a leading cause of death in humans (Fuster, 2014). However, varying forms of cardiovascular disease also pose a major threat to farmed animals such as fast-growing fish, broiler chickens and pigs (Olkowski et al., 1996; Poppe and Taksdal, 2000; Poppe et al., 2002; Poppe et al., 2003; Takle et al., 2006; van Essen, 2017). With regards to fast-growing fish, cardiovascular disease may be of specific concern in salmonids due to their high degree of morphological and physiological cardiac plasticity in response to environmental and anthropogenic factors (Gamperl and Farrell, 2004). The salmonid heart is composed of an inner spongy myocardial layer that is supplied with oxygen from venous blood returning to the lumen of the heart, as well as a well-developed outer layer of compact myocardium supplied with oxygenated arterial blood *via* the coronary circulation, which resembles the situation in mammals (Farrell, **1991**). Therefore, plastic cardiovascular changes associated with rearing in intensive aquaculture, combined with an impaired coronary circulation due to arteriosclerosis, have been suggested to increase the risk of myocardial dysfunction and death in farmed salmonids (Poppe and Taksdal, 2000; Poppe et al., 2002; Poppe et al., 2003; Takle et al., 2006).

Due to the focus of selective breeding programs in aquaculture with regards to traits such as rapid growth, late sexual maturation and

* Corresponding author.
E-mail address: jeroen.brijs@slu.se (J. Brijs).

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disease resistance, other important traits such as the functional morphology of various organs may easily have been neglected (Poppe et al., 2003). Cardiac morphology of intensively farmed salmonids has been observed to change from the normally distinct pyramidal ventricular structure found in wild fish to a more rounded shape with increased levels of fat deposition around the heart (Gamperl and Farrell, 2004; Poppe et al., 2003). It has been speculated that this morphological change could be due to a combination of factors such as selective breeding programs, rearing strategies, over-feeding and the reduced activity levels of farmed salmonids (Gamperl and Farrell, 2004; Poppe et al., 2003). However, further research is urgently needed to validate these hypotheses. Moreover, recent studies have demonstrated that stress in the farm environment can also be a contributing factor towards abnormal heart morphology, as chronically elevated levels of the stress hormone cortisol induces maladaptive pathological cardiac hypertrophy, remodeling and dysfunction (Johansen et al., 2011; Johansen et al., 2017). Since the form and function of the heart is intimately linked to whole animal performance (Claireaux et al., 2005; Farrell, 1991; Johansen et al., 2017; Poppe et al., 2003), it is crucial to identify and potentially remedy environmental, anthropogenic and physiological factors that contribute towards the development of abnormal heart morphology in farmed fish.

The presence of coronary arteriosclerotic lesions has also been linked to severe cardiac pathology in farmed salmonids (Poppe et al., 2007). Although coronary arteriosclerosis has sometimes been described as a 'fact of life' in salmonids, studies have shown that the enhanced growth rates associated with aquaculture and dietary composition can increase the risk of developing this condition (Farrell, 2002; Farrell et al., 1986; Farrell et al., 1990; Gamperl and Farrell, 2004; Saunders et al., 1992; Seierstad et al., 2005; Seierstad et al., 2008). Arteriosclerotic lesions in salmonids predominately appear in the main coronary artery, as lesion formation is initiated when the vascular endothelium of the artery is damaged during the overstretching that can occur with each heartbeat during periods of intense or stressful activity (Farrell, 2002; Saunders et al., 1992). Such lesions will inevitably impair coronary blood flow, and in severe cases may even completely occlude the main conduit for oxygenated blood to the myocardium (Farrell, 2002). Experimental occlusion of coronary blood flow in salmonids can impair ventral aortic blood pressure and elevate resting heart rate, presumably due to impaired cardiac contractility (Ekström et al., 2017; Farrell, 1987; Steffensen and Farrell, 1998). This has been demonstrated to constrain the ability of fish to cope with the metabolic demands associated with exercise, elevated temperatures and hypoxia (Ekström et al., 2017; Ekström et al., 2018; Ekström et al., 2019; Farrell and Steffensen, 1987; Gamperl et al., 1994; Gamperl et al., 1995; Steffensen and Farrell, 1998). It is also believed to predispose fish for cardiac failure during stressful situations such as those regularly found in aquaculture (*e.g.* grading, transportation and treatments for disease and parasites, Mercier et al., 2000; Poppe et al., 2003; Poppe et al., 2007). In fact, it has been suggested that the abovementioned cardiac morphological anomalies and coronary arteriosclerotic lesions may even underlie the recurrent and significant production losses of 10–20% in salmonid aquaculture (Brocklebank and Raverty, 2002; Mercier et al., 2000; Poppe et al., 2007).

As far as we are aware, previous evaluations of cardiovascular disease in farmed fish were performed over a decade ago (Farrell and Johansen, 1992; Farrell et al., 1986; Farrell et al., 1990; Poppe et al., 2003; Saunders et al., 1992; Seierstad et al., 2005; Seierstad et al., 2008). If anything, it is likely that during this period the incidence and severity of cardiovascular disease in farmed fish may have increased further due to the continual selection for an increased growth rate of fish in aquaculture (Ellis et al., 2015; Gamperl and Farrell, 2004; Poppe et al., 2003). Thus, from both an ethical and economical point of view, it is important to re-evaluate the current extent of this problem in salmonid aquaculture. Moreover, the environmental, anthropogenic and/or physiological factors that may promote the development of cardiovascular disease in modern production systems require a more systematic evaluation. Therefore, in the present study we used a wide range of cardiovascular disease indicators to determine the current prevalence and severity of cardiovascular disease in farmed rainbow trout (*Oncorhynchus mykiss*) from an open cage farm in the Baltic Sea, an open cage farm in a lake, and a land-based recirculating aquaculture system (RAS). We also used the same indicators to identify underlying factors promoting the development of cardiovascular disease in these fish, as well as experimentally and theoretically evaluating the functional significance of coronary obstructions on the cardiovascular physiology and performance of rainbow trout. Taken together, this study provides a broad analysis of the current prevalence, severity and consequences of cardiovascular disease in farmed fish, as well as some of the underlying factors promoting the development of the disease.

2. Material and methods

The present study consists of both field-based and laboratory-based investigations. The purpose of the field-based investigations was to evaluate the prevalence and severity of cardiovascular disease in farmed rainbow trout from different types of aquaculture facilities, as well as to identify the environmental, anthropogenic and physiological factors that may promote the development of the disease. The purpose of the laboratory-based investigations was to experimentally evaluate the functional significance of coronary obstructions on cardiovascular physiology and performance in rainbow trout.

2.1. Field-based investigations of cardiovascular disease in farmed rainbow trout

2.1.1. Experimental animals and aquaculture facilities

The prevalence and severity of cardiovascular disease in farmed rainbow trout were evaluated in three different types of aquaculture facilities, which included an open cage sea farm, an open cage lake farm and a land-based RAS. The open cage sea farm was located in the Baltic Sea near Brändö, Åland Islands, Finland (water salinity: ~5–6 ppt, average monthly water temperature: ~9 °C, monthly water temperature range: 0 to 19 °C, water temperature during sampling: ~6 °C) and 60 fish were sampled in total from 6 different cages (*i.e.* 10 fish per cage, sampled in November 2018). The open cage lake farm was located in Lake Ströms Vattudal near Strömsund, Sweden (water salinity: ~0–1 ppt, average monthly water temperature: ~6 °C, monthly water temperature range: 0 to 15 °C, water temperature during sampling: ~1 °C) and 30 fish were sampled in total from 3 different cages (*i.e.* 10 fish per cage, sampled in February 2019). The land-based RAS was located in Kungshamn, Sweden (water salinity of ~14–15 ppt, water temperatures maintained between 12 and 14 °C throughout the year, water temperature during sampling: ~13 °C) and 20 fish were sampled in total from 2 different indoor holding tanks (*i.e.* 10 fish per holding tank, sampled in April 2019).

Rearing conditions with respect to the environmental and anthropogenic factors investigated in the present study were relatively similar among the different cages at the open cage lake farm, as well as among the different holding tanks at the land-based RAS. However, at the open cage farm in the Baltic Sea, rearing conditions differed substantially among cages, which provided an opportunity to investigate the environmental and anthropogenic factors that potentially contribute towards the development of cardiovascular disease. To achieve this, we took advantage of the extensive experience of the personnel from that facility to select 6 different cages located in areas where they knew fish were subjected to different levels of water current, predation and boat traffic. With respect to water current, cages were either located in areas with low levels of water current (*e.g.* cages located in sheltered and protected bays) or high levels of water current (*e.g.* cages located in water passageways with high flows). With regards to predation, cages were either located in areas with low levels of predation (*e.g.* fish in

these cages seldomly exhibited wounds caused by seals, egrets and cormorants) or high levels of predation (*e.g.* fish in these cages frequently exhibited wounds caused by the abovementioned predators and protective seal culling measures were employed in these areas). Concerning boat traffic, cages were either located in areas with low levels of boat traffic (*e.g.* cages were in remote locations and only disturbed by personnel of the aquaculture facility), medium levels of boat traffic (*e.g.* cages were in locations where small, recreational boats regularly pass by) and high levels of boat traffic (*e.g.* cages were located near commercial ferry routes that regularly transport passengers and vehicles between the Åland islands). In addition, we noted the hatchery of origin. Fish were either sourced from hatchery 1, a 'normal' hatchery typically found in Scandinavia where fish were raised under natural temperature and light regimes (growth season: April to October, average water temperature during growth season: ~15 °C, monthly water temperature range: 5 to 22 °C, photoperiod range: 6:18 to 18:6-h light:dark throughout the year), or from hatchery 2, a hatchery that utilizes the warm spill water from a power plant and manipulated photoperiods to accelerate the early growth of trout fingerlings (water temperatures constantly maintained between 10 **and** 15 °C and the photoperiod was set to 19:5-h light:dark). Due to the manipulated temperature and light regimes, fish from hatchery 2 reached slaughter size (~2.5 to 3.5 kg) approximately 6 months faster than fish from hatchery 1. We also noted the commercial brand of feed pellets that were distributed to each cage, as two different brands of pellets were used. An overview of the environmental and anthropogenic factors that each sea cage was subjected to, as well as all of the physiological data that was collected from rainbow trout in the present study is reported in supplementary information 1–3.

All experimental protocols in the field-based investigations were performed at the respective aquaculture facilities and were in accordance with either the Åland Provincial government project approval committee (decision 2/2016 for protocols performed at the facility near Brändö, Åland Islands) or national regulations and covered by an ethical permit approved by the Regional ethical committee on animal research in Gothenburg, Sweden (5–8–18-12,466-2018 for protocols performed at facilities near Strömsund and Kungshamn, Sweden).

2.1.2. Field-based experimental protocol and sampling procedure

Rainbow trout were captured using dip nets from the open cages located at the Baltic Sea farm and lake farm (facilities near Brändö and Strömsund, respectively) or from indoor holding tanks in the land-based RAS (facility near Kungshamn). Upon capture, 10 trout were simultaneously subjected to a 30 min period of standardized confinement stress in a 500 l solid plastic container with a lid in order to assess their tolerance to stressful interventions (Johansen et al., 2011). The water within the container was refreshed every 5 min during this period to ensure sufficient water oxygenation and to avoid the build-up of metabolic end products.

Following the period of confinement stress, trout were euthanized with a sharp blow to the head and ~ 1 ml of blood was withdrawn from the caudal vessels using heparinized syringes. This procedure took less than 1 **min** for each fish and the blood samples were immediately placed on ice for further analyses (see 2.1.3.). Subsequently, body mass (g), fork length (mm) and gonad mass (g) of each fish was determined. From these measures, gonado-somatic index (GSI: gonad mass/body mass × 100) and condition factor were calculated (condition factor: (10^5 × body mass)/fork length^3). In addition, the ventricle and bulbus arteriosus were carefully removed and photographed from 2 different angles to determine aspects of 3-dimensional ventricular morphology (*see 2.1.4.*) using a Canon EOS Rebel XSi (Canon, New York, U.S.A.). The bulbus arteriosus was then carefully trimmed from the ventricle and placed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) at 4 °C for further analyses (*see 2.1.5.*). The remaining excess tissue (*i.e.* atrium) was then carefully trimmed from the ventricle from which the blood was removed *via* gentle squeezing and blotting with tissue paper. The ventricle was subsequently weighed to determine relative ventricular mass (RVM: ventricular mass/body mass × 100).

2.1.3. Blood and plasma analyses

Blood samples were **analyzed** for haematocrit (Hct, %) and haemoglobin concentration ([Hb], g dl^{-1}). Hct was determined as the fractional red cell volume after centrifugation of a subsample of blood in 80 μl heparinized microcapillary tubes at 10000 r.c.f. (relative centrifugal force) for 5 min in a Hct centrifuge (Haematokrit 210, Hettich, Tuttlingen, Germany). A handheld Hb 201^+ meter (Hemocue AB, Ängelholm, Sweden) was used to determine [Hb] and values were corrected for fish blood (Clark et al., 2008). Mean corpuscular haemoglobin concentration (MCHC, g dl^{-1}) was subsequently calculated as [Hb]/Hct ×100.

Following the haematological analyses, the remaining blood samples were centrifuged at 10000 rcf for 5 min in a microcentrifuge (Eppendorf 5415D, Eppendorf, Hamburg, Germany). The plasma was subsequently collected and frozen at −80 °C for analyses to determine the concentration of total cholesterol (mg dl^{-1}), plasma glucose (mmol l^{-1}) and plasma cortisol (ng ml^{-1}). Concentration of total cholesterol was determined from 10-fold diluted plasma samples using a cholesterol quantitation kit (MAK043, Sigma-Aldrich, St Louis, MO, USA), while plasma glucose was determined from 2.5-fold diluted plasma samples using a glucose assay kit (GAHK20, Sigma-Aldrich, St. Louis, MO, USA). Plasma cortisol concentration was determined by a radioimmunoassay (RIA) using a cortisol antibody (Code: S020; Lot: 1014–180,182, Guildhay Ltd., Guildford, Surrey, UK) as previously described by Young (1986) and validated by Sundh et al. (2011). As a tracer, tritiated hydrocortisone-[1,2,6,7–^{3}H(N)] (NET 396; NEN Life Sciences Products, Boston, Massachusetts, USA) was used and cortisol standards were prepared from hydrocortisone (Sigma, St. Louis, MO, USA). Radioactivity was determined with a Wallac 1409 liquid scintillation counter (LKB Instruments, Turku, Finland). Intra- and inter-assay coefficients of variation for this cortisol RIA has been shown to be 3.9% and 5.4%, respectively, with a detection limit of 0.7 ng ml^{-1} (Sundh et al., 2011).

2.1.4. Ventricular morphology analyses

As described by Poppe et al. (2003), height:width ratios and the alignment of the bulbus arteriosus were used to describe the 3-dimensional morphology of the ventricle for each fish (Fig. 1A–B). For measurements of the height:width ratio, photographs of the heart with the cranio-ventral surface facing up were analyzed using ImageJ 1.52 k software (National Institutes of Health, Maryland, U.S.A.). A contour of the heart was traced from the original image before drawing a line across the base to exclude the atrium and the bulbus arteriosus. The height of the ventricle was defined as the distance from the middle of the base to the apex, and the width was defined as the widest segment of the ventricle, parallel to the base (Fig. 1A). To determine the alignment of the bulbus arteriosus, the angle between the longitudinal ventricular axis and the axis of the bulbus arteriosus were determined from photographs of the hearts in lateral recumbency using ImageJ 1.52 k software (Fig. 1B).

2.1.5. Determining extent of arteriosclerosis in coronary arteries

After fixation in 4% formaldehyde overnight at 4 °C, the bulbus arteriosus was rinsed three times with 0.1 M PBS (duration of each rinse was 30 min) before being stored in a solution of PBS containing sodium azide (0.2% *w/v*) at 4 °C. The bulbus arteriosus was then dehydrated, cleared and embedded in paraffin wax for sectioning at 5 μm thickness on a Shandon Finesse ME microtome (Shandon Scientific Ltd., Cheshire, U.K.).

In a similar fashion to previously described procedures (Farrell and Johansen, 1992; Farrell et al., 1990), six serial sections of the coronary artery were taken from three sites located along the bulbus arteriosus. The sections were then stained using standard haematoxylin and eosin

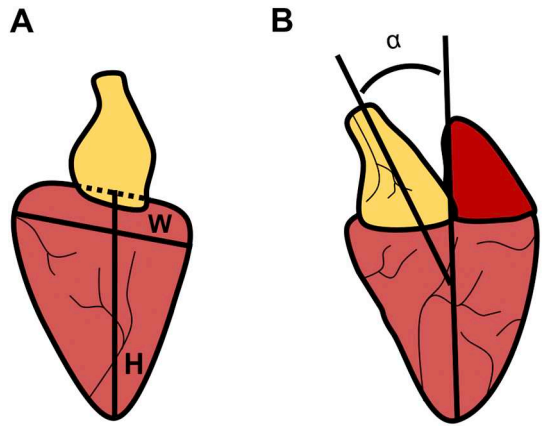

Fig. 1. Evaluating the ventricular morphology of farmed rainbow trout. Two numerical measurements were used to describe the ventricular morphology of farmed rainbow trout as described by Poppe et al. (2003), which included the height:width ratio of the ventricle (light red structure) and the alignment of the bulbus arteriosus (yellow structure). (A) The height (H) of the ventricle was defined as the distance from the middle of the base to the apex, whereas the width (W) was defined as the widest segment of the ventricle, parallel to the base. (B) The alignment of the bulbus arteriosus was determined by measuring the angle (α) between the longitudinal ventricular axis and the axis of the bulbus arteriosus from photographs of the hearts in lateral recumbency. **(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)**

staining procedures, and photographed using a Nikon DXM1200 microscope camera (Nikon Europe B.V. Badhoevedorp, Netherlands). Thus, eighteen arterial cross-sections were graded for the incidence and severity of arteriosclerosis in each fish. Coronary arteriosclerotic lesions in salmonids are generally characterized as an intimal proliferation of the vascular smooth muscle of the coronary artery with a disrupted elastic lamina (Farrell and Johansen, 1992; Farrell et al., 1990; Seierstad et al., 2005; Seierstad et al., 2008). These lesions resemble the early forms of arteriosclerotic lesions found in the coronary arteries of mammals, but lack the calcium and lipid inclusions typically found in mature mammalian lesions (Farrell, 2002). In the present study, coronary arteriosclerotic lesions were graded using a previously described grading system (Farrell and Johansen, 1992; Farrell et al., 1990). Briefly, the severity of arteriosclerosis was ranked from 0 to 5, with 0 representing an artery without arteriosclerotic lesions, 1 representing an artery with a small proliferation on the tunica intima (the inner cell layer of the vessel), 2 representing an artery with a large intimal proliferation, 3 representing an artery with multiple small intimal proliferations, 4 representing an artery with multiple large intimal proliferations, and 5 representing the 'catch-all' for severe lesions involving the tunica media (the middle layer of the vessel) and/or very large portions of the intima (Fig. 2). Lesion incidence (LI: number of cross sections with a grade $>$ 0 / total number of cross sections $\times$ 100) and lesion severity index (LSI: $((1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4) + (5 \times n_5))/(n_1 + n_2 + n_3 + n_4 + n_5)$, where n = the number of cross sections with a grade equivalent to the subscript) were calculated using formulas described by Farrell et al. (1990). In addition, the photograph of the cross section with the largest arteriosclerotic lesion was selected for every fish, from which the total inner area of the artery and area of the lesion were calculated using ImageJ 1.52 k software. This information was then used to calculate the maximum arterial blockage (%) for every fish.

2.2. Laboratory-based evaluations of the physiological effects of coronary obstruction

To evaluate whether coronary obstruction affects the ECG in a manner similar to myocardial ischemia in mammals, we re-**analyzed** previously recorded ECG traces from rainbow trout published by Ekström et al., 2017. These fish were obtained from Antens Laxodling AB, Alingsås, Sweden and kept in the aquarium facilities at the University of Gothenburg, Gothenburg, Sweden (n = 4, body mass = 2325 ± 186). ECG traces were analyzed before and after mechanical coronary occlusion in anaesthetized trout using the Scope View module in the LabChart Pro v7.3 software (ADInstruments, Castle Hill, Australia). Briefly, the ECG of anaesthetised trout was continuously recorded using surgically implanted sub-cutaneous electrodes before and during a 30 min occlusion of the main coronary artery, which was achieved by placing a vascular clamp around the artery (see Ekström et al., 2017 for details regarding anaesthesia, surgery and experimental protocols). By using the Scope View module in LabChart Pro v7.3, average ECG traces were calculated from 120 heart beats at different time points throughout the occlusion period (~ every 7 min). From these average traces, amplitudes and timing of the varying waveforms within the ECG such as the *P*-wave (atrial depolarization), *QRS*-complex (ventricular depolarization) and *T*-wave (ventricular repolarization) were determined, and from which clinical symptoms of myocardial ischemia could be identified (Munz et al., 2011; Preda and Burlacu, 2010; Rodríguez et al., 2006; Sun et al., 2013).

2.3. Statistical analyses

Statistical analyses were performed using SPSS Statistics 26 (IBM Corp., Armonk, NY, USA). To statistically analyse differences in body and blood/plasma parameters, as well as the differences in ventricular morphology/extent of arteriosclerosis in trout from the different aquaculture facilities, we used one-way ANOVAs with Tukey *post hoc* analyses (for body length, condition factor, plasma cholesterol, plasma glucose, plasma cortisol, RVM, ventricular height:width ratio, alignment of the bulbus arteriosus, LSI and maximum arterial blockage, as these parameters did not violate any of the assumptions for a one-way ANOVA); Welch ANOVAs with Games-Howell *post hoc* analyses (for body mass and MCHC, as these parameters violated the assumption of homogeneity of variances); one-way ANCOVAs with Bonferroni adjusted *post hoc* analyses (for Hct and [Hb], as body mass was linearly related to these parameters and thus included as a covariate); and a Kruskal-Wallis H test using Dunn's procedure with a Bonferroni correction for multiple comparisons (for GSI and LI, as these parameters were not normally distributed).

Multiple regression models were run on the data collected from trout sampled at the Baltic Sea farm to determine the relative contribution of environmental, anthropogenic and physiological factors to the total variance explained for parameters such as condition factor, heart size and shape, alignment of the bulbus arteriosus, prevalence and severity of arteriosclerosis, as well as plasma levels of cholesterol, glucose and cortisol. Environmental factors included water current (2 categorical levels: low *vs.* high levels of water current) and predation (2 categorical levels: low *vs.* high levels of predation). Anthropogenic factors included boat traffic (3 categorical levels: low, medium and high levels of boat traffic), hatchery of origin (2 categorical levels: hatchery 1 and 2), and the brand of commercial pellet the trout were fed (2 categorical levels: pellet brand 1 and 2). Physiological factors included condition factor and haematological indices, as well as plasma levels of cholesterol, glucose and cortisol. For each model, all factors that seemed to be linearly related (assessed by partial regression plots) to the dependent variable under investigation were initially included to make the full model. Factors were subsequently eliminated one-at-a-time using the backwards-selection strategy until only factors with statistically significant *p*-values remained. Regression coefficients and standard errors for the multiple regression models are reported in supplementary information 4.

To meet the assumptions of the abovementioned models, a logarithmic transformation was applied to values of plasma glucose and plasma cortisol, a natural logarithmic transformation was applied to

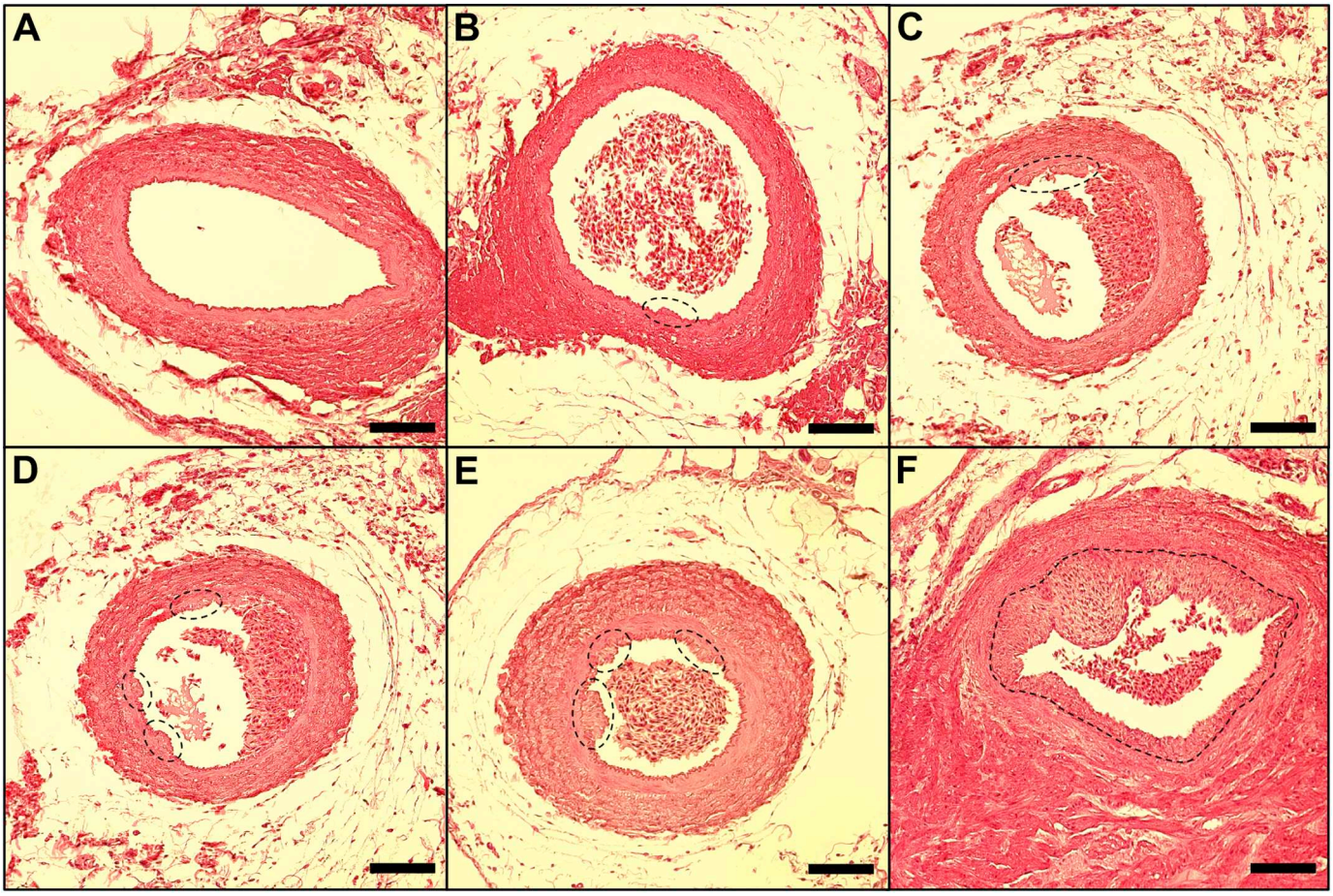

Fig. 2. Grading the severity of arteriosclerotic lesions in the coronary artery of rainbow trout. Sections of the coronary artery that demonstrate the varying degrees of arteriosclerosis in farmed rainbow trout, which range from (A) an artery with no lesions (grade 0), (B) an artery with a small singular lesion (grade 1), (C) an artery with a large singular lesion (grade 2), (D) an artery with multiple small lesions (grade 3), (E) an artery with multiple large lesions (grade 4), to (F) an artery with a catch-all for severe lesions (grade 5). Grades were assigned based on a previously described grading system (Farrell et al., 1990; Farrell and Johansen, 1992). Black bars in the bottom left corner of each panel represent 100 μm.

values of RVM, and a reflect and square transformation was applied to values of LI. F-, χ^2_2, and *p*-values obtained from the statistical analyses are reported throughout the text and all *p*-values < .05 were considered statistically significant.

3. Results

3.1. Field-based investigations of cardiovascular disease in farmed rainbow trout

3.1.1. Physiological status and stress response of trout following confinement

Condition factor of trout differed between the three different aquaculture facilities ($F_{2,107}$ = 16.618, p < .001) with the highest condition factor observed at the Baltic Sea farm (~1.71), followed by the lake farm (~1.58), and then the land-based RAS (~1.46, Table 1). However, differences were also observed in GSI (χ^2_2 = 58.024, p < .001), as trout at the Baltic Sea farm and land-based RAS had a higher GSI than trout at the lake farm (p < .001, Table 1). When accounting for these differences in GSI by subtracting gonad mass from body mass, condition factor of trout no longer differed between the Baltic Sea farm and lake farm, but both were still significantly higher than for trout at the land-based RAS (~1.60 *vs.* ~1.44, $F_{2,107}$ = 7.944, p = .001).

No significant differences were observed in Hct, [Hb] and MCHC of trout from the different facilities following confinement stress (Table 1). Yet, relatively minor differences were observed with regards to plasma levels of glucose (lake farm and land-based RAS > Baltic Sea farm) and cortisol (Baltic Sea farm ≥ land-based RAS ≥ lake farm) in trout following confinement stress (Table 1). Total cholesterol concentration in the plasma of trout also differed between the facilities ($F_{2,66}$ = 4.453, p = .015), as levels were significantly elevated in trout from the lake farm (Table 1). Visual observations revealed that 7% of trout from the Baltic Sea farm, 20% from the lake farm, and 5% from the land-based RAS could not tolerate the confinement stressor, as they were unresponsive to physical contact and floating upside down after 30 min (Table 2). It was observed that these fish generally displayed a higher Hct, a lower MCHC and higher total cholesterol concentration when compared with the respective mean values from each aquaculture facility (Table 2).

Multiple regression models revealed that out of the varying environmental and anthropogenic factors investigated at the Baltic Sea farm, factors such as the hatchery of origin and water current significantly contributed towards explaining some of the variation in the condition factor of trout ($F_{2,57}$ = 40.453, p < .001, R^2 = 0.587). Trout sourced from the hatchery with manipulated temperature and light regimes (hatchery 2) and/or were located in cages subjected to low levels of water current were generally associated with a higher condition factor than trout sourced from the 'normal' hatchery typically found in Scandinavia (hatchery 1) and/or were located in cages subjected to high levels of water current. The commercial pellet brand the trout were fed and condition factor significantly contributed towards

Table 1
Body and blood/plasma parameters of trout from the different aquaculture facilities. Mean ± s.e.m. of a range of body and blood/plasma parameters of trout from the open cage Baltic Sea farm, open cage lake farm and land-based RAS.

Measured variables	Baltic Sea farm	Lake farm	Land-based RAS	Statistical summary
Body parameters				
Body mass (g)	2730 ± 69^{a}	2609 ± 104^{a}	1338 ± 52^{b}	Welch's $F_{2,60.008} = 150.989$, $p < .001$
Fork length (mm)	543 ± 4^{a}	547 ± 7^{a}	449 ± 5^{b}	$F_{2,107} = 61.704$, $p < .001$
Condition factor (K)	1.70 ± 0.02^{a}	1.58 ± 0.03^{b}	1.46 ± 0.04^{c}	$F_{2,107} = 16.618$, $p < .001$
GSI (%)	4.33 ± 0.55^{a}	0.03 ± 0.00^{b}	1.12 ± 0.30^{a}	$\chi^2_2 = 58.024$, $p < .001$
Blood/plasma parameters after confinement stress				
Hct (%)	41.3 ± 0.9	39.3 ± 1.4	34.4 ± 1.2	$F_{2,104} = 1.357$, $p = .262$
[Hb] (g dl^{-1})	11.1 ± 0.2	10.6 ± 0.4	9.3 ± 0.3	$F_{2,105} = 2.218$, $p = .114$
MCHC (g Hb dl^{-1})	27.3 ± 0.4	27.0 ± 0.4	27.4 ± 0.9	Welch's $F_{2,50.018} = 0.601$, $p = .552$
Plasma cortisol (ng ml^{-1})	72.5 ± 7.6^{a}	45.5 ± 6.4^{b}	50.0 ± 5.0^{a,b}	$F_{2,105} = 4.803$, $p = .010$
Plasma glucose (mmol l^{-1})	6.4 ± 0.3^{a}	8.8 ± 0.6^{b}	7.8 ± 0.5^{b}	$F_{2,107} = 9.176$, $p < .001$
Plasma cholesterol (mg dl^{-1})	351 ± 13^{a}	402 ± 16^{b}	339 ± 17^{a}	$F_{2,66} = 4.453$, $p = .015$

Data are presented as mean ± s.e.m. and statistical differences ($p < .05$) between aquaculture facilities are represented with different lower-case letters.

explaining some of the variation in the levels of plasma glucose ($F_{2,55} = 18.913$, $p < .001$, $R^2 = 0.407$), whereas only the commercial pellet brand contributed towards explaining some of the variation in the levels of plasma cholesterol ($F_{1,28} = 4.664$, $p = .040$, $R^2 = 0.143$). The intensity of boat traffic significantly contributed towards explaining some of the variation in the plasma cortisol levels observed following confinement stress ($F_{1,56} = 16.397$, $p < .001$, $R^2 = 0.226$), as fish exposed to higher levels of boat traffic exhibited higher cortisol responses to confinement stress.

3.1.2. Ventricular morphology and explanatory factors

The relative size of the heart differed between trout from the different aquaculture facilities ($F_{2,107} = 20.411$, $p < .001$), as trout from the land-based RAS had a lower RVM (~0.08% of body mass) than trout from the Baltic Sea farm and lake farm (~0.10% of body mass, Fig. 3A). The ventricular shape, as determined from the ventricular height:width ratio, did not differ in trout from the different facilities (~1.05, $F_{2,107} = 0.66$, $p = .519$, Fig. 3B). However, the alignment of the bulbus arteriosus did differ ($F_{2,101} = 6.078$, $p = .003$), as the angle between the longitudinal ventricular axis and the axis of the bulbus arteriosus was ~3° greater in trout from the lake farm than from the Baltic Sea farm (Fig. 3C). It was observed that fish unable to tolerate the 30 min period of confinement stress generally displayed a greater angle between the longitudinal ventricular axis and the axis of the bulbus arteriosus when compared with the respective mean values from each aquaculture facility (Table 2).

Multiple regression models revealed that out of the varying environmental, anthropogenic and physiological factors investigated at the Baltic Sea farm, the plasma cortisol response observed following confinement stress significantly contributed towards explaining some of the variation in RVM ($F_{1,56} = 7.268$, $p = .009$, $R^2 = 0.115$), with RVM increasing in fish with a higher plasma cortisol response. Furthermore, the hatchery of origin significantly contributed or tended to contribute towards explaining some of the variation in ventricular height:width ratio ($F_{1,58} = 5.673$, $p = .021$, $R^2 = 0.089$) and the alignment of the bulbus arteriosus ($F_{1,52} = 3.906$, $p = .053$, $R^2 = 0.070$) in fish from the Baltic Sea farm. Trout from hatchery 2 generally had a slightly higher ventricular height:width ratio and a higher degree of misalignment of the bulbus arteriosus.

3.1.3. Prevalence and severity of arteriosclerosis

Arteriosclerotic lesions were detected in ~92% of fish at the Baltic Sea farm and in 100% of the fish at the other two facilities. Significant differences were observed in the incidence (LI: $\chi^2_2 = 15.376$, $p < .001$; Fig. 4) and severity of arteriosclerosis between the different aquaculture facilities (LSI: $F_{2,92} = 3.261$, $p = .043$, maximum arterial blockage: $F_{2,97} = 6.994$, $p = .001$; Fig. 5). The distribution of LI scores at the Baltic Sea farm and land-based RAS did not significantly differ, but both were significantly lower than the distribution of LI scores at the lake farm ($p < .001$, Fig. 4). Trout sampled at the lake farm had an LSI of ~3.9, which was significantly higher than that found at the land-based RAS (~3.1) but not at the Baltic Sea farm (~3.4, Fig. 5A). Furthermore, maximum arterial blockages were significantly higher at the lake farm (~32%) than at the Baltic Sea farm and land-based RAS (~19% and ~ 21%, respectively, Fig. 5B). It was observed that fish unable to tolerate the 30 min period of confinement stress generally displayed a higher LI and maximum arterial blockage when compared with the respective mean values from each aquaculture facility (Table 2).

Multiple regression models revealed that out of the varying environmental, anthropogenic and physiological factors investigated at the Baltic Sea farm, only the hatchery of origin significantly contributed towards explaining some of the variation in LI ($F_{1,58} = 7.937$, $p = .007$, $R^2 = 0.120$), LSI ($F_{1,52} = 6.579$, $p = .013$, $R^2 = 0.112$) and maximum arterial blockage ($F_{1,57} = 4.829$, $p = .032$, $R^2 = 0.078$). Trout from hatchery 2 were generally associated with a higher prevalence and severity of arteriosclerosis when compared with trout from hatchery 1.

3.2. Laboratory-based evaluations of the physiological effects of coronary obstruction

The effects of the arterial blockages on coronary blood flow caused by arteriosclerotic lesions can be modelled using Poiseuille's law (Poiseuille, 1840) assuming laminar blood flow and similar values of blood pressure, blood viscosity and coronary artery length in trout from the different aquaculture facilities. The average maximum arterial blockages of 19%, 32% and 21% seen in trout from the Baltic Sea farm, lake farm and land-based RAS, respectively (Fig. 5B), correspond to theoretical reductions in coronary blood flow of ~34%, ~54%, and ~ 38%, respectively (Fig. 6A). Furthermore, when coronary blood flow was experimentally occluded, clear changes in the ECG were observed in all four anaethetised trout (Fig. 6B). Specifically, the amplitude of the peak of the QRS-complex gradually decreased, while the time between the P-wave and the R-wave increased. In addition, the ST-segment of the ECG was distinctly elevated throughout the coronary occlusion (Fig. 6B).

4. Discussion

Abnormal cardiac morphology in farmed salmonids has previously been suggested to be a relatively overlooked and widespread problem in aquaculture (Poppe et al., 2003). Indeed, regardless of the type of aquaculture facility, the majority of sampled rainbow trout possessed a morphologically abnormal heart with rounded ventricles (*i.e.* mean height:width ratios of ~1.05 *c.f.* ~1.31 in wild trout in Poppe et al., 2003) and a high degree of vessel misalignment (*i.e.* mean angles of 28–31° between the longitudinal ventricular axis and the axis of the

Table 2
Comparison of cardiovascular disease indicators between trout that were and were not able to tolerate the confinement stressor from each aquaculture facility.

Measured variables	Sea farm	Lake farm	Land-based RAS
Sample size (n)			
Did not tolerate confinement stress	4	4	1
Did tolerate confinement stress	56	16	19
RVM (% of body mass)			
Did not tolerate confinement stress	0.12 ± 0.01	0.10 ± 0.01	0.07
Did tolerate confinement stress	0.10 ± 0.00	0.10 ± 0.00	0.08 ± 0.00
H:W ratio			
Did not tolerate confinement stress	1.12 ± 0.06	1.07 ± 0.02	1.32
Did tolerate confinement stress	1.06 ± 0.01	1.06 ± 0.03	1.02 ± 0.02
Angle (°)			
Did not tolerate confinement stress	35 ± 1	32 ± 3	34
Did tolerate confinement stress	28 ± 1	31 ± 1	30 ± 1
Lesion incidence (%)			
Did not tolerate confinement stress	99 ± 1	97 ± 3	100
Did tolerate confinement stress	67 ± 4	94 ± 4	66 ± 7
Lesion severity index (LSI)			
Did not tolerate confinement stress	3.5 ± 0.2	4.3 ± 0.4	3.0
Did tolerate confinement stress	3.4 ± 0.2	3.9 ± 0.3	3.1 ± 0.3
Arterial blockage (%)			
Did not tolerate confinement stress	26 ± 6	33 ± 11	39
Did tolerate confinement stress	19 ± 2	32 ± 4	20 ± 3
Haematocrit (%)			
Did not tolerate confinement stress	44.8 ± 4.8	42.8 ± 1.6	41
Did tolerate confinement stress	41.2 ± 0.9	39.3 ± 1.3	34.1 ± 1.2
Haemoglobin (g dl-1)			
Did not tolerate confinement stress	10.0 ± 1.1	11.0 ± 0.3	10.5
Did tolerate confinement stress	11.2 ± 0.2	10.9 ± 0.3	9.5 ± 0.2
MCHC (g dl-1)			
Did not tolerate confinement stress	25.0 ± 2.0	25.8 ± 1.0	25.6
Did tolerate confinement stress	27.4 ± 0.5	27.8 ± 0.3	28.1 ± 0.6
Total cholesterol (mg dl-1)			
Did not tolerate confinement stress	–	457 ± 48	401
Did tolerate confinement stress	354 ± 13	389 ± 15	335 ± 18
Plasma glucose (mmol l-1)			
Did not tolerate confinement stress	6.2 ± 2.2	9.2 ± 0.9	13.2
Did tolerate confinement stress	6.4 ± 0.2	8.2 ± 0.4	7.5 ± 0.4
Plasma cortisol (ng ml-1)			
Did not tolerate confinement stress	53.9 ± 27.8	28.1 ± 4.1	96.3
Did tolerate confinement stress	73.5 ± 7.8	24.9 ± 3.0	47.5 ± 4.7

Data are presented as mean ± s.e.m. except for the single trout that was unable to tolerate the confinement stressor from the land-based RAS where only the mean is displayed. Missing data is represented by '-' and is due to the fact that we only had a limited number of cholesterol quantitation kits (*i.e.* total cholesterol).

bulbus arteriosus *c.f.* ~23° in wild trout in Poppe et al., 2003). Interestingly, we also observed that fish unable to tolerate confinement stress (*i.e.* fish were unresponsive to physical contact and floating upside down after 30 min of confinement stress) appeared to have an even greater degree of vessel misalignment. This observation supports previous suggestions that individuals with morphologically abnormal ventricles and a high degree of vessel misalignment may have an impaired ability to cope with metabolically demanding or stressful situations (Claireaux et al., 2005; Poppe et al., 2003). However, due to the relatively low sample sizes, detailed statistical analyses of the observations regarding the individuals that were unable to tolerate the confinement stressor were not possible and therefore these observations must be interpreted with caution. Thus, further research investigating the effects of ventricular morphology and vessel misalignment on *in vivo* cardiovascular performance in farmed salmonids is clearly needed. When investigating the environmental, anthropogenic and physiological factors that could contribute to the development of abnormal cardiac morphology in fish at the Baltic Sea farm, only the hatchery of origin contributed towards explaining some of the variation observed in ventricular morphology and alignment of the bulbus arteriosus. Our findings therefore support, at least in part, the suggestion that either the specific breeding program and/or rearing strategy (*e.g.* the use of warm spill water from a power plant and manipulated photoperiods to accelerate the early growth of trout fingerlings) contributes towards the development of an abnormal heart shape (Poppe et al., 2003).

The relative heart mass of wild rainbow trout have previously been demonstrated to be greater than that of farmed trout (Graham and Farrell, 1992). This difference is considered to be related to differences in activity levels, as wild fish have more opportunity and need for intense activity than captive animals (Graham and Farrell, 1992). However, despite their potentially sedentary lifestyle, the ventricles of trout sampled at the Baltic Sea farm and lake farm in the present study were surprisingly large (RVM's of ~0.10% of body mass when sampled at 6 °C and 1 °C, respectively) and of similar size to that previously reported for wild trout of equal size (RVM's of ~0.09 and ~ 0.10% of body mass for land-locked and anadromous trout, respectively, when sampled at 7–10 °C, Graham and Farrell, 1992). The seemingly enlarged ventricles of trout sampled at these two facilities were most likely due

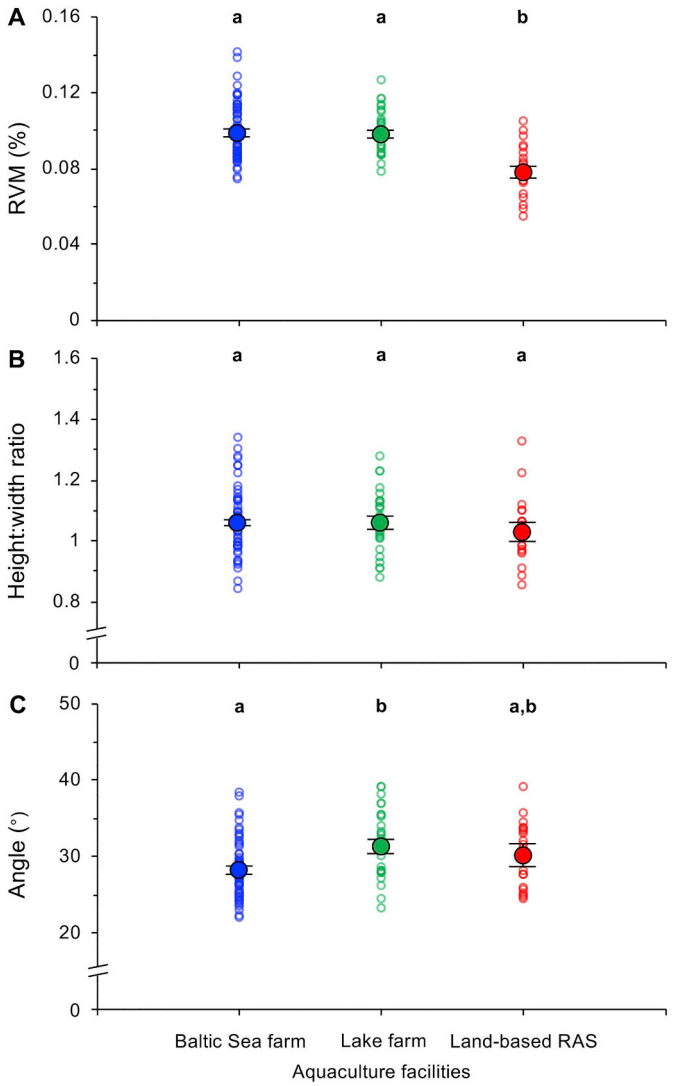

Fig. 3. Ventricular morphology of rainbow trout from three different types of aquaculture facilities. (A) Relative ventricular mass (RVM), (B) ventricular height:width ratio and (C) the alignment of the bulbus arteriosus of rainbow trout cultivated in an open cage Baltic Sea farm (blue circles), open cage lake farm (green circles) and a land-based RAS (red circles). Raw data points are plotted in all figures (open circles) and are overlaid with the mean ± s.e.m. (filled circles). In all figures, n = 60, 30 and 20 for the Baltic Sea farm, lake farm and land-based RAS, respectively, with the exception of (C) where n = 54 for the Baltic Sea farm due to poor quality photos preventing analysis in several cases. Statistical differences (p < .05) between the facilities are represented with different lower-case letters. **(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)**

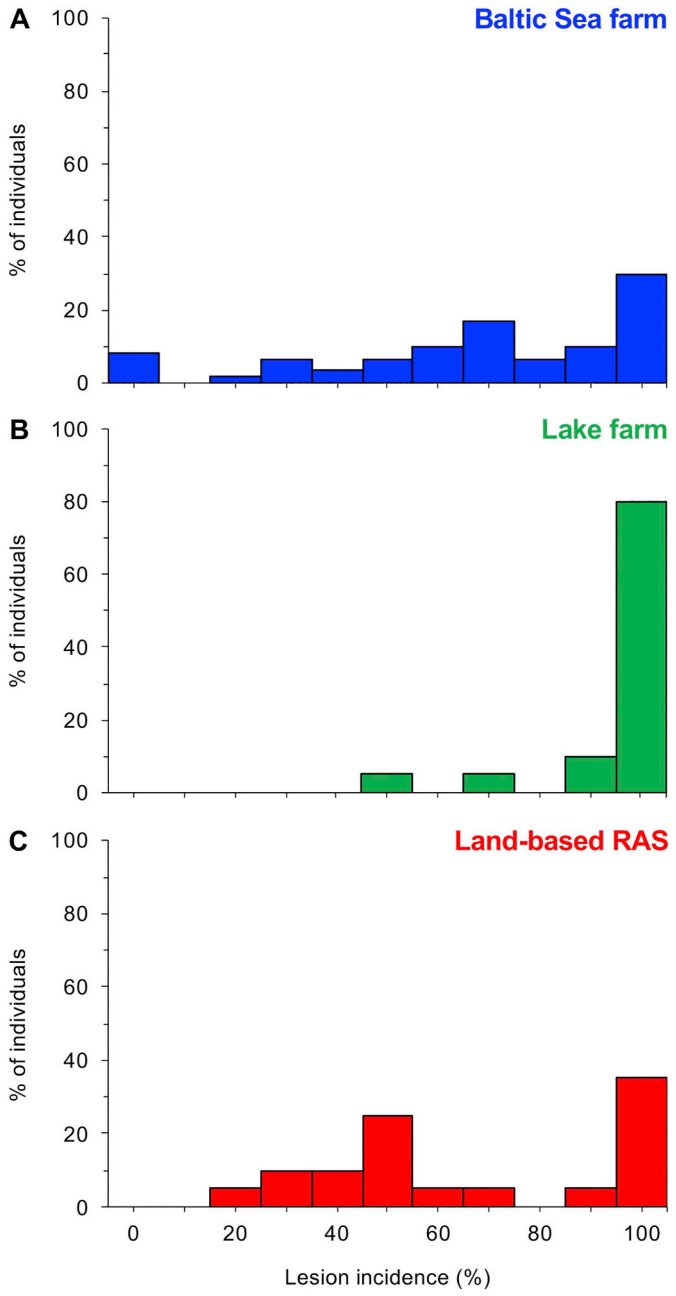

Fig. 4. Incidence of arteriosclerotic lesions in rainbow trout from three different types of aquaculture facilities. Proportion of individuals with varying degrees of lesion incidence (LI) at (A) an open cage Baltic Sea farm (n = 60), (B) an open cage lake farm (n = 20), and (C) a land-based RAS (n = 20). The distribution of LI scores was significantly higher (p < .001) in trout sampled from the open cage lake farm than from the Baltic Sea farm and land-based RAS.

to the cardiac hypertrophic response associated with cold-acclimation (especially for trout from the lake farm). For example, due to the high degree of cardiac plasticity in rainbow trout, individuals exposed to a 10 °C drop in temperature for 3–4 weeks were shown to increase RVM by 20–40% in order to partially offset the negative inotropic effect of cold temperatures on cardiac contractility (Farrell, 1991; Farrell et al., 1988; Graham and Farrell, 1989). However, at least in the case of trout sampled at the sea farm, this finding may also be related to stress- and cortisol-induced pathological cardiac enlargement (Johansen et al., 2011; Johansen et al., 2017). Importantly, similar to Johansen et al. (2011), we found that the cortisol stress response of trout from the Baltic Sea farm was significantly and positively related to ventricle size. Moreover, the variation in the cortisol stress response of these trout were in turn significantly related to the level of boat traffic in the rearing environment, which suggests that this anthropogenic factor may be a chronic stressor for fish in sea cages and warrants further investigation. Interestingly, RVM's of trout from the Baltic Sea farm that were unable to tolerate confinement stress (~0.12% of body mass) were similar to values deemed to represent pathologically enlarged hearts (RVM's of ~0.12% of body mass, Johansen et al., 2017). Johansen et al. (2017) clearly demonstrated that cardiovascular function was severely impaired in trout with pathologically enlarged hearts, as cardiac output scope was less than half that of trout with RVM's of ~0.09% of body mass. Thus, it could be speculated that an impaired cardiovascular function due to pathological cardiac enlargement may, at least in part,

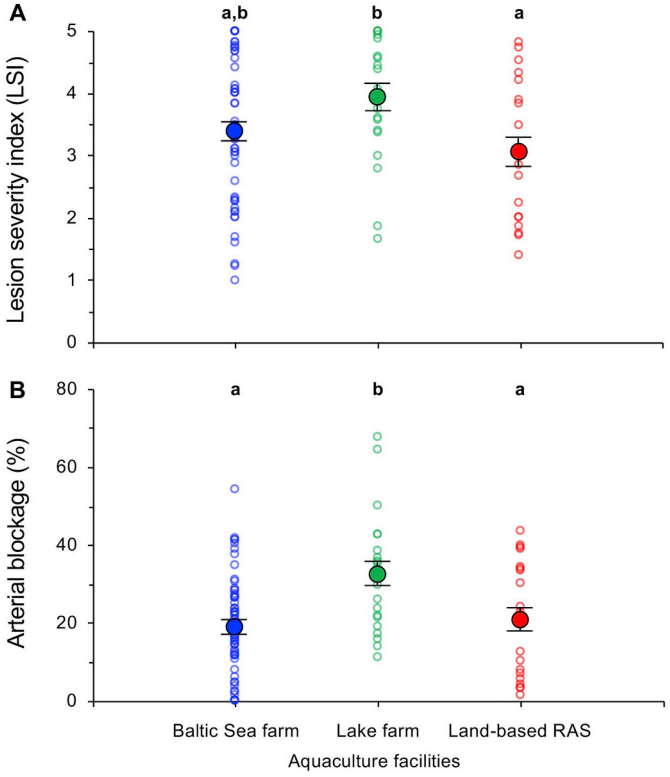

Fig. 5. Severity of arteriosclerotic lesions in rainbow trout from three different types of aquaculture facilities. (A) Lesion severity index (LSI) and (B) maximum arterial blockage caused by arteriosclerotic lesions in trout from an open cage Baltic Sea farm (n = 55, blue circles), an open cage lake farm (n = 20, green circles), and a land-based RAS (n = 20, red circles). Raw data points are plotted in all figures (open circles) and are overlaid with the mean ± s.e.m. (filled circles). Statistical differences (p < .05) between the facilities are represented with different lower-case letters. **(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)**

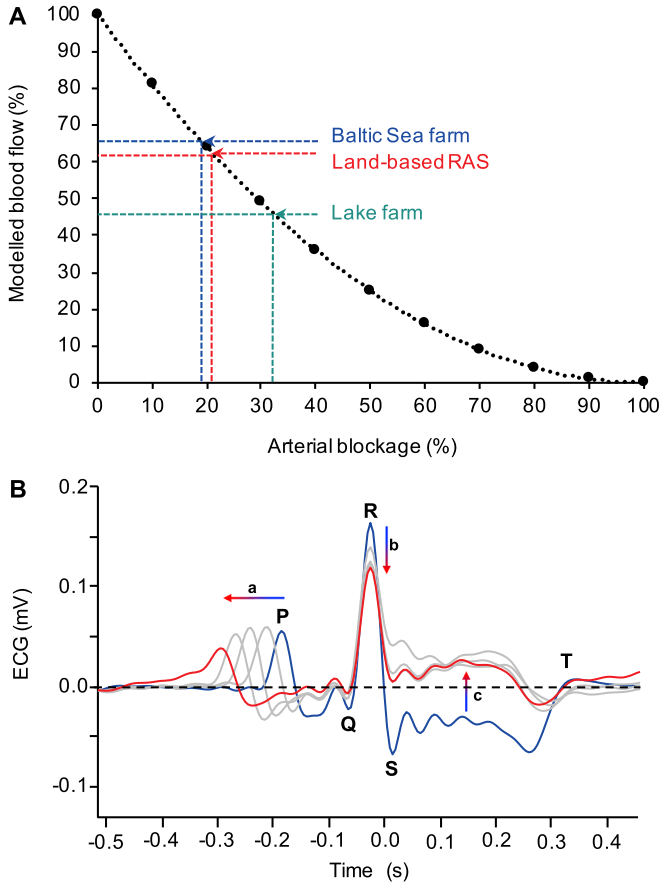

Fig. 6. The consequences of coronary obstruction on the blood flow and electrocardiogram of rainbow trout. According to Poiseuille's law, the black dashed line in (A) represents the theoretical reductions in laminar blood flow through a capillary caused by reductions in the radius of the vessel (*e.g. via* blockages) when assuming equal blood pressure, viscosity and capillary length. Coloured dashed lines indicate estimations of reduced coronary blood flow caused by arteriosclerotic lesions in rainbow trout from the open cage Baltic Sea farm (blue), open cage lake farm (green) and land-based RAS (red). (B) A subjectively healthy electrocardiogram (ECG) of a rainbow trout directly prior to the occlusion of the coronary artery (blue line). The ECG consists of the P-wave (atrial depolarization), the QRS-complex (ventricular depolarization) and the T-wave (ventricular repolarization). During a 30 min occlusion (from blue to red ECG trace), the time between the P-wave and R-wave increases (see a), the amplitude of the R-wave is gradually reduced (see b) and the ST-segment is elevated (see c). Grey lines in (B) represents ECG's at regular time periods throughout the occlusion period (~ every 7 min) and the black dashed line in (B) represents the isoelectric line. **(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)**

underlie the poor performances of trout from the Baltic Sea farm that were unable to tolerate confinement stress.

Lesion prevalence and severity in the coronary artery of salmonids is strongly correlated to fish size (R^2 > 0.95 for LI and LSI in cultured and wild Atlantic salmon, *Salmo salar,* Saunders et al., 1992), and thus growth or growth rate has been proposed to be the primary factor responsible for lesion development (Farrell, 2002; Saunders et al., 1992; Seierstad et al., 2005; Seierstad et al., 2008). When investigating the underlying factors that could potentially explain some of the variation in lesion prevalence and severity of trout at the Baltic Sea farm, we found that LI, LSI and maximum arterial blockage caused by the lesions were all higher in trout sourced from the hatchery that uses manipulated temperature and light regimes to accelerate the early growth of trout fingerlings. This indicates that the specific breeding program and/or rearing strategy could be major factors contributing towards the development of coronary arteriosclerosis in farmed salmonids, and suggests that further research in this area is warranted to potentially prevent or reduce the development of the disease in the future. In light of the strong relationship between fish size and arteriosclerosis, comparisons of lesion prevalence and severity between studies must take fish size into account, which is unfortunately difficult as the majority of previous studies do not report fish size. However, similar to previous studies, very few fish were lesion free and the incidence of lesions in affected trout (mean LI of 67–95%) were in the same range as previously reported values for similarly sized trout and salmon (LI of ~46–82%, Farrell and Johansen, 1992, Saunders et al., 1992). Despite the fact that growth rate most likely explains the majority of the variation in the prevalence and severity of arteriosclerosis (Farrell, 2002), we observed a significantly higher LI and LSI in trout sampled from the lake farm when compared to the other facilities. A potential explanation for this finding may, at least in part, be related to the elevated levels of total plasma cholesterol observed in trout from the lake farm, as this has previously been shown to be associated with an increased prevalence and severity of arteriosclerotic lesions in mature Atlantic salmon and Chinook salmon (*Oncorhynchus tshawytscha*) in freshwater (Eaton et al., 1984; Farrell et al., 1986). When evaluating the current severity of arteriosclerosis, it seems that the severity of arteriosclerotic lesions (LSI's of 3.1–3.9) and the arterial blockages that these lesions cause (19%–32% blockage) are higher than previously reported values for similarly sized farmed and wild trout (LSI of ~2.2–3.0, arterial blockage of ~11–15%, Farrell and Johansen, 1992). The high level of lesion severity observed in the present study is further

exemplified when compared to results from the most comprehensive investigation undertaken on lesion severity in farmed and wild salmonids (Saunders et al., 1992). When adjusting for the slightly different grading systems used (*i.e.* grade 3–4 and grade 5 lesions in the present study are regarded as grade 3 and grade 4 lesions, respectively, in Saunders et al., 1992), the severity of lesions in trout sampled in the present study were ~ 8% (Baltic Sea farm), ~28% (lake farm) and ~ 12% (land-based RAS) higher than expected for their respective size (see correlation equation for cultured salmon in Farrell, 2002 and Saunders et al., 1992). This apparent increase in the severity of arteriosclerotic lesions in trout over time is most likely linked with the continual enhancements in growth rate that have occurred in salmonid aquaculture over the last decades (Ellis et al., 2015; Gamperl and Farrell, 2004; Poppe et al., 2003).

In general, fish that were unable to tolerate confinement stress exhibited on average higher values for many of the cardiovascular disease indicators. It could be speculated that since the heart receives sufficient oxygen from the returning venous blood during routine conditions (Farrell et al., 1985), these seemingly healthy and well-fed fish were able to successfully perform routine day-to-day activities. However, when metabolically challenged, the oxygen demand of the heart would be expected to substantially increase, while the venous oxygen content decreases due to the increased tissue oxygen extraction (Farrell and Clutterham, 2003; Ekström et al., 2017; Farrell, 2002). This issue may have been compounded in these individuals during confinement stress, as cardiac function may be impaired due to their pathologically enlarged hearts and/or misaligned bulbus arteriosus (Johansen et al., 2017; Poppe et al., 2003). Moreover, indications of a greater degree of red blood cell swelling was also observed in these individuals (*i.e.* increased Hct and decreased MCHC, Nikinmaa, 1983), and at least with regards to trout sampled at the open cage farms, the negative effects of the low temperature on cardiac contractility (*i.e.* negative inotropic effect) and blood viscosity (*i.e.* increased workload on the heart and further restrictions to coronary blood flow) may have even further compounded the issue (Farrell, 1991; Graham and Farrell, 1989). During stress, these trout may have become increasingly reliant on their coronary circulation to sustain myocardial oxygenation and cardiac performance. The high prevalence and severity of arteriosclerosis in these individuals substantially reduced coronary blood flow (Fig. 6A), which may have led to myocardial ischemia and impaired cardiovascular function (Ekström et al., 2017; Ekström et al., 2018; Ekström et al., 2019; Farrell and Steffensen, 1987; Gamperl et al., 1994; Gamperl et al., 1995; Steffensen and Farrell, 1998). Moreover, the experimental coronary occlusion adversely affected the ECG, as the R-wave amplitude was reduced (indicative of impaired ventricular action potential generation), the S-T segment was elevated (a clinical sign for a heart infarct), and the time between the P-wave and the R-wave had increased (Munz et al., 2011; Preda and Burlacu, 2010; Rodríguez et al., 2006; Sun et al., 2013). Collectively, these findings support previous suggestions that heart infarcts may be related to stress-induced mortalities observed during some common farming practices (*i.e.* grading, transportation and treatments for disease and parasites; Mercier et al., 2000; Poppe et al., 2007). The next step would be to confirm our findings on-site at the aquaculture facilities. This can be done by implanting fish with bio-loggers that continuously record ECG (Brijs et al., 2018; Brijs et al., 2019) and/or *via* diagnostic ECG investigations on fish displaying symptoms of heart failure after being subjected to stressful husbandry practices.

5. Conclusions

The present study demonstrates the high prevalence and severity of cardiovascular disease in farmed rainbow trout that occurs throughout a range of different aquaculture facilities. Our findings also reveal that the hatchery of origin is an important factor underlying the variation observed in the development of cardiovascular disease. This indicates that the continual selection for an increased growth rate of fish in aquaculture (either *via* selective breeding programs or rearing strategies such as the use of manipulated temperature and light regimes during early rearing stages), coincides with a range of cardiac abnormalities and high prevalence/severity of coronary arteriosclerosis. We suggest that the physiological consequences of these cardiac abnormalities can impair cardiac oxygen supply during metabolically demanding activities, as well as alter cardiac electrical activity with ECG characteristics resembling those of acute myocardial ischemia in mammals. In light of these findings, we believe that further research regarding the effects of selective breeding programs and/or rearing strategies on the development of cardiovascular disease in farmed fish may provide a promising avenue for identifying methods or techniques to potentially prevent or reduce the development of this affliction in the future. Finally, further research into improving husbandry practices and rearing conditions to reduce the magnitude or frequency of metabolically demanding and/or stressful situations may be highly beneficial with regards to reducing the recurrent and significant production losses frequently reported in salmonid aquaculture. This will be especially important for open cage farms in the future, as average global temperatures and the frequency and severity of transient heat waves are predicted to increase, which will undoubtedly push the metabolic and cardiovascular limits of farmed salmonids (Ekström et al., 2018; Ekström et al., 2019; Ganguly et al., 2009).

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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References

Brijs, J., Sandblom, E., Axelsson, M., Sundell, K., Sundh, H., Huyben, D., Broström, R., Kiessling, A., Berg, C., Gräns, A., 2018. The final countdown: continuous physiological welfare evaluation of farmed fish during common aquaculture practices before and during harvest. Aquaculture 495, 903–911.

Brijs, J., Sandblom, E., Rosengren, M., Sundell, K., Berg, C., Axelsson, M., Gräns, A., 2019. Prospects and pitfalls of using heart rate bio-loggers to assess the welfare of rainbow trout (*Oncorhynchus mykiss*) in aquaculture. Aquaculture 509, 188–197.

Brocklebank, J., Raverty, S., 2002. Sudden mortality caused by cardiac deformities following seining of preharvest farmed Atlantic salmon (*Salmo salar*) and by cardiomyopathy of postintraperitoneally vaccinated Atlantic salmon parr in British Columbia. Can. Vet. J. 43, 129–130.

Claireaux, G., McKenzie, D.J., Gaylene, Genge A., Chatelier, A., Aubin, J., Farrell, A.P., 2005. Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. J. Exp. Biol. 208, 1775–1784.

Clark, T.D., Eliason, E.J., Sandblom, E., Hinch, S.G., Farrell, A.P., 2008. Calibration of a hand-held haemoglobin analyser for use on fish blood. J. Fish Biol. 73, 2587–2595.

Eaton, R.P., McConnell, T., Hivath, L.G., Black, W., Schwartz, R.E., 1984. Coronary myointimal hyperplasia in freshwater Lake Michigan salmon (genus *Oncorhynchus*): evidence for lipoprotein-related arteriosclerosis. Am. J. Pathol. 116, 311–318.

Ekström, A., Axelsson, M., Gräns, A., Brijs, J., Sandblom, E., 2017. Influence of the coronary circulation on thermal tolerance and cardiac performance during warming in rainbow trout. Am. J. Physiol. Regul. Integr. Comp. Physiol. 312, R549–R558.

Ekström, A., Axelsson, M., Gräns, A., Brijs, J., Sandblom, E., 2018. Importance of the coronary circulation for cardiac and metabolic performance in rainbow trout (*Oncorhynchus mykiss*). Biol. Lett. 14, 1–4.

Ekström, A., Gräns, A., Sandblom, E., 2019. Can't beat the heat? Importance of cardiac control and coronary perfusion for heat tolerance in rainbow trout. J. Comp. Physiol. B. 189, 757–769.

Ellis, T., Turnbull, J.F., Knowles, T., Lines, J., Auchterlonie, N., 2015. Trends during development of Scottish salmon farming: an example of sustainable intensification. Aquaculture 458, 82–99.
Farrell, A.P., 1987. Coronary flow in a perfused rainbow trout heart. J. Exp. Biol. 129, 107–123.
Farrell, A.P., 1991. From hagfish to tuna: a perspective on cardiac function in fish. Physiol. Zool. 64, 1137–1164.
Farrell, A.P., 2002. Coronary arteriosclerosis in salmon: growing old or growing fast? Comp. Biochem. Physiol. A 132, 723–735.
Farrell, A.P., Clutterham, S.M., 2003. On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. J. Exp. Biol. 206, 487–496.
Farrell, A.P., Johansen, J.A., 1992. Reevaluation of regression of coronary arteriosclerotic lesions in repeat-spawning steelhead trout. Arterioscler. Thromb. 12, 1171–1175.
Farrell, A.P., Steffensen, J.F., 1987. Coronary ligation reduces maximum sustained swimming speed in Chinook salmon (*Oncorhynchus tshawytscha*). Comp. Biochem. Physiol. A 87, 35–37.
Farrell, A.P., Wood, S., Hart, T., Driedzic, W.R., 1985. Myocardial oxygen consumption in the sea raven, *Hemitripterus americanus*: the effects of volume loading, pressure loading and progressive hypoxia. J. Exp. Biol. 117, 237–250.
Farrell, A.P., Saunders, R.L., Freeman, H.C., Mommsen, T.P., 1986. Arteriosclerosis in Atlantic salmon. Effects of dietary cholesterol and maturation. Arteriosclerosis 6, 453–461.
Farrell, A.P., Hammons, A.M., Graham, M.S., Tibbits, G.F., 1988. Cardiac growth in rainbow trout, *Salmo gairdneri*. Can. J. Zool. 66, 2368–2373.
Farrell, A.P., Johansen, J.A., Saunders, R.L., 1990. Coronary lesions in Pacific salmonids. J. Fish Dis. 13, 97–100.
Fuster, V., 2014. Global burden of cardiovascular disease: time to implement feasible strategies and to monitor results. J. Am. Coll. Cardiol. 64, 520–522.
Gamperl, A.K., Farrell, A.P., 2004. Cardiac plasticity in fishes: environmental influences and intraspecific differences. J. Exp. Biol. 207, 2539–2550.
Gamperl, A.K., Pinder, A., Boutilier, R., 1994. Effect of coronary ablation and adrenergic stimulation on in vivo cardiac performance in trout (*Oncorhynchus mykiss*). J. Exp. Biol. 186, 127–143.
Gamperl, A.K., Axelsson, M., Farrell, A.P., 1995. Effects of swimming and environmental hypoxia on coronary blood flow in rainbow trout. Am. J. Physiol. Regul. Integr. Comp. Physiol. 269, R1258–R1266.
Ganguly, A.R., Steinhaeuser, K., Erickson, D.J., Branstetter, M., Parish, E.S., Singh, N., Drake, J.B., Buja, L., 2009. Higher trends but larger uncertainty and geographic variability in 21st century temperature and heat waves. Proc. Natl. Academ. Sci. 106, 15555–15559.
Graham, M.S., Farrell, A.P., 1989. The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. Physiol. Zool. 62, 38–61.
Graham, M.S., Farrell, A.P., 1992. Environmental influences on cardiovascular variables in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Biol. 41, 851–858.
Johansen, I.B., Lunde, I.G., Røsjø, H., Christensen, G., Nilsson, G.E., Bakken, M., Øverli, Ø., 2011. Cortisol response to stress is associated with myocardial remodeling in salmonid fishes. J. Exp. Biol. 214, 1313–1321.
Johansen, I.B., Sandblom, E., Skov, P.V., Gräns, A., Ekström, A., Lunde, I.G., Vindas, M.A., Zhang, L., Höglund, E., Frisk, M., Sjaastad, I., Nilsson, G.E., Øverli, Ø., 2017. Bigger is not better: cortisol-induced cardiac growth and dysfunction in salmonids. J. Exp. Biol. 220, 2545–2553.
Mercier, C., Aubin, J., Lefrancois, C., Claireaux, G., 2000. Cardiac disorders in farmed adult brown trout, *Salmo trutta* L. J. Fish Dis. 23, 243–249.
Munz, M.R., Faria, M.A., Monteiro, J.R., Aguas, A.P., Amorim, M.J., 2011. Surgical porcine myocardial infarction model through permanent coronary occlusion. Comp. Med. 61, 445–452.
Nikinmaa, M., 1983. Adrenergic regulation of haemoglobin oxygen affinity in rainbow trout red cells. J. Comp. Physiol. 152, 67–72.
Olkowski, A.A., Kumor, L., Classen, H.L., 1996. Changing epidemiology of ascites in broiler chickens. Can. J. Anim. Sci. 76, 135–140.
Poiseuille, J.L.M., 1840. Recherches experimentales sur le moyements des liquides dans les tubes de tres petits diametres. Comptes Rend. Acad. D. Sc. 11, 961–1041.
Poppe, T.T., Taksdal, T., 2000. Ventricular hypoplasia in farmed Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 42, 35–40.
Poppe, T.T., Johansen, R., Torud, B., 2002. Cardiac abnormality with associated hernia in farmed rainbow trout *Oncorhynchus mykiss*. Dis. Aquat. Org. 50, 153–155.
Poppe, T.T., Johansen, R., Gunnes, G., Tørud, B., 2003. Heart morphology in wild and farmed Atlantic salmon Salmo salar and rainbow trout (*Oncorhynchus mykiss*). Dis. Aquat. Org. 57, 103–108.
Poppe, T.T., Taksdal, T., Bergtun, P.H., 2007. Suspected myocardial necrosis in farmed Atlantic salmon, *Salmo salar* L.: a field case. J. Fish Dis. 30, 615–620.
Preda, M.B., Burlacu, A., 2010. Electrocardiography as a tool for validating myocardial ischemia-reperfusion procedures in mice. Comp. Med. 60, 443–447.
Rodríguez, B., Trayanova, N., Noble, D., 2006. Modeling cardiac ischemia. Ann. N. Y. Acad. Sci. 1080, 395–414.
Saunders, R.L., Farrell, A.P., Knox, D.E., 1992. Progression of coronary arterial lesions in Atlantic salmon (*Salmo salar*) as a function of growth rate. Can. J. Fish. Aquat. Sci. 49, 878–884.
Seierstad, S.L., Poppe, T.T., Koppang, E.O., Svindland, A., Rosenlund, G., Frøyland, L., Larsen, S., 2005. Influence of dietary lipid composition on cardiac pathology in farmed Atlantic salmon, *Salmo salar* L. J. Fish Dis. 28, 677–690.
Seierstad, S.L., Svindland, A., Larsen, S., Rosenlund, G., Torstensen, B.E., Evensen, Ø., 2008. Development of intimal thickening of coronary arteries over the lifetime of Atlantic salmon, *Salmo salar* L., fed different lipid sources. J. Fish Dis. 31, 401–413.
Steffensen, J.F., Farrell, A.P., 1998. Swimming performance, venous oxygen tension and cardiac performance of coronary-ligated rainbow trout, *Oncorhynchus mykiss*, exposed to progressive hypoxia. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 119, 585–592.
Sun, X., Cai, J., Fan, X., Han, P., Xie, Y., Chen, J., Xiao, Y., Kang, Y.J., 2013. Decreases in electrocardiographic R-wave amplitude and QT interval predict myocardial ischemic infarction in Rhesus monkey with left anterior descending artery ligation. PLoS One 8, e71876.
Sundh, H., Calabrese, S., Jutfelt, F., Niklasson, L., Olsen, R.-E., Sundell, K., 2011. Translocation of infectious pancreatic necrosis virus across the intestinal epithelium of Atlantic salmon (*Salmo salar* L.). Aquaculture 321, 85–92.
Takle, H., Baeverfjord, G., Helland, S., Kjorsvik, E., Andersen, O., 2006. Hyperthermia induced atrial natriuretic peptide expression and deviant heart development in Atlantic salmon *Salmo salar* embryos. Gen. Comp. Endocrinol. 147, 118–125.
Van Essen, G.J., 2017. Cardiovascular proportionality of modern pigs: Are we breaking the allometric scaling laws? Retrieved from. http://hdl.handle.net/1765/95778.