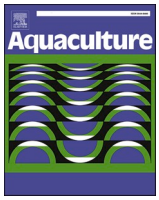
Aquaculture 581 (2024) 740482

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Ischemia-induced alterations in the electrocardiogram of salmonid fish

Lucas A. Zena [a,*], Andreas Ekström [a], Daniel Morgenroth [a], Tristan McArley [a], Albin Gräns [b], Michael Axelsson [a], Ida B. Johansen [c], Erik Sandblom [a]

[a] Department of Biological and Environmental Sciences, University of Gothenburg, 413 90 Gothenburg, Sweden
[b] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, 532 23 Skara, Sweden
[c] Faculty of Veterinary Medicine Department of Preclinical Sciences and Pathology, Norwegian University of Life Sciences, Ås, Norway

ARTICLE INFO

Keywords:
Cardiac biomarkers
Hypoxic bradycardia
Myocardial ischemia
Fish welfare
Coronary arteriosclerosis

ABSTRACT

Cardiovascular diseases such as coronary arteriosclerosis are widespread and constitute severe health and welfare problems for both farmed and wild salmonid fish. However, effective tools for rapid screening and analysing heart diseases in fish do not currently exist. Electrocardiogram (ECG) recordings are widely used for screening and diagnosing numerous cardiac pathologies in humans, but the use of ECG techniques to diagnose and characterize cardiac abnormalities in fish is still in its infancy. In this study, we induced myocardial ischemia in anaesthetized rainbow trout by surgical coronary artery ligation. Additionally, we experimentally manipulated the fish's heart rate and environmental oxygen availability by altering gill water flow and oxygen saturation (*i.e.*, no flow, normoxic flow and hyperoxic flow), and analyzed changes in the ECG profile in detail. The main ECG abnormalities observed in fish with ligated coronaries in normoxia were atrioventricular blocks, prolonged QRS duration, reduced QRS amplitude and changes in the ST-segment such as the presence of early repolarization pattern. Furthermore, when gill water flow was stopped, fish exhibited pronounced hypoxic bradycardia, which alleviated all ECG abnormalities in coronary ligated fish. This is the first study to provide a detailed characterization of electrocardiographic markers of myocardial ischemia in fish. Our study shows that hypoxic bradycardia improves cardiac electrical conductivity, presumably by reducing mismatches in myocardial oxygen supply and demand. Yet, the importance of avoiding hypoxic bradycardia in experimental and biomedical studies on anaesthetized fish is highlighted as it can potentially lead to incorrect ECG interpretations.

1. Introduction

It is well known that farmed salmonid fishes (*Salmonidae spp.*) develop various heart diseases, which result in poor health and welfare as well as substantial economic losses in aquaculture (Garseth et al., 2018). For instance, both wild and farmed salmonids develop coronary arteriosclerosis, a process where vascular smooth muscle cells proliferate within the lumen of the coronary artery, restricting the flow of oxygenated and nutrient-rich blood to the heart muscle (Brijs et al., 2020; Dalum et al., 2016, 2017; Farrell, 2002; Poppe et al., 2003). The prevalence and severity of coronary arteriosclerosis is thought to be particularly high in farmed salmonids. For example, 92–100% of individuals sampled from different commercial rainbow trout farms in Sweden and Finland exhibited coronary lesions, but the underlying causes and implications of these abnormalities on fish performance and cardiac function are not well understood (Brijs et al., 2020). Experimental surgical ligation of the main coronary artery results in significant heart muscle inflammation and impaired cardiac pumping capacity (Morgenroth et al., 2021; Zena et al., 2021). Moreover, increased coronary flow is particularly important during metabolically challenging events (*e.g.*, exercise and/or environmental warming or hypoxia) that increase cardiac workload and oxygen demand (Ekström et al., 2018; Ekström et al., 2017; Morgenroth et al., 2021; Steffensen et al., 1998). Thus, in farmed fish, coronary arteriosclerosis likely reduces the heart's resilience to stressful farming events such as crowding, transportation, handling and disease treatment (Brijs et al., 2020; Frisk et al., 2020). Indeed, recent histopathological examinations of hearts from Atlantic salmon (*Salmo salar*) that died suddenly during a stressful farm event revealed myocardial degeneration compatible with ischemia (Poppe et al., 2021). However, diagnostic tools for early detection of myocardial ischemia in salmonids, which would aid to unravel underlying causes and facilitate precautionary measures and mitigate

* Corresponding author.
E-mail address: lucas.zena@bioenv.gu.se (L.A. Zena).

https://doi.org/10.1016/j.aquaculture.2023.740482
Received 25 September 2023; Received in revised form 7 December 2023; Accepted 13 December 2023
Available online 16 December 2023

mortality, are currently unavailable.

In humans, prolonged deprivation of oxygen supply to the myocardium can lead to substantial cardiomyocyte death and necrosis, referred to as myocardial infarction (Vogel et al., 2019). Patients with myocardial infarction who present with chest pain are usually diagnosed based on abnormalities in the electrocardiogram (ECG) (Thygesen et al., 2018; Vogel et al., 2019). The ECG represents the collective electrical activity of the heart and consists of three main components in healthy individuals; the P wave representing depolarization of the atria prior to atrium contraction, the QRS complex representing depolarization of the ventricles that precedes ventricular contraction (systole), and the T wave representing ventricular repolarization associated with ventricular relaxation (diastole). The atrial repolarization wave (Ta wave) is generally not visible in the ECG due to its low magnitude and frequent concealment by the subsequent QRS complex.

The ECG of fish exhibit many fundamental similarities with the human ECG (Vornanen and Hassinen, 2016) and consists of a P wave, QRS complex and T wave (Brijs et al., 2020; Nomura and Ibaraki, 1969; Ueno et al., 1986; Vornanen and Hassinen, 2016). Largely due to these similarities, zebrafish have emerged as a tractable animal model system for studies of cardiovascular diseases. For example, the ECG of zebrafish has been widely used in various physiological screening studies, such as drug toxicity and cardiac remodelling and repair after cardiac injury (Chablais et al., 2011; Le et al., 2022; Milan et al., 2006; Yu et al., 2010). Cardiac remodelling studies in zebrafish have used various techniques to induce cardiac injury including ventricle resection, as well as cryo- and laser injury (Stewart et al., 2022). However, these methods do not accurately mimic an actual event of myocardial ischemia. The most accurate simulation of acute myocardial ischemia involves the occlusion of one or more coronary arteries. Nonetheless, this procedure can be impractical in most fish models utilized in cardiovascular development and disease studies due to their small body size (Potts et al., 2021).

Human patients with myocardial infarction typically exhibit an elevation of the segment between the S and T waves; this is referred to as ST-elevation myocardial infarction (STEMI). STEMI usually occurs as a result of transmural ischemia affecting the entire thickness of the ventricular wall, which in humans is comprised mostly of compact myocardium perfused by the coronary circulation (Vogel et al., 2019). In contrast, most teleost fishes, including zebrafish, lack a well-developed compacta and predominantly rely on the diffusion of oxygen from venous blood into the inner spongy myocardium (Hu et al., 2001; Tota, 1983). Thus, ECG markers of ischemia may exhibit distinct differences between mammals and fishes. In fact, cardiac resection and cryoinjury of the zebrafish heart induce ST segment depression (Liu et al., 2016). Although ST segment depression can also be associated with acute ischemia in humans, it is not specific and may also be present in patients with non-ischemic events (Pollehn et al., 2002).

In contrast to most teleosts, salmonids such as trout and salmon possess an outer compact ventricular layer that represents 20–50% of the ventricular mass and receives oxygenated blood *via* the coronary circulation (Farrell et al., 2012; Tota, 1983; Tota et al., 1983). Despite the widespread problem with coronary arteriosclerosis in salmonid fish, only a few studies have employed ECG recording techniques in salmonids in conjunction with protocols to experimentally induce myocardial ischemia and determine how the heart's electrical activity is affected (Brijs et al., 2020; Ekström et al., 2017; Wallbom et al., 2023; Zena et al., 2021). For instance, detailed examination of the ECG of two anaesthetized rainbow trout subjected to 30-min coronary artery occlusion showed similar ECG alterations as those found in mammals with myocardial ischemia, including S-T segment elevation and reduced R-wave amplitude (Brijs et al., 2020; Ekström et al., 2017). A gradual prolongation of the PR interval (*i.e.*, atrioventricular conduction delay), which is another telltale sign of myocardial ischemia in mammals (Thygesen et al., 2018), was also identified in one of the coronary artery ligated fish (Brijs et al., 2020). Although electrical conduction abnormalities are widely recognized as complications of acute myocardial infarction in humans (Thygesen et al., 2018) and the fact that ECG recordings in fish are widely used in biomedical research, there is still no uniformity in the electrocardiographic criteria used to characterize various heart diseases in fish.

Being a highly aerobic organ, the heart can encounter conflicting demands when exposed to low environmental oxygen levels (hypoxia). In adult mammals exposed to hypoxia, an increase in heart rate (tachycardia) seems to assist in compensating for reduced arterial oxygenation to sustain systemic oxygen delivery (Joyce and Wang, 2022). However, the heart as such would likely benefit from slowing down (bradycardia) to reduce myocardial oxygen demand. In fact, this is commonly observed in most adult fishes, including salmonids, which exhibit a reflex bradycardia when exposed to acute hypoxia, a phenomenon that has intrigued comparative physiologists for decades (Joyce and Wang, 2022; Morgenroth et al., 2021; Randall, 1982; Smith and Jones, 1978). It is generally thought that hypoxic bradycardia may improve myocardial oxygenation by increasing blood residence time in the lumen of the heart, which in turn increases the time for oxygen extraction by the spongy myocardium (Farrell, 2007). Even so, there is currently no clear evidence supporting the benefits of hypoxic bradycardia for cardiac oxygenation and function in fish. Detailed examination of ECG markers of myocardial ischemia could potentially be a useful tool to help unravel this.

In the current study, we used bipolar ECG leads to perform high-resolution recordings of the heart's electrical activity in anaesthetized rainbow trout (*Oncorhynchus mykiss*), before and after coronary artery ligation. The primary research objective was to characterize ECG alterations in detail and identify potential biomarkers of myocardial ischemia in a fish species that relies heavily on the coronary circulation. We then implemented various protocols to manipulate water flow across the gills, including reduced or completely ceased water flow to induce hypoxic bradycardia, as well as increased water flow and oxygen content (*e.g.*, hyperoxia) to induce tachycardia. This allowed us to address the hypothesis that bradycardia alleviates, while tachycardia worsens, any myocardial ischemia-specific ECG changes in fish. Finally, we performed a detailed histological and gross morphological examination of the ischemic heart to link cardiac morphology to electrophysiological function.

2. Material and methods

2.1. Experimental animals

Rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792, average body mass: 871 ± 34 g) of mixed sexes were obtained from a local fish farm (Vänneåns Fiskodling AB, Sweden) and maintained at the University of Gothenburg in holding tanks supplied with recirculating aerated freshwater at 10 °C under a photoperiod of 12:12 h for at least four weeks prior to the experiments. They were fed three times a week with commercial fish pellets. The experimental procedures were covered by ethical permit #5.8.18–10,907/2020 issued by the regional animal ethics committee in Gothenburg.

2.2. Surgery and instrumentation

Fish were anaesthetized in freshwater (10 °C) containing tricaine methanesulfonate (MS-222; 150 mg l^{-1}) buffered with $NaHCO_3$ (300 mg l^{-1}) and placed on a v-shaped trough on a surgery table with the ventral side up. The gills were irrigated continuously with 10 °C aerated water containing MS-222 (75 mg l^{-1}) buffered with $NaHCO_3$ (150 mg l^{-1}). Electrocardiogram electrodes were prepared by soldering 23-gauge hypodermic needles, with the beveled edge blunted, onto insulated stainless-steel wires. Two electrodes were implanted subcutaneously close to the pectoral fins on each side of the heart and sutured in place. The negative electrode was implanted close to the right pectoral fin, while the positive electrode was implanted close to the left pectoral fin.

A third electrode (*i.e.*, also positive) was implanted in between and approximately 3 cm caudal to the pectoral fins, forming a triangle in relation to the rainbow trout heart (*i.e.*, Einthoven's triangle). A fourth ground electrode was kept submerged in the surgical table water to optimize the signal to noise ratio. Electrocardiogram signals were amplified using a differential amplifier (Bio Amp FE231, ADInstruments, Sydney, Australia) and sampled at a rate of 1 kHz using LabChart v7.3.7 software (ADInstruments, Sydney, Australia).

2.3. Experimental protocol and data analyses

Leads I and II were recorded in real time, while lead III was calculated using the ECG lead setup from LabChart (lead II – lead I). In the bipolar lead I, both electrodes were positioned cranial to the heart, with the negative electrode placed close to the right pectoral fin and the positive electrode placed close to the left pectoral fin. In lead II, the negative electrode was positioned close to the right pectoral fin (cranial to the heart), while the positive electrode was placed caudal to the heart. The experimental protocol (see Fig. 1 for a detailed summary of the experimental procedures) was conducted in different steps as follows: a) The ECG signals were first collected for ~5 min with the fish positioned with its ventral side up while the gills were artificially ventilated with high water flow (~5 L min^{-1}). b) The fish was then positioned on its left lateral side on a flat wet foam placed on the surgery table and the ECG was recorded continuously while the coronary artery was exposed in the isthmus on the right side. The gills were continuously irrigated with

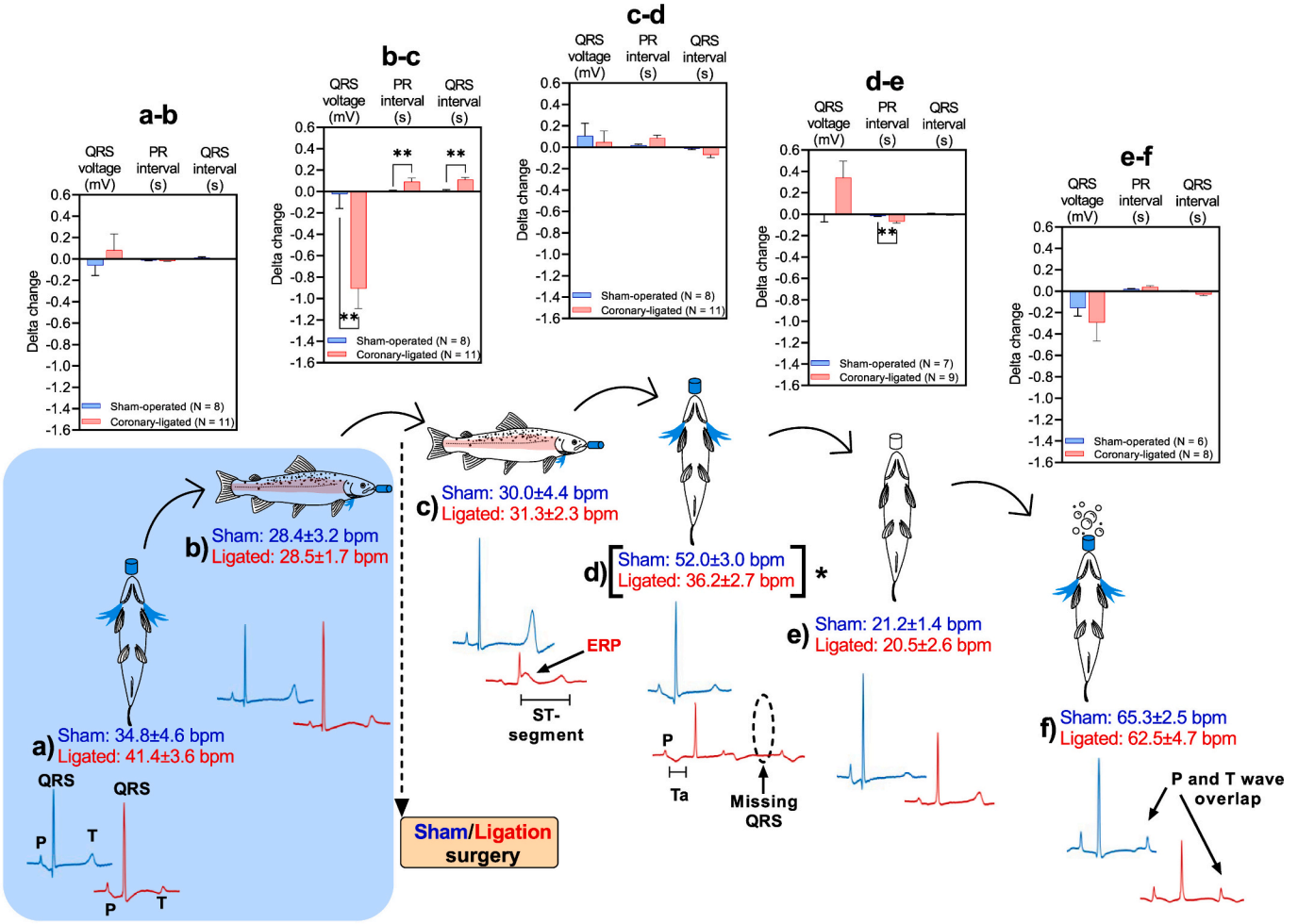


Fig. 1. Electrocardiography abnormalities following coronary ligation or sham operation in trout. Plots show changes in QRS amplitude, PR interval, and QRS interval associated with different artificial ventilation regimes. The experimental protocol (see Material and Methods) was conducted in 6 different experimental steps (indicated by a to f) with different artificial gill perfusion/ventilation regimes (low flow indicated by a small diameter tube, high flow with a large tube, zero flow with an open tube, and hyperoxia with bubbles). The corresponding heart rate (in beats per minute, bpm) during each experimental step are depicted for each experimental group. Transition point a-b indicates changes in ECG parameters obtained when artificial ventilation changed from high (a) to low (b), with the fish positioned on its left lateral side. Note, in points a and b fish had not yet undergone sham-operation and coronary-ligation surgery (blue shaded square). Point c indicates changes in ECG parameters observed immediately after coronary ligation when artificial perfusion was maintained low. Transition point c-d, the fish were again positioned ventral side up, and artificial ventilation changed from low (c) to high (d). Transition point d-e indicates when artificial ventilation was turned off for ~1 min. In transition point e-f, high artificial ventilation was combined with hyperoxic gas (>250% air saturation). Asterisks denote statistical difference between sham-operated and coronary-ligated fish. Brackets and asterisks denote statistically significant differences in heart rate between sham-operated and coronary-ligated fish. * = $P < 0.05$, ** = $P < 0.01$. Rainbow trout drawings show schematic representations of each water flow regime with representative ECG waveforms. Note the upward convex pattern marking the end of the QRS complex in c, which represents an early repolarization pattern (ERP), causing changes in the ST-segment—the portion of the ECG from the end of the QRS complex to the beginning of the T wave— and the missing QRS complex following the P wave and the presence of an atrium repolarization wave (Ta) for coronary-ligated fish in d. Additionally, note that T waves may be difficult to identify at high heart rates during hyperoxia because they are superimposed on the following P wave (f). The ECG traces were derived from 1-min ECG recordings for each regimen and obtained from Lead II for both sham-operated and coronary-ligated fish. The QRS complex on Lead II is negative and was inverted to facilitate visualization and interpretation of the results. Data are means±s.e.m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

aerated water with anaesthetics and diverted over the gill arches on the left side with reduced water flow adjusted so that visualization of the coronary artery on the right side was possible (*i.e.*, low water flow over the gills on the right-side). c) In one experimental group, the main coronary artery was ligated permanently using a silk suture (coronary-ligated). A second group was treated identically except that the coronary artery was only exposed but not ligated (sham-operated). After the surgical procedure, the ECG was recorded for approximately 7–10 min while water flow over the gills remained low. d) The fish was again placed ventral side up on the v-shaped surgery table, and the gills on both sides were fully irrigated (~5 L min^{-1}). e) Water flow was then turned off for ~1 min to trigger a hypoxic reflex bradycardia (Neiffer and Stamper, 2009). f) Water flow was then turned on (~5 L min^{-1}) again, such that the heart rate increased back to baseline levels. After this, pure O_2 was bubbled into the anaesthetic water creating increasingly hyperoxic water (up until ~250% air saturation) perfusing the gills. The experiment was concluded when the fish's heart rate stabilized, and the second-degree atrioventricular (AV) block observed in coronary ligated fish disappeared (see results), which consistently occurred at approximately 250% air saturation. At the end of the experiments, ECG electrodes were removed, and fish were marked with a coloured plastic bead attached to the dorsal fin for identification. The fish were then allowed to recover in their holding tank for 10 days.

From each ECG recording, we averaged sequential heart beats over one-minute periods that did not contain any obvious ectopic beats or other artifacts, to calculate heart rate and measure the QRS complex voltage in mV, as well as the PR interval and QRS duration in seconds. The PR and QRS interval were averaged while the QRS voltage was summed from all 3 leads.

2.4. Casting of the coronary vasculature and histopathology

To confirm complete ligation of the coronary artery, fish were, after recovering for 10 days from surgery, anaesthetized and positioned on their left lateral side on a flat surgery table covered with wet foam. Deep anesthesia was maintained by irrigating the gills with water containing MS-222 at a concentration of 150 mg l^{-1} buffered with 300 mg l^{-1} of $NaHCO_3$. An incision (4 cm) was made on the lateral side behind the pectoral fin to expose the celiacomesenteric artery, which was dissected free from surrounding tissue. The vessel was cannulated with a PE-50 cannula and 0.2 mL of heparin solution (5000 IU ml^{-1}) was injected. The fish was then placed with its ventral side up with the heart exposed allowing video recording of the beating heart. An incision was then made in the atrium to drain the blood, and a peristaltic pump (Minipuls 3 Peristaltic Pump, model 312, France) was used to infuse a vasodilating buffer solution (4 mg of papaverine in phosphate-buffered saline +50 IU ml^{-1} of heparin) through the celiacomesenteric artery cannula. When the blood in the coronary vascular tree had been rinsed out, as indicated by clear fluid appearing in the exposed heart, a silicone injection compound (Microfil®, Flow Tech Inc., Boulder, Colorado) was injected through the celiacomesenteric artery until complete filling of the coronary arteries was visually confirmed. A 2–0 silk suture was then tied around the entire ventral aorta, including the main coronary artery, to prevent the Microfil from draining out of the coronary vasculature. All hearts were visually inspected and filmed/photographed *in situ* before and after Microfil injection. After Microfil injection, the heart was covered with PBS-soaked gauze to prevent it from drying and was allowed to sit for approximately 30 min to allow the Microfil to cure. The heart was then removed and stored in 4% paraformaldehyde (4% in PBS) at 4 °C for 24 h and later transferred to 70% ethanol.

All of the hearts were subsequently photographed for gross morphology inspection using a Canon EOS 40D camera (Canon EF 100 mm f/2.8 L Macro IS USM Lens) mounted in a lightening unit (Kaiser Fototechnik RB 218 N HF, Buchen, Germany). For this, a petri dish was half-filled with agar containing a hole in the center, which allowed the positioning of the heart in the desired orientation for photographing. The petri dish was filled with 70% ethanol completely submerging the heart. Subsequently, fixed hearts were prepared for histological examination by standard paraffin wax techniques and stained with hematoxylin and eosin (see Zena et al., 2021 for details).

2.5. Statistics

Statistical analyses were performed using GraphPad prism (v. 9.3.1) and R software v. 1.1.383 (http://www.R-project.org/). First, we assessed the effect of changing water flow over the gills on heart rate after sham and coronary ligation surgery by fitting a linear mixed model using the 'lmer' function in the lme4 package in R. If significant general effects were found, comparisons among treatments were further investigated using a Bonferroni multiple comparison test. To allow a robust statistical comparison of the different experimental steps we calculated delta values for each ECG parameter (*i.e.*, PR interval, QRS interval and QRS amplitude) associated with each of the experimental steps changing water flow/gill perfusion applied to the fish (see Fig. 1). That is, delta was calculated for all ECG parameters as follows: a-b) the transition from high to low water flow across the gills; b-c) transition from before to after sham/ligation surgery when water flow across the gills was maintained low; c-d) transition from low to high water flow across the gills; d-e) transition from high to no water flow across the gills (water flow was turned off for ~1 min); e-f) changing water oxygen saturation from normoxia (100% air saturation) to hyperoxia (>250% air saturation) under high water flow across the gills. A *t*-test for parametric data and a Mann-Whitney test for non-parametric data were then applied to compare delta values between sham-operated and coronary-ligated fish within each transition step for PR interval, QRS interval and QRS amplitude. Mortality was assessed by log-rank test (Kaplan Meier). All values are presented as means±s.e.m. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Effect of coronary ligation on heart rate responses to changing gill water flow

We found a significant overall effect of changes in gill water flow on the heart rate of anaesthetised fish across treatment groups ($F_{(3,\ 45)} = 71.412$; $P < 0.001$; Fig. 1). While coronary ligation had no significant effect on routine heart rate ($F_{(1,\ 16.3)} = 3.128$; $P = 0.09$; Fig. 1), there was a significant interaction effect of coronary ligation status at different water flow regimes ($F_{(3,\ 44.9)} = 3.875$; $P = 0.015$; Fig. 1). When the artificial ventilation was changed from low to high water flow, the sham fish exhibited a large increase in heart rate (from 30.0 ± 4.4 to 52.0 ± 3.0 bpm; $P < 0.001$; Fig. 1c-d), while heart rate did not change in ligated fish (31.3 ± 2.3 *vs* 36.2 ± 2.7 bpm, Fig. 1c-d). The unaltered heart rate in the coronary-ligated group was explained by the appearance of a second-degree AV block with a fixed ratio of 2 P waves for each QRS complex (*i.e.*, a 2:1 conduction ratio) in most ligated fish at elevated gill water flow (8 out of 11 fish), which manifested as skipped heart beats and a clearly compromised capacity to increase the mean heart rate (Fig. 2). However, given that the sinoatrial cycle length is shorter than the QT interval, each P wave occurred either during or prior to the preceding T wave (Suppl. Fig. 1). This suggests that when the impulse reached the ventricle, the ventricular muscle was refractory, causing the 2:1 block. Furthermore, due to the prolonged PR interval (first-degree block), atrial repolarization, which usually has minimal impact on the normal rhythm, could be clearly distinguished within the PR interval in some ligated fish (Fig. 1 and Suppl. Fig. 1).

Both sham-operated and coronary-ligated fish showed a clear bradycardia when water flow was turned off for ~1 min (sham: from 52.0 ± 3.0 to 21.2 ± 1.4 bpm, $P < 0.001$; ligated: from 36.2 ± 2.7 to 20.5 ± 2.6 bpm, $P < 0.001$; Fig. 1d-e and 2e). Hyperoxia combined with high water flow over the gills did not affect heart rate in sham fish

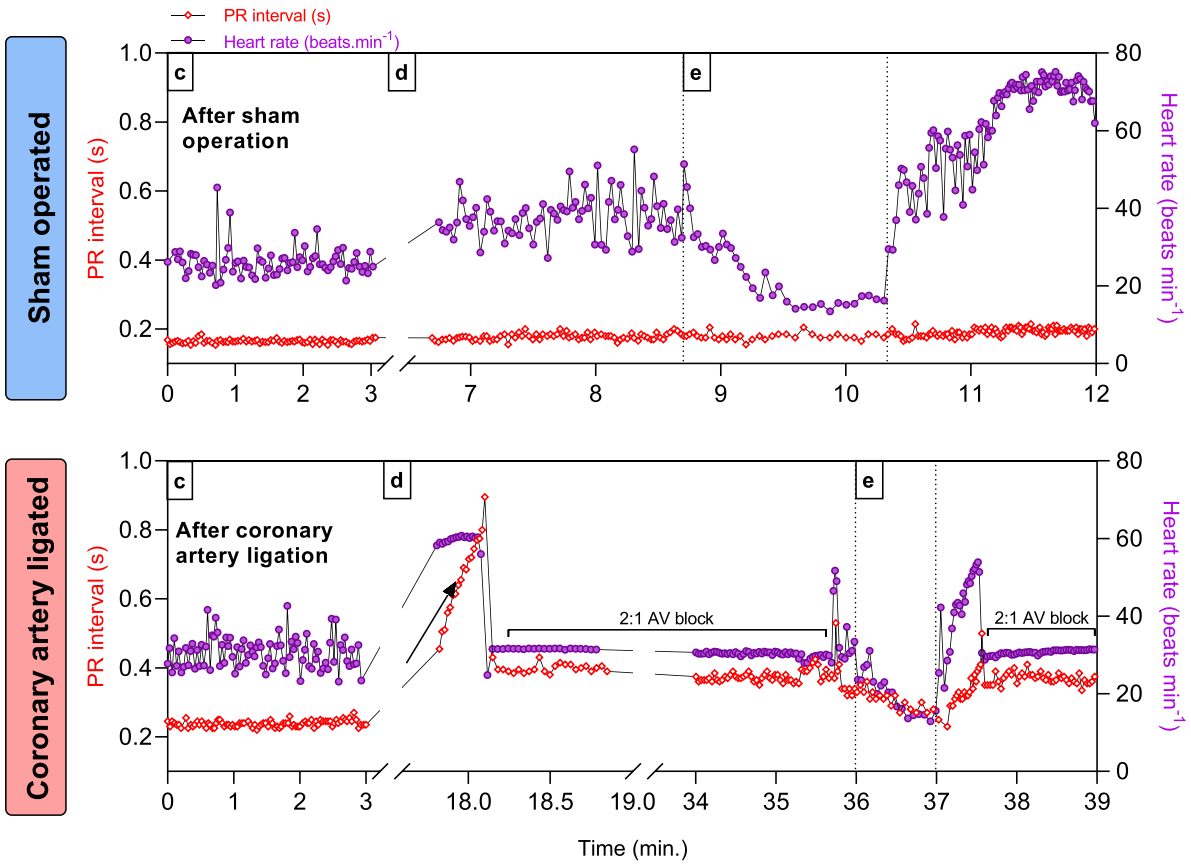


Fig. 2. Atrioventricular (AV) conduction in trout. Representative PR interval and heart rate recordings following changes in water flow over the gills from one sham-operated (top panel) and one coronary-ligated (lower panel) rainbow trout (*Oncorhynchus mykiss*) reported in Fig. 1. Plots show changes in heart rate and PR interval associated with changes in artificial ventilation. The letter designations (c to e) are the same as specified in Fig. 1. Sham/ligation surgery combined with low gill water flow (c). Heart rate increased after increasing artificial ventilation (d). The PR interval remained relatively constant in the sham fish while gradually prolonged in the ligated fish (arrow) until it turned into a 2:1 atrioventricular (AV) block, causing the heart rate to collapse. Turning off artificial ventilation for ~1 min (e) resulted in reflex bradycardia (time interval between dashed lines). Note that the PR interval remained constant in the sham fish during bradycardia, while the PR interval gradually declined as the heart rate decreased in the ligated fish. When artificial ventilation was turned back on (second dashed line), a 2:1 AV block was again observed in the coronary-ligated fish. Red diamonds represent the instantaneous PR interval, while purple circles represent the heart rate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compared to normoxia (65.3 ± 2.5 *vs* 52.0 ± 3.0 bpm; $P = 0.22$; Fig. 1d and f), whereas hyperoxia in ligated fish caused an increase in heart rate compared with normoxia (from 36.2 ± 2.7 to 62.5 ± 4.7 bpm; $P < 0.001$; Fig. 1d and f). Importantly, the improved capacity of coronary-ligated fish to increase heart rate with high gill water flow combined with hyperoxia occurred because the previously observed second-degree AV block in normoxia did not occur under hyperoxic conditions.

3.2. Effect of coronary ligation on ECG characteristics

In normoxia, coronary artery ligation significantly affected all of the recorded ECG characteristics (Fig. 1). Firstly, there was an atrioventricular conduction delay as indicated by a prolonged PR interval (ligated: 0.09 ± 0.03 *vs* sham: 0.005 ± 0.01 s; U = 7.0, $P = 0.001$; Fig. 1b-c). Secondly, the electrical signal also propagated slower across the ventricle myocardium as indicated by a prolonged QRS complex duration (ligation: 0.11 ± 0.02 *vs* sham: 0.005 ± 0.01 s; $t = 3.805$, df = 17, P = 0.001; Fig. 1b-c), and the QRS amplitude was reduced (ligated: −0.90 ± 0.2 *vs* sham: −0.02 ± 0.1 mV; $t = 3.805$, df = 17, P = 0.001; Fig. 1b-c).

None of the ECG characteristics after ligation surgery were affected by increased artificial ventilation (Fig. 1c-d). Yet, the PR interval showed a trend to increase further in coronary-ligated fish when these fish exhibited a second-degree AV block (ligated: 0.086 ± 0.03 *vs* sham: 0.02 ± 0.01 s; $t = 1.946$, df = 17, $P = 0.07$; Fig. 2d). Induced bradycardia by ceasing water flow resulted in a slight increase in QRS amplitude in ligated fish, although this change was not statistically different compared to sham fish (ligated: 0.34 ± 0.16 *vs* sham: −0.0004 ± 0.07 mV; U = 14, P = 0.07), and caused significantly faster atrioventricular conduction as indicated by a larger reduction in PR interval compared to sham fish (ligated: −0.07 ± 0.02 *vs* sham: −0.01 ± 0.004 s; U = 7, $P = 0.008$; Fig. 1d-e). In contrast, the QRS complex duration did not differ between sham and ligated fish (sham: 0.003 ± 0.004 *vs* ligated: −0.001 ± 0.008 s; $t = 0.5054$, df = 14, $P = 0.62$; Fig. 1d-e). Except for preventing or eliminating second-degree AV block as mentioned above, none of the other ECG characteristics were affected by hyperoxia (Fig. 1e-f and Suppl. Fig. 2).

Fig. 2 shows the typical changes in PR interval and heart rate in a representative sham-operated fish (upper panel) and a coronary-ligated fish (lower panel). Sham-operated fish exhibited increases in heart rate with increased artificial ventilation and bradycardia following removal of artificial ventilation. As the heart rate changed, the PR interval remained constant across changes in gill water flow. In contrast, in coronary-ligated fish (lower panel) a gradual prolongation of the PR interval occurred with increased water flow over the gills before culminating in a tachycardia-dependent second-degree AV block. Stopping the gill water flow instantaneously reduced heart rate and PR interval and any second-degree AV block disappeared.

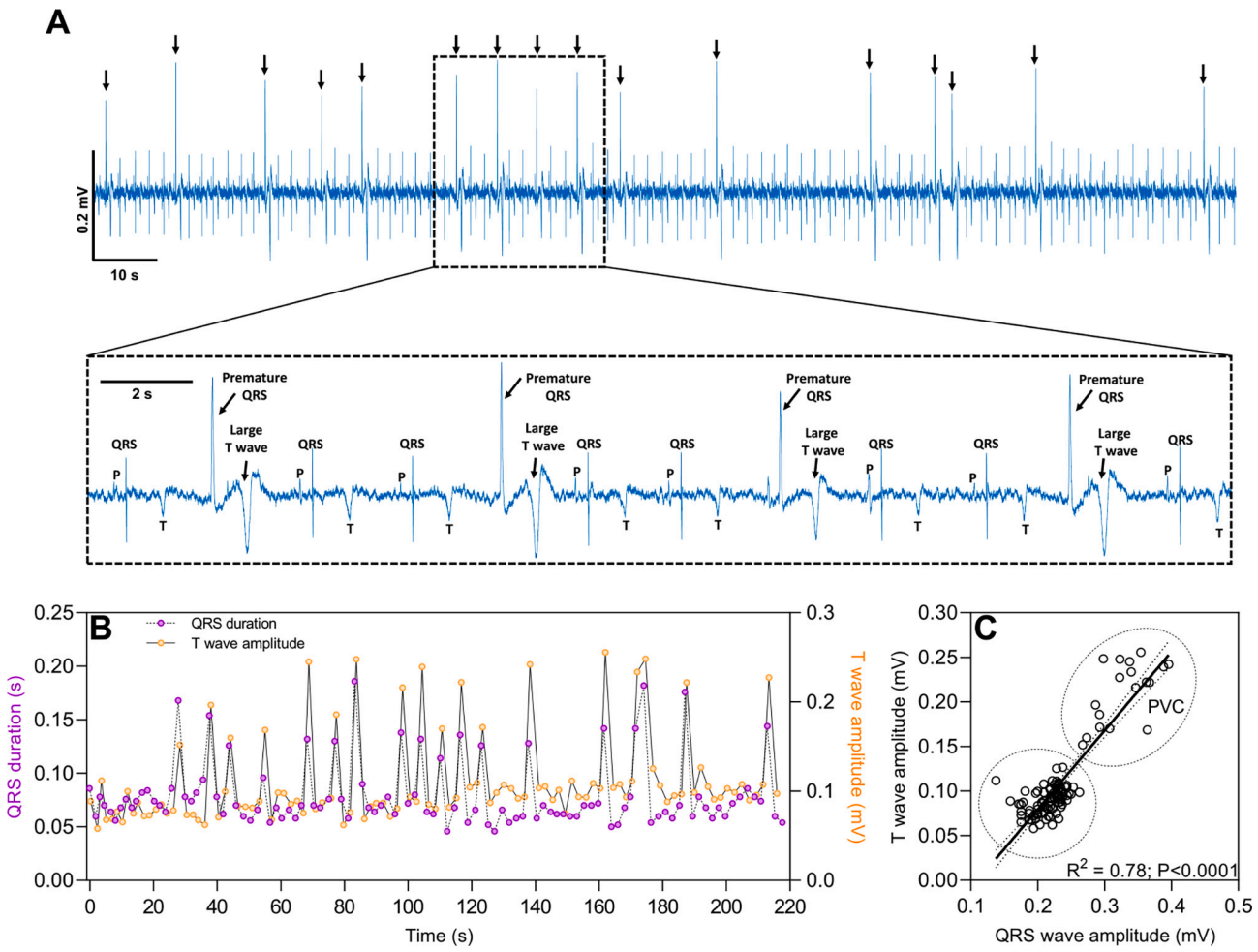


Fig. 3. Premature QRS complexes following coronary ligation in trout. One representative anaesthetized rainbow trout after coronary ligation showing premature QRS complexes followed by the presence of large T waves. (A) The dashed square shows a magnified ECG containing 4 Premature QRS complexes followed by its respective large T wave, as well as regular heartbeats showing P waves, QRS complexes, and T waves. Note that the premature QRS complex occurs earlier than expected in the cardiac cycle, it is abnormal in shape (*e.g.*, larger amplitude), not preceded by a P wave, and has a longer duration compared to the normal QRS (B). Note that premature QRS complexes are prolonged compared to typical QRS complex. The T wave amplitude directly correlates with the QRS amplitude (C). Vertical arrows in A point all the premature QRS complexes developed followed coronary ligation.

3.3. Less common electrocardiographic abnormalities in coronary ligated fish

Premature QRS complexes appeared after coronary ligation in one fish. Due to its occurrence earlier than expected in the cardiac cycle, the QRS complex displayed an abnormal shape, characterized by a larger amplitude and longer duration compared to the normal QRS. Moreover, a premature QRS was typically not preceded by a P wave (Fig. 3; Salyer, 2007). In addition, the T waves that followed each of the premature QRS complexes had larger amplitude compared to regular T waves (Fig. 3B). Moreover, premature QRS amplitudes correlated positively with corresponding large T waves (Fig. 3C). None of the sham-operated fish exhibited any signs of premature QRS complex.

Another less common ECG abnormality found after coronary artery ligation was the presence of a 2:1 AV block associated with irregular QRS amplitude that reversed direction around the baseline and changed voltage (Suppl. Figs. 3 and 4).

3.4. Effect of coronary ligation on gross morphology and histopathology of the heart

All 8 sham-operated fish survived up to the 10th day of the experiment, while only 6 out of 11 coronary artery ligated fish survived the first 10 days (Fig. 4). Two of the dead ligated fish were euthanized due to physical signs of illness; that is, one on day 10 due to clinical signs including exophthalmia and swollen body, and the other on day 5 due to sideways swimming behaviour. One surviving fish from the coronary-ligated group showed signs of a ventricular aneurysm (located at the base of the ventricle; Fig. 5B and Suppl. video 2). Histopathologically, the aneurysm revealed massive inflammatory cell infiltration in the spongy myocardium of the ventricle (Fig. 5D and E). At least four of the deceased fish in the ligated group showed clear signs of hemopericardium at necropsy, out of which two fish exhibited dilated atria with clotted blood, and another fish exhibited dilated atrium with clotted blood and a ruptured ventricle (Fig. 5C and Suppl. video 3).

4. Discussion

We present a comprehensive study on electrocardiographic abnormalities, along with morpho-histological characteristics associated with myocardial ischemia in a salmonid fish. Coronary artery ligation resulted in various ECG abnormalities; notably a prolonged QRS complex, atrioventricular conduction delays, and a reduction in QRS amplitude. These changes directly resemble ECG alterations observed in humans during a heart attack (Thygesen et al., 2018). The presence and sometimes severity of ECG abnormalities in coronary-ligated trout was also

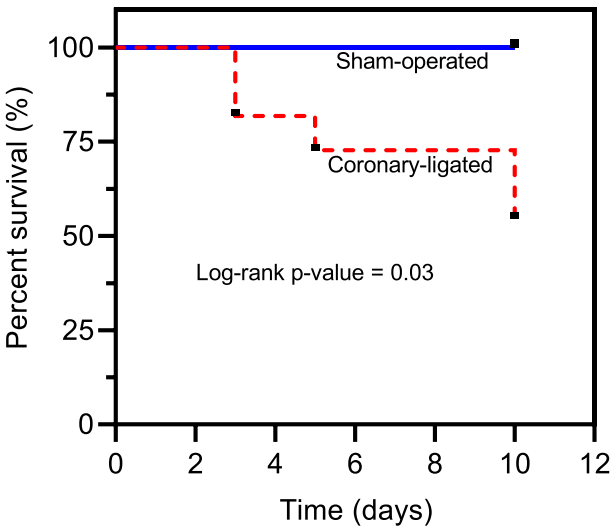


Fig. 4. Kaplan–Meier survival curves over 10 days following coronary ligation or sham operation in trout. Survival in rainbow trout after sham surgery and coronary artery ligation. All sham-operated fish survived until the 10th day. Kaplan–Meier curves were compared using Log-rank (Mantel–Cox) Test with Chi^2 analysis.

found to be highly influenced by the fish's heart rate. Specifically, when the heart rate was high (tachycardia), most trout with ligated coronaries exhibited a fixed ratio of 2 P waves to 1 QRS (*i.e.*, a 2:1 atrioventricular block), instead of the usual 1:1 relationship, along with other ECG alterations. However, all observed ECG alterations were alleviated or disappeared when the heart rate was lower (bradycardia). Importantly, however, when coronary-ligated fish were ventilated with hyperoxic water and exhibited a significantly higher heart rate compared to normoxia, the 2:1 AV block generally disappeared indicating improved myocardial electrical conduction during hyperoxia (Fig. 1). Furthermore, during the 10-day post-operative recovery period, coronary-ligated fish experienced some mortality, with necropsy findings revealing hemopericardium resulting from atrium/ventricle rupture. These findings and their implications for fish biomedical research, as well as fish mortality in aquaculture are discussed in greater detail below.

4.1. Mortality following coronary ligation

Although myocardial damage caused by ischemic episodes is typically irreversible in adult mammals, this is not the case for some fish species, including the rainbow trout, which have the ability to undergo cardiac remodelling and restore cardiac function even after complete coronary ligation (Zena et al., 2021). Even so, this capacity is likely size- (or age) dependent as the relative proportion of compact myocardium receiving coronary flow increases with increasing body size in salmonid fishes (Poupa et al., 1974). Indeed, the mortality observed here in coronary ligated fish (45%) with a body mass of ~900 g contrasts with our previous studies on smaller rainbow trout where there was no mortality in coronary ligated individuals weighing ~250 g (Zena et al., 2021). This suggests that larger fish may have a reduced capacity to recover from ischemic cardiac injury, which may be attributed to an increasing proportion of ventricular compact myocardium with fish body mass (Farrell et al., 2012). This observation is highly relevant and consistent with the observation that substantial mortality associated with myocardial lesions and injury in the farming industry typically occurs in large fish at the end of the production cycle (Frisk et al., 2020; Poppe et al., 2021; Poppe et al., 2007; Sommerset et al., 2021; Yousaf et al., 2013).

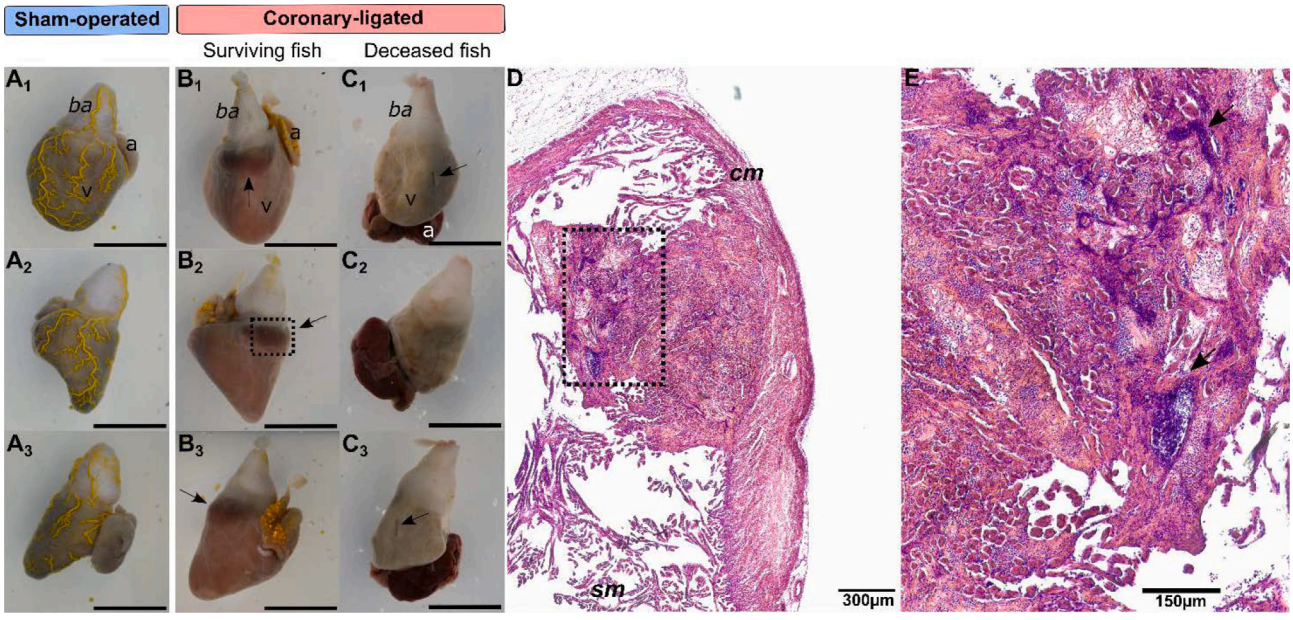


Fig. 5. Clinical signs and gross morphology of representative hearts from surviving and deceased trout over 10 days following coronary ligation or sham operation. Ventral (A-C_1) and lateral (right, A-C_2, and left, A-C_3) views of sham-operated (A_1-A_3) and coronary-ligated (B_1-B_3 and C_1-C_3) rainbow trout hearts. Panel A shows pictures of the heart of a sham-operated fish with the coronary circulation perfused with Microfil silicone casting material (yellow). Panel B shows pictures of the heart of a survived coronary-ligated fish in which coronary ligation prevented the coronary vessels from being filled with Microfil (note the presence of the microfil inside the atrium instead). A slight dilation of the base of the heart suggests a ventricle aneurysm (indicated by arrows). Panel C shows pictures of the heart of a deceased coronary-ligated fish. Microfil injection into the cardiovascular system was not possible for deceased fish. 4 out of 5 deceased fish exhibited hemopericardium, and one fish (C) exhibited ventricular rupture (indicated by arrows). Scale bar: 1 cm. a: atrium, v: ventricle, and *ba*: *bulbus arteriosus*. Histopathology of the aneurysm region in B_2 showing a large inflammatory response (D). Magnified picture of the boxed region in D showing large amounts of various inflammatory cells (black arrows) in the area of the aneurysm (E). Bright-field images in D and E were stained with Hematoxylin and eosin. *cm*, compact myocardium; *sm*, spongy myocardium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Gross cardiac lesions and disorders such as pale hearts and hemopericardium are very common in salmonids suffering from cardiac inflammatory diseases such as cardiomyopathy syndrome and heart and skeletal muscle inflammation (Garseth et al., 2018; Kongtorp et al., 2004). Similarly, some of the deceased fish in the coronary artery ligated group in the present study exhibited a pale heart and blood accumulation inside the pericardium; the latter likely resulting from atrial, *sinus venous* or ventricular rupture. Cardiac rupture can also result from acute myocardial infarction in humans and is obviously associated with high mortality rates (Hao et al., 2019; Kondo et al., 2016). Previous studies have found compromised cardiac contractility and impaired stroke volume in coronary-ligated salmonids (Morgenroth et al., 2021; Steffensen et al., 1998). These effects are likely caused by a reduction in compact myocardium mass and myocardial interstitial collagen content, which compromise structural integrity and function of the heart (Zena et al., 2021). Consequently, an impaired cardiac systolic function may cause blood to pool in the venous circulation leading to increased central venous pressure and cardiac preload (Sandblom and Axelsson, 2007; Sandblom and Gräns, 2017), which in connection with an already fragile ventricular wall (*e.g.*, aneurysm) may increase the risk of cardiac rupture.

4.2. Myocardial ischemic-like ECG changes during different levels of artificial gill ventilation

We identified two primary changes in the QRS morphology that emerged shortly after coronary artery ligation. First, there was always a prolonged QRS complex. Although we did not observe a distinct ST-segment elevation, which is common during myocardial infarction in mammals, the prolonged QRS complex in rainbow trout with occluded coronaries somewhat resembled an ST-segment elevation. Alternatively, this alteration could be more accurately described as an early repolarization pattern (ERP), which manifested as either terminal QRS slurring (a gradual transition from the QRS segment to the ST segment), or the presence of a positive deflection on the terminal QRS complex (known as notching) resulting in a concave upward ST-segment elevation (Oka et al., 2019). Secondly, another distinct QRS alteration in coronary ligated fish was a reduction of the QRS amplitude, which suggests extensive myocardial injury causing loss of viable myocardium mass, as shown in humans (Kobayashi et al., 2017; Tan et al., 2015). Reduced QRS amplitude is consistent with previous studies on anaesthetized rainbow trout subjected to temporary (30 min) coronary artery occlusion (Brijs et al., 2020; Ekström et al., 2017). Thus, QRS prolongation and amplitude depression represent two reliable indicators that may potentially be used to diagnose coronary obstruction in salmonid fish.

In addition to alterations in QRS characteristics, coronary ligation also caused an abnormal prolongation of the PR interval, which can be characterized as a first-degree AV block, as previously suggested by Brijs et al. (2020). Although the fish heart is thought to lack the distinct nodal and conducting structures found in mammals (Šolc, 2007), functional studies suggest the presence of a conducting pathway analogous to the conducting system of the mammalian heart (Sedmera et al., 2003). Consequently, similar to mammals, our data suggest myocardial ischemia disrupts the electrical conduction system of the trout heart, leading to severe atrioventricular conduction disturbances.

Even though fish were slightly bradycardic as gill water flow was reduced during the coronary ligation procedure, most of the ECG abnormalities observed after coronary ligation remained when gill water flow was increased again. Heart rate increased substantially with increased water flow over the gills but only in sham-operated fish, while coronary-ligated fish showed only a moderate increase in heart rate from the increased water flow. Indeed, our detailed ECG analysis showed that the AV block in ligated fish worsened when water flow over the gills was increased and fish typically transitioned from a first-degree AV block with abnormally slow conduction through the AV canal to a second-degree AV block with a fixed ratio of 2 P waves for each QRS complex (2:1); also known as “advanced heart block” (Barold, 1997; de Medina et al., 1978). This latter conduction disturbance was the main reason for the lack of (or diminished) increase in heart rate observed in many of the ligated fish with increasing gill water flow. We also verified that the precipitation of more advanced heart blocks were always preceded by a gradual lengthening of the PR interval until a QRS complex was missing following a P wave. This pattern resembles type 1 second-degree AV block found in humans (Vogler et al., 2012). However, in all eight coronary-ligated fish that developed a 2:1 atrioventricular block, the non-conducted P wave (the P wave before the missing QRS complex) occurred during or prior to the T wave of the preceding QRS complex. This observation suggests that the ventricle had not completed repolarized before the atrium contracted a second time (Suppl. Fig. 1). Consequently, the 2:1 atrioventricular conduction most likely resulted from an extended ventricular refractory period surpassing the sinus rate, causing a phenomenon known as pseudo 2:1 atrioventricular block (Kelleher et al., 2022; Rosenbaum and Acunzo, 1991). Furtheremore, some coronary ligated fish exhibited a clear atrium repolarization wave (known as the Ta wave) that occurred concurrently with second-degree AV block. Ta waves are typically undetectable as they are often hidden within the QRS complex, unless there is a malfunction in the conduction system, such as atrioventricular block (Childers, 2011). Consequently, the appearance of the Ta wave in the ECG of rainbow trout could serve as an additional cardiac indicator for diagnosing ischemic heart disease in fishes.

The prevalence of second-degree AV blocks induced by myocardial ischemia at high heart rates (*i.e.*, tachycardia-dependent AV block) may be the underlying mechanism for the bradycardia and cardiac collapse often observed at critically high temperatures in fish (Ekström et al., 2019; Haverinen and Vornanen, 2020). In various teleost fishes, including salmonids, the atrioventricular region is formed by a ring of vascularized compact myocardium named the atrioventricular canal (Icardo, 2017; Icardo and Colvee, 2011). Indeed, the occurrence of rhythm disorders, including atrioventricular conduction disturbances in all coronary-ligated fish in the current study (*e.g.*, first and second-degree AV blocks), clearly illustrates the importance of the coronary circulation for maintaining the electrical conduction pathway across the atrioventricular canal in the salmonid heart.

4.3. Changes in ECG characteristics in coronary ligated fish during hypoxic and hyperoxic conditions

A transiently terminated gill water flow led to a predicted and pronounced bradycardia in both sham and coronary-ligated fish, most likely reflecting a classic hypoxic bradycardia found in most fishes. Even so, the bradycardia allowed more time for recovery from the refractory state in the ventricle and abolished the second-degree AV block in coronary-ligated fish. Thus, the reflex bradycardia induced by the stopped water flow across the gills appeared to have, at least temporary, benefits to the electrical conduction capacity of the ischemic fish heart. This may be achieved by extending the end-diastolic filling time, which allows more time for luminal oxygen diffusion. In addition, the bradycardia may transiently reduce the overall oxygen demand of the heart, thereby reducing the mismatch between myocardial oxygen supply and demand alleviating the ischemic ECG abnormalities. This observation aligns with the idea that hypoxic bradycardia directly contributes to improved myocardial oxygenation in fishes (Farrell, 2007; Joyce and Wang, 2022).

The artificially ventilated coronary-ligated trout exposed to acute hyperoxia developed a pronounced tachycardia. As myocardial oxygen consumption may increase while the time for luminal oxygen diffusion should decrease whenever heart rate increases, the coronary ligated fish exhibiting tachycardia should be more susceptible to developing second-degree AV block. However, only one ligated fish developed second-degree AV block after exposure to hyperoxia. It lasted only a few minutes and normalized as soon as hyperoxia levels in the anaesthetic

solution increased to higher levels (>250 air saturation). The prevention of a second-degree AV block during hyperoxic conditions suggest that putatively elevated oxygen levels in the luminal venous blood were sufficient to improve cardiac function and electrical conduction capacity, regardless of elevated heart rates and potentially increased cardiac oxygen demand.

5. Conclusions

In conclusion, the current methodology, which involved ECG recordings and analyses to detect abnormal myocardial conduction patterns, has the potential to be integrated into models designed to diagnose cardiac health and predict the impact of stressful aquaculture conditions and mortality risk of farmed salmonids. This parallels the use of electrocardiographic predictors in human patients with a high cardiovascular risk, demonstrating potential applications in forecasting long-term survival rates. Moreover, our findings on the pronounced impacts of alterations in artificial gill water flow and oxygenation on heart rate and ECG characteristics in rainbow trout is particularly relevant when establishing standard practices for using ECG as a diagnostic tool for cardiovascular diseases in farmed fish. These findings also underscore the need to carefully consider these methodological aspects to prevent misinterpretation of heart rate and ECG data in biomedical research utilizing fish as models (*e.g.*, zebrafish) for various cardiovascular diseases.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.740482.

Funding

This work was supported by: Helge Ax:son Johnsons Stiftelse (LAZ), The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Svenska Forskningsrådet Formas; 2019–00299 to ES). NordForsk (DigiHeart project #103385 to IBJ, AG, and ES).

CRediT authorship contribution statement

Lucas A. Zena: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Andreas Ekström:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Daniel Morgenroth:** Writing – review & editing, Methodology, Investigation. **Tristan McArley:** Writing – review & editing, Methodology, Investigation. **Albin Gräns:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Conceptualization. **Michael Axelsson:** Writing – review & editing, Visualization, Supervision, Conceptualization. **Ida B. Johansen:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Erik Sandblom:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

References

Barold, S.S., 1997. American College of Cardiology/American Heart Association guidelines for pacemaker implantation after acute myocardial infarction. What is persistent advanced block at the atrioventricular node? Am. J. Cardiol. 80, 770–774. https://doi.org/10.1016/S0002-9149(97)00513-4.

Brijs, J., Hjelmstedt, P., Berg, C., Johansen, I.B., Sundh, H., Roques, J.A.C., Ekström, A., Sandblom, E., Sundell, K., Olsson, C., Axelsson, M., Gräns, A., 2020. Prevalence and severity of cardiac abnormalities and arteriosclerosis in farmed rainbow trout (Oncorhynchus mykiss). Aquaculture 526, 735417. https://doi.org/10.1016/j.aquaculture.2020.735417.

Chablais, F., Veit, J., Rainer, G., Jawiska, A., 2011. The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. BMC Dev. Biol. 11 https://doi.org/10.1186/1471-213X-11-21.

Childers, R., 2011. Atrial repolarization: its impact on electrocardiography. J. Electrocardiol. 44, 635–640. https://doi.org/10.1016/j.jelectrocard.2011.07.031.

Dalum, A., Tangen, R., Falk, K., Hordvik, I., Rosenlund, G., Torstensen, B., Koppang, E. O., 2016. Coronary changes in the Atlantic salmon Salmo salar L: characterization and impact of dietary fatty acid compositions. J. Fish Dis. 39, 41–54. https://doi.org/10.1111/jfd.12321.

Dalum, A.S., Kristthorsdottir, K.H., Griffiths, D.J., Bjørklund, K., Poppe, T.T., 2017. Arteriosclerosis in the ventral aorta and epicarditis in the bulbus arteriosus of Atlantic salmon (Salmo salar L). J. Fish Dis. 40, 797–809. https://doi.org/10.1111/jfd.12561.

de Medina, E.O.R., Bernard, R., Coumel, P., Damato, A.N., Fisch, C., Krikler, D., Mazur, N.A., Meijler, F.L., Mogensen, L., Moret, P., Pisa, Z., Wellens, H.J., 1978. Definition of terms related to cardiac rhythm. WHO/ISFC Task Force. Eur J Cardiol 8, 127–144.

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. https://doi.org/10.1152/ajpregu.00536.2016.

Ekström, A., Axelsson, M., Grans, A., Brijs, J., Sandblom, E., 2018. Importance of the coronary circulation for cardiac and metabolic performance in rainbow trout (Oncorhynchus mykiss). Biol. Lett. 14 https://doi.org/10.1098/rsbl.2018.0063.

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 Biochem. Syst. Environ. Physiol. 189, 757–769. https://doi.org/10.1007/s00360-019-01243-7.

Farrell, A.P., 2002. Coronary arteriosclerosis in salmon: growing old or growing fast? Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 132, 723–735.

Farrell, A.P., 2007. Tribute to P. L. Lutz: a message from the heart - why hypoxic bradycardia in fishes? J. Exp. Biol. 210, 1715–1725. https://doi.org/10.1242/jeb.02781.

Farrell, A.P., Farrell, N.D., Jourdan, H., Cox, G.K., 2012. A perspective on the evolution of the coronary circulation in fishes and the transition to terrestrial life. Ontogeny and Phylogeny of the Vertebrate Heart. 75–102.

Frisk, M., Høyland, M., Zhang, L., Vindas, M.A., Øverli, Ø., Johansen, I.B., 2020. Intensive smolt production is associated with deviating cardiac morphology in Atlantic salmon (Salmo salar L.). Aquaculture 529. https://doi.org/10.1016/j.aquaculture.2020.735615.

Garseth, H., Fritsvold, C., Svendsen, J.C., Bang Jensen, B., Mikalsen, A.B., 2018. Cardiomyopathy syndrome in Atlantic salmon Salmo salar L.: a review of the current state of knowledge. J. Fish Dis. 41, 11–26. https://doi.org/10.1111/jfd.12735.

Hao, W., Lu, S., Guo, R., Fan, J., Zhen, L., Nie, S., 2019. Risk factors for cardiac rupture complicating myocardial infarction: a PRISMA meta-analysis and systematic review. J. Invest. Med. 67, 720–728. https://doi.org/10.1136/jim-2018-000841.

Haverinen, J., Vornanen, M., 2020. Reduced ventricular excitability causes atrioventricular block and depression of heart rate in fish at critically high temperatures. J. Exp. Biol. 223 https://doi.org/10.1242/jeb.225227.

Hu, N., Joseph Yost, H., Clark, E.B., 2001. Cardiac morphology and blood pressure in the adult zebrafish. Anat. Rec. 264, 1–12. https://doi.org/10.1002/ar.1111.

Icardo, J.M., 2017. Heart morphology and anatomy. Fish Physiol. 36, 1–54. https://doi.org/10.1016/bs.fp.2017.05.002.

Icardo, M., Colvee, E., 2011. The atrioventricular region of the teleost heart. A Distinct Heart Segment 242, 236–242. https://doi.org/10.1002/ar.21320.

Joyce, W., Wang, T., 2022. Regulation of heart rate in vertebrates during hypoxia: a comparative overview. Acta Physiol. 234 https://doi.org/10.1111/apha.13779.

Kelleher, S.T., Prendiville, T., Carroll, A., 2022. Long QT syndrome presenting as fetal bradycardia and 2:1 atrioventricular block in a preterm infant. Arch. Dis. Child. Fetal Neonatal Ed. 0, 2022. https://doi.org/10.1136/archdischild-2021-323281.

Kobayashi, A., Misumida, N., Aoi, S., Kanei, Y., 2017. Low QRS voltage on presenting electrocardiogram predicts multi-vessel disease in anterior ST-segment elevation myocardial infarction. J. Electrocardiol. 50, 870–875. https://doi.org/10.1016/j.jelectrocard.2017.06.012.

Kondo, T., Nagasaki, Y., Takahashi, M., Nakagawa, K., Kuse, A., Morichika, M., Sakurada, M., Asano, M., Ueno, Y., 2016. An autopsy case of cardiac tamponade caused by a ruptured ventricular aneurysm associated with acute myocarditis. Leg. Med. 18, 44–48. https://doi.org/10.1016/j.legalmed.2015.12.002.

Kongtorp, R.T., Taksdal, T., Lyngøy, A., 2004. Pathology of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon Salmo salar. Dis. Aquat. Organ. 59, 217–224. https://doi.org/10.3354/dao059217.

Le, T., Zhang, J., Nguyen, A.H., Trigo Torres, R.S., Vo, K., Dutt, N., Lee, J., Ding, Y., Xu, X., Lau, M.P.H., Cao, H., 2022. A novel wireless ECG system for prolonged monitoring of multiple zebrafish for heart disease and drug screening studies. Biosens. Bioelectron. 197, 113808 https://doi.org/10.1016/j.bios.2021.113808.

Liu, C.C., Li, L., Lam, Y.W., Siu, C.W., Cheng, S.H., 2016. Improvement of surface ECG recording in adult zebrafish reveals that the value of this model exceeds our expectation. Sci. Rep. 6, 1–13. https://doi.org/10.1038/srep25073.

Milan, D.J., Jones, I.L., Ellinor, P.T., MacRae, C.A., 2006. In vivo recording of adult zebrafish electrocardiogram and assessment of drug-induced QT prolongation. Am.

J. Physiol. - Hear. Circ. Physiol. 291, 269–273. https://doi.org/10.1152/ajpheart.00960.2005.
Morgenroth, D., McArley, T., Gräns, A., Axelsson, M., Sandblom, E., Ekström, A., 2021. Coronary blood flow influences tolerance to environmental extremes in fish. J. Exp. Biol. jeb.239970 https://doi.org/10.1242/jeb.239970.
Neiffer, D.L., Stamper, M.A., 2009. Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. ILAR J. 50, 343–360. https://doi.org/10.1093/ilar.50.4.343.
Nomura, S., Ibaraki, T., 1969. Electrocardiogram of the rainbow trout and its radio transmission. Japanese J. Vet. Sci. 31, 135–147.
Oka, E., Iwasaki, Y.K., Maru, Y., Fujimoto, Y., Hagiwara, K., Hayashi, H., Yamamoto, T., Yodogawa, K., Hayashi, M., Shimizu, W., 2019. Prevalence and significance of an early repolarization electrocardiographic pattern and its mechanistic insight based on cardiac magnetic resonance imaging in patients with acute myocarditis. Circ. Arrhythmia Electrophysiol. 12, 1–8. https://doi.org/10.1161/CIRCEP.118.006969.
Pollehn, T., Brady, W.J., Perron, A.D., Morris, F., 2002. The electrocardiographic differential diagnosis of ST segment depression. Emerg. Med. J. 129–135.
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. Organ. 57, 103–108. https://doi.org/10.3354/dao057103.
Poppe, T.T., Taksdal, T., Bergtun, P.H., 2007. Suspected Myocardial Necrosis in Farmed Atlantic Salmon, *Salmo salar* L: A Field Case, pp. 615–620.
Poppe, T.T., Reed, A.K., Hofossæter, M., Wisløff, H., 2021. Novel myocardial pathology in farmed salmonids. J. Fish Dis. 1–4 https://doi.org/10.1111/jfd.13486.
Potts, H.G., Stockdale, W.T., Mommersteeg, M.T.M., 2021. Unlocking the secrets of the regenerating fish heart: comparing regenerative models to shed light on successful regeneration. J. Cardiovasc. Dev. Dis. 8, 1–18. https://doi.org/10.3390/jcdd8010004.
Poupa, O., Gesser, H., Jonsson, S., Sullivan, L., 1974. Coronary-supplied compact shell of ventricular myocardium in salmonids: growth and enzyme pattern. Comp. Biochem. Physiol. Part A Physiol. 48, 85–88.
Randall, D., 1982. The control of respiration and circulation in fish during exercise and hypoxia. J. Exp. Biol. 100, 275–288. https://doi.org/10.1242/jeb.100.1.275.
Rosenbaum, M.B., Acunzo, R.S., 1991. Pseudo 2:1 atrioventricular block and T wave alternans in the long QT syndromes. J. Am. Coll. Cardiol. 18, 1363–1366. https://doi.org/10.1016/0735-1097(91)90560-V.
Salyer, S.W., 2007. Chapter 2 - cardiology. In: Essential Emergency Medicine for the Healthcare Practitioner. Saunders, pp. 37–96. https://doi.org/10.1016/B978-1-4160-2971-7.X1000-8.
Sandblom, E., Axelsson, M., 2007. The venous circulation: a piscine perspective. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 148, 785–801. https://doi.org/10.1016/j.cbpa.2007.08.036.
Sandblom, E., Gräns, A., 2017. Form, function and control of the vasculature. Fish Physiol. 36, 369–433. https://doi.org/10.1016/bs.fp.2017.06.001.
Sedmera, D., Reckova, M., DeAlmeida, A., Sedmerova, M., Biermann, M., Volejnik, J., Sarre, A., Raddatz, E., McCarthy, R.A., Gourdie, R.G., Thompson, R.P., 2003. Functional and morphological evidence for a ventricular conduction system in zebrafish and Xenopus hearts. Am. J. Physiol. Heart Circ. Physiol. 284, 1152–1160. https://doi.org/10.1152/ajpheart.00870.2002.
Smith, F.M., Jones, D.R., 1978. Localization of receptors causing hypoxic bradycardia in trout (Salmo gairdneri). Can. J. Zool. 56, 1260–1265. https://doi.org/10.1139/z78-181.
Šolc, D., 2007. The heart and heart conducting system in the kingdom of animals: a comparative approach to its evolution. Exp. Clin. Cardiol. 12, 113–118.
Sommerset, I., Bornø, B., Jensen, B.B., Brun, E., 2021. The Health Situation in Norwegian Aquaculture 2020.
Steffensen, J.F., Farrell, A.P., Farrell, T., 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. https://doi.org/10.1016/S1095-6433(97)00470-4.
Stewart, K.M.R., Walker, S.L., Baker, A.H., Riley, P.R., Brittan, M., 2022. Hooked on heart regeneration: the zebrafish guide to recovery. Cardiovasc. Res. 118, 1667–1679. https://doi.org/10.1093/cvr/cvab214.
Tan, N.S., Goodman, S.G., Yan, R.T., Tan, M.K., Fox, K.A.A., Gore, J.M., Brieger, D., Steg, P.G., Langer, A., Yan, A.T., 2015. Prognostic significance of low QRS voltage on the admission electrocardiogram in acute coronary syndromes. Int. J. Cardiol. 190, 34–39. https://doi.org/10.1016/j.ijcard.2015.04.085.
Thygesen, K., Alpert, J.S., Jaffe, A.S., Chaitman, B.R., Bax, J.J., Morrow, D.A., White, H. D., Corbett, S., Chettibi, M., Hayrapetyan, H., Roithinger, F.X., Aliyev, F., Sujayeva, V., Claeys, M.J., Smajić, E., Kala, P., Iversen, K.K., El Hefny, E., Marandi, T., Porela, P., Antov, S., Gilard, M., Blankenberg, S., Davlouros, P., Gudnason, T., Alcalai, R., Colivicchi, F., Elezi, S., Baitova, G., Zakke, I., Gustiene, O., Beissel, J., Dingli, P., Grosu, A., Damman, P., Juliebo, V., Legutko, J., Morais, J., Tatu-Chitoiu, G., Yakovlev, A., Zavatta, M., Nedeljkovic, M., Radsel, P., Sionis, A., Jemberg, T., Müller, C., Abid, L., Abaci, A., Parkhomenko, A., 2018. Fourth universal definition of myocardial infarction (2018). Circulation 138, e618–e651. https://doi.org/10.1161/CIR.0000000000000617.
Tota, B., 1983. Vascular and metabolic zonation in the ventricular myocardium of mammals and fishes. Comp. Biochem. Physiol. Part A Physiol. 76, 423–437. https://doi.org/10.1016/0300-9629(83)90442-5.
Tota, B., Cimini, V., Salvatore, G., Zummo, G., 1983. Comparative study of the arterial and lacunary systems of the ventricular myocardium of elasmobranch and teleost fishes. Am. J. Anat. 167, 15–32. https://doi.org/10.1002/aja.1001670103.
Ueno, S., Yoshikawa, H., Ishida, Y., Mitsuda, H., 1986. Electrocardiograms recorded from the body surface of the carp, Cyprinus carpio. Comp. Biochem. Physiol. – Part A Physiol. 85, 129–133. https://doi.org/10.1016/0300-9629(86)90473-1.
Vogel, B., Claessen, B.E., Arnold, S.V., Chan, D., Cohen, D.J., Giannitsis, E., Gibson, C.M., Goto, S., Katus, H.A., Kerneis, M., Kimura, T., Kunadian, V., Pinto, D.S., Shiomi, H., Spertus, J.A., Steg, P.G., Mehran, R., 2019. ST-segment elevation myocardial infarction. Nat. Rev. Dis. Prim. 5, 1–20. https://doi.org/10.1038/s41572-019-0090-3.
Vogler, J., Breithardt, G., Eckardt, L., 2012. Bradyarrhythmias and conduction blocks. Rev. Española Cardiol. Eng. Ed. 65, 656–667. https://doi.org/10.1016/j.rec.2012.01.027.
Vornanen, M., Hassinen, M., 2016. Zebrafish heart as a model for human cardiac electrophysiology. Channels 10, 101–110. https://doi.org/10.1080/19336950.2015.1121335.
Wallbom, N., Zena, L.A., McArley, T.J., Ekström, A., Axelsson, M., Gräns, A., Sandblom, E., Morgenroth, D., 2023. Increased reliance on coronary perfusion for cardiorespiratory performance in seawater-acclimated rainbow trout. J. Exp. Biol. 1–11 https://doi.org/10.1242/jeb.244733.
Yousaf, M.N., Koppang, E.O., Skjødt, K., Hordvik, I., Zou, J., Secombes, C., Powell, M.D., 2013. Comparative cardiac pathological changes of Atlantic salmon (Salmo salar L.) affected with heart and skeletal muscle inflammation (HSMI), cardiomyopathy syndrome (CMS) and pancreas disease (PD). Vet. Immunol. Immunopathol. 151, 49–62. https://doi.org/10.1016/j.vetimm.2012.10.004.
Yu, F., Li, R., Parks, E., Takabe, W., Hsiai, T.K., 2010. Electrocardiogram signals to assess zebrafih heart regeneration: implication of long QT intervals. Ann. Biomed. Eng. 38, 2346–2357. https://doi.org/10.1007/s10439-010-9993-6.
Zena, L.A., Ekström, A., Gräns, A., Olsson, C., Axelsson, M., Sundh, H., Sandblom, E., 2021. It takes time to heal a broken heart: ventricular plasticity improves heart performance after myocardial infarction in rainbow trout, *Oncorhynchus mykiss*. J. Exp. Biol. 224 https://doi.org/10.1242/jeb.243578.