

# Changes in heart rate during electrical stimulation of the atrium in rainbow trout (*Oncorhynchus mykiss*) at low temperature

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## Abstract

In this work, we investigated the effect of high heart rate (HR) in vivo on the electrical properties and pumping functions of the heart ventricle of the rainbow trout (*Oncorhynchus mykiss*) at low ambient temperatures. HR was altered by atrial pacing. The electrocardiogram (ECG) parameters and hemodynamic parameters of the heart ventricle of rainbow trout adapted to a temperature of 5–7 °C were studied from the normal sinus rhythm (21.6 ± 4.9 bpm) to the maximum possible HR. Results show that a HR of about 60 bpm is the upper limit of the normal functional activity of the ventricle of the heart. An increase in heart rate up to 60 bpm leads to an increase in the PQ interval and QRS complex, a decrease in the QT interval on the ECG, and a violation of the rhythmic activity of the heart (i.e., the occurrence of extrasystole), as well as to a considerable change in the hemodynamic parameters of the ventricle of the heart and a decrease in its contractile properties. After a period of ventricular extrasystole for several minutes (10–15 min), the activity of the sinus node resumes but with a lower HR compared with the initial HR. The duration of the QRS complex recovers to the initial one, and the PQ and QT intervals increase. Maximum systolic pressure and end-diastolic pressure also return to their original values after extrasystolic contraction. After the experimental extrasystole, the phenomenon of the absence of an increase in HR is observed. The arising extrasystole probably has a functional nature and is one of the mechanisms of electromechanical homeostatic control in the heart.

**Keywords:** heart rate, electrocardiogram, hemodynamics, heart ventricle, temperature, rainbow trout

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## Introduction

In fish, the functional activity of the heart during adaptation to environmental conditions is realised primarily by increasing heart rate (HR) and changing cardiac output (Farrell et al., 2009). At the same time, HR is one of the factors limiting adaptation to the changing needs of the body (Farrell et al., 2009; Casselman et al., 2012; Anttila et al., 2014; Badr et al., 2016).

Due to global warming, studies of the physiological activity of the heart of fish were carried out in the range from the optimal (preferred) temperature to the upper temperature limit, which causes a lethal outcome. In Atlantic salmon acclimatised to a temperature of 12 °C, cardiac collapse starts at 21–23 °C with HR of about 150 bpm (Anttila et al., 2014). In roach acclimatised to 18 °C in summer, when the temperature reaches 28.1 ± 0.5 °C, HR increases from 61 ± 5 bpm to maximum values of 150 ± 7 bpm (Badr et al., 2016). In rainbow trout at an upper lethal temperature of 23–25 °C, HR reaches its maximum level of 120 bpm

(Farrell et al., 1996). Prolonged temperatures below 5 °C reduce trout's activity and survival in winter (Matthews and Berg, 1997).

In fish, an increase in temperature above the optimum causes disturbances in heart rhythm and conduction, leading to cardiac collapse (Jayasundara and Somero, 2013; Anttila et al., 2014; Vornanen et al., 2016; Haverinen et al., 2017).

Studies on the effect of temperature on the cardiac activity of fish were carried out from the optimal temperature of the species and a high temperature. However, the functional activity of the heart of fish under conditions of a low temperature boundary of the habitat has been insufficiently studied. At the same time, the lower limit of the temperature of the aquatic environment leads to irreversible consequences for each species of fish. For example, in roach acclimatized to 18 °C in summer, a temperature drop to 6 °C is the lowest temperature limit for their survival (Badr et al., 2016).

We chose rainbow trout as our research object. The trout can remain active at approximately 4–24 °C (Keen et al., 2017). The maximum stroke volume and the power of the heart ventricle are observed around the optimal temperature of 15 °C. At temperatures above 15 °C, the maximum stroke volume decreases, and the power of the heart ventricle decreases. At 5–7 °C, a decrease in contractility and a deterioration in the pumping function of the ventricle of the heart are observed (Kibler et al., 2020a).

This work aims to study the effect of atrial electrical stimulation on the electrical properties and pumping functions of the heart ventricle of the rainbow trout (*Oncorhynchus mykiss*) in vivo under conditions of low environmental temperatures.

Studying the function of the heart with maximum acceleration of the rhythm in fish in a habitat with a low temperature will provide understanding on the regularities of the electrophysiological stability of the heart of fish in extreme conditions.

## Materials and methods

The study was carried out in accordance with the international rules for the treatment of experimental animals (Guide for the Care and Use of Laboratory Animals. National Academy of Sciences (Washington, USA, 2011) and was approved by the local Ethical Committee of the Institute of Physiology, Komi Science Centre, Ural Branch, RAS. Experiments were carried out on 14 rainbow trout (*Oncorhynchus mykiss*) of both sexes and adapted for several weeks to the water temperature of 5–7 °C in pond farm cages. The fish were immobilised and fixed in a water-filled tray on their backs. A rubber hose was inserted into the oral cavity, through which to provide artificial respiration, and river water at 5–7 °C

was continuously passed through the gills under low pressure using a pump (submersible pump XL-3340). The body temperature of the experimental animals during the study was maintained within the range of 5–7 °C.

HR was changed by pacing with a CEECX-3 pacemaker. The atrium was stimulated by stimuli lasting for 2 mins at an amplitude of 2–4 V in the range of 10 bpm increments from sinus rhythm to the maximum HR at which disorders in the heart activity occurred. During electrical stimulation, a period of extrasystole was initially observed, and then a period of partial restoration of sinus rhythm. The upper heart rate limits at which cardiac abnormalities occurred were about 60 bpm.

The pressure in the ventricular cavity of the heart was measured directly using a single-lumen catheter (inner diameter = 1 mm) filled with heparinised saline solution, inserted through the ventricular wall. The intraventricular pressure was determined with an ISO-TEC transducer (Hugo Sachs Elektronik) that was connected to the Prucka Mac-Lab 2000 system (General Electric).

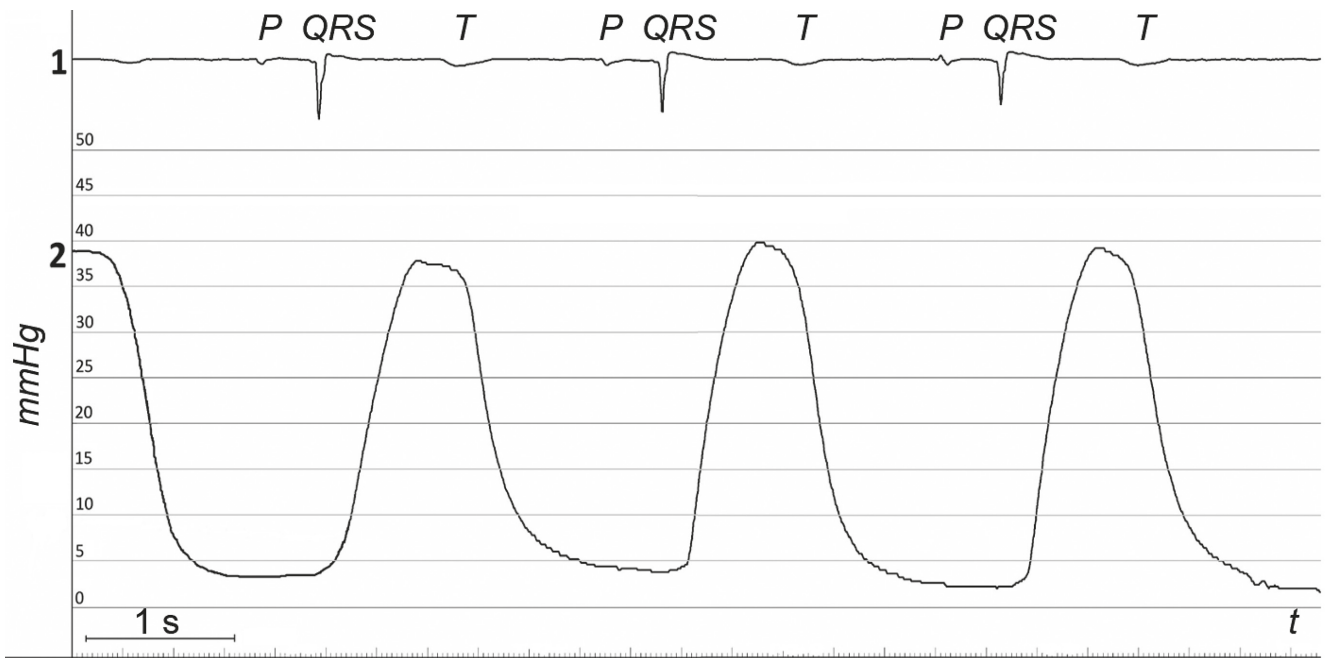
The electrocardiogram (ECG) was recorded in bipolar leads: I, right-left pectoral fins; II, right pectoral — left pelvic fins; and III, left pectoral — left pelvic fins. ECG and changes in pressure in the ventricle were recorded synchronously using the Prucka Mac-Lab 2000 system. The following hemodynamic parameters were measured: maximum systolic pressure of the ventricle (MSVP), end-diastolic pressure of the ventricle (EDVP), maximum rate of the ventricular pressure rise ( $dp/dt_{max}$  — isovolumic contractility index), and maximum rate of ventricular pressure fall ( $dp/dt_{min}$  — isovolumic relaxation index).

Results were statistically analysed through the BIO-STAT 4.03 software using the Wilcoxon and Mann-Whitney criteria. Differences were recognised as significant at the significance level of  $p < 0.05$ . Data were presented as mean  $\pm$  standard deviation ( $M \pm \sigma$ ).

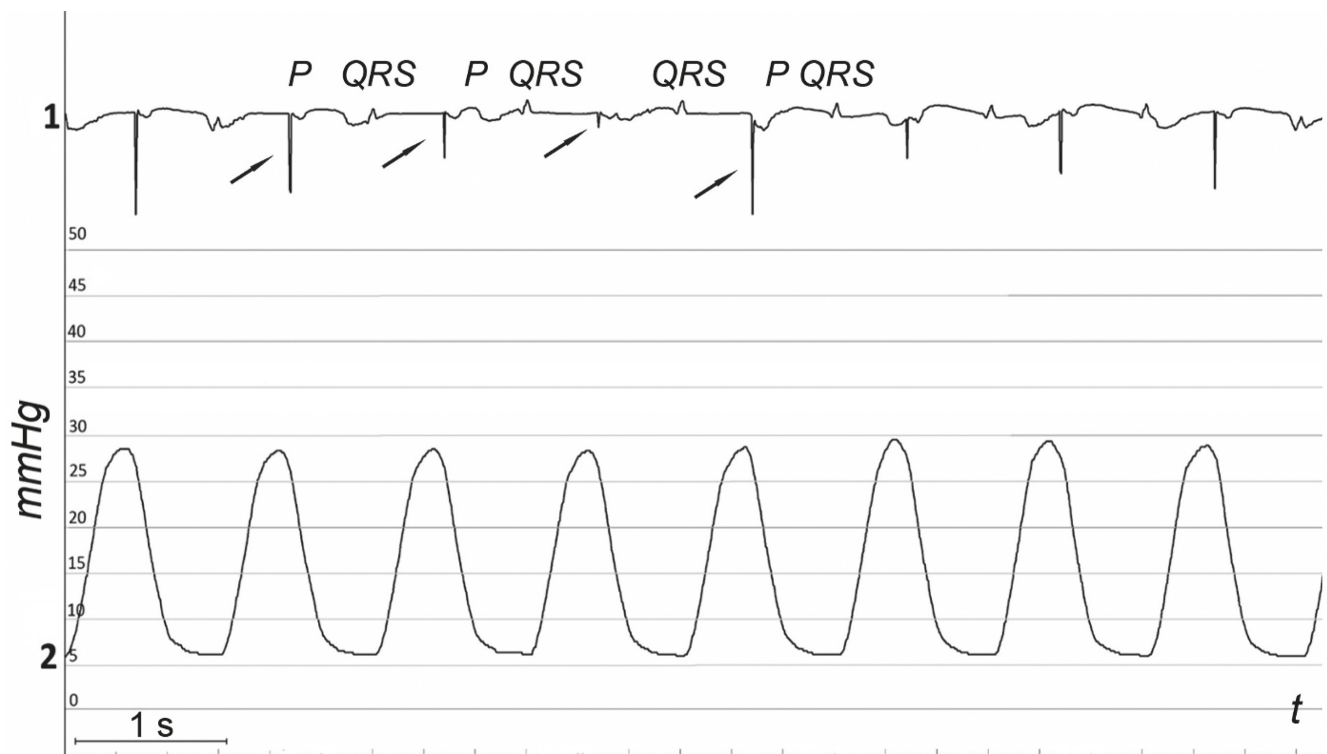
## Results

The HR in rainbow trout with sinus-atrial rhythm at an ambient temperature of 5–7 °C was  $21.6 \pm 4.9$  bpm. On the ECG in lead II (Fig. 1), the durations of the PQ interval, QRS complex and QT interval were  $205.0 \pm 45.4$  ms,  $102.7 \pm 12.5$  ms and  $1208.2 \pm 249.4$  ms, respectively. With an increase in the frequency of stimulation, an increase in the PQ interval and QRS complex and a decrease in the QT interval were observed (Fig. 2). The limiting frequency of stimulation at which the HR was not assimilated was 60 bpm. At 60 bpm, the durations of the PQ interval, QRS complex and QT interval were  $285.00 \pm 69.14$  ms ( $p < 0.05$ ),  $148.3 \pm 12.5$  ms ( $p < 0.05$ ) and  $506.6 \pm 93.0$  ms ( $p < 0.05$ ), respectively.

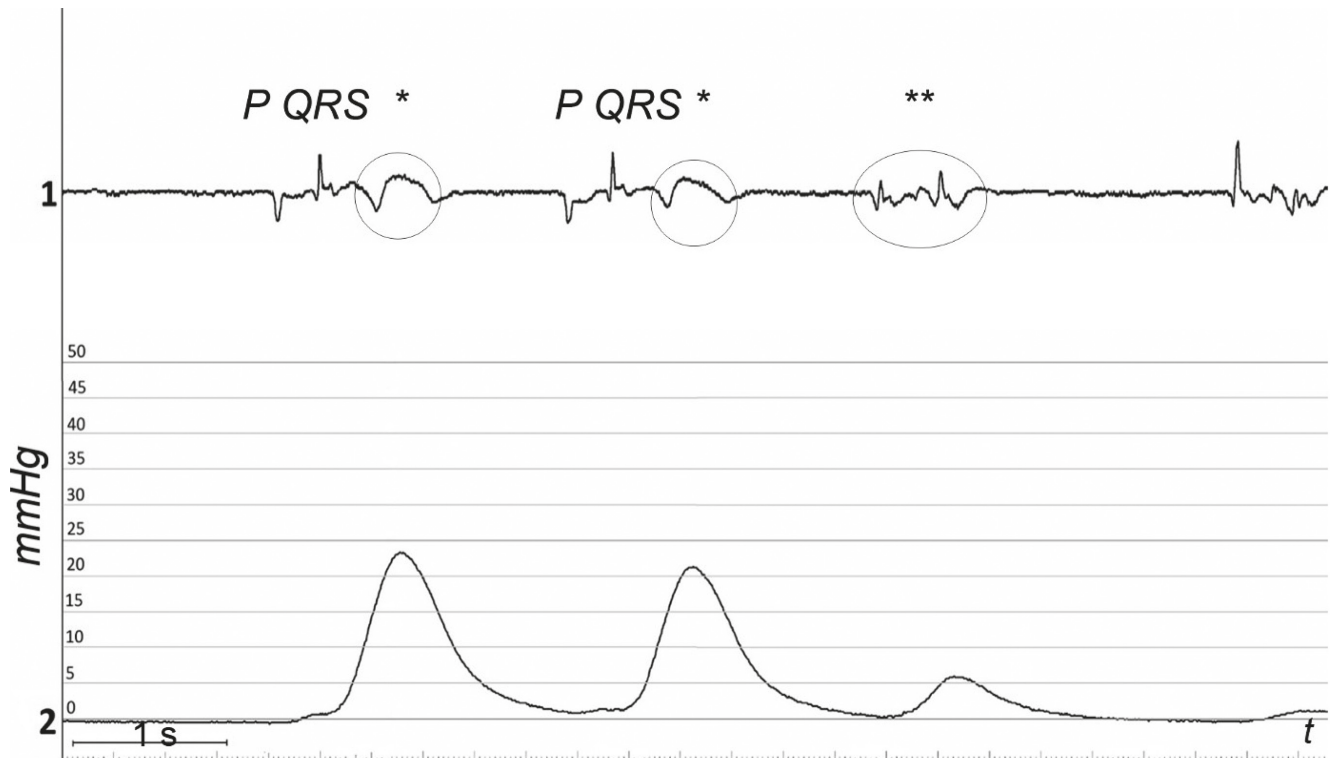
At a frequency of an artifact heart rhythm of about 60 bpm, significant disturbances in the electrical activ-



**Fig. 1.** Representative electrocardiograms recorded in lead II (right pectoral–left pelvic fins) (1) and intraventricular pressure (2) in the ventricle of the heart in rainbow trout at the initial sinus-atrial rhythm. *t* — time in seconds.



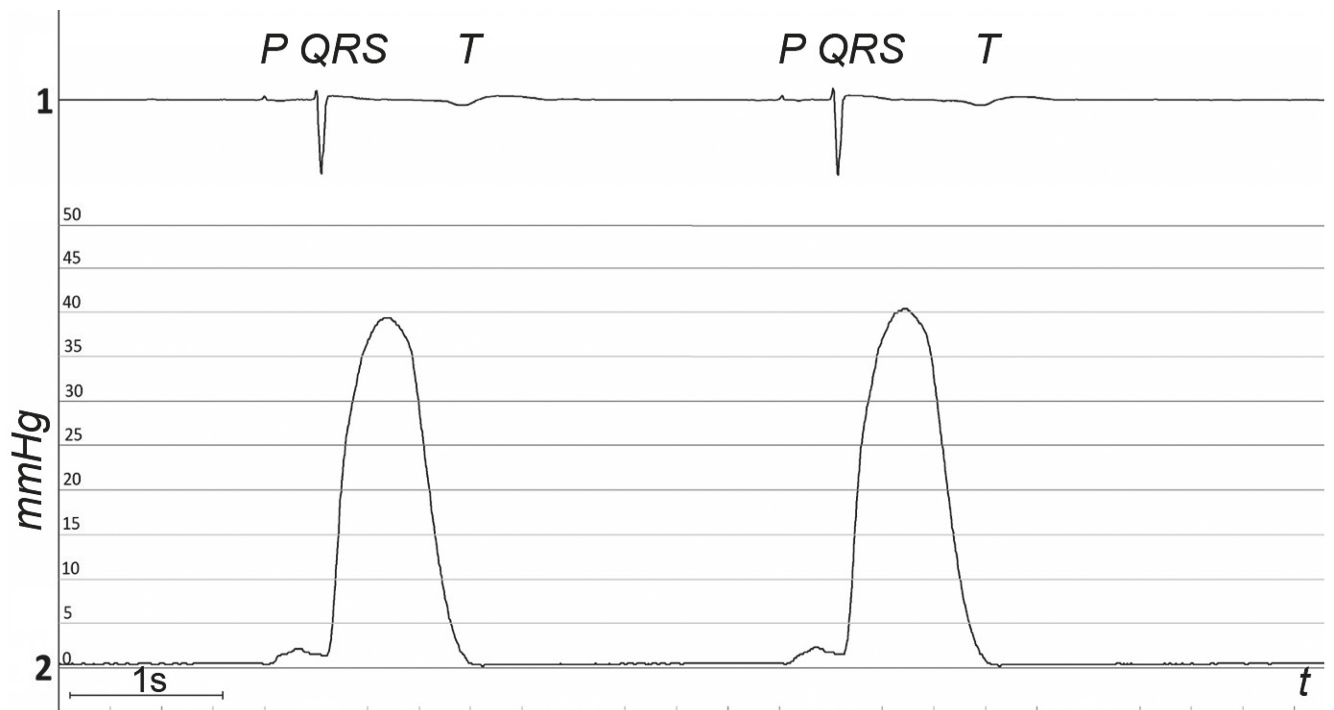
**Fig. 2.** Representative electrocardiograms recorded in lead II (right pectoral — left pelvic fins) (1) and intraventricular pressure (2) under the atrial artificial electrical stimulation at HR 60 bpm of the rainbow trout. Arrows indicate stimulating impulse. The T wave of the ECG is flattened. *t* — time in seconds. (Explanation in the text)



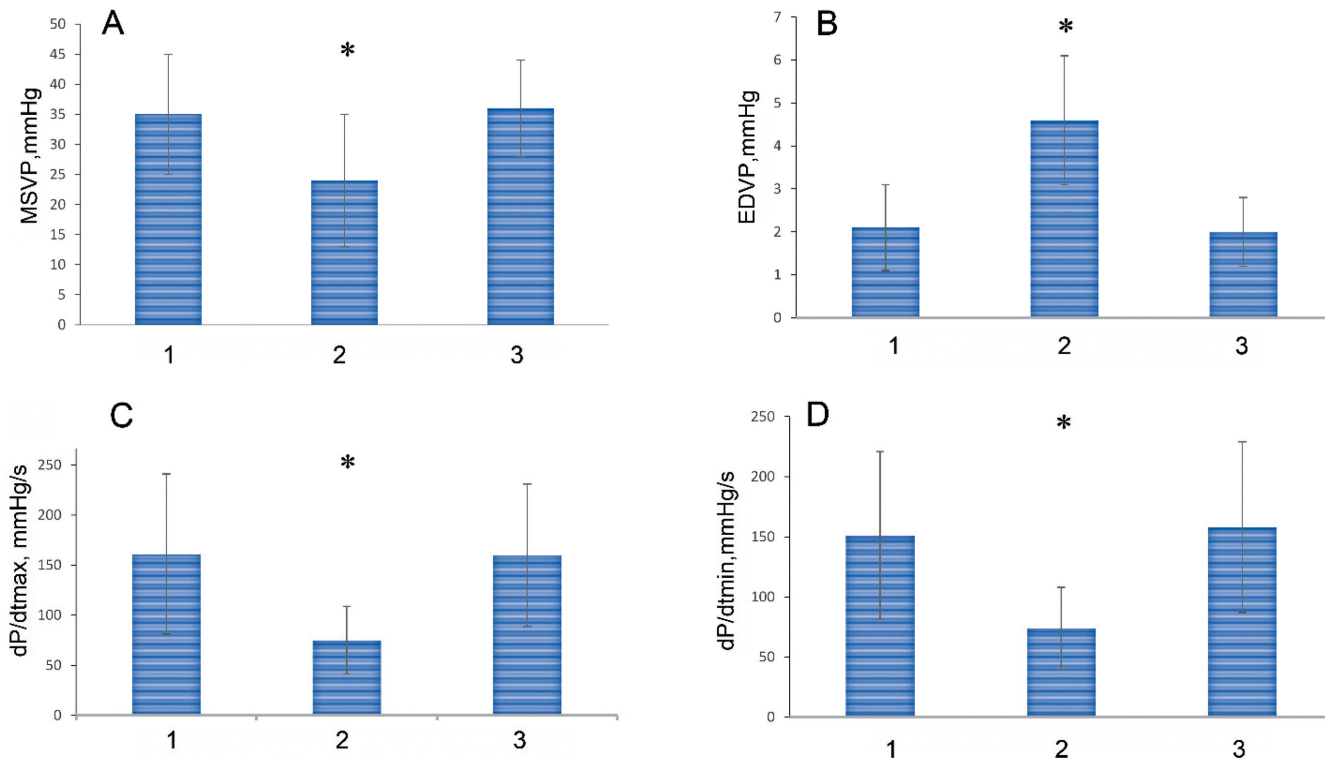
**Fig. 3.** Representative electrocardiograms recorded in lead II (right pectoral — left pelvic fins) (1) and intraventricular pressure (2) with rhythm disturbance.

\*Early ventricular extrasystoles.

\*\*Atrial extrasystoles. Following atrial extrasystoles, the pause is excessively lengthened. t — time in seconds.



**Fig. 4.** Representative electrocardiograms recorded in lead II (right pectoral — left pelvic fins) (1) and intraventricular pressure (2) in rainbow trout after pacing during the recovery of spontaneous sinus-atrial rhythm. t — time in seconds. (Explanation in the text)



**Fig. 5.** Modified Fig. 1 (Kibler et al., 2020a). Hemodynamic parameters of the heart ventricle in rainbow trout with a sinus-atrial rate and an atrium pacing rhythm of 60 bpm.

A — the maximum systolic pressure of the ventricle (MSVP); B — end-diastolic pressure of the ventricle (EDVP); C — the maximum rate of the ventricular pressure rise ( $dP/dt_{max}$ ); D — the maximum rate of the pressure fall ( $dP/dt_{min}$ ). 1 — initial sinus-atrial rhythm, 2 — atrium pacing rhythm of 60 bpm, 3 — sinus-atrial rhythm (15 minutes after the period of ventricular extrasystole). \*  $p < 0.05$  — to the initial sinus rhythm.

ity of the heart occurred, and these disturbances were characterised by instabilities in rhythm and conduction. During the period of extrasystole, the systolic pressure in the ventricle ranged from 24 mmHg/s to 5 mmHg/s (Fig. 3).

After extrasystole, spontaneous electrical activity of the sinus-atrial node resumed in 50 % of fish (Fig. 4). However, along with the restoration of the work of the sinus-atrial node, there was a slowdown in heart rate ( $17.5 \pm 3.3$  bpm) compared to the initial sinus rhythm ( $21.6 \pm 4.9$  bpm). Moreover, the phenomenon of the absence of an increase in HR was observed. The duration of the PQ interval was  $352.9 \pm 40.1$  ms ( $p < 0.05$ ); the QT interval increased to  $1405.4 \pm 287.0$  ms ( $p < 0.05$ ). The duration of the QRS complex ( $103.2 \pm 14.2$  ms) was restored to the duration of the initial rhythm ( $102.7 \pm 12.5$  ms).

In relation to the initial rhythm of  $21.6 \pm 4.9$  bpm, an increase in HR to 60 bpm led to a drop in MSVP, maximum rate of increase and decrease in pressure in the ventricle and increase in EDVP (Fig. 5). In the postextrasystolic period, with spontaneous sinus-atrial rhythm in fish, an almost complete recovery of haemodynamic parameters was observed (Figs. 4 and 5). With a spontaneous sinus rhythm of  $17.5 \pm 3.3$  bpm, the maximum systolic pressure of the ventricle was  $36.0 \pm 8.1$  mm

Hg; the end-diastolic pressure of the ventricle was  $2.0 \pm 0.8$  mm Hg; the maximum rate of pressure increase  $dP/dt_{max}$  was  $160.2 \pm 71.3$  mm Hg/s; and the maximum rate of pressure drop  $dP/dt_{min}$  was  $158.0 \pm 71.1$  mm Hg/s.

## Discussion

With an increase in HR up to 60 bpm, significant increases in the duration of the PQ interval and the QRS complex were revealed, which indicated a slowdown in the rate of conduction of excitation from the atrium to the ventricle. The QT interval of the rainbow trout ECG, which reflects the average duration of the action potential (AP) in the ventricular myocardium (Fogoros, 2009), is significantly shortened at high HR frequencies. The dynamics of AP duration are largely determined by  $K^+$  currents aimed at maintaining  $Ca^{2+}$  homeostasis. In most fish species, the main current responsible for the shortening of APs is  $I_{Kr}$  (Vornanen et al., 2002). A decrease in the duration of the QT interval of the ECG due to increased HR is probably associated with decreased duration of AP at 5–7 °C because of a change in  $I_{Kr}$  density.

In the rainbow trout we studied, the upper limit of HR after the occurrence of disturbances in the heart had a rhythm of 60 bpm at 5–7 °C. The relatively high



HR in rainbow trout is due to the high density of  $I_{Na}$  in the heart (Haverinen and Vornanen, 2004). At the same time, under conditions of changes in the ambient temperature,  $I_{Na}$  limits the propagation of electrical excitation (Vornanen et al. 2014). It is possible that the limiting factor for the upper HR limit to about 60 bpm in rainbow trout is the disruption of the function of voltage-dependent sodium channels.

In the heart of fish, no highly organised conduction system is observed. Therefore, the dominant direction of movement of electrical excitation is determined by the uniform movement of the activation wave from the primary foci of excitation located near the atrioventricular opening along the wall of the ventricle of the heart (Shmakov and Roschevsky, 1997). In rainbow trout with atrial stimulation above 50 bpm, desynchronisation occurs in the spatiotemporal relationship of depolarisation processes in the form of an inversion of the direction of movement of the activation wave from the dorsoventral basal region to the ventrocaudal basal region (Kibler et al., 2020b). This phenomenon probably affects the biomechanical activity of the myocardium. A previous study (Nuzhny et al., 2018) showed that high HR causes an uneven distribution of the load on the myocardium and unloading of the pressure of the apex of the heart, which leads to the occurrence of ventricular tachycardia and polymorphic ventricular extrasystoles. In rainbow trout, the occurrence of ventricular extrasystole in response to an increase in HR of up to 60 bpm can also be considered as a result of the redistribution of mechanical stress in the wall of the ventricle of the heart. In this regard, the mechanical interaction between early- and late-activated muscle elements causes an increase in the duration of the calcium transition and the duration of the AP along the excitation wave (Solovyova et al., 2006). After a period of extrasystole, this phenomenon leads to a significant increase in the QT interval and a slower HR compared with the original sinus rhythm.

A previous study (Kibler et al., 2020a) showed that an increased HR in the rainbow trout of up to 60 bpm leads to a significant change in the hemodynamic parameters of the ventricle of the heart and a decrease in contractile properties. In our experiment, when the heart rhythm was disturbed, the intraventricular pressure was unstable and varied within wide limits. With the resumption of the activity of the sinus-atrial node after extrasystole, the hemodynamic parameters of the ventricle of the heart were restored to the initial level (Fig. 5). Slowing down the HR to  $17.5 \pm 3.3$  bpm in relation to the initial rhythm of  $21.6 \pm 4.9$  bpm did not have a significant effect on the hemodynamic parameters of the heart.

Thus, the phenomenon of the impossibility of accelerating the heart rate after extrasystole was first discovered by us in rainbow trout. This phenomenon is ob-

served in people with cardiovascular diseases, in whose heart the fetal gene program is re-expressed, and the process of myocardial remodeling develops in the direction of decreasing the scale of structural heterogeneity (Naiditsch, 2006; Nechesova et al., 2008). The heart of fish, which has incomplete morphofunctional ontogenesis in comparison with mammals, can be considered an organ at the stage of embryonic development. The role of the spongy myocardium in ensuring the functional state of the fish heart and its electrophysiological and contractile functions are still controversial. A noncompact (spongy) myocardium occurs in humans when normal embryogenesis in the heart is disturbed and it can also be formed under the influence of various factors throughout life. The question of whether it is possible to use the fish heart as a model for experimental study of the mechanisms of cardiac arrhythmias, heart failure, and thanatogenesis in humans with noncompact cardiomyopathy remains unanswered.

Thus, an artifact of a HR of about 60 bpm at a low temperature (5–7 °C) in rainbow trout causes ventricular extrasystole. After extrasystole, the resumption of the activity of the sinus-atrial node occurs with a lower HR compared to the initial HR. Hemodynamic parameters of the heart, decreasing with extrasystole, return to the values at the initial sinus-atrial rhythm. After the experimental extrasystole, the phenomenon of the absence of an increase in HR is observed. The arising extrasystole probably has a functional nature and is one of the mechanisms of electromechanical homeostatic control in the heart.

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