

# Seasonal changes of electrophysiological heterogeneities in the rainbow trout ventricular myocardium

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## ABSTRACT

**Introduction:** Thermal adaptation in fish is accompanied by morphological and electrophysiological changes in the myocardium. Little is known regarding seasonal changes of spatiotemporal organization of ventricular excitation and repolarization processes. We aimed to evaluate transmural and apicobasal heterogeneity of depolarization and repolarization characteristics in the rainbow trout in-situ ventricular myocardium in summer and winter conditions.

**Methods:** The experiments were done in summer-acclimatized (SA, 18°C, n = 8) and winter-acclimatized (WA, 3°C, n = 8) rainbow trout (*Oncorhynchus mykiss*). 24 unipolar electrograms were recorded with 3 plunge needle electrodes (eight lead terminals each) impaled into the ventricular wall. Activation time (AT), end of repolarization time (RT), and activation-repolarization interval (ARI, a surrogate for action potential duration) were determined as dV/dt min during QRS-complex, dV/dt max during T-wave, and RT-AT difference, respectively.

**Results:** The SA fish demonstrated relatively flat apicobasal and transmural AT and ARI profiles. In the WA animals, ATs and ARIs were longer as compared to SA animals ( $p \leq 0.001$ ), ARIs were shorter in the compact layer than in the spongy layer ( $p \leq 0.050$ ), and within the compact layer, the apical region had shorter ATs and longer ARIs as compared to the basal region ( $p \leq 0.050$ ). In multiple linear regression analysis, ARI duration was associated with RR-interval and AT in SA and WA animals. The WA animals additionally demonstrated an independent association of ARIs with spatial localization across the ventricle.

**Conclusion:** Cold conditions led to the spatial redistribution of repolarization durations in the rainbow trout ventricle and the formation of repolarization gradients typically observed in mammalian myocardium.

## 1. Introduction

Spatiotemporal organization of ventricular excitation in vertebrates is determined by the spread of activation wave(s) and nonuniform repolarization. The former is determined by the morphology of conduction fibers, which sets mainly endo-to-epicardial activation sequence in most vertebrates. The nonuniformities of repolarization manifest as so-called repolarization gradients (differences in action potential durations between ventricular regions). At least, interventricular, apicobasal, and transmural repolarization gradients can be discerned in the ventricular myocardium (Arteyeva et al., 2013; Meijborg et al., 2014). The magnitude of these gradients varies significantly across animal species

and experimental conditions. Though the transmural gradient is elusive (Boukens et al., 2017), careful measurements usually demonstrate at least the small difference in repolarization timing between sub-endocardial and subepicardial regions, and the electrophysiological nature of these differences was shown long ago (Antzelevitch et al., 1991).

Ventricular myocardium in fish has spongy and compact muscle layers (Duran et al., 2015; Farrell et al., 2009; Kochova et al., 2015; Pieperhoff et al., 2009) expressed to a different extent in different species. The spongy layer is considered as a predecessor of a His-Purkinje system (Jensen et al., 2012), while the compact layer bears the most contractile load and is related to coronary vasculature (Farrell et al.,

**Abbreviations:** ARI, activation-repolarization interval; AT, activation time; IQR, interquartile range; RT, end of repolarization time; SA, summer-acclimatized; WA, winter-acclimatized.

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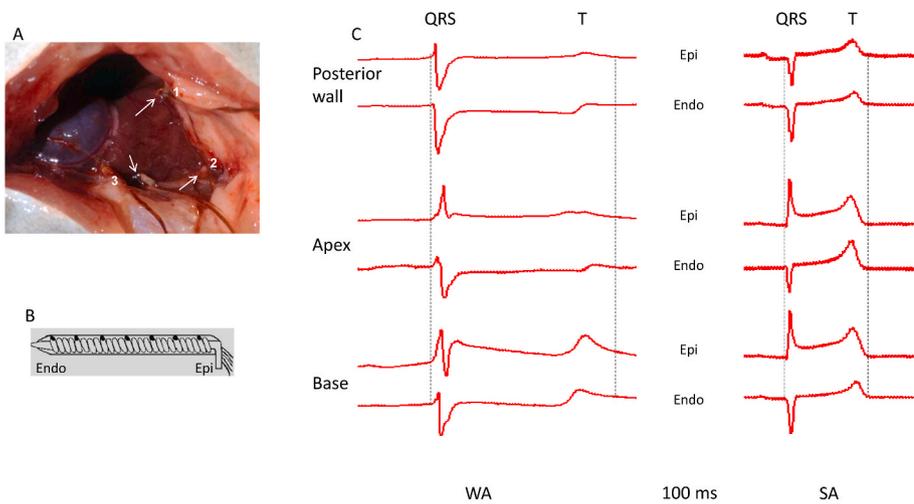
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**Fig. 1. Representative subepicardial (epi) and subendocardial (endo) electrograms recorded in the ventricle in the WA and SA rainbow trout.** A) Positions of placement of the plunge electrodes (white arrows) in the posterior wall near the atrioventricular junction (1), apex (2), and base (3). B) Schematic of the plunge needle electrode with eight leads. C) Unipolar electrograms recorded in epicardial and endocardial leads in different locations. Vertical dotted lines indicate QRS-complex onsets and the latest endings of T-waves (margins of QT-intervals). See prolonged QRS and QT-intervals in WA fish. The predominant polarity of QRS-complexes is negative in the subendocardial layers and positive in the subepicardial layers reflecting endo-to-epi activation spread in the base and apex of both WA and SA fish. In the SA fish, the T-waves were uniform across the ventricular myocardium. In contrast, the WA fish demonstrated different T-wave polarity and/or morphology in different layers and areas of the ventricle reflecting heterogeneous repolarization.

2009). It might be expected that the presence of the two different muscle layers would cause the development of the transmural repolarization gradient. However, the previous works did not demonstrate the difference in action potential duration between the inner and outer layers (Vaykshnorayte et al., 2011a; Patrick et al., 2011).

Thermal adaptation in fish is critical for survival, and the function of the heart is essential for this process (Farrell et al., 2009). Thermal adaptation modifies heart morphology and functional characteristics (Keen et al., 2017). Specifically, lowering the ambient temperature leads to a relative decrease of compact myocardium and the development of fibrosis in the ventricular wall, which could result in uncoupling of the compact and spongy layers (Keen et al., 2016; Klaiman et al., 2011). The temperature-dependent changes of cardiac electrophysiological properties that determine heart rate and conduction were shown to play an important role in seasonal acclimatization (Vornanen, 2016). However, the contractile function of the myocardium depends on coordination of the intraventricular electrical properties (Markhasin et al., 2012), but little is known up to date about seasonal changes of spatiotemporal patterns of ventricular depolarization and repolarization. It might be expected that such changes involve the ventricular electrophysiological patterns on different anatomical axes. In the present study, we tested this hypothesis by evaluating apicobasal and transmural profiles of depolarization and repolarization timing in summer-acclimatized (SA) and winter-acclimatized (WA) rainbow trout.

## 2. Materials and methods

The investigation was carried out in accordance with the *Guide for the Care and Use of Laboratory Animals*, 8th Edition published by the National Academies Press (US) 2011, and was approved by the institutional ethical committee.

Eight SA [18°C, August, median body weight 594 (IQR 354–800) g] and eight WA [3°C, April, median body weight 690 (IQR 490–700) g,  $p = 0.673$  vs SA] rainbow trout (*Onchorhynchus mykiss*) were studied at the fishery farm (located in Kirov Region, Russia, 58°40' N; 50°47' E). The animals were taken out from the fishery ponds immediately before the experiments. The measurements were performed at an ambient temperature approximately 2°C higher than the temperature of acclimatization. Fish were stunned by a sharp blow at the head and the spine was cut. After that, the heart was exposed via the ventral surface. The animals were studied under supraventricular (presumably, sinus) rhythm verified by 'limb' lead ECG. Unipolar electrograms were recorded from the ventricular walls of spontaneously beating hearts as described earlier (Vaykshnorayte et al., 2011a). In brief, three plunge needle electrodes were inserted in the ventricular wall at the apex,

anterior part of the base, and in the posterior region adjacent to the atrioventricular junction (Fig. 1, panel A). Each needle electrode bore eight lead terminals (Fig. 1, panel B) connected to the amplifiers of a custom-designed system for electrophysiological contact mapping (16 bits; bandwidth 0.05 to 1000 Hz; sampling rate 4000 Hz).

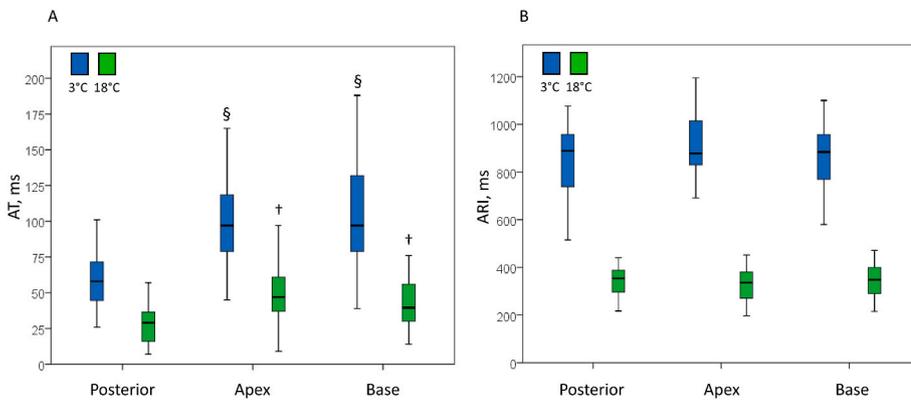
In unfiltered electrograms in each ventricular lead, activation time (AT) and end of repolarization time (RT) were determined as the instants of  $dV/dt$  minimum during QRS-complex and  $dV/dt$  maximum during T-wave, respectively (Coronel et al., 2006). An activation-repolarization interval (ARI, a surrogate for the action potential duration) was determined as the time differences between the RT and AT. The length of a plunge needle electrode was selected to match the thickness of the ventricular wall. Two outermost and two innermost lead terminals were referred to as compact layer and spongy layer terminals, respectively. In order to get one value for each layer, the data obtained from the two corresponding leads were averaged.

Data are expressed as medians and interquartile ranges (IQR). Statistical analysis was performed with the SPSS package (IBM SPSS Statistics 23, SPSS, Inc., Chicago, Illinois, USA). Wilcoxon and Friedman tests were applied for paired and multiple repeated comparisons, respectively. Mann-Whitney test was performed for comparisons between the WA and SA animals. Associations of ARI durations with electrophysiological (RR-interval and AT) and spatial (transmural and apicobasal positions) factors were evaluated with multivariate regression analysis (enter method). The electrophysiological factors, transmural positions, and apicobasal positions were tested as scale, ordinal and nominal variables, respectively. The differences were considered significant at  $p < 0.05$ .

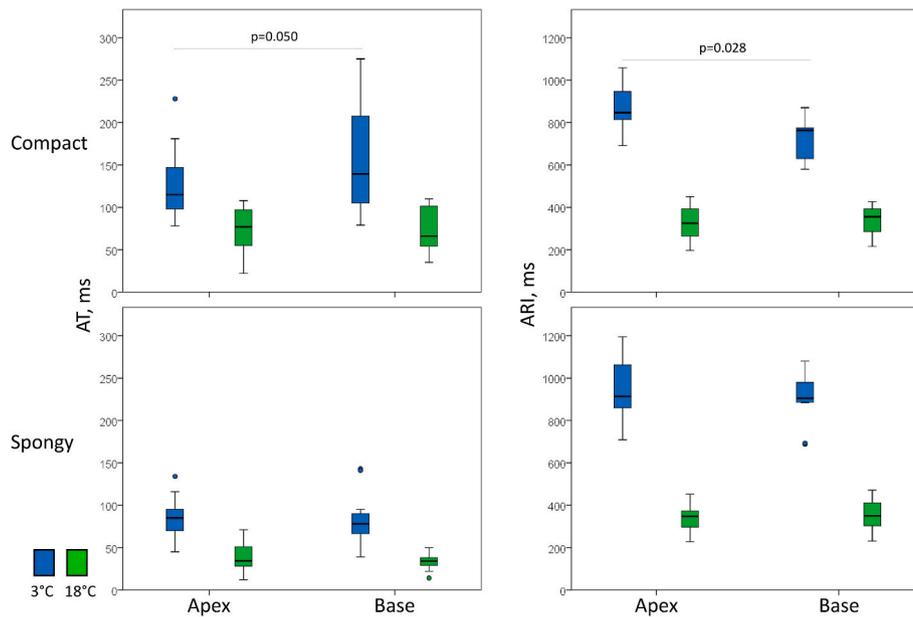
## 3. Results

The changes in ambient temperature caused significant effects on cardiac electrophysiological properties in rainbow trout. Fig. 1, panel C displays representative electrograms recorded in different ventricular areas in the subepicardial and subendocardial regions. RR-interval was longer in the WA as compared to SA animals [median 4045 (IQR 2360–5230) ms vs 1991 (IQR 1406–2767) ms,  $p = 0.029$ , respectively,  $n = 8$  for both groups]. At all studied sites, ATs and ARIs were significantly greater in cold conditions ( $p < 0.001$ ).

We first analyzed parameters of ventricular depolarization and repolarization in different ventricular areas averaged over wall thickness. An earliest ventricular activation was consistently observed in the posterior wall near the atrioventricular junction in the SA and WA fish. From this region, activation wave spread to the apex and anteriorly resulting in that the ATs in the apex and anterior base were significantly



**Fig. 2.** Activation times (AT, panel A) and activation-repolarization intervals (ARI, panel B) averaged over the entire thickness of the ventricular wall in different areas of the ventricle in SA (18°C, n = 8) and WA (3°C, n = 8). See longer temporal parameters in the WA animals ( $p \leq 0.001$  for all comparisons) and significantly earlier activation of the posterior wall near atrioventricular junction (Posterior) as compared to the heart apex (Apex) and anterior base (Base). See also a relatively flattened ARI distribution across the above areas both in SA and WA animals. † $p = 0.008$  vs Posterior, § $p = 0.012$  vs Posterior.



**Fig. 3.** Apicobasal distribution of activation times (AT) and activation-repolarization intervals (ARI) separately in the compact and spongy layers in SA (18°C, n = 8) and WA (3°C, n = 8) animals. See the opposite distribution of ATs and ARIs in the compact layer of WA animals.

longer as compared to the posterior wall both in SA and WA fish (Fig. 2, panel A). Repolarization duration distribution on the apicobasal axis was relatively uniform. The ARI durations did not differ between the three ventricular areas in both groups of animals (Fig. 2, panel B).

Then, separately in the compact (subepicardial) and spongy (subendocardial) layers, we compared the timing of depolarization and repolarization between the apex and base (Fig. 3). The SA fish had a relatively uniform distribution of both ATs and ARIs in both layers with no statistically significant differences being observed between the apical and basal regions. The WA animals had a similar uniform apicobasal distribution of depolarization and repolarization timing in the spongy layer. However, apicobasal differences were observed in the compact layer in the WA fish, namely, the apical region had a shorter AT and longer ARI as compared to the subepicardial basal region.

On the transmural axis both in SA and WA rainbow trout, activation wave spreads from endocardium to epicardium. Fig. 4 demonstrates that the subendocardial ATs were shorter than the subepicardial ATs at both temperatures. The transmural profile of ARIs was relatively flat at 18°C (Fig. 5). However, in the WA animals, a significant transmural difference in ARI durations was observed in all studied ventricular areas with the subepicardial ARIs being shorter than the subendocardial ARIs (Fig. 5).

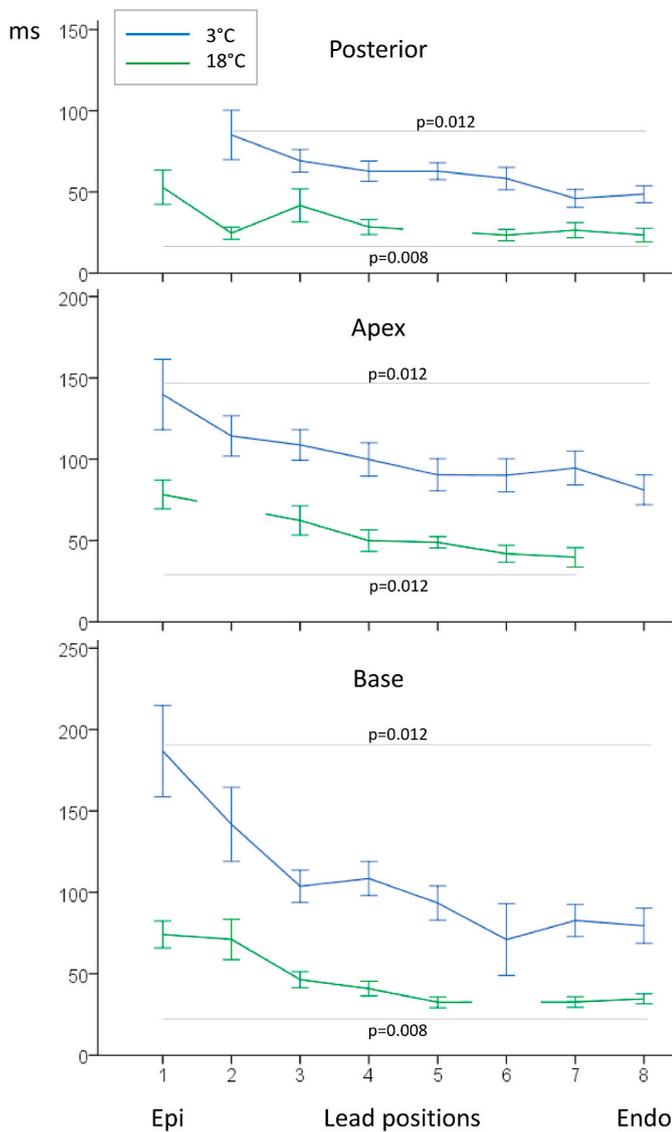
In multivariate regression analysis (Table 1), we tested the association of ARI with the factors expected to affect repolarization duration:

RR-interval, AT, and position on the transmural and apicobasal axes. The ARI was associated positively with RR-interval and negatively with AT both in the SA and WA animals (Fig. 6). However, the ARI in the WA fish also demonstrated a significant association with the spatial position independently of RR-interval and AT (Table 1). Since AT and ARI were inversely related to each other, the distribution of RT (the sum of AT and ARI) was relatively uniform across the ventricular myocardium (Table 2).

#### 4. Discussion

The present study demonstrated the development of electrophysiological heterogeneities in the rainbow trout heart in cold conditions. A significant difference in the repolarization durations across the ventricular wall (shorter epi vs longer endo) was formed in the WA animals. This transmural repolarization gradient was opposite to the transmural AT sequence (shorter endo vs longer epi). Furthermore, lowering the ambient temperature was associated with the development of the opposite apicobasal activation and repolarization duration gradients in the compact layer of the ventricle.

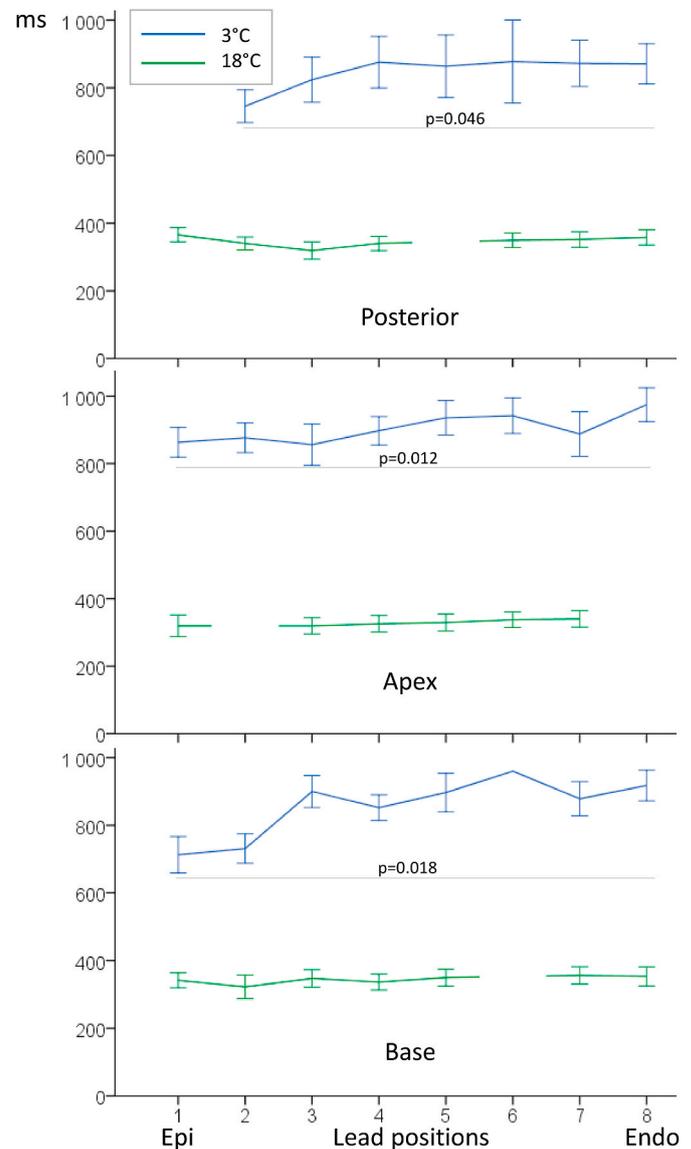
Cold conditions led to slowing the electrophysiological processes, which manifested as the decreased heart rate (prolonged cardiac cycle length estimated as RR-intervals), prolonged ventricular activation



**Fig. 4.** Transmural profiles of activation times (AT) in SA (18°C, n = 8) and WA (3°C, n = 8) rainbow trout in the posterior wall near the atrioventricular junction (Posterior), apex (Apex), and anterior base (Base). The numbers from 1 to 8 on the horizontal axis indicate leads on the intramural needle from the epicardium (lead 1) to the endocardium (lead 8). The missing points relate to bad quality signals from some leads. See a progressive increase of ATs from endocardium to epicardium especially in the WA animals. P-values indicate statistically significant differences between the inner- and outermost leads.

(delayed ATs), and repolarization (prolonged ARIs). Prolonged ventricular activation is likely due to the reported accumulation of connective tissue in the ventricular wall caused by cold acclimation (Klaiman et al., 2011; Keen et al., 2016). Prolongation of repolarization and heart rate decrease was observed despite the expected temperature compensation (Haverinen and Vornanen, 2007; Aho and Vornanen, 2001; Hassinen et al., 2008; Abramochkin and Vornanen, 2015). The mechanism of ARI prolongation cannot be explained on the basis of the obtained data, but theoretically, it should result from an altered balance between depolarizing and repolarizing currents in favor of depolarization.

Probably, the most important finding of the present study is that in cold conditions repolarization is prolonged nonuniformly. This heterogeneous response resulted in the development of repolarization duration gradients opposite to the activation sequence in the corresponding



**Fig. 5.** Transmural profiles of activation-repolarization intervals (ARI) in SA (18°C, n = 8) and WA (3°C, n = 8) rainbow trout in the posterior wall near the atrioventricular junction (Posterior), apex (Apex), and anterior base (Base). The numbers from 1 to 8 on the horizontal axis indicate leads on the intramural needle from the epicardium (lead 1) to the endocardium (lead 8). The missing points relate to bad quality signals from some leads. See a flat ARI distribution in the SA fish and a progressive increase of ARIs from epicardium to endocardium in the WA fish. P-values indicate statistically significant differences between the inner- and outermost leads.

directions. On the apicobasal axis, ARIs were longer in the apex than in the base within the external (compact) layer of the myocardium. On the transmural axis, ARIs progressively increased from epicardium to endocardium. Previously, our group reported that acute heart cooling induced predominant prolongation of repolarization in the apical region of the heart both on the epicardial surface of amphibian (Valkshnorayte et al., 2007) and mammalian (Azarov et al., 2008) heart. In the present study, we found that a similar change developed in seasonal acclimatization in fish hearts and that this effect is limited to the compact myocardial layer.

This is the first, to our knowledge, observation of a transmural direction of activation spread in the fish heart. In contrast to pike (Vaykshnorayte et al., 2011a), the activation wave in the rainbow trout ventricle propagated not only along the walls but also from

**Table 1**  
Associations of ARI durations with electrophysiological and spatial factors in SA (18°C) and WA (3°C) rainbow trout.

Predictor	SA, n = 8		WA, n = 8	
	B (95% CI) <sup>a</sup>	p	B (95% CI)	p
RR	14.77 (9.17 – 20.36)	<0.001	64.32 (49.13 – 79.50)	<0.001
AT	-0.95 (-1.40 – -0.49)	<0.001	-0.82 (-1.51 – -0.14)	0.019
Transmural position <sup>b</sup>	-2.15 (-6.73 – 2.43)	0.355	12.23 (1.57 – 22.89)	0.025
Apicobasal position <sup>c</sup>	6.35 (-5.17 – 17.88)	0.278	46.53 (14.92 – 78.14)	0.004

<sup>a</sup> - regression coefficient and 95% confidence interval.  
<sup>b</sup> - ordinal variable from epi to endo.  
<sup>c</sup> - nominal variable, AT – activation time, RR – RR-interval in ECG.

endocardium to epicardium in a similar way as in other vertebrates. This activation pattern was found both in the SA and WA fish, but in the WA animals, a steeper AT gradient was usually observed, especially across a transition region between the compact and spongy layers (see the bottom panel in Fig. 4). Though other explanations are not excluded, this activation delay could be due to the accumulation of connective tissue in this area (Klaiman et al., 2011; Keen et al., 2016). It can be expected that relative development of the spongy and compact layers in different fish species affects activation patterns and specifically might account for the presence or absence of the transmural activation pattern. In mammals, the transmural gradient of repolarization durations has also been recorded (Arteyeva et al., 2013; Meijborg et al., 2014); however, it is noteworthy that it is much easily found in isolated myocardial preparations than in a whole heart (Boukens et al., 2017). In the present study, it was shown that the transmural repolarization duration gradient can also be observed in the fish ventricle, and this gradient is subject to temperature-induced changes since it was demonstrated only in the WA but not SA animals.

The differences in repolarization durations observed across ventricular myocardium can be due to regional intrinsic electrophysiological differences (expression of channel proteins etc) and electrotonic interaction between adjacent sites (Vaykshnorayte et al., 2011b). The latter results in that the closely spaced myocardial regions tend to finish the repolarization process at the same time. It means that the earlier activated regions would have longer action potential durations, while those activated later would have shorter action potential durations. In other words, electrotonic interaction would result in the inverse relationship between ATs and ARIs with no prerequisites of any intrinsic spatial differences in ion transport machinery. Importantly, such a mechanism suggests that the differences in repolarization durations between the

regions should be comparable with corresponding differences in activation times. However, in some cases, the observed difference in repolarization durations is far greater than the AT difference. This situation suggests that the nonuniformities of repolarization could not be entirely ascribed to the electrotonic interaction and there are some intrinsic differences between the regions in question, which could develop during ontogeny or form due to remodeling processes.

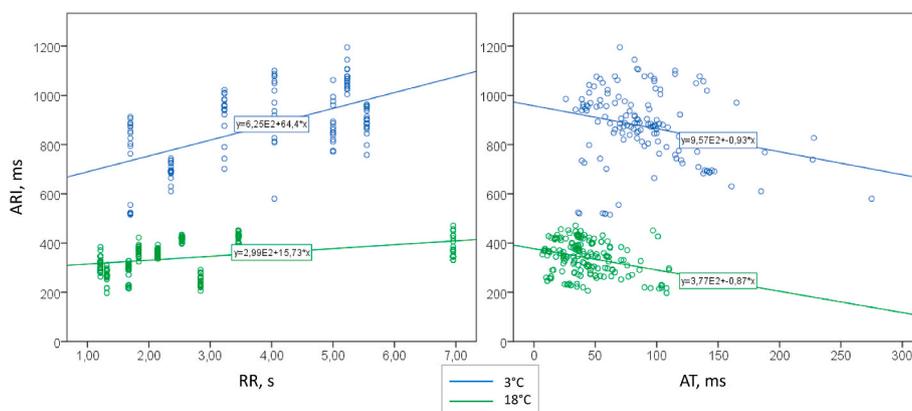
To find out whether the ARI distribution in the WA and SA rainbow trout was governed only by electrotonic interaction or any seasonal remodeling process is involved, we performed multivariate regression analysis (Table 1 and Fig. 6). As tested predictors, it included RR-interval, AT, and a spatial location on the transmural and apicobasal axes. If the spatial location is independently associated with the ARI duration, it suggests the presence of intrinsic repolarization heterogeneities. We found such an association in the WA but not SA animals that could be explained by myocardial electrical remodeling during cold adaptation resulting in the development of transmural and apicobasal heterogeneities of repolarization durations.

The obtained data suggest that the cold-induced changes are related to the increased hemodynamical load in cold conditions. At least part of the observed WA vs SA differences was related to the compact myocardial layer that is considered as a predecessor of contractile myocardium (Jensen et al., 2012). Moreover, apicobasal as well as transmural repolarization distribution in the WA fish is spatially inversely related to the activation sequence, i.e., the earlier the AT, the longer the ARI, and vice versa. This activation-repolarization pattern can facilitate the contraction process, since optimal coordination of the contractile elements requires that activation and therefore contraction proceeds from “slow” to “fast” contracting muscle elements that have long and short action potential duration, respectively (Markhasin et al., 2012).

Limitations of the study. The seasonal changes in the electrophysiological properties of the rainbow trout heart were probably due to thermal adaptation. However, our experimental settings did not allow us

**Table 2**  
Apicobasal and transmural distributions of RTs (medians and interquartile ranges) in SA (18°C, n = 8) and WA (3°C, n = 8) rainbow trout.

		Posterior	Apex	Base	p
18°C	Compact	378 (330–429)	382 (336–447)	404 (358–454)	0.889
	Spongy	380 (331–430)	378 (333–435)	380 (333–446)	0.767
	P	0.813	0.575	0.173	
3°C	Compact	820 (728–936)	944 (917–1082)	893 (855–961)	0.091
	Spongy	987 (814–1018)	1004 (961–1163)	993 (966–1057)	0.866
	P	0.116	0.012	0.028	



**Fig. 6.** Scatter plots of activation-repolarization intervals duration (ARI) vs RR-interval and activation time (AT) with regression equations in SA (18°C, n = 8) and WA (3°C, n = 8). See a significant direct relationship between ARI and RR (left) and an inverse relationship between ARI and AT (right).

to demonstrate adaptation changes directly. The animals were kept in the fishery ponds according to industrial standards, where some variation of ambient water conditions cannot be excluded. The fact that the measurements were performed at the temperature of acclimatization did not permit distinguishing between long-term thermal adaptation and direct (acute) effects of temperature. The damage to the central nervous system might cause functional effects on the cardiovascular system including a significant decrease in heart rate. The above considerations warrant cautious interpretation of the obtained data.

## 5. Conclusion

Thus, the present study demonstrated that the cold conditions could induce the changes in the spatiotemporal organization of the ventricular electrophysiological properties leading to the development of repolarization gradients of a mammalian type. It warrants further investigation of the mechanism and functional significance of the found seasonal electrophysiological changes.

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## CRedit authorship contribution statement

**Marina A. Vaykshnorayte:** Methodology, Investigation, Formal analysis, draft writing. **Vladimir A. Vityazev:** Methodology, Investigation, Writing – review & editing. **Jan E. Azarov:** Conceptualization, Funding acquisition, Methodology, Investigation, Formal analysis, draft writing.

## 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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