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ORIGINAL PAPER

Transcript expression of inward rectifier potassium channels of Kir2 subfamily in Arctic marine and freshwater fish species

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Abstract

Inward rectifier K^+ (Kir2) channels are critical for electrical excitability of cardiac myocytes. Here, we examine expression of Kir2 channels in the heart of three Gadiformes species, polar cod (*Boreogadus saida*) and navaga (*Eleginus nawaga*) of the Arctic Ocean and burbot (*Lota lota*) of the temperate lakes to find out the role of Kir2 channels in cardiac adaptation to cold. Five boreal freshwater species: brown trout (*Salmo trutta fario*), arctic char (*Salvelinus alpinus*), roach (*Rutilus rutilus*), perch (*Perca fluviatilis*) and pike (*Esox lucius*), and zebrafish (*Danio rerio*), were included for comparison. Transcript expression of genes encoding Kir2.1a, − 2.1b, − 2.2a, − 2.2b and − 2.4 was studied from atrium and ventricle of thermally acclimated or acclimatized fish by quantitative PCR. Kir2 composition in the polar cod was more diverse than in other species in that all Kir2 isoforms were relatively highly expressed. Kir2 composition of navaga and burbot differed from that of the polar cod as well as from those of other species. The relative expression of Kir2.2 transcripts, especially Kir2.2b, was higher in both atrium and ventricle of navaga and burbot (56–89% from the total Kir2 pool) than in other species (0.1–11%). Thermal acclimation induced only small changes in cardiac Kir2 transcript expression in Gadiformes species. However, Kir2.2b transcripts were upregulated in cold-acclimated navaga and burbot hearts. All in all, the cardiac Kir2 composition seems to be dependent on both phylogenetic position and thermal preference of the fish.

Keywords Fish heart · Inward rectifier potassium channels · Kir2 paralogues · Thermal acclimation · RT-qPCR

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Abbreviations

AP	Action potential
bp	Base pair(s)
cDNA	DNA complementary to RNA
DNase	Deoxyribonuclease
Dnaja2	DnaJ (hsp40) subfamily A member 2
dNTP	Deoxyribonucleoside triphosphate
gDNA	Genomic DNA
I_{K1}	Inward rectifying potassium current
I_{Na}	Sodium current
Kir	Inward rectifier potassium channel
PCR	Polymerase chain reaction
qPCR	Quantitative real-time polymerase chain reaction
RT	Reverse transcriptase
WGD	Whole genome duplication

Introduction

Temperature is a critical environmental factor for ectothermic animals by determining the rate of biochemical reactions and physiological processes of organisms (Precht

et al. 1955). Most fishes are ectotherms and physiologically adapted to tolerate temperature changes within species-specific limits. The thermal tolerance limits and thermal preferenda of fish vary at different stages of life history and differ for different activities like metabolism, reproduction, growth, locomotion and digestion (Clark et al. 2013; Fry 1971; Hofmann and Fischer 2002; Holt and Jorgensen 2015).

High temperatures depress heart rate and cardiac output and thereby the aerobic performance of the fish (Eliason et al. 2013; Farrell 2009). In terms of global warming, temperature-dependence of cardiac function might be particularly critical for survival, distribution and abundance of fish in future climates. Recent findings suggest that the high temperature-induced depression of heart rate in fish may be caused by the failure of electrical excitation of the heart (Badr et al. 2016; Vornanen 2016).

Contraction of atrial and ventricular myocytes of the vertebrate heart are activated by a small depolarization of sarcolemmal from the resting membrane potential of about − 80 mV to the threshold (about − 65 mV) of the cardiac action potential (AP). Once generated, AP propagates in orderly manner through the cardiac syncytium and maintains pump function of the heart under changing physiological and environmental conditions including temperature changes (Harris 1941; Ramanathan et al. 2006; Vornanen 2017). In working cardiac myocytes, electrical excitability, i.e., the ease with which cardiac myocytes are triggered to fire propagating (all-or-none) APs by the flow of transmembrane ion currents, critically depends on the balance between the inward (depolarizing) Na^+ current (I_{Na}) and the outward (repolarizing) K^+ current (I_{K1}) (Milstein et al. 2012, Varghese 2016). In atrial and ventricular myocytes, I_{K1} is the main ion current that determines the negative resting membrane potential, and therefore the strength of depolarization needed to bring membrane potential to the firing threshold of the cardiac AP. In atrial myocytes, the acetylcholine-sensitive inward rectifier current (I_{KAch}) may also contribute to the resting membrane potential (Abramochkin et al. 2014; Molina et al. 2007). At the threshold potential, the density of the depolarizing I_{Na} exceeds the density of repolarizing I_{K1} (and I_{KAch}) and results in generation of propagating AP (Varghese 2016). Because I_{Na} and I_{K1} have opposite effects on cardiac excitability, differences in heat-resistance between I_{Na} and I_{K1} may result in thermal deterioration of electrical excitability (Vornanen 2016, 2017). Indeed, I_{Na} is depressed at temperatures, where I_{K1} continues to increase generating a mismatch between I_{Na} and I_{K1}: the diminished I_{Na} may be unable to depolarize the membrane to action potential threshold, since the increased resting leakage of K^+ through I_{K1} channels effectively resist it. This may result in a failure of electrical excitation of myocytes in particular in the ventricle (Vornanen 2016).

Previous studies have shown that the response of I_{K1} to chronic temperature changes is variable between fish species and cardiac chambers and may be partly phylogenetically determined (Abramochkin and Vornanen 2015; Badr et al. 2017; Haverinen and Vornanen 2009). In some species, like crucian carp (*Carasssius carassius*), roach (*Rutilus rutilus*), burbot (*Lota lota*) and European perch (*Perca fluviatilis*) ventricular I_{K1} is upregulated under cold-acclimation, while in others (e.g., bluefin tuna, *Thunnus thynnus*) it may be unresponsive to thermal acclimation or depressed in the chronic cold (e.g., rainbow trout, *Oncorhynchus mykiss*) (Galli et al. 2009; Hassinen et al. 2007; Haverinen and Vornanen 2009; Vornanen et al. 2002). Since I_{K1} is critical for temperature-dependence of electrical excitation of cardiac myocytes, we decided to examine transcript expression of the Kir2 channels, which generate the cardiac I_{K1}, in species which differ in regard to temperature preferences/tolerances. To this end, we compared mRNA expression of the cardiac Kir2 channels in three closely related species of the order Gadiformes: polar cod (*Boreogadus saida,* Lepechin, 1774; Gadiformes, Gadidae), navaga (*Eleginus nawaga*, Pallas 1811; Gadiformes, Gadidae) and burbot (*Lota lota*, Linnaeus, 1758; Gadiformes, Lotidae). These are regarded as stenothermic species, but they differ in their thermal preferences and thus the extent of their stenothermicity varies. The polar cod is the most stenothermic being a cryopelagic species that prefers cold and icy waters of the Arctic Ocean. The navaga also live in Arctic Ocean, but they are less stenothermic, and flourish in the White Sea where water temperatures in summer may exceed 15 °C. The burbot is the only gadiform species that has managed to invade the freshwater lakes of temperate latitudes. Burbot have retained some characteristics of their stenothermic relatives but are probably less stenothermic than polar cod or navaga as indicated by better heat-resistance of electrical excitability in ventricular myocytes of burbot in comparison to those of navaga and polar cod (Abramochkin et al. 2019). For comparative purposes, Kir2 expression of some other boreal species: brown trout (*Salmo trutta fario*, Salmoniformes), arctic char (*Salvelinus alpinus*, Salmoniformes), European perch (*Perca fluviatilis*, Perciformes), Northern pike (*Esox lucius*, Esociformes) and roach (*Rutilus rutilus*, Cypriniformes), as well as the model species zebrafish (*Danio rerio*, Cypriniformes) are provided. Functional studies on cardiac AP and K^+ currents of several freshwater teleost species has shown that phylogenetic differences exist in the ability to adjust the electrical function of the cardiac myocytes to different temperatures (Haverinen and Vornanen 2009). Based on those findings, it was hypothesized that the cardiac Kir2 composition of teleost fish is dependent on both phylogeny and thermal preferences of the fish.

Materials and methods

Animals

Polar cod were caught on 16th and 21st of September 2014 from Admiralty peninsula (75°21′N, 55°02′E, water temperature − 1 °C, Table 1) and Perseus elevation (77°37′N, 43°37′E, water temperature 0 °C) of the Barents Sea (Russia) during the expedition of joint Russian–Norwegian ecosystem survey (Barents ecosystem survey, BESS). The polar cod were caught from the two locations in the same season and are all regarded as cold-acclimatized despite of the one centigrade difference in the habitat temperature. Navaga were caught on 20th and 21st of March (water temperature − 0.9 °C) and on 9th of September (water temperature 8–9 °C) 2012 near The White Sea Biological Station of Lomonosov Moscow State University (66°19′50″N, 33°40′06″E) located at the Kandalaksha Bay of The White Sea (Russia). The burbot were caught in February 2014 from the lake Pyhäselkä (Finland). Perch, roach and pike were caught from local lakes near the University of Eastern Finland in April and September 2017, July and September 2015, and August and November 2010, respectively. Brown trout and arctic char were obtained from Kontiolahti and Enonkoski fish farms, respectively. Fish were randomly divided in two groups and maintained in 500-l stainless steel tanks at + 4 and + 18 °C under 12:12 light:dark photoperiod for at least 4 Weeks before experiments. Fish were fed three times a week with aquarium fish food (Tetra, Melle, Germany). All experiments were conducted with the permission of the National Committee for Animal Experimentation (permission ESAVI/2832/04.10.07/2015).

RNA extraction

Fish were stunned by a blow to the head and the spine was cut at the neck. Atrium and ventricle of navaga and polar cod were excised and stored in RNAlater solution (Invitrogen, supplied by Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) for the delivery of samples to Joensuu (Finland), and the tissues of other fish species were frozen in liquid nitrogen immediately after excision of the tissue. In the laboratory, the samples were stored at − 80 °C until used in experiments. The RNA was extracted by TRI Reagent Solution (Thermo Scientific, Vilnius, Lithuania) and stored at − 80 °C (for RNA concentrations see Table S1). The quality and quantity of DNA and RNA was checked by agarose gel electrophoresis and NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), respectively. The RNA was treated with RNase-free DNaseI (Thermo Scientific or Promega, Madison, Wisconsin, USA) to degrade the genomic DNA potentially present in the RNA samples.

Cloning and sequencing

Genomic DNA was extracted using phenol:chloroform extraction (Sambrook et al. 1989), quantified by NanoDrop and qualified by agarose gel electrophoresis. DNase-treated RNA was translated to cDNA by RevertAid Premium reverse transcriptase or Super Script IV reverse transcriptase (Thermo Scientific) using random hexamers. 1–2 µl of the cDNA or 50 ng of gDNA was used as a template in 25 µl polymerase chain reaction (PCR-I) including the final concentration of 0.2 mM dNTP mix, 0.2 mM primers (synthesized by Invitrogen, Glasgow, UK) presented in Table 2,

Table 1 Thermal tolerances, acclimation/acclimatization temperatures and body weights (mean ± SE) of the fish

Species	Thermal tolerance	Cold acclimation/acclimatization			Warm acclimation/acclimatization		
		Temperature	*N*	Weight (g)	Temperature	*N*	Weight (g)
Arctic char	0–23 (Elliott and Elliott 2010)	+ 4	6	96.0 ± 10.4	+ 18	6	104.4 ± 11.4
Polar cod	− 1.4 to 17.1 (Drost et al. 2016)	− 1	5	61.6 ± 1.0	0[a]	5	52.9 ± 1.4
Brown trout	0–25 (Elliott and Elliott 2010)	+ 4	6	425.0 ± 64.7	+ 18	6	323.2 ± 122.6
Burbot	3–32 (Hofmann and Fischer 2002)	+ 4	5	259.8 ± 29.6	+ 18	5	318.9 ± 87.5
Navaga	− 1 to > 15[b] (DeVries and Steffensen 2005)	− 0.9	6	41.7 ± 11.4	+ 8 to 9	5	94.0 ± 9.4
Perch	0–32 (Horoszewicz 1973)	+ 4	6	64.5 ± 16.8	+ 18	6	31.2 ± 8.4
Pike	0–33 (Hokansson 1977)	+ 4	6	1086.7 ± 205.3	+ 18	3	182.2 ± 67.1
Roach	0–35 (Cocking 1959)	+ 4	6	61.6 ± 7.1	+ 18	6	12.9 ± 3.3
Zebrafish	11–42 (Cortemeglia and Beitinger 2005)	+ 18	5[c]	0.71 ± 0.05	+ 28	5[c]	0.68 ± 0.03

[a]Polar cod is not really acclimatized to warm temperatures, but they were collected from two different locations with different temperatures and for clarity polar cod from warmer habitat are marked as warm-acclimatized

[b]No experimental data is available for the thermal tolerance of navaga, but they are able to live in the White Sea where water temperature may exceed 15 °C during summer

[c]Each sample constituted from cardiac tissue collected from 5 fish, thus the total number of fishes used was 25

Table 2 Primer pairs (in 5′–3′ orientation) used in cloning and qPCR

Gene	Primers for cloning	bp	Primers for qPCR	bp
Arctic char (*Salvelinus alpinus*)				
Kir2.1aa	F: ATGGGAAGTGTGCGGGCCAAC	1284	F: GCCAACCGCTACAGCATAGT	150
	R: TCATATTTCAGATTCTCGTCTTAGGG		R: ATTGCAGTGACCGTCCTTCT	
Kir2.1ab	–	–	F: GCCAACCGCTACAGCATAGT	150
			R: ATTGCAGTGACCGTCCTTCT	
Kir2.1b	F: CCAGGTGCAACAGCAGCAGG	1652	F: GAGGTGGCCCTTGAAAAAGT	133
	R: CCGTTAGGAGCAAAGGTCAAAC		R: CAATGCCGTTCTCAATAGCA	
Kir2.2aa	F: ATGAGTGTGGGGCGTCTCAACCGTTACA	1344	F: GTCCACTCTATGGGATTGGT	101
	R: TCATATCTCCGACTCCCTGCGGTAT		R: CTGTGTGGTCATGGCAGTC	
Kir2.2ab	F: ATGAGTGTGGGGCGTCTCAACCGTTACA	1335	F: AGTCCACTCTATGGGATCAGG	101
	R: TCATATCTCCGACTCCCTGCGGTAT		R: TGCGTGGTCATGGCGGTT	
Kir2.2ba	–		F: GTGTCCTCGGAGGAAGAAGG	129
			R: GCCGTTCTTGTTCACGAAG	
Kir2.2bb	–		F: GTGTCCTCGGAGGAAGAAGG	129
			R: GCCGTTCTTGTTCACGAAG	
Kir2.4	F: CGCTTTGTGGGCAAGGAC	725	F: TTCGTCAACATGAGCGAGAG	103
	R: GGTGAGACCAARAAGATGCG		R: GGAAGGAGAGGCAGAAGATG	
DnaJa2	F: CCAAATGCTGGHGACAARTTCAA	437	F: TGCAGCTGAGCAAGAATGTT	110
	R: ACWGACTGCATCTGTTGDACCAT		R: GATCATGATGCGCATACCAC	
Polar cod (*Boreogadus saida*)				
Kir2.1a	F: TGGCCGACATCTTCACCAC	696	F: TTGGAGAACAATGGCCAGAAGT	121
	R: TCCACCATGCCCTCCAGGAT		R: GACACTCGTCGGTCACGTA	
Kir2.1b	F: ATGGGGAGCGTGCGWAGCCA	911	F: CTGCAGAGCGAGAGGCAGATGT	121
	R: TCCACCATGCCCTCCAGGAT		R: GGCACTCCTCGGTGACGTA	
Kir2.2a	F: ATGACYGCDGCCAGYCGGGCCAAC	624	F: CGCCTTTCTGTTCTCCATCG	103
	R: CGCCACATCAGACTCAGCTTGCCGT		R: GGACTGGAAGACCACCATG	
	F: GTCTTCATGGTGGTGGCBCAGTCCAT	785		
	R GCCTGSAGYCTGTCAAAGTCATGC			
Kir2.2b	F: ATGAGYGTTGGAMGGASCCMCCACTA	1117	F: ACACCGACGCCTTCACGC	82
	R: AKGTCGAACTCTTCYGRTCVTSGAT		R: GTGGTCTGCGTCTCCACGGA	
Kir2.4	F: CGCTTTGTGGGCAAGGAC	502	F: TTCGAGCCGTGCTTCCTCC	110
	R: TTGCGCTTCTTGGGCTTGG		R: GGACACTCCTCGGTTATGCT	
DnaJa2	F: AAGGAGCTGTACGACCGCTA	237	F: AAGGAGCTGTACGACCGCTA	93
	R: CTTGCTGAGCTGAAGTTTGG		R: CCGAAGATGTGGGAGAAGATGT	
Brown trout (*Salmo trutta fario*)				
Kir2.1aa	F: ATGGGAAGTGTGCGRGCCAA	1284	F: GCCAACCGCTACAGCATAGT	150
	R: TYATATTTCWGATTCTCGYCTYAG		R: ATTGCAGTGACCGTCCTTCT	
Kir2.1ab	F: ATGGGAAGTGTGCGRGCCAA	1278	F: GCCAACCGCTACAGCATAGT	150
	R: TYATATTTCWGATTCTCGYCTYAG		R: ATTGCAGTGACCGTCCTTCT	
Kir2.1ba	F: ATGGGGAGCGTGCGWAGCCAR:	1275	F: GAGGTGGCCCTTGAAAAAGT	133
	CTCCTCCAGTGTTGCRTTCTCA		R: CAATGCCGTTCTCAATAGCA	
Kir2.1bb	F: ATGGGGAGCGTGCGWAGCCA	1281	F: GAGGTGGCCCTTGAAAAAGT	133
	R: CTCCTCCAGTGTTGCRTTCTCA		R: CAATGCCGTTCTCAATAGCA	
Kir2.2aa	F: ATGAGTGTGGGGCGTCTCAACCGTTACA	1332	F: GTCCACTCTATGGGATTGGT	101
	R: TCATATCTCCGACTCCCTGCGGTA		R: CTGTGTGGTCATGGCAGTC	
Kir2.2ab	F: ATGAGTGTGGGGCGTCTCAACCGTTACA	1335	F: GTCCACTATATGGGATCAGG	101
	R: TCATATCTCCGACTCCCTGCGGTA		R: CTGCATAGTCATGGCGGTT	
Kir2.2ba	F: TAGCTGAGTCTGGCTCGGTT	1345	F: GTGTCCTCGGAGGAAGAAGG	129
	R: AATATATTGTCAAAGGTYTAAGGTC		R: GCCGTTCTTGTTCACGAAG	

Table 2 (continued)

Gene	Primers for cloning	bp	Primers for qPCR	bp
Kir2.2bb	F: GGCTGAGCCTGRCTTGGTC	1341	F: GTGTCCTCGGAGGAAGAAGG	129
	R: GAAAGGTCAAATGTCAAAGGTC		R: GCCGTTCTTGTTCACGAAG	
Kir2.4	F: AACGTCACCTTCGTCAACAT	402	F: TTCGTCAACATGAGCGAGAG	103
	R: CACAATGCACTGCAAAACG		R: GGAAGGAGAGGCAGAAGATG	
DnaJa2	–	–	F: TGCAGCTGAGCAAGAATGTT	110
			R: GATCATGATGCGCATACCAC	
Burbot (*Lota lota*)				
Kir2.1a	F: ATGGGAAGTGTGCGGGCCAAC	917	F: AAGAGCCACCTCGTGGAAG	101
	R: GTCATGGCRGTSGCYTCSACCATGCC		R: ACGTCGATGTCCGTCTGGT	
Kir2.1b	F: ATGGGGAGCGTGCGWAGCCA	1271	F: TGGAGGAGGAGTTTGAGGAA	123
	R: GACACGGGATCCTCCGTTCTT		R: TGTCTTCGGAGTCTGACACG	
Kir2.2a	F: GGTGCCGCAACCGCTTYGTCAAGAA	1033	F: TCATCAAACCTCGGATCACC	102
	R: GCCTGSAGYCTGTCAAAGTCATGC		R: AAACCAGGAAAATCCGATCC	
Kir2.2b	F: ATGAGYGTTGGAMGGASCCMCCACTA	1108	F: GATGACGACACGTTCACTCCTTGC	83
	R: AKGTCGAACTCTTCYGRTCVTSGAT		R: GTCGTCTGCGTCTCAACCGA	
Kir2.4	F: AACGTCACCTTCGTCAACAT		F: TTCCTCCAGGTCAACAGCTT	101
	R: TTGCGCTTCTTGGGCTTGG	494	R: CAGGGGACACTCTTCGGTTA	
DnaJa2	–	–	F: AAGGAGCTGTACGACCGCTA	93
			R: CCGAAGATGTGGGAGAAGATGT	
Navaga (*Eleginus navaga*)				
Kir2.1a	–	–	F: ATCTTCTGCCTGGCCTTCAT	102
			R: GCACTTCTGGCCATTGTTCT	
Kir2.1b	F: ATGGGGAGCGTGCGWAGCCA	908	F: GGTCTTCTGCTTCTCCTTCC	101
	R: TCCACCATGCCCTCCAGGAT		R: ACATCTGCGTGTCGCTCTG	
Kir2.2a	F: CTGGGTCATAGCCCTGCT	819	F: AGAAGAACGTCTACAAGGTGG	124
	R: TTGGAGTTGGAGCTGGG		R: TTGGAGTTGGAGCTGGG	
Kir2.2b	F: ATGAGYGTTGGAMGGASCCMCCACTA	1058	F: CTGTCCTCTGAGATCCTGTG	93
	R: AKGTCGAACTCTTCYGRTCVTSGAT		R: CTTGTGAAAGTGTGCGTAGTC	
Kir2.4	F: AACGTCACCTTCGTCAACAT	494	F: TCCTCCAGGTCAACAGCTTC	100
	R: TTGCGCTTCTTGGGCTTGG		R: AGGGGACACTCCTCGGTTAT	
DnaJa2	–	–	F: TGGAGAGCAGGGGCTACGAG	93
			R: AAGCCGAACAGTCCTCCACCA	
Perch (*Perca fluviatilis*)				
Kir2.1a	F: CATCATCGGCGCCGTCATGGCCAAGAT	917	F: CACGGGGACTTGGAAAATAA	108
	R: GTCATGGCRGTSGCYTCSACCATGCC		R: GTAGCCGTAGCCGATAGTGG	
Kir2.1b	F: ACACAGCGCAAACATCAGAG	1161	F: GGTAGTGGCCCTCTGTTACG	109
	R: AGGCAAAGAATCCACATTGC		R: AGTGGTTTGGGTCTCCACTG	
Kir2.2a	F: GGTGCCGCAACCGCTTYGTCAAGAA	781	F: TTAGCCTTTTGGGTCATTGC	106
	R: TCCAGGATSACCACRATCTCAAAGTC		R: AGGCAGCCACAAATCCATTA	
Kir2.2b	F: AATACCACAGCCGGTTTGTC	1117	F: GCTAACATTCGGCCTGTCAT	120
	R: GCCTGCTCATCCACTAGCTC		R: AAAGCTGCCACAAAGGTGTT	
Kir2.4	F: AACGTCACCTTCGTCAACAT	478	F: CAGCGCTACCTTAACGACCT	114
	R: TTGCGCTTCTTGGGCTTGG		R: GAAGGCAAATCCAAAGAGCA	
	F: GARGARGARGTCATGAGGCT	795		
	R: CACAATGCACTGCAAAATGA			
DnaJa2	F: CCAAATGCTGGHGACAARTTCAA	417	F: GACCGCTATGGAGAACAAGG	108
	R: ACWGACTGCATCTGTTGDACCAT		R: ACCACCCATGAATCCAAAAA	

Table 2 (continued)

Gene	Primers for cloning	bp	Primers for qPCR	bp
Pike (*Esox lucius*)				
Kir2.1a	–	–	F: AACAAGGAGGAGATGGACGA R: GGCACGGTAGCCTGGTTAT	101
Kir2.1b	–	–	F: GCTTCTGAAAATGGCTCTGC R: AAGGTGGACTCGATGGACTG	106
Kir2.2a	–	–	F: TCATGTGGAGGGTAGGGAAC R: CTCCCCTTCATCTGTGATCC	95
Kir2.2b	–	–	F: AGTCTCCCCACTCACCATTG R: GGCTTCAACCATTCCTTCAA	124
Kir2.4	–	–	F: CAACGAGGACATCAATGTGG R: GAAGAACGGCGACTCATTGT	103
DnaJa2	–	–	F: TGCACCGATTGTAATGGAGA R: TGGACCTCCAGGATTTTCAC	100
Roach (*Rutilus rutilus*)				
Kir2.1a	–	–	F: TGGAGAATGGAAGTCCCAAG R: AGCGATAGCCATAGCCGATA	102
Kir2.1b	F: TGGCGTTGGATGATGGTCCT R: TCATCCTCYGTTTCCTCTTTATC	926	F: TTCCTGTTTTCCGTGGAGAC R: CCAAGATGCTCTGGAAAACC	106
Kir2.2a	–	–	F: GAAGAGGAGGACGACGACAG R: TCTGCAGTCGTTCGAAATCA	100
Kir2.2b	–	–	F: TAAGGTGGGCTACTCGCACT R: GCCAGATTCCAAGGATTTGT	100
Kir2.4	–	–	F: CTTCGTCAACATGAGCGAGA R: AGAGGGTGAACACCACGAAC	101
DnaJa2	–	–	F: GTTATGGGGAACAGGGTCTG R: ACCCATGAAGCCAAACAAAC	101
Zebrafish (*Danio rerio*)				
Kir2.1a	–	–	F: GTGGCCCTTTCAAACAAAGA R: GCCTGGCTGTGTTCAGAGT	104
Kir2.1b	–	–	F: CGGAGGATGATGATGATGAC R: AAGCTGTGCTTTTGACATCG	102
Kir2.2a	–	–	F: CCAGAACGGATAAAGCCAGA R: CCTTTGTTCTGTGCATCGAG	102
Kir2.2b	–	–	F: CGGTGCCAACTTCTGCTAT R: GTCTCTAGCTCAGTCCCCCT	100
Kir2.4	–	–	F: CTGCAGATCTCCTCCTCTGT R: AGGAGTCTTGTCGAGGTGGT	103
DnaJa2	–	–	F: CTATGGGGAACAGGGTCTGC R: GTCCACCCATGAAACCAAAC	104

Genes that were not accessible in sequence databases were cloned. The length (bp) of the product amplified with each primer pair is represented

and 0.02 U/µl DyNAzyme EXT polymerase (Thermo Scientific). Cycling conditions for all PCR-reactions were as follows: initial denaturation at 94 °C, one or four cycles at 94 °C for 30 s, at 40 °C for 30 s and at 72 °C for 0.5–2 min (depending on the length of the product), followed by 30 cycles at 94 °C for 30 s, at 55–65 °C (depending on the melting temperature of the primers) for 30 s and at 72 °C for 60–90 s, and final extension at 72 °C for 5 min. If no PCR products were observed in the agarose gel electrophoresis after the first PCR, 1 µl of the PCR reaction mix was used as a template in the second PCR amplification (PCR-II) in a reaction mix identical to PCR-I. The amplification consisted of an initial denaturation at 96 °C for 2 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing

at 55–65 °C for 30 s (temperature depending on the melting temperature of the primers) and extension at 72 °C for 0.5–2 min (time depending on the length of the product), and by final extension at 72 °C for 5 min. The PCR products were separated in 0.8% agarose gel, and the nucleotide chain of desired length was extracted from the gel using GeneJET Gel Extraction Kit (Thermo Scientific), ligated to pGEM-T Easy vector (Promega Madison, Wisconsin, USA) and transformed to *Escherichia coli* DH5α cells. Plasmids containing the insert were purified from the cultured cells using the GeneJET Plasmid Extraction Kit (Thermo Scientific), and sequenced by GATC Biotech AB (Köln, Germany). The Kir2 sequences obtained were transformed to corresponding protein sequences using Transeq (www.ebi.ac.uk/Tools/st/emboss_transeq/).

For the phylogenetic analysis, Kir2 sequences for cod (*Gadus morhua*), stickleback (*Gasterosteus aculeatus*), Atlantic salmon (*Salmo salar*), sea squirt (*Ciona intestinalis*) and human were searched from GenBank and Ensembl databases and aligned with the Kir2 sequences of the studied species using ClustalOmega (http://www.ebi.ac.uk/Tools/msa/clustalo/). The neighbor-joining phylogenetic tree was constructed using SimplePhylogeny (www.ebi.ac.uk/Tools/phylogeny/simple_phylogeny/) and graphical presentation of the tree was prepared with Geneious 10.0. The bacterial K^+ channel KirBac3.1 was used to root the tree.

Quantitative measurements of transcripts

Transcript expression of Kir2 channels was measured by quantitative RT-PCR (qPCR) from atrial and ventricular tissue of the fish using established methods (Hassinen et al. 2007, 2008). An aliquot of 1–2 µg of total RNA (see Table S1) was DNase-treated and converted to cDNA by Maxima H Minus First Strand cDNA Synthesis Kit (Thermo Scientific) using a mixture of random hexamer and oligo (dT) primers. A control cDNA reaction, containing all other components except the RT enzyme, was prepared from every sample. Amplification was performed from every sample in triplicates using Maxima SYBR Green qPCR Master Mix (Thermo Scientific), primers presented in Table 2 (final concentration 0.3 µM) and AriaMx Real-Time PCR System (Agilent Technologies Inc., Santa Clara, CA, USA). The amplification consisted of an initial denaturation step at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 58–60 °C for 30 s, and extension at 72 °C for 30 s. The fluorescence was read after each extension step, and the melting curve analysis was performed in the end of PCR amplification to verify the accuracy of the PCR products. Agilent AriaMX (version 1.3) results were exported into Microsoft Excel for further analysis. The specificity of the primers was confirmed either by sequencing the qPCR products or by testing whether the primers were able to amplify the unspecific Kir2 plasmids. Only specific primers were used in the qPCR analysis. DnaJ (hsp40) subfamily A member 2 (dnaja2) (GenBank accession number MH362821 for arctic char, MH409958 for polar cod, MF095118 for brown trout, JX193859 for burbot, KF312377 for navaga, MH362833 for perch, XM_010897761 for pike, KX430029 for roach and NM_213493 for zebrafish) was used as a reference gene, since the transcript expression of this gene has been observed to be more stable between atrium and ventricle (Hassinen et al. 2015) and less temperature dependent than the commonly used reference genes of β-actin or ribosomal proteins (Vornanen et al. 2005). The transcript abundance of Kir2 genes was normalized to the expression level of DnaJA2 in every sample.

Statistics

The results are represented as means ± s.e.m. Statistically significant differences between Kir2 gene expressions were assessed at the 5% level ($P < 0.05$) using one-way ANOVA after checking normality of distribution and equality of variances and making necessary transformation of variables. Paired comparisons between two means were done by Tukey's (equal variances) or Dunnett's T3 (unequal variances) post hoc tests. Differences in Kir2 expression levels between cold- and warm-acclimated fish were tested using two-tailed Student's *t* test for independent samples.

Results

Kir2 sequences

To determine the expression of all cardiac Kir2 genes, sequences of Kir2 isoforms were mined from GenBank and Ensembl databases for all studied species. If sequences were not available, at least partial coding sequences were PCR cloned (37 genes in total, for accession numbers see Fig. 1) from cardiac cDNA or genomic DNA of the fish. The phylogenetic analysis of the cloned teleost Kir2 genes revealed that they were orthologues to Kir2.1 (gene name *kcnj2*), Kir2.2 (*kcnj12*) or Kir2.4 (*kcnj14*) genes (Fig. 1). Several Kir2 paralogues were present in all studied species. Notably, the salmonid species (Atlantic salmon, arctic char and brown trout) had more Kir2 paralogues than the other species. Atlantic salmon and brown trout had 4 paralogues for Kir2.1 and Kir2.2 (Fig. 1), while the arctic char had three Kir2.1 paralogues. The four salmonid Kir2.1/Kir2.2 paralogues were named as Kir2.1- or Kir2.2aa, − ab, − ba and − bb, respectively (Fig. 1, Table 2). Two paralogues were found for Kir2.1 (Kir2.1a and Kir2.1b) and Kir2.2 (Kir2.2a and − b) in all other fish species. Only one gene encoding Kir2.4 was found in each

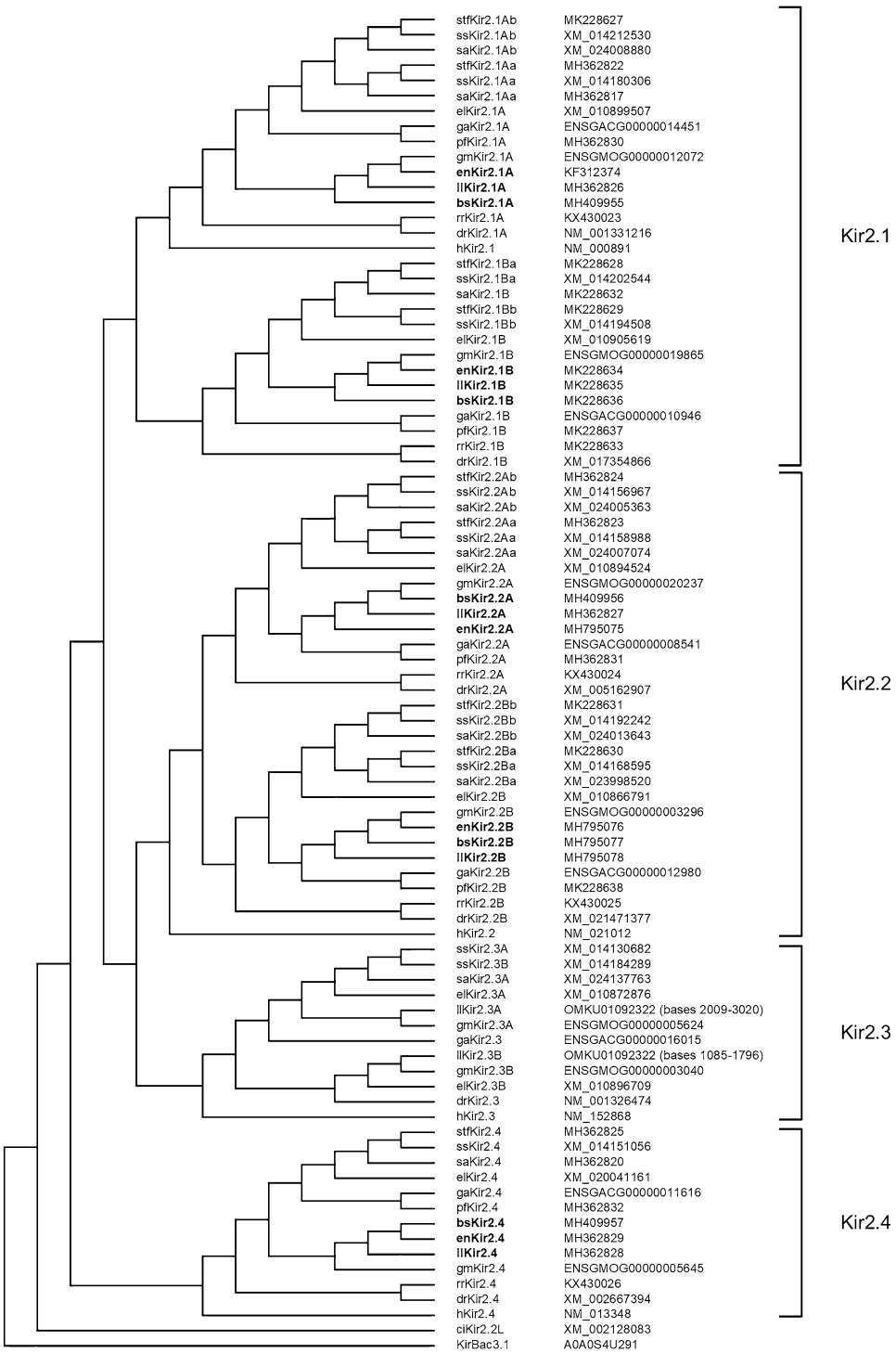

Fig. 1 Neighbour-joining tree of Kir2 nucleotide sequences. The tree was rooted with KirBac3.1 channel of *Ralstonia solanacearum*. Accession numbers of the sequences are from GenBank or Ensembl for human and fish genes and from UniProt for KirBac3.1. *Boreogadus saida*, bs; *Ciona intestinalis*, ci; *Danio rerio*, dr; *Esox lucius*, el; *Eleginus navaga*, en; *Gasterosteus aculeatus*, ga; *Gadus morhua*, gm; *Homo sapiens*, h; *Lota lota*, ll; *Perca fluviatilis*, pf; *Rutilus rutilus*, rr; *Salvelinus alpinus*, sa; *Salmo salar*, ss; *Salmo trutta fario*, stf

species, suggesting that no paralogues exist for Kir2.4 in the genomes of these fish.

Interspecies differences in Kir2 composition

The expression levels of Kir2 genes, normalized to the expression of the reference gene DnaJA2, are not directly comparable among species because the basal expression level of DnaJA2 may vary between species. To this end, interspecies comparisons were made based on the relative (percentage) expression of Kir2.1, Kir2.2 and Kir2.4 isoforms without making any difference between paralogous genes in the statistical analysis (for more detailed expression of different paralogues see *Atrial and ventricular isoform*

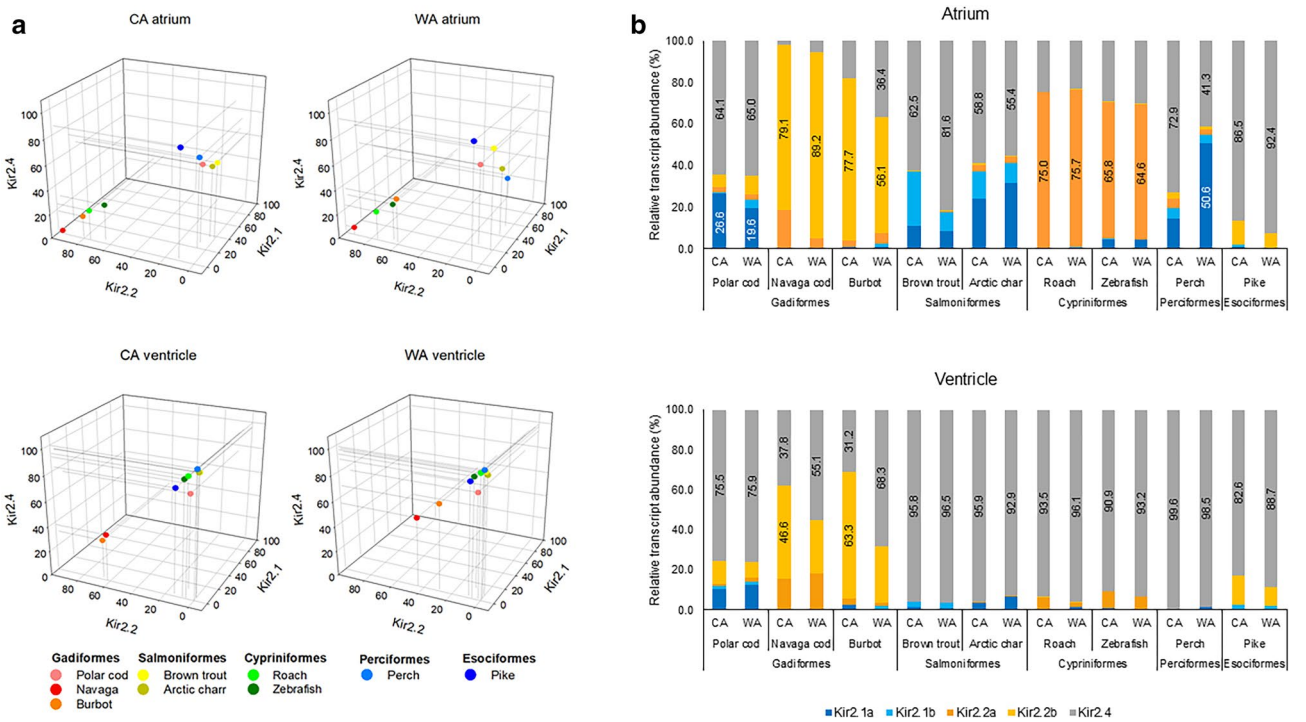

Fig. 2 Percentage abundances of different Kir2 transcripts in atrium and ventricle of thermally acclimated/acclimatized fish. Transcript abundance of Kir2 genes were normalized to the expression of DnaJA2 after which the percentage abundances of each Kir2 transcripts were calculated. **a** A 3D-graph of Kir2.1 (Kir2.1a and −b combined), Kir2.2 (Kir2.2a and −b combined) and Kir2.4 transcript abundances in cold- and warm-acclimated fish atrium (upper graphs) and ventricle (lower graphs). **b** Bar charts showing relative expression of each Kir2 isoform in atrium and ventricle of thermally acclimated fish. Percentage abundance for the dominating isoform in each cardiac chamber is indicated. If the transcript abundances of two most expressed isoforms were statistically equal, both values are represented. The sample size (*n*) is five for polar cod, burbot and zebrafish; six for arctic char, brown trout, navaga, perch, cold-acclimated pike and roach, and three for warm-acclimated pike

composition). The 3D-plots (Fig. 2a) show that Kir2 composition clearly differed between species and phylogenetic groups especially in the atrium. Atrial Kir2 composition was similar in navaga, burbot (Gadiformes species), roach and zebrafish (Cypriniformes species). Within this group of four species the relative expression of Kir2.2 transcripts was similar (61.2–97.2% of the total Kir2 transcript pool, $P > 0.05$) and significantly higher than in the other species (0.44–11.4%, $P < 0.05$). Of note, Gadiformes and Cypriniformes species expressed a different Kir2.2 paralogue, Kir2.2b and Kir2.2a, respectively (Fig. 2b). In the other group of 5 species, Kir2.4 was the main atrial isoform apart from the warm-acclimated perch, where Kir2.1 (50.6%) and Kir2.4 (41.3%) were expressed to similar extent. Kir2.1 channels were expressed to some extent also in the atrium of navaga (0.7%), burbot (1.1-2.6%), pike (0.7–2.2%), roach (0.2–0.9%) and zebrafish (4.6–4.7%) (Fig. 2b).

The ventricular Kir2 expression profile was more uniform across species than the atrial Kir2 expression (Fig. 2a). Kir2.4 was clearly the major ventricular isoform comprising 75.5–99.6% of the total Kir2 transcripts in all other species except navaga and burbot (Fig. 2b). The latter two species differed from other species in having significantly more of Kir2.2 especially in the cold-acclimated fish (62.0% in navaga and 66.2% in burbot) ($P < 0.05$). Because of warm-acclimation the relative proportion of Kir2.4 transcripts increased from 31.2 to 68.3% in burbot and from 37.8 to 55.1% in navaga; therefore, the ventricular Kir2 composition of the warm-acclimated burbot and navaga became akin to that of other species (Fig. 2a). However, the Kir2.2 expression of navaga and burbot ventricle was still significantly higher than in other species ($P < 0.05$). Kir2.2 expression was particularly low in Salmoniformes and perch representing less than 0.4% of the total Kir2-transcripts. Kir2.1 was expressed most strongly in polar cod (12.1–13.9%), but clearly less in other species (0.1–7.0%).

Atrial and ventricular Kir2 composition

To compare the total Kir2 transcript levels between atrium and ventricle, transcript abundances of different Kir2 isoforms normalized to the expression of reference gene DnaJA2 were summed up and proportioned to the total Kir2 abundance of the cold-acclimated atrium in each species

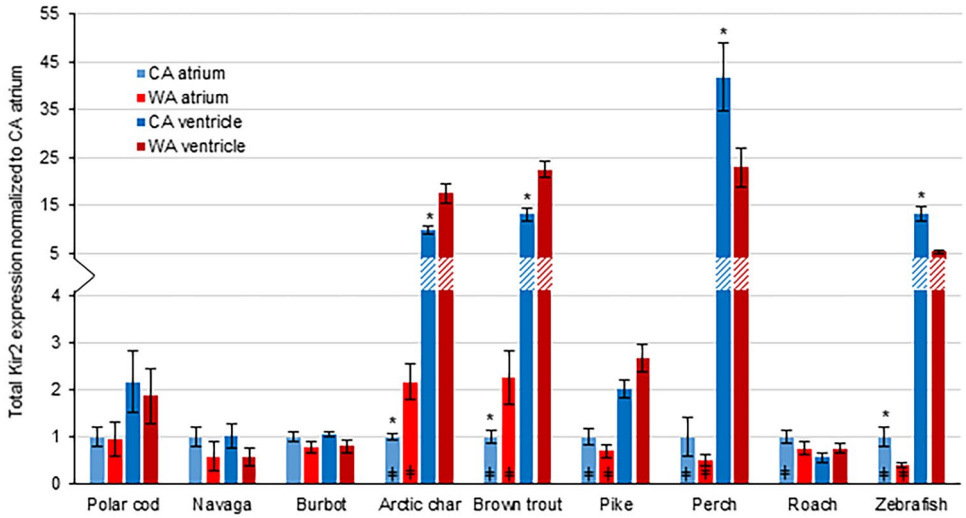

Fig. 3 The combined total expression level of all Kir2 transcripts (mean ± s.e.m.) in cold- and warm-acclimated fish cardiac atrium and ventricle. In each species, expression levels of different Kir2 channels were normalized to the DnaJA2 transcript abundance after which they were proportioned to the Kir2 expression of the cold-acclimated atrium of the same species to make the fold changes of total Kir2 abundances between atrium and ventricle as well as between acclimation temperatures comparable between species. An asterisk (*) and alveolar click letter (ǂ) indicates statistically significant difference ($P < 0.05$) between cold- and warm-acclimated fish, and between atrium and ventricle, respectively

(Fig. 3). By this means, the total Kir2 expression in CA atrium was set to one in all species, and the fold changes of Kir2 total expressions in cardiac chambers and thermal acclimation were comparable between species. There were prominent quantitative and qualitative differences in Kir2 expression between atrium and ventricle. However, Gadiformes species were clearly an exception in this respect: the total Kir2 level was similar in atrium and ventricle in all three species ($P > 0.05$). Furthermore, in burbot and polar cod the Kir2 expression profiles were nearly identical in the two chambers. In the roach, the total Kir2 abundance was similar in atrium and ventricle of the warm-acclimated fish, while in the cold-acclimated roach Kir2 expression was lower in ventricle than in atrium ($P < 0.05$). The total Kir2 abundance was higher in ventricle of brown trout (13.0- and 10.0-fold in cold-acclimated and warm-acclimated ventricle, respectively), arctic char (9.7- and 8.1-fold), pike (2.0- and 3.8-fold), perch (41.7- and 46.3-fold) and zebrafish (13.3- and 13.2-fold).

Transcript expression of Kir2.1, − 2.2 and − 2.4 were quantified also at the paralogue level. In Salmoniformes, genes encoding Kir2.1aa and − ab as well as − ba and − bb shared so high identity that their expressions were quantified with primer pairs binding to both paralogues and were reported as "Kir2.1a" and "Kir2.1b", respectively. Paralogues for salmonid Kir2.2 were analyzed in the same way. Generally, all Kir2 isoforms of each species were expressed to some extent in both atrium and ventricle of the respective species (Figs. 4, 5 and S1). However, different Kir2 isoforms showed striking variation in expression, the most highly expressed isoform being 100–10,000 times more abundant than the least expressed isoform in each cardiac chamber (Figs. 4 and 5). Usually there were one or two dominating isoforms, whereas the other ones were expressed only in trace amounts. In ventricle, Kir2.4 was clearly the dominating isoform in 7 out of 9 species (polar cod, brown trout, arctic char, roach, zebrafish, perch and pike) (Fig. 4). In two Gadiformes species (navaga and burbot), ventricular Kir2 composition differed from that of other species, in that the Kir2.2 isoforms were highly expressed. In the ventricle of navaga, the expression of Kir2.2a, Kir2.2b and Kir2.4 were similar and they were the most abundant Kir2 isoforms in both acclimation temperatures. In the burbot ventricle, Kir2.2b and Kir2.4 were almost equally expressed and they were the dominant ventricular Kir2 isoforms.

Kir2 isoform diversity was larger and phylogenetic differences more obvious in the atrial than ventricular tissue (Fig. 5). For simplicity, only the cold-acclimated/cold-acclimatized fish are compared here. Among the Gadiformes species, the atrial Kir2 composition of polar cod differed from that of navaga and burbot. Kir2.2b was the most highly expressed Kir2 isoform in the atrium of navaga and burbot, whereas Kir2.4 and Kir2.1a were the dominating atrial isoforms in the polar cod atrium. Differently from the Gadiformes species, the atria of the Cypriniformes species (roach, zebrafish) strongly expressed Kir2.2a, whereas expression of the Kir2.2b paralogue was very low. Relatively high expression of Kir2.1 isoforms was typical for the atrium of the Salmoniformes species and the perch. In the brown trout, the most highly expressed atrial Kir2 isoforms

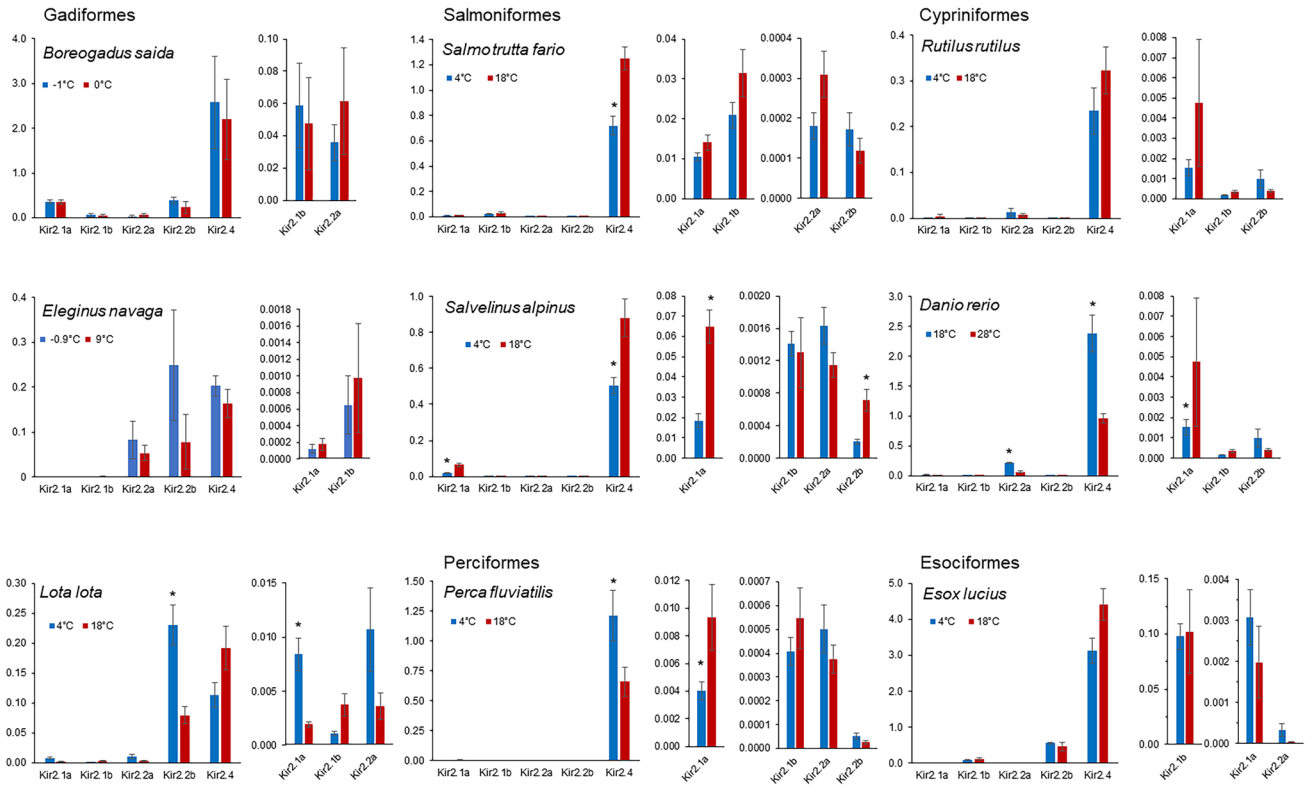

Fig. 4 Transcript expression of Kir2.1, Kir2.2 and Kir2.4 isoforms in thermally acclimated/acclimatized fish ventricle. Kir2 transcript levels were normalized to the mRNA level of the reference gene DnaJA2. The results are mean ± s.e.m. from 5 to 6 fish (except for warm-acclimated pike, where $n = 3$). Data for each sample was the mean of three technical replicates. An asterisk indicates statistically significant difference ($P < 0.05$) between cold and warm-acclimated/acclimatized fish

were Kir2.4 and Kir2.1b. In the arctic char, Kir2.4 was the dominating atrial isoform. In pike, the atrial Kir2 composition resembled that of the ventricle, Kir2.4 being the most expressed Kir2 isoform in both cardiac chambers. Actually, the pike was the only species in which Kir2.4 was by far the most abundant atrial isoform.

Effects of temperature

Based on the total Kir2 expression, the species can be divided in three groups: no change in expression (Gadiformes, pike and roach), increased expression (Salmoniformes) and decreased expression (perch and zebrafish) after acclimation/acclimatization to warm (Fig. 3). Even though thermal acclimation/acclimatization did not induce any changes in the total cardiac Kir2 expression of the Gadiformes species, minor changes were evident for some isoforms. In the polar cod, Kir2.1b expression was significantly higher in warm-acclimated than in cold-acclimated atrium ($P < 0.05$) (Fig. 5). It should be noted, however, that Kir2.1b represents only 0.5–4.1% of the total atrial Kir2 transcripts in this species (Fig. 2). In the atrium of navaga Kir2.1b, Kir2.2a and −b were more abundant in cold-acclimatized than warm-acclimatized fish ($P < 0.05$). In burbot, Kir2.2b transcripts were more abundant in both atrium and ventricle of cold-acclimated than warm-acclimated fish. Moreover, Kir2.1a transcripts were significantly more abundant in cold-acclimated than warm-acclimated burbot ventricle ($P < 0.05$).

In brown trout and arctic char (Salmoniformes), the total Kir2 expression increased in warm-acclimation both in atrium and ventricle (Fig. 3). In both species, Kir2.1a, Kir2.2a and Kir2.4 were more abundant in warm-acclimated than in cold-acclimated atrium and Kir2.4 was more abundant in warm-acclimated than cold-acclimated ventricle ($P < 0.05$). In addition, arctic char showed elevated ventricular Kir2.1a and Kir2.2b transcript expression in warm-acclimation ($P < 0.05$).

Warm-acclimation decreased the total Kir2 transcripts in perch and zebrafish ($P < 0.05$). In the perch ventricle, the dominant Kir2.4 isoform was significantly lower in warm-acclimated fish. In warm-acclimated zebrafish ventricle, the lower total Kir2 expression was a result of decreased expression of Kir2.1a, Kir2.2a and Kir2.4. In zebrafish atrium, the same genes were reduced but only the change in Kir2.2a was statistically significant ($P < 0.05$).

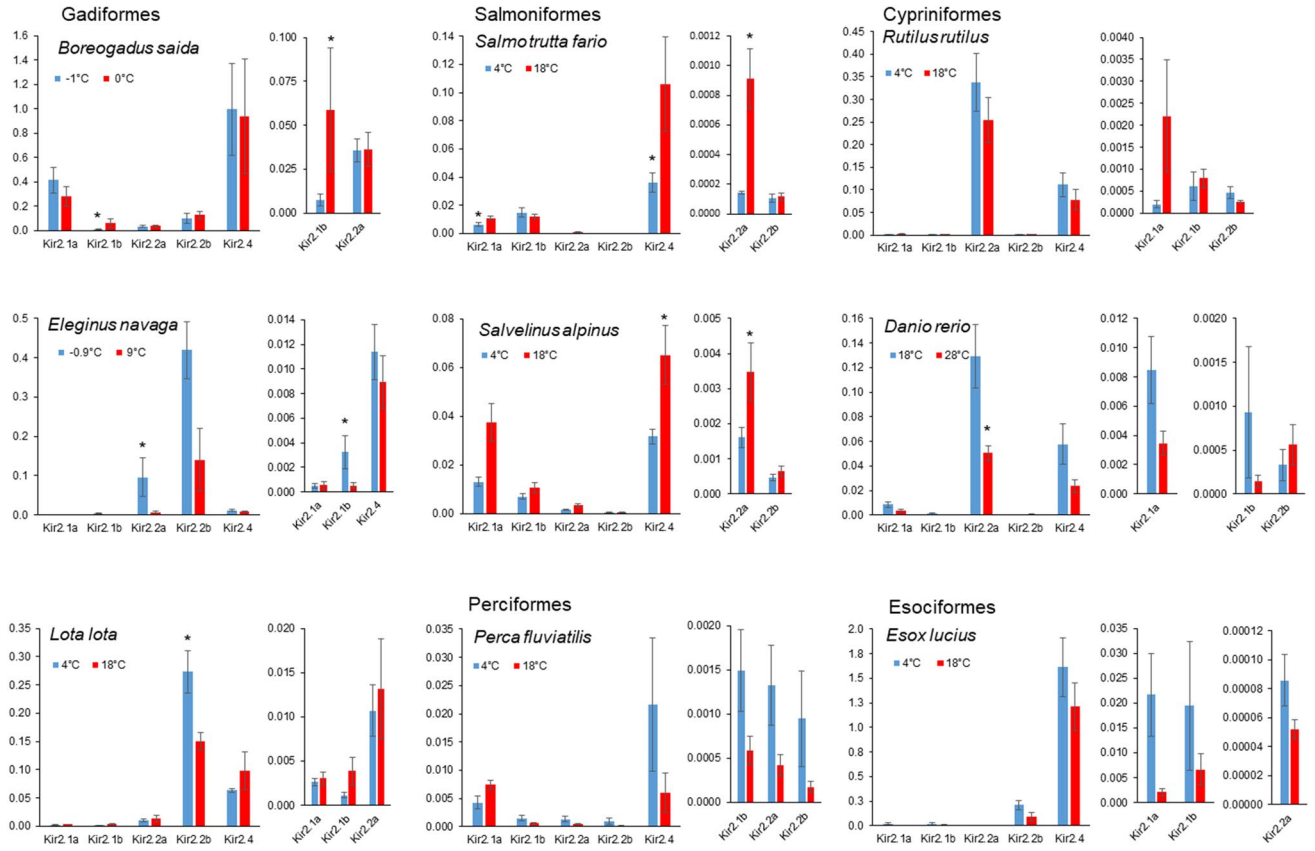

Fig. 5 Transcript expression of Kir2.1, Kir2.2 and Kir2.4 isoforms in thermally acclimated/acclimatized fish atrium. Kir2 transcript levels were normalized to the mRNA level of the reference gene DnaJA2. The results are mean ± s.e.m. from 5 to 6 fish (except for warm-acclimated pike, where $n = 3$). Data for each sample was the mean of three technical replicates. An asterisk indicates statistically significant difference ($P < 0.05$) between cold and warm-acclimated/acclimatized fish

Pike and roach were the only species where thermal acclimation had no effect on Kir2 expression either in atrium or ventricle.

Discussion

Comparison of Kir2 channel composition and expression in the heart of nine teleost fish species representing different fish groups and different thermal habitats/preferences revealed four salient features. (1) There is no typical cardiac Kir2 composition, which is common for all studied fish species. This is particularly true for the atrium. (2) There seems to be a strong phylogenetic component in Kir2 composition of fish hearts especially in the atrium. (3) Thermal acclimation/acclimatization affects cardiac Kir2 expression in species- and isoform-specific manner. (4) The presence of gene paralogues for cardiac Kir2 channels is a common feature for all studied fish species. These findings support our hypothesis that fish cardiac Kir2 composition depends on phylogenetic relationship of the species, whereas the hypothesized dependence of Kir2 composition on thermal preferences of the fish species was less clear.

Kir2 composition

The Gadiformes order is particularly interesting, since it provides a research setting to compare Kir2 composition and expression among closely related species but with differing thermal preferences. The cod-like fishes—including the freshwater burbot—are generally regarded as cold stenothermic species (Müllman 1939). However, they are not equally stenothermic, but sooner form a continuum from the most stenothermic polar cod to somewhat less stenothermic navaga and the least stenothermic burbot (Hofmann and Fischer 2002; Hölker et al. 2004; Nagel et al. 2011; Pääkkönen and Marjomäki 2000). The cardiac Kir2 composition of Gadiformes fish differs from that of the other studied species in two ways. First, the total Kir2 expression level is similar in atrium and ventricle, and there are relatively small differences in Kir2 isoform composition between the two cardiac chambers.

In other species, atrial and ventricular Kir2 expression shows more distinct quantitative and qualitative chamber-specific differences. The physiological function of Kir2 channels/I_{K1} current on electrical excitability is stabilization of membrane potential: high density of I_{K1} sets membrane potential close to the equilibrium potential of K^+ ions and reduces spontaneous excitability in particular in ventricular myocytes (Zaritsky et al. 2001). Generally, the density of I_{K1} is much lower in atrium than ventricle; this makes atrial muscle more easily excitable by the sinoatrial pacemaker, while the higher density of I_{K1} in the ventricle may prevent ventricular arrhythmia (Dhamoon and Jalife 2005). However, the physiological importance of the outward I_{K1} is not solely determined by the total Kir2 abundance but is also critically dependent on the Kir2 isoform composition. All Kir2 channels provide repolarizing outward K^+ current, but they pass K^+ efflux with different ease. To our knowledge, the rectification properties of Kir2 isoforms have been studied only in crucian carp (C*arassius carassius*) and zebrafish. According to these studies Kir2.1 is weakly inwardly rectifying, i.e., it passes more outward K^+ current than Kir2.4 and Kir2.2a channels (Hassinen et al. 2008, 2015). Kir2.2b is the most strongly rectifying and therefore the least repolarizing channel (Hassinen et al. 2008, 2015). Notably, the strong inward rectifier Kir2.2b is more abundantly expressed in atrium than ventricle in the hearts of Gadiformes species. Consistent with this the density of I_{K1} is lower in atrial than ventricular cardiomyocytes of the navaga (Abramochkin and Vornanen 2015) and burbot (Haverinen and Vornanen 2009). Generally, fish hearts have clearly more of the weakly rectifying Kir2.4 channels in ventricle than atrium. Second, the relative proportion of Kir2.2b is high in both cardiac chambers of Gadiformes—especially in navaga and burbot—whereas in the hearts of other teleost species Kir2.2b is minimally expressed. The presence of this strong inward rectifier may reduce the density of I_{K1} in burbot and navaga ventricle in comparison to ventricles of other fish species. It is also obvious that among the three Gadiformes species navaga and burbot are mutually more similar than either of them with the polar cod. The latter species differs from navaga and burbot by expressing more Kir2.4 and Kir2.1a in both cardiac chambers. Whether the differences within the Gadiformes species reflect phylogenetic distances or thermal preferences between species remains to be shown.

Another striking similarity in Kir2 composition was noticed among Cypriniformes: roach and zebrafish have very similar Kir2 expression pattern both in atrium and ventricle. Kir2 expression pattern of Salmoniformes species (brown trout, arctic char) is also similar, but they share similarities also with the perch and the polar cod.

Temperature and Kir2 composition and expression

Interestingly, thermal habitats/preferences of the fish are not strongly reflected in the cardiac Kir2 composition. Indeed, the cryopelagic polar cod has a similar atrial Kir2 composition as the more eurythermic Salmoniformes species and the perch. In the ventricle, the stenothermic Gadiformes group expresses more Kir2.2 and Kir2.1 than the members of other fish orders.

Thermal acclimation had variable effects on Kir2 transcript expression in different species. In Salmoniformes species, the total transcript expression of Kir2 isoforms was reduced in cold-acclimation. This may be a common pattern of thermal compensation of Kir2 channels/I_{K1} current for Salmoniformes species, because also the I_{K1} current is depressed in the cold-acclimated rainbow trout (*Oncorhynchus mykiss*) (Hassinen et al. 2007; Haverinen and Vornanen 2009; Vornanen et al. 2002). In Gadiformes species as well as in pike and roach, the total cardiac Kir2 transcript expression was similar in both acclimation temperatures. Consistent with this, thermal acclimation did not change the I_{K1} current of pike atrium and ventricle and burbot atrium (Haverinen and Vornanen 2009). However, in burbot ventricle and in both cardiac chambers of the roach, I_{K1} is elevated in cold-acclimation (Haverinen and Vornanen 2009) even if the Kir2 total expression level remains unaltered (present study). In burbot, cold-acclimation increases the expression of Kir2.2b suggesting that the isoform switching from the dominance of Kir2.4 in warm-acclimation to the dominance of Kir2.2b produces the increase in I_{K1} current under cold-acclimation. This kind of isoform switching is not seen in the roach, thus the mechanism of I_{K1} induction in the cold-acclimated roach remains unresolved. In perch, Kir2 expression as well as I_{K1} current is enhanced in cold-acclimated ventricle, but not in atrium (Haverinen and Vornanen 2009). In zebrafish, the total Kir2 expression is higher in cold-acclimated than in warm-acclimated fish heart. It seems that thermal acclimation-induced differences in cardiac I_{K1} current density are achieved by changing either the total Kir2 abundance or the Kir2 isoform composition. In the crucian carp heart the expression of Kir2.2a and −b depends on thermal acclimation, Kir2.2a is prevailing at warm (+18 °C) and Kir2.2b at cold (+4 °C) (Hassinen et al. 2008). Thus, it seems that Kir2.2b is a cold-adapted Kir2.2 isoform, which is supported also by the increased Kir2.2b expression in cold-acclimated navaga and burbot heart. To understand the importance of isoform switching in thermal acclimation of fish I_{K1}, more studies are needed about the temperature sensitivity of the functional properties of different Kir2 isoforms. Collectively, the present findings are in accordance with the previous electrophysiological studies, which showed that the response of fish cardiac I_{K1} current to chronic temperature stress is more strongly determined by phylogeny than thermal preferences of the species (Haverinen and Vornanen 2009).

Kir2 diversity

A whole genome duplication (WGD) occurred in the common ancestor of teleost fishes some 320–350 million years ago (the teleost-specific WGD) (Christoffels et al. 2006; Taylor et al. 2001), and additional WGDs has happened in salmonids, carp and other fish groups more recently (Allendorf and Thorgaard 1984; Larhammar and Risinger 1994; Macqueen and Johnston 2014; Pasquier et al. 2016). Although many of the paralogue genes generated by the genome duplications may have been lost in evolution, a significant number of paralogues still exist in fish genomes (Jegla et al. 2009). Indeed, paralogues were found for Kir2.1 and Kir2.2 genes in all studied species. For example, in the heart of brown trout four paralogues for both Kir2.1 and Kir2.2 were expressed increasing total number of the Kir2 genes (one Kir2.4) in the heart to nine. The genome duplications have generated diversity in fish cardiac Kir2 gene population, which exceeds that of the mammals (Jegla et al. 2009). Mammalian hearts usually express Kir2.1, Kir2.2, Kir2.3 and Kir2.4 channels (Anumonwo and Lopatin 2010; Coetzee et al. 1999). In mammalian ventricular myocytes, Kir2.1 channels prevail whereas Kir2.2 and Kir2.3 are smaller components of the Kir2 family (Zaritsky et al. 2001). For example, in the right ventricle of the human heart, Kir2.1, Kir2.2, and Kir2.3 transcripts constitute 47, 29, and 24% of the total Kir2 transcripts, respectively (Gaborit et al. 2007). While the large Kir2 gene diversity is probably useful for adaptation of fish to different aquatic habitats, it forms a significant challenge for experimenters who study gene expression in response to environmental toxicants or use fish as a model for human cardiac pathophysiology or drug screening. When expression of fish cardiac Kir2 is studied, it cannot be taken granted that fish hearts express the same Kir2 gene products as the mammalian hearts. For example, Kir2.1 is the principal isoform in mammalian ventricles, while it is very weakly expressed in fish ventricle. Even though Kir2.4 seems to be the major ventricular Kir2 isoform in most fishes, it is not safe to assume to be true for all fish species, as indicated by the abundant presence of Kir2.2 channels in burbot and navaga ventricles. The situation is even more complicated in the case of the atrial muscle, where Kir2 composition is even more diverse between species. Even if zebrafish is still the most common fish model for developmental biology, toxicology and drug screening, many other fish species are increasingly used, e.g., for modeling human diseases (Schartl 2014). The high diversity of cardiac Kir2 composition in different fish species demonstrates the importance of analyzing the gene expression profile of each species before using them as a model for human cardiac toxicology, disease or drug screening.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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