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# Functional and Structural Differences in Atria Versus Ventricles in Teleost Hearts

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Additional information is available at the end of the chapter

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## 1. Introduction

Across vertebrates, the fish heart is structurally relatively simple. The heart of teleosts is unique in structure, composed of four chambers in series: venous sinus, atrium, ventricle and bulbus arteriosus. The two chambers acting as pumps are the atrium and ventricle, a simplified version of that seen in tetrapods. These chambers develop from a simple linear tube [1] and differ not only morphologically but also physiologically with different characteristic rates of contractility [2]. Fish heart design is reflective of the needs for oxygen delivery to working skeletal muscle in an often oxygen poor environment. The heart is the first definitive organ to develop and become functional, as embryological survival depends on its proper function. The vertebrate-specific development of multiple chambers with enough muscle to generate higher systemic pressures allowed for perfusion of larger and more complex tissues.

Fish show great variability in the development of the heart and modification of heart performance capacities to meet tissue perfusion demands imposed by differences in life history. Research has tended to focus on inotropic or chronotropic responses of the heart to stress such as temperature in larger fish such as trout, or on development through the use of zebrafish mutants. Between these sources much has been revealed about the contractile properties of the two chambers. This research will be reviewed in the context of roles of the atrium and ventricle in fish in achieving variability in myocardial contractility.

## 2. Gene expression

In mammals, 23% of cardiac-specific genes show differences in expression between the atria and the ventricle [3]. Key morphogenetic events during heart ontogenesis are conserved

across vertebrates [4]. Gene expression programs specific to each chamber are likely regulating key aspects of chamber formation. The focus on fish heart developmental patterns has primarily been due to the use of zebrafish as a model organism of vertebrate development. The morphological differences created during chamber development are guided by changes in gene expression patterns [1], a process well conserved in vertebrates, regardless of whether there are one or two atria or ventricles. Genetic screens in zebrafish have revealed genes and pathways underlying development in the heart. Since many stages of development are conserved across vertebrates, this information has generated considerable insight into congenital heart defects in humans. In the context of this chapter, timing and localization of gene expression can also contribute to our understanding of the differentiation of chambers both in morphology as well as through variation in contractility.

The T-box genes (Tbx) are responsible, in large part, for patterning that distinguishes chamber myocardium from non-chamber myocardium [5]. Subregionalization is important for atrial or ventricular identity, either via selective transcription or repression of genes. The physical interaction of Nkx2.5, Gata4 and Tbx5 in particular activates the expression of chamber-specific genes to stimulate the differentiation of primary myocardial cells into chamber myocardium [1]. By the time cells are restricted to cardiac lineage, they are already destined for either the atrium or the ventricle. During early gastrulation, myocardial progenitors already have axial coordinates that lead to chamber assignment [6]. There are two sources of these myocardial progenitors, or two heart fields. In the first heart field, cardiomyocyte differentiation initiates in the ventricle and allows continuous addition of new cardiomyocytes to the venous pole of the heart tube progressing towards the atrium [7]. Differentiation at the arterial pole in the second heart field occurs only in the later stages of cardiac development. By 72 hours post-fertilization (hpf) individual heart chambers are morphologically differentiated with the ventricular wall being thicker and ventricular cardiomyocytes being larger than those of the atrium [8].

The chambers express different subsets (or paralogs) of sarcomeric proteins such as myosin heavy chains (MHC) and myosin light chains (MLC) [9]. Variation in paralog expression (frequently referred to in the literature as isoforms) may confer differing contractile properties to the atrium and the ventricle, such as with altered MHC expression being correlated with altered myofibrillar ATPase activity (crucian carp - [10]). MHC expression is chamber-specific by 22 hpf, preceding any obvious chamber formation but corresponding to the initiation of the heart beat [6]. These isoforms are not only divided spatially but also by differences in timing of expression. Atrial-specific myosin gene expression is induced after ventricle-specific myosin gene expression in zebrafish [11]. This time-dependent expression may be due to repression of the promoters of these genes [1].

The timing of these developmental pathways is critical, as the development of one chamber can influence the other. Ventricular morphology can change with developmental atrial dysfunction seen with zebrafish mutations in atrial-specific MHC [11]. For example, in the *wea* mutant, a lack of a functional atrial MHC results in defects in atrial myofilament organization and contractility. Despite the mutation being seen only in a chamber-specific protein, the ventricle changes as well in response to these atrial defects. Effective embryonic circula-

tion requires appropriate relative sizes of the atrium and ventricle. Bone morphogenetic protein (BMP) signaling has been shown to be involved with the regulation of chamber size in zebrafish [12]. However, mutations affecting BMP signaling only reduce atrium size but not the ventricle. During this stage of development, the size of chambers may be relatively independent of one another.

### 3. Morphology

In fish the heart is arranged in series with the ventricle primarily filled by contraction of the atrium [13], rather than the mammalian system in which the thin-walled atrium contributes only a small amount to ventricular filling under resting conditions. While the atrium and ventricle compose the main muscular components of the fish heart, there are other chambers with important roles in guiding appropriate blood flow through the heart. Generally in fish blood flows through the sinus venosus to the atrium to the ventricle and then out to the bulbus arteriosus. More recently, the atrioventricular region and conus arteriosus have been identified as morphologically discrete segments present throughout modern teleosts as well [14, 15].

#### 3.1 General morphology

As with most vertebrates, the cardiac tube is S-shaped with a dorsally positioned atrium and ventrally positioned ventricle to allow for appropriate momentum of the bloodstream towards the arterial pole. The morphology of the heart sets up an alternating arrangement of slow conducting segments (e.g. inflow tract, atrioventricular region and outflow tract) with fast conducting contractile segments (atrium and ventricle). Blood first enters the thin-walled sinus venosus. This is an independent, but non-muscular chamber in fish that acts as a drainage pool for the venous system. The composition of the sinus venosus varies greatly between species, from primarily connective tissue in *Danio rerio* (zebrafish) to mainly myocardium in *Anguilla anguilla* (European eel) to even smooth muscle cells in *Cyprinus carpio* (carp) [16, 17, 18]. The presence of the sinus venosus has thus been suspected to have allowed the development of the atrium as the principal driving force for ventricular filling [19]. Blood then moves into the atrium, an active contractile chamber in fish that again shows considerable variability in size and shape between species [18]. A general trend throughout teleost species is the similarity in volume of the two main muscular chambers is indicative of the importance of the role of the atrium in filling the ventricle. While atrial volume is closer in size to that of the ventricle in fish, the atrium is still less developed muscularly and possibly representative of a more primitive heart [20-22].

Separating the atrium and the ventricle is the atrioventricular region, a ring of cardiac tissue supporting the AV valves. The degree of isolation of this region from the surrounding musculature varies between species [23] but in all species it plays a critical role in regulating the pattern of electrical conduction. The ventricle has a mass and wall thickness up to five times that of the atrium [18]. The thickly muscular ventricle varies in terms of structural organiza-

tion, histology, coronary distribution and relative mass between species as well. This variation reflects the fact that different modes of heart performance are attributed to different ventricle types since this chamber is responsible for the active cardiac output [18]. This classification will be discussed further in this section. From the ventricle, blood moves into the outflow tract leading into the beginning of the dorsal aorta. In more basal fish there are two segments: the proximal muscular conus arteriosus and the distal arterial-like bulbus arteriosus [24]. While later teleosts were thought to have lost the conus arteriosus, more recent evidence shows it is a distinct segment in many families [14, 15]. The bulbus arteriosus is more prominent and serves as the main component of the outflow tract. This elastic chamber expands to store large portions of the stroke volume, and gradually releases a steady non-damaging flow to gill vascular [18]. The role of these segments is variable between species and has been reviewed in more detail [25].

The AV-region, bulbus arteriosus and conus arteriosus are unique to fish though homologous regions may be seen in early embryological stages of higher vertebrates. While, the atrium and ventricle are present across vertebrates, their diversity in morphology and function and how they contribute to contractility will be the focus of the remainder of this Chapter.

### 3.2. Myocardial arrangement

The teleost myocardium is composed of two distinct layers: compact and spongy. The atrium tends to be primarily spongy, with more open space between fibers and greater variability in orientation [26]. The external rim is myocardium surrounding a complex network of pectinate muscles. Collagen provides support, being predominant both in surrounding the myocardium and the internal trabecular network [15]. Meanwhile, while the ventricle composition varies greatly between species, the periphery of the ventricle tends to be primarily compact tissue with the luminal being primarily spongy [15, 27]. The compact layer is composed of typically well-organized fibers composing well-arranged bundles covering the inner ventricle. The spongy layer is composed of long finger-like branching cells in close proximity to luminal fluids. Not all fish species have distinct layers in the ventricle to the same degree as the salmonids and scombroids in which most of the experiments characterizing the morphology of the chambers has been done. For example, the relatively sedentary flatfish has a heart with virtually no lumen and is composed almost entirely of spongy tissue [28]. These phenotypes have been subdivided into four subclasses (Types I-IV). A Type I ventricle consists of an entirely spongy ventricle, while Type II has both compact and spongy myocardium but with coronary vessels restricted to the compact layer. Type III and Type IV have vascularized trabeculae, with coronary vessels penetrating spongy and compact myocardium. Types III and IV are distinguished from one another by Type III having less than 30% of the heart mass composed of compact myocardium (exemplified by many elasmobranchs) and Type IV representing ventricles with greater amounts of compact myocardium [29].



### 3.3. Cardiomyocytes

On the cellular level, cardiomyocytes are more cuboidal in the ventricle compared to the more squamous shape seen in the atrium [30]. Ventricular myocytes from ectothermic vertebrates more closely resemble myocytes from neonatal mammals rather than adults in terms of structure and function. They are long ( $>100\ \mu\text{m}$ ), thin ( $<10\ \mu\text{m}$ ) and generally lacking in t-tubules (trout - [18]; trout - [31]; neonatal rabbit - [32]). These myocytes appear in electron microscopy images to have less extensive sarcoplasmic reticulum (SR) than mammals [33] but some peripheral couplings with sarcolemmal membrane are observed [34]. Atrial muscle cells contain well-developed sarcomeric reticulum, peripheral couplings but no t-tubules [35]. The narrow extended shape of teleost cardiomyocytes from both chambers decreases the diffusion distance from the sarcolemma to the center of the cell [36-38]. This high surface-to-volume ratio of the cells may be related to increased efficacy of E-C coupling. These morphological distinctions contribute to the chamber-specific contractile properties which will be discussed further in this Chapter.

### 3.4. Lifestyle demands

These morphological differences between species are reflective of a diversity of lifestyle demands. Active fish have a larger relative heart mass than that of less active species; the relative heart mass in the skipjack tuna is 10 times larger than in the sluggish flounder [39]. However, a similar atrium to ventricle mass ratio exists in species with very different lifestyles (0.2 in active Atlantic salmon, Atlantic cod and inactive winter flounder - [28]). More active fish also show a difference in whole heart shape with active fish (e.g. salmonid and scombrid families) exhibiting pyramid-shaped hearts whereas inactive fish hearts are more rounded and sac-like in shape [40]. The pyramidal shape normally correlates with a thicker myocardial wall and high cardiac output. This allows for high levels of stroke work and the maintenance of relatively high blood pressures [18].

The amount of compact myocardium is generally increased in fish with greater cardiac output demands. With pyramidal ventricles, the myocardial wall is normally formed primarily by the compact layer, which is complex and thicker in these higher performance fish [18] although this phenotype is also found in the relatively sedentary Antarctic teleosts [26]. This suggests that shape and degree of compact myocardium do not always correlate with lifestyle and in fact the shape of the chamber does not always correlate with the inner architecture [41]. This active phenotype may also display plasticity, as trout that are rendered inactive will display an abnormal rounded shape [42]. This may also be the case in aquaculture conditions, where fish become less active and may be overfed [43]. The proportion of compact myocardium has also been shown to vary with seasons [18] and growth [44].

Active fish rely on coronary circulation to allow for increased cardiac output. More compact tissue in the ventricle generally requires greater  $\text{O}_2$  perfusion. In general, sluggish species lack this coronary circulation and can rely completely on  $\text{O}_2$  contained in the venous circulation [45]. Hence thicker ventricles seen in more active fish have a more developed coronary distribution to the compact myocardium, and a greater percentage of compact myocardium. Very active fish like scombroids and elasmobranchs also have a coronary supply to the

spongy myocardium and atrium [46]. However, this increased demand for O<sub>2</sub> supply for cardiac function means that these species are at a disadvantage in hypoxic water. The more primitive hagfish heart composed almost entirely of spongy myocardium can meet cardiac ATP requirements for resting conditions even in anoxic water [47].

#### 4. Filling patterns

Atrial filling occupies a substantial proportion (up to 75%) of the cardiac cycle and as discussed above, the atrial chamber volume in fish may be comparable to that of the ventricle. Substantial debate exists in the literature as to the mechanism of both atrial and ventricular filling. Though noninvasive echocardiography has greatly contributed to research on flow patterns, it does require anaesthetization and restraint of the fish. Because the heart is acutely sensitive to venous pressure, and venous pressure is acutely sensitive to gravitational forces and extramural pressure, considerable variation may be seen from study to study. Certain studies have shown a more monophasic filling of both atrium and ventricle via a vis-a-tergo mechanism (e.g. trout - [48]). In this model, ventricular pressure is derived from arterial flow and venous capacitance is the primary determinant of cardiac output [13]. Other studies have demonstrated a more biphasic filling pattern via a vis-a-front mechanism in which cardiac suction plays a prominent role in ventricular pressure (e.g. elasmobranchs - [49]). Even triphasic patterns have been observed, with a third wave of filling possibly involving sinus contraction (e.g. leopard shark - [49]).

These filling patterns of the chambers may be both a reflection of phylogeny and external conditions. Less active fish such as the sea raven require above-ambient filling pressures that may require vis-a-tergo filling [50]. The venous pressure control mechanisms are not seen to be as developed in elasmobranchs as in teleosts resulting in a greater necessity of vis-a-front filling [51]. Meanwhile with increased stroke volumes during exercise resulting in more positive filling pressures, teleosts may further shift to a vis-a-tergo cardiac filling mode for increased activity [52]. However, the relative proportion of early to late phase filling (or passive vs. atrial active) suggests that the late phase is greater than the early phase in biphasic [49] and the third phase in triphasic does not change this pattern. With late phase (active atrial contraction) playing such an important role regardless of filling mode, the trend still holds that fish hearts are still more dependent on atrial contraction for ventricular filling than mammals.

#### 5. Physiological differences between chambers

It has been proposed that a key regulator of cardiac performance is the end diastolic volume (EDV) in both mammals and fish. Many of the morphological differences between chambers may have evolved in order to meet the high physiological demands of variation in metabolic rate and increased tolerance to a wide range of environmental conditions. Several of these will be highlighted in this section.

### 5.1. Force generation

Cardiac output may be modulated through variations in stroke volume and heart rate, but the relative contributions vary substantially among fish species. In general fish respond to greater hemodynamic loads by increasing cardiac output mainly through increased stroke volume rather than heart rate. Stroke volume can vary up to 2-3 fold with exercise (salmonids - [53]) but not all species exhibit the same ability [54-55]. End systolic volume (ESV) of the fish heart relative to stroke volume is very small [56-57] unlike that of the mammalian heart. However, end diastolic volume in fish is greatly determined by atrial contraction rather than central venous pressure seen in mammals [13]. The modulation of atrial contractility and Frank-Starling mechanisms are thus particularly important in determining ventricle filling.

The whole heart displays a greater sensitivity to filling pressure than in mammals, in part attributed to greater myocardial extensibility coupled with an increase in myofilament calcium sensitivity over a large range of sarcomere lengths [58]. The Starling principle dictates that the strength of contraction is, in part, a function of the length of the muscle fiber (and hence sarcomere length) prior to contraction (i.e. preload). Stroke volume increases as a function of end diastolic volume since muscle fibers and sarcomeres within the fibers are stretched towards their optimum. Between various species of fish there is considerable variation in length-dependent sensitivity. For example, flounder heart is very sensitive to filling pressures, needing smaller increases in preload to achieve higher values of stroke volume. This more pronounced Starling curve is further impacted by the high distensibility of its chambers based on pressure-volume curves [28]. This length-dependent strength of contraction is also directly affected by temperature in certain teleost species (e.g. trout- [58]). With fish cardiac myocytes showing enhanced length-dependent activation, and calcium sensitivity increasing with sarcomere length [59], calcium handling is an important consideration in the basis of the length dependence of the strength of cardiac contraction in fish. The unique properties of E-C coupling in the chambers of the fish heart will be discussed in greater detail further in this Chapter. The reduced relative duration of diastole seen with active atrial contraction in fish may have evolved in response to the relatively slow rate of the excitation-contraction coupling processes.

The importance of strong atrial contraction is reflected in the fact that atrial muscle generates greater maximal force ( $F_{max}$ ) than ventricular muscle in a variety of teleost species (e.g. rainbow trout- [60]; yellowfin tuna - [61]; brown trout - [62]). Although systolic blood pressure measurements show the atrium to be a weaker pump, the higher force generated in excised strips suggests the relative weakness of the atrium may simply be reflective of the anatomical difference in wall thickness between the chambers. The greater  $F_{max}$  of the atrial cardiomyocytes individually does not account for the difference in overall force generation by the greater quantity of cardiomyocytes in the thicker ventricle wall.

Cardiac performance is directly related to the contractile properties of cardiomyocytes. As mentioned previously, ventricular and atrial muscles express different paralogs (or isoforms) of myosin heavy chain (MHC) [63]. The maximum rate of force produced by muscle fibers is determined to a large degree by MHC paralog content. Alterations in paralog composition therefore are associated with changes in myofibrillar function as well as myosin



ATPase activity [64]. Because of this, the myosin ATPase reaction is higher in atrial than in ventricular cardiomyocytes [65]. Across vertebrates, from the primitive dogfish right up to mammals, the atrium has a higher concentration of MHC protein content than the ventricle [4]. The increased levels of MHC may be contributing to the increased  $F_{\max}$  of individual atrial cardiomyocytes relative to ventricular. However, MHC expression pattern differences between chambers may also be species-specific. In goldfish, both chambers have electrophoretically similar MHC while in other fish species such as pike, ventricular myocardium exhibited electrophoretically faster MHC [65]. Linking chamber-specific isoform usage with functional differences across a broader phylogenetic range as well as temperatures may help to clarify the importance of this heterogeneity.

## 5.2. Action potential

As in other vertebrates, the teleost action potential can be divided into a rapid upstroke of the action potential (phase 0), a rapid initial repolarization of the AP (phase 1) followed by a plateau phase (phase 2). The plateau phase is followed by repolarization of the action potential (phase 3) returning it to the resting membrane potential (phase 4). Teleost atrial and ventricular myocytes also have distinct AP morphologies with the atrial action potential having a shorter plateau phase and a slower repolarization of the AP. Among the ionic currents contributing to the differences in fish atrial and ventricular AP morphologies, ventricular myocytes have prominent inwardly rectifying potassium current  $I_{K1}$  and a small  $I_{Kr}$  while the opposite is true for atrial myocytes [66-67]. Other currents expected to affect the AP plateau such as the L-type calcium current ( $I_{Ca}$ ) or the  $Na^+/Ca^{2+}$  exchanger (NCX) current ( $I_{NCX}$ ) have not been compared systematically in the teleost heart.

Action potentials have been recorded in a number of teleost species (carp - [68]; trout - [34]; zebrafish - [69]) with a considerable variability in AP morphology with the atrial AP duration ranging from 197 ms in carp atrium at 27 °C to 281 ms in trout atrium at 15 °C, 601 ms in carp at 11 °C and 1284 ms at 4 °C and ventricular AP duration ranging from 407 ms at 10 degrees to 143 ms at 20 °C in trout ventricle [71-72] and from 1042 ms at 11 °C to 737 ms at 18 °C in Burbot ventricle [70,72], likely reflecting species-dependent differences as well as variations in physiological-experimental conditions such as temperature or salinity. Although these variations in AP-duration are larger than 10-fold, they allow the teleost heart to adapt to a wide temperature range [18] while the mammalian heart becomes refractory when temperature is lowered. The greater adaptability of the teleost heart to temperature is also reflected in the properties of ionic channels and ion transporters that determine excitability and the action potential shape. For example the change in trout NCX activity is only weakly modified by temperature changes ( $Q_{10} = 1.2 - 1.5$ ) as compared to mammals ( $Q_{10} = 2.5$ ) [73-74] and calcium uptake and leak from the sarcoplasmic reticulum is slowed in parallel, resulting in preservation of calcium release from the SR in trout [75-76]

In recent years, action potentials have also been recorded in myocytes isolated from teleost atrial and ventricular myocytes with several reports showing that the resting membrane potential in atrial myocytes is significantly more depolarized than in ventricular myocytes [66,

77]. It was proposed that this could be due to the low  $I_{K1}$  density in trout atrial myocytes [66]. However, membrane potentials recorded in atrial tissue preparations [71] are comparable to values recorded in trout ventricular preparations [70, 78] under comparable experimental conditions. Studies have showed that the resting membrane potential recorded with current-clamp technique in trout atrial myocytes was highly sensitive to the holding current applied [70]. Moreover, recordings of potassium conductance in these myocytes suggested that the loss of the resting membrane potential in isolated trout atrial myocytes is likely due to the lack of activation of the acetyl choline-activated potassium current ( $I_{KACh}$ ).

### 5.3. Excitation-contraction coupling

Calcium handling is an important consideration as the primary basis for regulation of cardiac contraction in all species, including fish, since it is determined by delivery and removal of calcium to and from cardiac troponin C (cTnC), the thin filament protein which senses changes in cytosolic  $Ca^{2+}$  concentrations and initiates the contractile process. Therefore, the amplitude and kinetics of the cytosolic calcium transient will modulate the kinetics and force of contraction, and this in turn will set the limit for the contraction frequency. Mammalian cardiac muscle has intracellular calcium stores in the SR in close proximity to T-tubules that provide the majority of the calcium that binds to cTnC. In ectotherms, the activation of calcium release from the SR and its contribution to contractile activation is controversial with some reports showing that the majority of calcium is derived from extracellular space [79, 36, 34, 58] while others report a considerable contribution of the SR both to the activation of contraction [38, 80] and to calcium removal from the cytosol during relaxation [58, 80-81]. Interestingly, calcium handling by the SR has also been reported to differ between fish atrial and ventricular myocytes and these differences as well as species dependent differences are likely to account for part of this controversy on the role of the SR in the teleost heart. Additionally, a significant number of reports examining the role of the SR in the teleost heart relied heavily on pharmacological inhibition of the SR calcium release channel (also termed ryanodine receptor or RyR2) in multicellular tissue preparations. As discussed below, this approach may not be optimal to evaluate the contribution of the SR to the activation of contraction if other calcium delivering mechanisms are capable of compensating for inhibition of the RyR2. Instead, quantification of the contribution of each mechanism involved in the delivery and removal of calcium to and from cTnC is necessary to estimate its capacity and contribution.

### 5.4. Activation of contraction

In mammalian cardiomyocytes the key contributors to the activation of contraction are the L-type calcium channel that mediates calcium entry across the sarcolemma and the SR, which releases the majority of the calcium from the SR. During the upstroke of the action potential, reverse mode NCX may also contribute [82]. Since the initial reports on  $I_{Ca}$  measurements in fish cardiomyocytes [73, 83-84] there is now substantial information on  $I_{Ca}$  density in atrial and ventricular myocytes from a number of fish species under different experimental conditions [77, 85]. Again, differences in experimental conditions and species

are abundant and there are substantial differences in the measurements of calcium entry though  $I_{Ca}$  among species at different temperatures; Burbot :  $I_{Ca}$ ,  $0.81 \pm 0.13$  pA pF<sup>-1</sup> at 4°C, and  $1.35 \pm 0.18$  pA pF<sup>-1</sup> at 11°C [55]; trout: 1, 2 and 4 pA pF<sup>-1</sup> at 7, 14 and 21 °C, respectively [77]. Among the experimental conditions that have may have the largest impact on  $I_{Ca}$  measurements, ruptured-patch technique with a high (5 mM) EGTA-buffered patch pipette solution has been shown to result in overestimation of  $I_{Ca}$  when compared to measurements performed with low EGTA (25 µM) or perforated patch-clamp technique [75]. Therefore, estimations of the contribution of the calcium carried by  $I_{Ca}$  to the activation of contraction also varies from as little as 25-30% in trout atrial myocytes using perforated patch technique [75,86], to more than 50% in zebrafish ventricular myocytes subjected to perforated patch-clamp technique [87] and close to 100% using ruptured patch-clamp technique with EGTA buffered patch pipettes in carp ventricular myocytes [37] and in trout atrial myocytes subjected to  $\beta$ -adrenergic stimulation [87]. The latter does not, however, necessarily imply that  $I_{Ca}$  is the sole source of calcium delivery in these preparations but merely indicates that it is sufficiently large to activate contraction if no other calcium sources are available. As mentioned above, chamber specific differences in the  $I_{Ca}$  density have not been systematically investigated in the teleost heart.

Theoretically, reverse mode NCX could also contribute to the activation of contraction at depolarized membrane potentials and/or when sodium channels produce a large local increase in the subsarcolemmal Na concentration. Several studies have shown that fish cardiomyocytes have a high NCX activity than mammalian cardiomyocytes, and the direct contribution of the NCX to the activation of contraction has been estimated to range from 25-45% under physiological conditions [86-87, 90]. However, the relative contribution of  $I_{NCX}$  to total sarcolemmal calcium entry depends critically on the membrane potential and the intracellular Na<sup>+</sup> concentration [74, 85-86]. Moreover, there is evidence that reverse mode NCX can trigger calcium release from the SR in trout atrial myocytes [90].

Finally, calcium release from the SR has also been reported to contribute to the activation of contraction in fish cardiac myocytes with a contribution that varies between 0 and more than 50% depending on species, tissue and experimental temperature. Some of the first publications to examine the effects of ryanodine, a compound that modulates the opening of the SR calcium release channel, on contraction in multicellular tissue preparations were [91-92] but the reported effects on contraction were only marginal at physiological temperatures and stimulation frequencies. Ryanodine was, however, reported to inhibit post rest potentiation, a phenomenon caused by calcium release from the SR [78], and in 1992 it was reported to have a strong effect on contraction in trout [93] and skipjack tuna [94] at 25 °C, suggesting a temperature- and/or species-dependent component of the effect of ryanodine. In 1998, it was shown that steady-state and maximal SR calcium content in trout ventricular myocytes was about 10 times higher than values reported in human cardiomyocytes and that uptake rates were sufficiently fast for the SR to participate in the regulation of calcium handling on a beat-to-beat basis [75] and in 1999 it was shown that brief membrane depolarizations were capable of eliciting calcium release from the SR in trout atrial myocytes corresponding to a contribution of up to 50% of the total calcium required for the activation of contraction [80].

Subsequently it was shown that this contribution of the SR to the activation of contraction was equally important at 21 and 7°C in trout atrial myocytes [95]. These findings were confirmed in trout using simultaneous recordings of membrane currents and calcium transients [89] and with confocal calcium imaging, which also demonstrated that local spontaneous calcium release from the SR (known as calcium sparks in mammalian cardiomyocytes) are also present in trout atrial and ventricular myocytes as well as in zebrafish ventricular myocytes [96]. This study also showed that the spark frequency in trout atrial myocytes was comparable to that recorded in human atrial myocytes under similar experimental conditions [97], but twice as fast as in trout ventricular myocytes. In line with this, the frequency of spontaneous membrane depolarizations, induced by SR calcium release, were also about 6-fold more frequent in trout atrial than ventricular myocytes, suggesting that SR calcium is more labile in atrial than ventricular myocytes. A higher contribution of the SR  $\text{Ca}^{2+}$  release has also been reported for the tuna heart [38, 67].

Experiments attempting to identify the triggers of SR calcium release showed that both  $\text{ICa}$  and reverse mode NCX are capable of triggering calcium release from the SR [90] and, unexpectedly, this study showed that the gain of  $\text{I}_{\text{Ca}}$  and  $\text{I}_{\text{NCX}}$ -induced calcium release was similar in trout atrial myocytes. Moreover, it was shown that the relative contribution of the two mechanisms depended strongly on the membrane potential and the intracellular  $\text{Na}^+$ -concentration. Considering that an intracellular  $[\text{Na}^+]$  between 13 and 14 mM has been reported in trout cardiomyocytes [98] reverse mode NCX could may be expected to contribute significantly to the triggering of calcium release from the SR in trout. While in mammals  $[\text{Na}^+]_i$  is higher in atrial than ventricular cells [99], no difference has been seen between chambers in rainbow trout [98]. However, with loss of function of NCX in mutant zebrafish (tre mutant) more cardiac fibrillation is seen in the atrium than the ventricle [100]. This suggests that the atrium is more susceptible to spontaneous  $\text{Ca}^{2+}$  release from the SR as a result of disruptions of normal calcium transients.

### 5.5. Relaxation of contraction

Under steady-state conditions, the calcium delivered to the myofilaments during the activation of contraction has to be removed from the cytosol during relaxation. Moreover, if equilibrium is to be maintained, the calcium entering across the sarcolemma must be extruded from the cell and calcium released from intracellular organelles must be re-sequestered by the same organelle. Based on the estimates of sarcolemmal calcium entry in the fish heart, sarcolemmal calcium extrusion would therefore be expected to vary between 50 and 100% in fish cardiomyocytes depending on the species and tissue. In the mammalian heart, forward mode NCX generally accounts for the vast majority of the sarcolemmal calcium extrusion [101] and experiments using rapid caffeine application in 0Na/0Ca-solution showed that this is also true for trout ventricular myocytes [84]. Assuming that the tail current elicited upon repolarization of the membrane potential is primarily carried by forward mode NCX, the time integral of this current can be used to estimate calcium extrusion by the NCX [75]. With this approach calcium extrusion by the NCX was estimated to account for about 55% of the total increase in calcium during activation of contraction suggesting that intracellular recy-



cling of calcium during contraction must account for about 45% of the total calcium transient [86]. On the other hand, measurements of the NCX activity showed a forward NCX-rate under physiological conditions of  $36 \text{ amol pF}^{-1} \text{ s}^{-1}$ , which is sufficient to remove the total calcium required for the activation of contraction in a few hundred ms [76].

In agreement with the notion that calcium cycling by the SR takes place on a beat-to-beat basis and could contribute to the activation of contraction, measurements of SR calcium load with rapid caffeine application and specific protocols used to measure steady-state and maximal SR calcium loading, showed that the SR calcium content in trout ventricular and atrial myocytes can be up to 10 times higher than in mammalian myocytes [58,61,77,84], and that the rate of SR calcium uptake under physiological conditions is also sufficient to remove the total calcium required for the activation of contraction between beats at physiological beating rates and temperatures in trout atrial and ventricular myocytes [75,95]. In the presence of  $\beta$ -adrenergic stimulation, the relationship between SR calcium uptake and the cytosolic calcium level was shifted towards lower calcium concentrations, but when normalized to the total calcium transport SR calcium uptake was only slightly increased from 35 to 41% by  $\beta$ -adrenergic stimulation [102].

It is interesting to note that the SR calcium load in fish cardiomyocytes is more than 10-fold higher than the amount of calcium released on each beat [84, 95, 103]. The reason for such an excessive SR calcium load compared to the calcium requirements on a beat-to-beat basis remains elusive, but it definitely provides fish species with an intracellular calcium reservoir that allows the heart to function under adverse conditions where SR calcium refilling is impaired for extended periods of time. This also explains why the inhibition of SR calcium uptake (with cyclopazonic acid) or increasing SR calcium leak (with ryanodine) may have a much slower effect on contraction in fish than in mammalian hearts.

Atrial cardiomyocytes have both greater SR  $\text{Ca}^{2+}$  loads [105-106] and make greater use of SR  $\text{Ca}^{2+}$  than ventricular cardiomyocytes [60, 105]. The degree of atrio-ventricular difference in SR  $\text{Ca}^{2+}$  load and usage is also species specific, being larger in rainbow trout than crucian carp or burbot [105].

The reliance on intracellular  $\text{Ca}^{2+}$  leads to more rapid contraction kinetics of the fish atrium due to faster calcium release and reuptake rates via the SR relative to ventricular tissue in a variety of teleost species (e.g. rainbow trout - [60]; burbot - [105]). Lowering of cytosolic  $\text{Ca}^{2+}$  concentration in this manner is via sarco-endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA), and thus the higher SERCA protein expression found in atrial contributes to this shorter duration of isometric contraction relative to ventricular myocardium [107]. In addition, the strength of contraction in teleost atrial muscle has greater ryanodine sensitivity than that in ventricles [60, 62] and is similar in magnitude to what is typically seen in many mammals [82].

Interestingly, the maximal capacity and steady-state SR  $\text{Ca}^{2+}$  load does not necessarily correlate with SERCA activity or ryanodine sensitivity in all ectotherms. Inverse relationships between ryanodine sensitivity and SR  $\text{Ca}^{2+}$  load have been found in rainbow trout, burbot and crucian carp [105]. While atrial contraction is more strongly inhibited by ryanodine



than ventricular contraction [60], the increased activity of SR  $\text{Ca}^{2+}$  ATPase may indicate that atria are able to load the SR with  $\text{Ca}^{2+}$  in the time between contractions. These factors may contribute to a faster recovery of myocardial contractility or increased mechanical restitution rates in atrial muscle. This may mean increased importance of SR  $\text{Ca}^{2+}$  for contraction during periods of environmental stress. More studies are necessary to clarify the role of SR  $\text{Ca}^{2+}$  handling in fish cardiomyocytes, especially with the species-specific and chamber-specific vagaries.

Differences in chamber-specific expression of the contractile element responsible for calcium sensitivity may also guide differences between contractility of the atrium and ventricle. Myofilament  $\text{Ca}^{2+}$  sensitivity is a critical factor in regulating cardiac contractility. The cardiac TnT subunit of the zebrafish troponin complex has been shown to be required for the contraction of both the atrium and the ventricle [106] as it is in mammals. However, zebrafish possess two copies of the gene for cardiac troponin C, with one demonstrated to be predominantly expressed in the heart of the zebrafish embryo. Loss of function of this gene results in abnormal atrial and ventricular chamber morphology, but the second cTnC gene enables function to be regained in the atrium [107]. This suggests a possible difference in expression of cTnC between chambers that could potentially lead to variation in calcium sensitivity. Gene duplication has led to multiple copies of sarcomeric proteins, and variation between these fish-specific paralogs may be a method of coping with varying environmental conditions.

## 6. Environmental influences

The interspecific variation of fish cardiac anatomy and physiology is guided by evolutionary adaptation to different habitats, modes of life and activity levels. Because ectothermic fish are exposed to different temperatures either seasonally or via the vertical thermocline, temperature-dependent regulation of cardiac contractility is crucial. Many fish face unpredictable temperature changes, meaning that fish hearts must have intrinsic mechanisms or rely on post-translational modifications to protect against acute temperature changes when there is not enough time for transcriptional regulation to take effect [108, 34].

Cardiac performance may change through independent changes in stroke volume and heart rate, with relative contributions of each varying substantially among fish. During exercise, more athletic fish modulate cardiac output preferentially by increasing SV, while less active species preferentially modulate HR in different species [54-55]. With environmental hypoxia, reflex bradycardia occurs and increased stroke volume is necessary to maintain cardiac output [109-110]. Higher temperatures have been shown to increase heart rate while colder temperatures decrease stroke volume in crucian carp [10]. For this review, the impact of temperature on cardiac contractility will be focused on. Mechanisms of control of both stroke volume and heart rate must be considered when looking at temperature sensitivity of the fish heart.

There appears to be seasonal variation with the activity of pacemaker cells (marine plaice - [111]). Heart rate is maintained despite the lower temperatures without the need for extrinsic modification. Changes in heart rate seen with temperature are not solely dependent on pacemaker cells. Therefore, changes that are seen may be due to ion channel temperature sensitivity. Chronic temperature stress can also modify  $K^+$  channel conductances and reduce action potential duration to enable higher heart rates (trout - [112]). Thermal acclimation modifies functional properties and subunit composition of  $K_{IR2}$  channels [113]. Cold temperatures induce decreased  $I_{K1}$ , possibly increasing the excitability of cardiomyocytes but increased  $I_{Kr}$  leading to limited AP duration and decreased refractoriness of the heart [114]. These changes in  $I_K$  appear when it is the predominant current, namely in ventricular cardiomyocytes [66] while the changes in  $I_{Kr}$  appear in both atrial and ventricular cardiomyocytes [114]. The density in particular of ventricular  $I_{K1}$  increases in warm-acclimated vs. cold acclimated fish (trout - [113]).

The plasticity of E-C coupling in fish has been suggested to be based, at least in part, on these temperature-induced changes in  $I_K$  [114]. These modifications lead to changes in  $Ca^{2+}$  influx and hence variation in E-C coupling.  $Ca^{2+}$  pumps and ion channels themselves are also known to be sensitive to acute temperature change [103]. The large SR calcium stores and low calcium sensitivity of ryanodine receptors have been proposed to play a critical role in maintaining contraction at lower temperatures than mammals [81]. Higher SR  $Ca^{2+}$  content may be necessary to initiate and maintain SR  $Ca^{2+}$  release events at lower temperatures [80,115].

Different species may have variations in response to temperature depending on their evolutionary history. Eurythermal fish such as trout must be able to cope with seasonal temperature fluctuations as well as more acute temperatures through the thermal gradient. Other stenothermal species are only able to cope with a more limited temperature range, though this range may be considered on the extreme end of the range for a eurythermal fish (e.g. Antarctic fish). Interspecies variation in excitation-contraction coupling seen with temperature acclimation may be indicative of the capacity for thermal niche expansion. This substantial variation in SR  $Ca^{2+}$  release relative to SL  $Ca^{2+}$  influx ratio exists between species as well as being dependent on acute and chronic temperature changes. Cold-tolerant active fish have a larger capacity to store  $Ca^{2+}$  in the SR than mammals (e.g. active teleosts - [115]; scombroids - [116]). The atrio-ventricular differences in  $Ca^{2+}$  loading are also more pronounced in cold-acclimated than warm acclimated fish (trout - [112]).

However, the contribution of the SR to cytosolic  $Ca^{2+}$  management varies with temperature in fish hearts. With cold acclimation, SR function is also enhanced more strongly in atrial than ventricular myocytes (trout - [60]). More cold tolerant species may also possess greater SR  $Ca^{2+}$  content to begin with (bluefin tuna and mackerel - [112]). The degree of modification of  $I_{Ca}$  to offset the negative effects of colder temperatures appears to be species specific, with many teleosts such as trout demonstrating relatively temperature-insensitive  $Ca^{2+}$  flux [103] whereas certain scombroids show significant reductions in  $I_{Ca}$  with decreasing temperatures [112]. This temperature sensitivity may differ between atrial and

ventricular cardiomyocytes, with ventricular maximum SR  $\text{Ca}^{2+}$  load varying only in the ventricle of most scombrids [112].

As previously mentioned, isoform compositional changes can lead to variation in contractility. Modulation of MHC isoform expression patterns with temperature also leads to changes in myofibrillar ATPase activity (crucian carp - [10]). This may also be a species-related difference, since not all species display the same changes. The degree to which each chamber varies is still unclear. Differing paralog usage between the chambers may be linked to temperature-induced  $\text{Ca}^{2+}$  sensitivity differences. Examples of this may exist in important of the contractile element. Troponin I, the inhibitory subunit of troponin, has multiple paralogs expressed in cardiac tissue. The relative expression of these paralogs in the ventricle shifts with temperature, possibly leading to changes in the calcium affinity of the entire troponin complex [117]. However, while paralog profiles vary between tissues (heart, slow skeletal) no information is known about the atrium. TnC isoform differences between chambers [107] may also lend insight into  $\text{Ca}^{2+}$  sensitivity changes with temperature as the affinity of cTnC in trout is temperature dependent [118]. As previously mentioned, the very presence of multiple fish-specific paralogs of important components of the contractile element may be indicative of how fish have evolved mechanisms to thrive in variable environments. Incorporating the chamber-specific variation with temperature response reveals the complexity of these mechanisms in achieving survival.

## 7. Summary

The unique structure of the fish heart accommodates the variability in function that have allowed species to exploit many different environments. The increased importance of the atrial contraction in guiding changes in cardiac output indicates that some of this physiological versatility may be due to atrio-ventricular differences. These two main chambers of the fish heart differ in terms of contractile properties from the molecular level of E-C coupling and AP morphology, and the whole heart morphology and function reflect this. The fish heart is representative of early embryological stages of higher vertebrates, these studies on both the development and functioning of chamber-specific properties lends insight into proper functioning of the heart not only in fish. However, the degree of atrio-ventricular differences vary between species making it very difficult to generalize species-specific results across phylogeny. The species-specific differences in these two chambers lend insight into how evolutionary history guides responses to environmental factors such as ambient temperature. Fish-specific genome duplications may have allowed for multiple chamber-specific genes with variable functions that allow for flexibility in function. Further work is required to determine how genome duplication has shaped the structure and function of the fish heart and to clarify how this wide-range of species-specific phenotypes has been maintained.

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