J Comp Physiol B
DOI 10.1007/s00360-016-1034-9

ORIGINAL PAPER

Characterization of the functional and anatomical differences in the atrial and ventricular myocardium from three species of elasmobranch fishes: smooth dogfish (*Mustelus canis*), sandbar shark (*Carcharhinus plumbeus*), and clearnose skate (*Raja eglanteria*)

Julie Larsen[1] · Peter Bushnell[2] · John Steffensen[1] · Morten Pedersen[3] · Klaus Qvortrup[4] · Richard Brill[5,6]

Received: 12 February 2016 / Revised: 25 August 2016 / Accepted: 13 September 2016

Abstract We assessed the functional properties in atrial and ventricular myocardium (using isolated cardiac strips) of smooth dogfish (*Mustelus canis*), clearnose skate (*Raja eglanteria*), and sandbar shark (*Carcharhinus plumbeus*) by blocking Ca^{2+} release from the sarcoplasmic reticulum (SR) with ryanodine and thapsigargin and measuring the resultant changes in contraction–relaxation parameters and the force–frequency relationship at 20 °C and 30 °C. We also examined ultrastructural differences with electron microscopy. In tissues from smooth dogfish, net force (per cross-sectional area) and measures of the speeds of contraction and relaxation were all higher in atrial than ventricular myocardium at both temperatures. Atrial-ventricular differences were evident in the other two species primarily in measures of the rates of contraction and relaxation. Ryanodine-thapsigargin treatment reduced net force and its maximum positive first derivative (i.e., contractility), and increased time to 50 % relaxation in atrial tissue from smooth dogfish at 30 °C. It also increased times to peak force and half relaxation in clearnose skate atrial and ventricular tissue at both temperatures, but only in atrial tissue from sandbar shark at 30 °C; indicating that SR involvement in excitation–contraction (EC) coupling is species- and temperature-specific in elasmobranch fishes, as it is in teleost fishes. Atrial and ventricular myocardium from all three species displayed a negative force–frequency relationship, but there was no evidence that SR involvement in EC coupling was influenced by heart rate. SR was evident in electron micrographs, generally located in proximity to mitochondria and intercalated discs, and to a lesser extent between the myofibrils; with mitochondria being more numerous in ventricular than atrial myocardium in all three species.

Keywords Atria · Cardiac · Force–frequency · Heart · Temperature · Ventricle

Abbreviations

CICR	Ca^{2+}-induced Ca^{2+}-release
$+dF/dt_{max}$	Maximum rate of force development (mN mm^{-2} s^{-1})
$-dF/dt_{min}$	Maximum rate of relaxation (mN mm^{-2} s^{-1})
DMSO	Dimethyl sulfoxide
EC	Excitation–contraction
ECG	Electrocardiogram
G	Glycogen storage granules
h	Hours
Hz	Hertz
ICD	Intercalated discs
kHz	Kilohertz
M	Mitochondria
min	Minutes

Communicated by H.V. Carey.

✉ Richard Brill
rbrill@vims.edu

[1] Marine Biological Section, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark

[2] Department of Biology, Indiana University South Bend, 1700 Mishawaka Avenue, South Bend, IN 46634-7111, USA

[3] Department of Science and Environment, Roskilde University, Universitetsvej 1, 4000 Roskilde, Denmark

[4] Department of Biomedical Sciences/CFIM, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen, Denmark

[5] Behavioral Ecology Branch, Ecosystems Processes Division, Northeast Fisheries Science Center, National Marine Fisheries Service, NOAA, Sandy Hook, NJ, USA

[6] Virginia Institute of Marine Science, PO Box 1346, Gloucester Point, VA 23062, USA

Published online: 29 September 2016

mm	Millimeter
mN	MilliNewtons
ms	Millisecond
MHC	Myosin heavy chains
NCX	Na^+–Ca^{2+} exchanger
nm	Nanometers
P	Probability
s	Seconds
SL	Sarcolemma
SERCA	SR Ca^{2+}-ATPase
SR	Sarcoplasmic reticulum
Z	Z-line

Introduction

The mechanisms involved in ventricular filling in elasmobranch fishes are similar to those of other vertebrates (Lai et al. 1996, 2004; Brill and Lai 2016). Across vertebrate species, ventricular filling occurs primarily as a result of venous pressure returning blood to the heart, with atrial contraction supplying the final fraction (~10–30 %) of end-diastolic ventricular volume (Lo et al. 1999; Bootman et al. 2006; Cotter et al. 2008). Atrial contraction has, moreover, been proposed to provide appropriate adjustments in end-diastolic ventricular volume (i.e., the "final kick", Korajoki and Vornanen 2012) so as to match cardiac output (through changes in stroke volume) and rates of circulatory system oxygen delivery to metabolic oxygen requirements (Farrell and Jones 1992; Aho and Vornanen 1999). This requires an appropriate atrial-ventricular delay in the conduction of the electrical signal stimulating contraction, and that the atrial myocardium contract and relax prior to ventricular systole (i.e., the time course of contraction and relaxation in atrial myocardium be shorter than in ventricular myocardium). Recordings of chamber-specific electrical activity (shown in ECG records) and echocardiographic assessments of chamber-specific flow and filling patterns have shown that these occur in elasmobranch fishes, as they do in other vertebrates (Lai et al. 1996, 1998, 2004). A shorter atrial contraction also provides a longer time for atrial filling during atrial diastole.

The contraction and relaxation of vertebrate myocardium is controlled by the rise and fall (respectively) of intracellular free calcium (Ca^{2+}), which binds to troponin C and removes the inhibition between the thin and thick filaments (Bers 2002; Gillis and Klaiman 2011). Increases in intracellular Ca^{2+} result from a combination of an influx of extracellular (i.e., trans-sarcolemmal) Ca^{2+} and that released from the sarcoplasmic reticulum (SR) (Galli and Shiels 2012; Shiels and Galli 2014). In mammals, SR Ca^{2+} release is the predominate mechanism and occurs in response to the initial increase in intracellular free Ca^{2+} (the so-called "Ca^{2+} induced Ca^{2+} release", "CICR") (Bers 2002). In contrast, the degree of involvement of trans-sarcolemmal Ca^{2+} influx in excitation–contraction (EC) coupling in ectothermic vertebrates is generally considered to be of greater importance than SR Ca^{2+} release (Tibbits et al. 1992; Galli and Shiels 2012; Gamperl and Shiels 2014; Shiels and Galli 2014; Shiels and Sitsapesan 2015), albeit with some exceptions. SR Ca^{2+} release has a significant role in excitation–contraction (EC coupling) in species capable of exceptional high heart rates, blood pressures, or cardiac outputs (e.g., tunas and varanid lizards) (Keen et al. 1992; Shiels et al. 1999; Galli et al. 2006). The same has been found in atrial and ventricular myocytes isolated from rainbow trout (*Oncorhynchus mykiss*) under elevated temperatures, or under conditions simulating those requiring higher contractility (e.g., during stress or exercise) (Shields et al. 2002a; Cros et al. 2014). To complete the cardiac cycle, relaxation of cardiac myocytes occurs when intracellular Ca^{2+} is pumped back into the SR by the SR Ca^{2+}-ATPase (SERCA) and actively transported across the sarcolemma by the Ca^{2+} pump and the Na^+–Ca^{2+} exchanger (NCX) (Bers 2002).

The general predominance of SL Ca^{2+} in EC coupling in teleost ventricular myocardium is adequate because of fishes' lower heart rates, cardiac outputs, and smaller myocardial cells (i.e., cells with a higher surface to volume ratio) compared to mammals (Farrell 1991; Farrell and Jones 1992; Tibbits et al. 1991; Vornanen et al. 2002b). As pointed out in recent reviews (Shiels and Galli 2014; Brill and Lai 2016), the relative importance of trans-sarcolemmal and SR Ca^{2+} movements to EC coupling in elasmobranch myocardium has received much less attention than in other vertebrates. Nonetheless, the mechanisms underlying the action potential and EC coupling at least in spiny dogfish (*Squalus acanthias*) myocardium appear largely congruent with other vertebrates (Maylie et al. 1994; Maylie and Morad 1979, 1981, 1995; Brill and Lai 2016). In elasmobranch myocytes (because of their small size and lack of a T-tubule system), trans-sarcolemmal calcium movement is generally considered sufficient for EC coupling, with CICR from the SR playing a minor role (Maylie et al. 1979; Thomas et al. 1996). Supportive of this conclusion is the observation that contraction of ventricular myocytes from spiny dogfish is blocked by nifedipine (a calcium channel blocker primarily affecting voltage-gated L-type calcium channels) (Näbauer and Morad 1992). The anatomical presence of SR in ventricular myocytes from spiny dogfish has, however, been qualitatively described as "considerable" and "frequently observed" (Maylie et al. 1979), implying a functional role of SR Ca^{2+} release under some circumstances. SR Ca^{2+} storage capability in the heart from little skate (*Raja erinacea*) has also been reported to be more developed than in hearts from teleost fishes (Driedzic and Gesser 1988).

In addition to species-specific differences, atrial and ventricular myocardium evince tissue-specific differences that extend from the molecular to the anatomical level, EC coupling and action potential (AP) morphology, and functional properties (Aho and Vornanen 1999; Bootman et al. 2006; Galli and Shiels 2012; Genge et al. 2012, 2013; Shiels and Galli 2014). For example, across a range of vertebrate species, atrial myocardium has been shown to contain up to three times the SR volume of ventricular myocardium, as well as significantly greater SR Ca^{2+} loads and SERCA activity (Haverinen and Vornanen 2009; Korajoki and Vornanen 2012, 2014; Shiels and Galli 2014). The role of SR Ca^{2+} release to EC coupling has been frequently assessed using ryanodine (a specific blocker of the SR Ca^{2+} release channel) often in combination with thapsigargin (a specific inhibitor of the SR Ca^{2+} ATPase pump) with the measure of SR involvement being the fractional reduction in the force of contraction following treatment (e.g., Rousseau et al. 1987; Sagara and Inesi 1991; Shiels and White 2005). By this measure, atrial myocardium in some (but not all) ectothermic and endothermic species evinces a larger reliance on SR Ca^{2+} release than ventricular myocardium (reviewed in Shiels and Galli 2014).

In mammals, contraction of the atrium is thought to provide up to ~30 % of the end-diastolic ventricular volume under stressful conditions (Bootman et al. 2006). Likewise, in both teleost and elasmobranch fishes, digital angiography, echo-Doppler cardiography, and simultaneous pressure measurement in the atrium and ventricle have shown that ventricular filling is biphasic. The first phase occurs due to blood flow into the ventricle during atrial diastole, and the second during atrial systole (Lai et al. 1998; Cotter et al. 2008; Brill and Lai 2016). Atrial contraction is, therefore, an important determinant of end-diastolic volume. End-diastolic volume is, in turn, a controlling factor in stroke volume through the well-known Frank–Starling mechanism (i.e., the ability of the heart to increase its force of contraction and stroke volume in response to an increase in end-diastolic volume), and therefore cardiac output. All this requires appropriate timing of atrial and ventricular contraction–relaxation cycles, including that the contraction–relaxation cycle be more rapid in atrial than ventricular myocardium. This is reflected in the shorter action potential and the larger dependence on SR Ca^{2+} release in atrial myocardium (Vornanen and Tuomennoro 1999; Vornanen et al. 2002a).

Within species, the relative importance of trans-sarcolemmal and SR Ca^{2+} to EC coupling is affected by both acute temperature change and temperature acclimation (Korajoki and Vornanen 2009, 2012, 2014; Methling et al. 2012). This occurs in addition to tissue-specific (i.e., atrial and ventricular) (Genge et al. 2012, 2013) and species-specific differences in the relative importance of trans-sarcolemmal and SR Ca^{2+} to EC coupling (Galli 2006; Galli et al. 2006, 2009; Galli and Shiels 2012; Shiels et al. 1999). The former happens primarily through the effect of temperature on the gating properties of the SR Ca^{2+}-release channels (i.e., the so-called "ryanodine receptors") as described in detail by Shiels and Galli (2014) and Shiels and Sitsapesan (2015). Fish occupying shallow marine inshore environments at temperate latitudes (and equivalent freshwater habitats) can be exposed to both acute and seasonal changes in water temperature, oxygen levels, and pH (Baumann et al. 2015; Breitburg et al. 2015a, b; Hofmann et al. 2011; Reum et al. 2014). Accordingly, they must be able to regulate cardiac output (i.e., heart rate and stroke volume) and the contractility of cardiac myocytes appropriately to satisfy metabolic oxygen requirements (both of the tissue downstream and the myocardium itself) under a broad range of environmental conditions (Olson and Farrell 2006). To this end, acclimation to low temperature has been shown to increase the importance of SR Ca^{2+} release in rainbow trout as demonstrated by a greater reduction of contractile force following application of ryanodine (Keen et al. 1994; Aho and Vornanen 1999), as well to increase the activity of the SR Ca^{2+} pump (Aho and Vornanen 1998, 1999; Korajoki and Vornanen 2012). Although myocardial tissues from other species (e.g., crucian carp, *Carassius carassius* and burbot, *Lota lota*) show either a decrease or no change in SR Ca^{2+} pump activity (respectively) with low temperature acclimation, responses are tissue-specific (Korajoki and Vornanen 2014). In teleost fishes, moreover, the SR Ca^{2+} release channel is temperature dependent with a higher degree of involvement following increases in temperature which are generally accompanied by increases in heart rate and metabolic oxygen requirements (Shiels and Farrell 1997; Shiels et al. 2002a; Galli and Shiels 2012).

Based on the similarity of EC coupling mechanisms in elasmobranch fishes to those of other vertebrates (Brill and Lai 2016), we suspect the responses of elasmobranch myocardium to temperature change will be broadly similar to those of other ectotherms. Subsequent to their major diversification in the Permian (250×10^6 years ago), however, members of the Elasmobranchii (i.e., sharks and rays) have come to occupy almost all aquatic environments (from the pelagic zone to the abyssal depths, and even into freshwaters); to inhabit latitudes from the tropics to the high Arctic (Klimley 2013). They also display an equivalent broad diversity of body morphologies, life styles, life histories, feeding and reproductive strategies, and energetics (Klimley 2013). Given this, and the broad diversity of the functional characteristics in the cardiovascular systems of elasmobranch fishes (Brill and Lai 2016), we contend that the functional properties of their atrial and ventricular myocardium are likely to show a level of diversity equivalent to

those seen in other ectothermic vertebrates. Previous studies of cardiac function have, however, often used one elasmobranch species (e.g., spiny dogfish) as a representative for all.

Our primary objectives were therefore:

1. to determine if differences exist in the functional characteristics of atrial and ventricular myocardium, including the involvement of SR Ca^{2+} release in the EC coupling at different temperatures;
2. to provide a cross-species examination of the involvement of SR Ca^{2+} release in EC coupling in elasmobranch myocardium at different temperatures and contraction frequencies.

For clarity we divide our results into three parts: (1) absolute values, (2) relative change following treatment with ryanodine and thapsigargin, and (3) force–frequency data.

These aspects of myocardial function are well studied in teleost, but not elasmobranch fishes. This lack of information on elasmobranch myocardial function is exemplified by the extensive reviews of the effects of temperature on myocardial function and compensatory mechanisms in fishes (Vornanen et al. 2002a; Gamperl and Shiels 2014), and the functional roles of SR in vertebrate hearts (Shiels and Galli 2014), all of which include data only on teleost fishes. Likewise, the review of force–frequency relationships by Shiels et al. (2002b) contains the only reference currently available (to the best of our knowledge) concerning the force–frequency relationships in elasmobranch myocardium, that of Driedzic and Gesser (1988). Similarly, the role of adrenergic stimulation maintaining cardiac function during acute reductions in temperature, hypoxia, and acidosis is well described in teleost fishes (reviewed by Gamperl and Shiels 2014), but likewise remains comparatively less studied in elasmobranch fishes, with the exception of studies by Davie and Farrell (1991) and Davie and Franklin (1992).

We investigated the relative roles of extracellular (i.e., trans-sarcolemmal) and intracellular (i.e., SR) sources of Ca^{2+} to EC coupling in atrial and ventricular tissue taken from three phylogenetically divergent elasmobranch species (representing two orders and three families): smooth dogfish [*Mustelus canis*, family Triakidae (houndsharks), order Carcharhiniformes (ground sharks)], sandbar shark [*Carcharhinus plumbeus,* family Carcharhinidae (requiem sharks), order Carcharhiniformes (ground sharks)], and clearnose skate [*Raja eglanteria*, family Rajidae (skates), order Rajiformes (skates and rays)]. These three species have sympatric distributions over the continental and insular shelves and within the tidal estuaries of the northeast and mid-Atlantic US east coast. They thus reside in inshore and near shore environments occupied by ~35 % of the 882 chondrichthyan species for which habitats are known (Compagno 1990). Smooth dogfish, sandbar shark, and clearnose skate do not, however, generally occupy the same areas in the northwest Atlantic Ocean at the same time of year because of differences in their thermal ecologies (Collette and Klein-MacPhee 2002; Grubbs and Musick 2007; Grubbs et al. 2007; Conrath and Musick 2008). In the mid-Atlantic Bight (the area from which the individuals used in this study were captured), smooth dogfish occur over a temperature range of ~5–28 °C, but are most common at 10–22 °C (Kiraly et al. 2003); sandbar shark occupy warm (~23–27 °C) coastal and adjacent offshore waters (Compagno 1984; McCandless et al. 2007; Conrath and Musick 2008; Able and Fahay 2010); and clearnose skate occur over a temperature range from ~9–30 °C, but prefer ~9–21 °C (McEachran and Musick 1975; Schwartz 1996; Collette and Klein-MacPhee 2002). We also note that these three species are of different ecomorphotypes as defined by Compagno (1990). Smooth dogfish is a relatively sluggish demersal species primarily feeding on decapod crustaceans; sandbar shark is an obligate ram ventilating active demersal species feeding mainly on bony fishes, small sharks, cephalopods, gastropods, shrimps, blue crabs and mantis shrimp (Medved and Marshall 1981; Medved et al. 1985; Stillwell and Kohler 1993); and clearnose skate is a largely sedentary benthic species feeding primarily on decapod crustaceans, bivalves, polychaete worms, squids, and fishes.

Materials and methods

Experimental animals

All animal capture, care, and experimental protocols and procedures were approved by the College of William and Mary Institutional Animal Care and Use Committee (protocol IACUC-2015-03-23-10286-rwbril; Cardiac function in elasmobranch fishes), followed the ethical standards of the Virginia Institute of Marine Science, and were in accordance with applicable laws and regulations. Experiments on cardiac tissue collected from smooth dogfish, sandbar shark, and clearnose skate were conducted at the Virginia Institute of Marine Science, Eastern Shore Laboratory (Wachapreague, VI, USA) during May through August in 2013, 2014, and 2015. Specimens were caught using standard recreational hook-and-line fishing gear either in the coastal tidal lagoon system adjacent to the Eastern Shore Laboratory or immediately offshore. Smooth dogfish and clearnose skate were maintained in indoor tanks with water continuously recirculated through a biofilter and ultraviolet sterilizer. Water temperature (20 ± 1 °C) reflected the ambient temperatures in the areas from which they were

captured. Sandbar sharks were kept in an outdoor circular fiberglass tank (~8 m diameter and ~2 m deep), which was shaded with a black mesh cloth awning to protect the animals from direct sunlight. The tank was continuously supplied with filtered seawater from the adjacent tidal lagoon, and the water within the tank was continuously recirculated through a biofilter and ultraviolet sterilizer. Because of the large volume of water required to hold sandbar shark (an obligate ram ventilating species), it was not feasible to control water temperature (which ranged from ~24–26 °C and reflected ambient temperatures in the areas from which the fish were captured). Salinity in all holding tanks ranged from 30–34 PSU. Animals were housed for 1–8 weeks before use in an experiment and fed approximately every other day.

Isolated heart strip procedures

Animals were euthanized with an overdose of sodium pentobarbital (~350 mg kg^{-1} body mass, which is approximately ten times the anesthetic dose) and then weighed. The heart was then immediately removed, and the conus arteriosus and atria separated from the ventricle. The ventricle was weighed and both it and the atria were placed in cold oxygenated elasmobranch Ringers solution (concentrations in mM: 360 urea, 280 NaCl, 90 trimethylamine oxide, 10 $CaCl_2 \cdot 2H_2O$, 5 $MgCl \cdot 6H_2O$, 5 glucose, 12 KCl, 4.5 $NaHCO_3$, 0.5 NaH_2PO_4, pH 7.8). The formulation was based on Thompson and O'Shea (1997) and Cooper and Morris (2004). The Ca^{2+} level we employed is well above in vivo plasma concentrations in elasmobranch fishes reported by Marshall et al. (2012) (2.3–3.3 mM) and that used in a previous study of the force–frequency relationships of elasmobranch fishes (Driedzic and Gesser 1988). In preliminary trials, however, we found that under our experimental conditions elasmobranch myocardial strips were unresponsive to electrical stimulation at calcium concentrations in the in vivo range. We checked the Ringers solution prior to use and did not see any indication of Ca^{2+} precipitation (e.g., cloudiness or settled flocculent).

Four myocardial strips (~1 mm × 10 mm) were cut from both the atria and ventricle obtained from each individual. The myocardial strips were mounted between stationary tissue clips and Fort 10 force transducers, and lowered into 10 or 14 ml organ baths (Myobath II; World Precision Instruments, Sarasota, FL USA) filled with oxygenated Ringers solution. The output signals from the force transducers were amplified with a Transbridge 4 M amplifier (World Precision Instruments), digitized at a 1 kHz sampling frequency using Daqbook 120 A/D system (Measurement and Computing Corp., Norton, MA, USA), and recorded with custom designed software developed using Dasylab version 9 (Measurement and Computing Corp.). Myocardial strips were stimulated by square wave pulses (5 ms duration) generated with a Grass SD9 (constant voltage) stimulator (Astro-Med Inc., West Warwick, RI, USA). The average (and range) of stimulus amplitudes were: smooth dogfish 70 V (20–110 V), sandbar shark 103 V (60–110 V), and clearnose skate 83 V (40–110 V). Immediately after mounting, the strips were tensioned to remove slack and allowed to rest (i.e., not stimulated) for 30 min. They were then stimulated at 0.2 Hz and further tensioned until a maximum response was measured. Stimulus amplitudes were subsequently adjusted to produce maximal force of contractions after which the strips were paced at 0.2 Hz for an additional 20 min before trials were initiated.

We assessed the contribution of SR Ca^{2+} to force generation in two of the chambers by measuring the effects of the addition of ryanodine and thapsigargin (Ascent Scientific, Princeton, NJ, USA), with final reagent concentration in chambers being 10 and 2.5 μM, respectively. The other two chambers received equivalent volumes of 0.9 % saline (100 or 140 μl, depending on chamber volume) or dimethyl sulfoxide (DMSO, 32 or 45 μl, depending on chamber volume) that were the solvents for ryanodine and thapsigargin, respectively. The latter are henceforth referred to as "control myocardial strips". (The added solvent volumes were ~1 and ~0.3 % of chamber volumes, respectively.) The data used for these assessments were those recorded immediately before, and 40 min after the addition of the reagents for both control myocardial strips and those treated with ryanodine and thapsigargin. Data from the control myocardial strips were used to account to any changes in measured parameters occurring over time.

To generate force–frequency data, stimulus frequency was increased in 0.2 Hz increments every 60 s, until contractions were no longer stable at a given frequency. This relatively short duration at stimulus frequencies above 0.2 Hz was chosen specifically to minimize any effects of fatigue or constraints on force development due the rate of oxygen diffusion from the surrounding Ringers solution to the interior of the muscle strips. Preliminary observations showed that net force stabilized within this stimulus period. Data at all stimulus frequencies were the mean of values taken from ten contractions. All tests were carried out at both 20 and 30 °C on separate sets of myocardial strips from all three species. A subset of measurements was also obtained at 25 °C using tissue from sandbar shark, as they were maintained close to this temperature during their time in captivity.

After each experiment, length (in mm) and wet mass (in mg) of each muscle strip were recorded and the cross-sectional area (in mm^2) calculated assuming that the strip was cylindrical and muscle density = 1.06 mg mm^{-3}:

$$\text{Cross-sectional area} = (\text{mass}/1.06)/\text{length}. \quad (1)$$

The net force produced (in milliNewtons, mN) relative to cross-sectional area (mm^2) was calculated using 1 mg force = 9.8066×10^{-3} mN.

Data analysis and statistical procedures

Software developed in Matlab (The Mathworks Inc., Natick, MA, USA) was used to calculate the following values from each contraction recorded in the digital data record: net force (mN mm^{-2}), time to peak force (ms), time to half relaxation (ms), the maximum first derivative of force (i.e., maximum rate of force development, $+dF/dt_{max}$, mN mm^{-2} s^{-1}), and the minimum first derivative of force (i.e., maximum rate of relaxation, $-dF/dt_{min}$, mN mm^{-2} s^{-1}). The values of these parameters and their relative (%) change due to ryanodine-thapsigargin treatment, or addition of 0.9 % saline and DMSO in control myocardial strips, were calculated using the mean of values taken from ten contractions.

As recommended by Weissgerber et al. (2015) for presentation and analysis of small sample sizes from continuous data, we present the values for body mass, ventricular mass, relative ventricular mass, contraction–relaxation parameters, and relative changes resulting from the addition of ryanodine and thapsigargin or 0.9 % saline and DMSO (in control myocardial strips) as box plots (produced using Sigmaplot, ver. 11.2), include the individual data points to allow visual comparison of the distributions and scatter, and employ nonparametric statistical procedures. Pairwise differences in the median values of relative ventricular mass and the contraction–relaxation parameters were analyzed using the nonparametric Mann–Whitney rank sum test (Paleontological Statistics, PAST, ver. 3.09) with significance assumed when $P < 0.05$. Significant differences between values obtained at 20 and 30 °C, within species and tissue type, were tested similarly. Significant differences between species, but within tissue type and at the same temperature, were tested using Kruskal–Wallis One Way Analysis of Variance on Ranks procedure, with all possible pairwise multiple comparison done using Dunn's Method (Sigmaplot, ver. 11.2). The effects of the addition ryanodine and thapsigargin on median values of contraction–relaxation parameters were likewise analyzed using the nonparametric Mann–Whitney rank sum test, with $P < 0.05$ taken as indicating significant differences in all cases.

Protocol for electron microscopy

Atrial and ventricular myocardial tissue samples (~1 mm × 1 mm) were isolated from one smooth dogfish, three sandbar shark, and two clearnose skate; then immediately fixed in a 2 % glutaraldehyde in 0.05 M sodium phosphate buffer (pH 7.4). The fixative was changed after 24 h. The samples were refrigerated until shipped to the University of Copenhagen where they were subsequently rinsed three times in 0.15 M sodium cacodylate buffer (pH 7.4) and post-fixed in 1 % osmium tetroxide with 0.05 M potassium ferricyanide in 0.12 M sodium cacodylate buffer (pH 7.4) for 2 h. The samples were then dehydrated in a graded series of ethanol concentration, transferred to propylene oxide, and embedded in Epon according to standard procedures (Luft 1977). Sections (~60 nm thick) were cut with a Reichert-Jung Ultracut E microtome and collected on copper grids with Formvar supporting membranes. Sections were stained with uranyl acetate and lead citrate and examined with a Philips CM 100 transmission electron microscope (Philips, Eindhoven, The Netherlands), operated at an accelerating voltage of 80 kV. Digital images were recorded with a Veleta digital slow scan 2 k × 2 k pixel charge coupled device camera and the associated software package (Olympus Soft Imaging Solutions GMBH, Münster, Germany).

Results

Body mass, ventricular mass, and relative ventricular mass

There was general overlap in the ranges of body mass, ventricular mass, and relative ventricular mass [i.e., the ratio of ventricular mass (g) to body mass (kg)] of the individuals available for our study (Fig. 1). There were also no significant differences in the median values for relative ventricular mass from smooth dogfish, sandbar shark, and clearnose skate.

Functional differences in atrial and ventricular myocardium within species

Atrial and ventricular myocardium from smooth dogfish showed the most consistent differences. These encompassed both net force and the parameters relating to rates of force development and relaxation (i.e., time to peak force, time to half relaxation, $+dF/dt_{max}$, and $-dF/dt_{min}$) at both 20 and 30 °C (Fig. 2). Atrial and ventricular myocardium from sandbar shark and clearnose skate also showed significant differences, but only in parameters relating to rates of force development and relaxation, and not at all temperatures (Fig. 2). More specifically, atrial tissue showed significantly shorter times to peak force and times to half relaxation in both species at 20 °C, as did atrial tissue from clearnose skate at 30 °C. Likewise, $+dF/dt_{max}$ (i.e., "contractility") was significantly higher in atrial than ventricular myocardium from sandbar shark and clearnose skate only

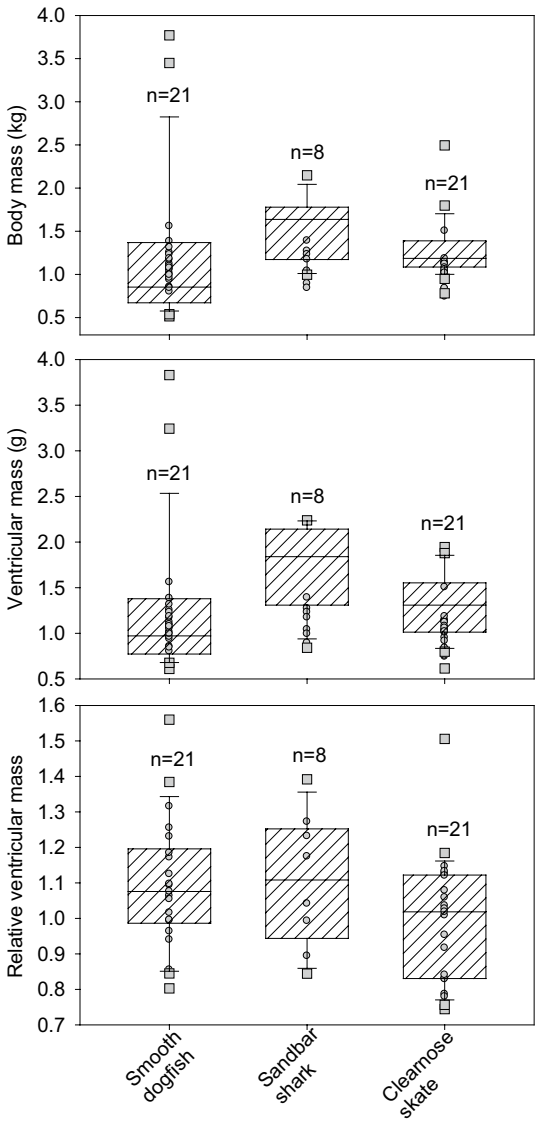


Fig. 1 The body masses, ventricular masses, and relative ventricular masses [i.e., the ratios of ventricular mass (g) to body mass (kg)] of the smooth dogfish, sandbar shark, and clearnose skate used in our study. The *solid lines* within the *boxes* mark the median values, the boundaries of the *boxes* the 25th and 75th percentiles, and the *whiskers* (*error bars*) above and below the boxes the 90th and 10th percentiles. Data points between the 90th and 10th percentiles are shown as *circles* and those outside this interval as *squares*. The number of individuals from which data were obtained ("*n*") are shown above each *bar*

at 30 °C (Fig. 2); and maximum rate of relaxation (i.e., $-dF/dt_{min}$) was higher in atrial tissue from the former species only at 30 °C (Fig. 2). We also note that atrial and ventricular myocardial tissue from sandbar shark demonstrated significant differences in all measured parameters at 25 °C (Fig. 2), which was the test temperature most closely approximating the temperature in their holding tank.

Effect of temperature within tissue types

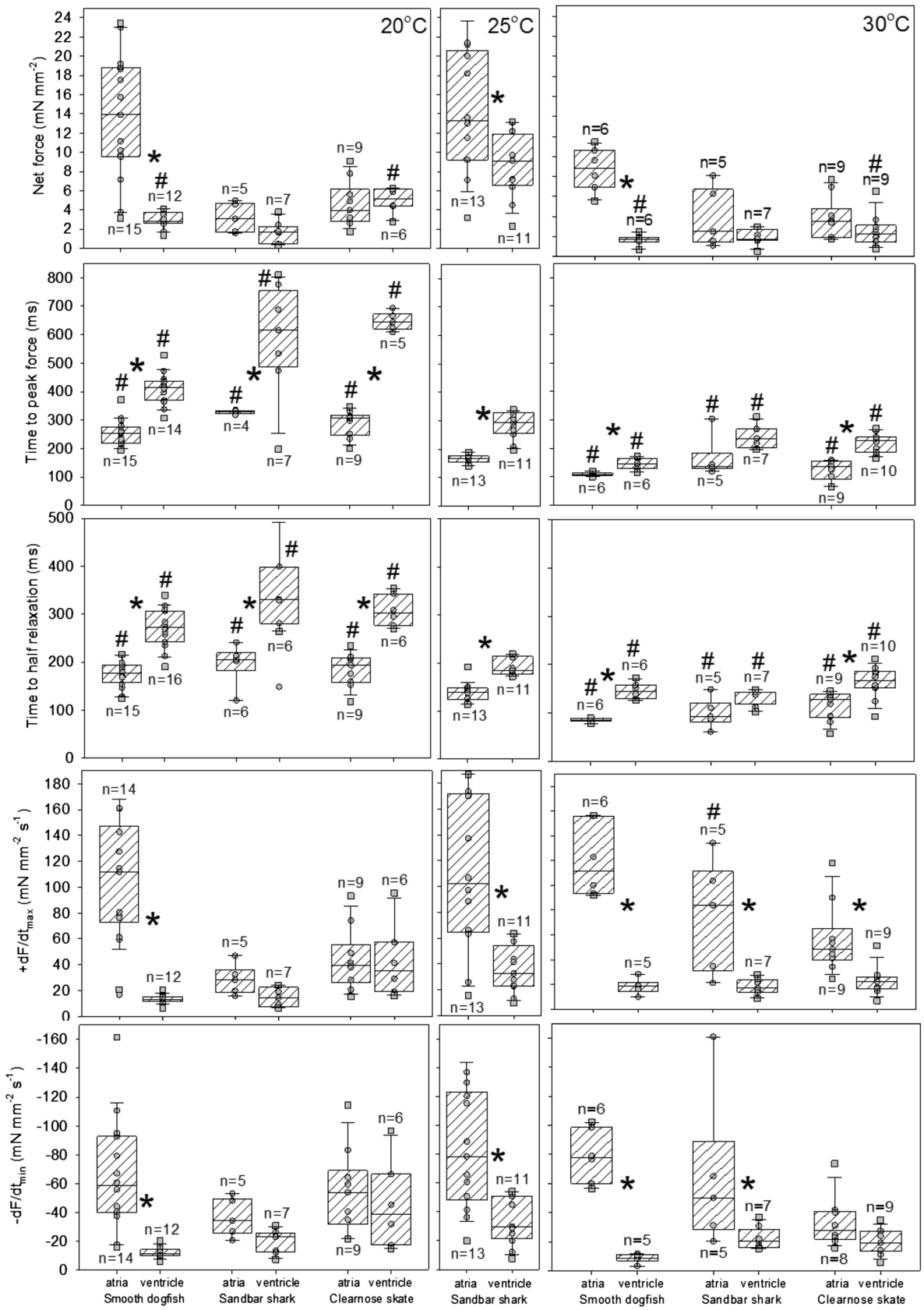
As expected, higher temperature generally resulted in decreased times to peak force and half relaxation in both atrial and ventricular myocardium in all three species (Fig. 2). In contrast, there were negative effects of increasing temperature on net force in ventricular myocardium from smooth dogfish and clearnose skate, but not in either myocardial tissue from sandbar shark; and no effect of temperature on $+dF/dt_{max}$ and $-dF/dt_{min}$ in any of the three elasmobranch species (Fig. 2). The effects of temperature (on those parameters where an increase of temperature had a significant effect) were calculated as Q_{10} values (i.e., the fractional change per 10 °C temperature change, Prosser 1973) from those instances where data were available for two temperatures (20 and 30 °C) from the same individual. Median Q_{10} values ranged from 1.5 to 2.5 (Fig. 3). The Kruskal–Wallis test showed only a marginally significant difference ($P = 0.049$) between median Q_{10} values, and the Mann–Whitney pairwise comparison with Bonferroni corrected P values was unable to isolate specific pairwise differences.

Functional differences between species within tissue types and temperature treatments

There were functional differences between species within tissue type (i.e., atrial or ventricular tissue) and temperature treatment (Table 1). The most numerous significant differences in functional properties, occurring in nine of 30 possible pairwise comparisons at 20 °C, and eight of 30 possible pairwise comparisons at 30 °C, were between myocardial tissue from smooth dogfish and the other two species. Time to 50 % relaxation was the only functional property where atrial and ventricular tissue from smooth dogfish demonstrated no significant differences with those of the other two species at both 20 and 30 °C (Table 1). In contrast, there were only two significant differences when comparisons are made between the functional properties of ventricular myocardium from sandbar shark and clearnose skate at 20 °C, and no significant differences when comparisons were made between the functions properties of ventricular myocardium from sandbar shark and clearnose skate at 30 °C.

Relative changes after treatment with ryanodine and thapsigargin

Treatment with ryanodine and thapsigargin resulted in both tissue- and species-specific changes in functional properties; and a temperature effect was also observed. Most notably the effect of ryanodine and thapsigargin was especially


20°C
25°C
30°C
Net force (mN mm-2)
Time to peak force (ms)
Time to half relaxation (ms)
+dF/dtmax (mN mm-2 s-1)
-dF/dtmin (mN mm-2 s-1)
atria ventricle
Smooth dogfish
Sandbar shark
Clearnose skate

◂**Fig. 2** The absolute values for contraction–relaxation parameters [net force (mN mm^{-2}), time to peak force (ms), time to half relaxation (ms), the maximum first derivative of force (i.e., maximum rate of force development, $+dF/dt_{max}$, mN mm^{-2} s^{-1}) and the minimum first derivative of force (i.e., maximum rate of relaxation, $-dF/dt_{min}$, mN mm^{-2} s^{-1})] measured in atrial and ventricular tissue at 20 and 30 °C. Stimulus frequency was 0.2 Hz. The *solid line* within the *box* marks the median value, the boundaries of the box the 25th and 75th percentiles, and the whiskers (*error bars*) above and below the box the 90th and 10th percentiles. Data points between the 90th and 10th percentiles are shown as *circles* and those outside this interval as *squares*. Significant differences between median values for atrial and ventricular myocardium are indicated by *asterisk* symbol, and significant differences between median values at 20 and 30 °C for atrial or ventricular tissue are indicated by *hash symbol*. The number of individuals from which data on the various parameters were obtained ("*n*") are shown above or below each *bar*

large on net force produced by atrial tissue from smooth dogfish at 30 °C; removal of the contribution of SR Ca^{2+} release to EC coupling resulted in a median reduction of 66 % at 30 °C (Fig. 4). This is more than twice the reduction in net force occurring in control myocardial strips (25 % at 30 °C), and the net difference (41 %) is considered the contribution of SR Ca^{2+} to net force development (Galli and Shiels 2012). Atrial myocardium from smooth dogfish also showed a significant decrease in $+dF/dt_{max}$ at 30 °C, as well as significant (albeit small) increases in time to half relaxation at both 20 and 30 °C (Fig. 4); the latter implying a contribution SR Ca^{2+} uptake to relaxation. Atrial myocardium from sandbar shark likewise showed a significant increase in time to half relaxation at 30 °C (Fig. 4); again indicating a significant contribution SR Ca^{2+} uptake to relaxation at the higher temperature. Atrial myocardium from clearnose skate showed small increases in time to peak force at both 20 and 30 °C, whereas both atrial and ventricular myocardium showed increases in time to half relaxation at both temperatures (Fig. 4). The median fractional change in time to half relaxation in response to the application of ryanodine and thapsigargin in clearnose skate was, however, significantly larger in atrial and ventricular myocardium at 20 °C (~29 %) than the changes displayed by these tissues at 30 °C (Fig. 4).

Force–frequency relationship

Atrial and ventricular myocardium from all three species had generally negative force–frequency relationships at both 20 and 30 °C (Fig. 5). There were, however, notable species-specific differences in the maximum stimulus frequencies at which coordinated contractions were maintained, especially at 30 °C; these were 2.4 Hz in myocardium from smooth dogfish, compared to 1.4 and 1.0 Hz in tissue from sandbar shark and clearnose skate, respectively. The species-specific differences in the maximum stimulus frequencies at which coordinated contractions could be maintained were, however, smaller at 20 °C. These were 1.0 Hz in myocardium from smooth dogfish and sandbar shark; compared to 0.6 Hz in tissue from clearnose skate.

In contrast to the differences in contraction–relaxation parameters between atrial and ventricular myocardium at 0.2 Hz stimulus frequency (Fig. 2), the changes in contraction–relaxation parameters occurring with increases in stimulus frequency were generally similar in atrial and ventricular myocardium; with the exception in time to half relaxation in atrial and ventricular myocardium from smooth dogfish at 20 °C (Fig. 5). In this case, the former showed a steep decrease in time to half relaxation with increases in stimulus frequency above 0.6 Hz, whereas the latter did not. The effects of exposure to ryanodine and thapsigargin on the absolute values of the contraction–relaxation parameters, which were prominent in atrial tissue from smooth dogfish at 30 °C (Fig. 2), were not reflected in the changes in contraction–relaxation parameters accompanying increased stimulus frequencies (Fig. 5). In brief, blocking of SR Ca^{2+} release and uptake had no obvious effects on the changes in contraction and relaxation parameters accompanying increases in stimulus frequency in both atrial and ventricular myocardium from smooth dogfish, sandbar shark, or clearnose skate (Fig. 5).

Transmission electron microscopy

In smooth dogfish, the presence of the SR was more obvious in atrial than ventricular myocardium. In the former, the SR was generally located in close proximity to mitochondria (M) and intercalated discs (ICD), and to a lesser extent between the myofibrils (MF) (Fig. 6). In ventricular myocardium, the SR was generally found between the mitochondria (Fig. 7). Mitochondria and glycogen storage granules (G) were less abundant in atrial than ventricular myocardium (Figs. 6, 7, respectively). In the latter, glycogen storage granules were generally in close proximity of the mitochondria (Fig. 7, panels 6 and 7). The distributional and abundance patterns of SR and mitochondrial in the atrial and ventricular myocardium of sandbar shark (Figs. 8, 9, respectively) were similar to those of smooth dogfish. The SR was more obvious in the atrial than ventricular tissue, whereas mitochondria were more numerous in the latter. In atrial tissue, the SR was again generally in close proximity to the mitochondria and intercalated discs and between the myofibrils (Fig. 8). In clearnose skate atrial tissue, the SR was likewise more noticeable and mitochondria less numerous compared to ventricular myocardium (Figs. 10, 11, respectively).

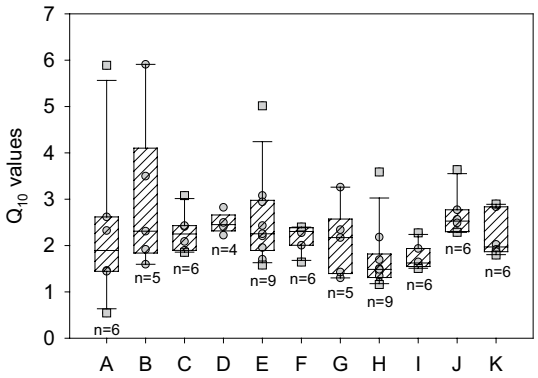


Fig. 3 The effects of temperature (on those parameters where an increase of temperature had a significant effect) calculated as Q_{10}s (i.e., the fractional change per 10 °C temperature change, Prosser 1973). The *X*-axis code letters are: *A* net force smooth dogfish ventricle, *B* net force clearnose skate ventricle, *C* time to peak tension smooth dogfish ventricle, *D* time to peak tension sandbar shark ventricle, *E* time to peak tension clearnose skate ventricle, *F* time to half relaxation smooth dogfish atria, *G* time to half relaxation sandbar shark atria, *H* time to half relaxation clearnose skate atria, *I* time to half relaxation smooth dogfish ventricle, *J* time to half relaxation sandbar shark ventricle, *K* time to half relaxation clearnose skate ventricle. The *solid line* within the *box* marks the median value, the boundaries of the box the 25th and 75th percentiles, and the whiskers (*error bars*) above and below the box the 90th and 10th percentiles. Data points between the 90th and 10th percentiles are shown as *circles* and those outside this interval as *squares*. The number of individuals from which data on the various parameters were obtained ("*n*") are shown below each *bar*

Discussion

Functional differences between atrial and ventricular myocardium in elasmobranch fishes

Our first objective was to determine the basic contraction–relaxation characteristics of atrial and ventricular myocardium of three elasmobranch species. More specifically, we wanted to determine whether the extensive functional differences between atrial and ventricular myocardium observed in other vertebrate groups, including cyclostomes and teleost fishes (Aho and Vornanen 1999; Asgrimsson et al. 1995; Bottinelli et al. 1995a; Genge et al. 2012, 2013; Korajoki and Vornanen 2012; Shiels et al. 1999; Vornanen and Haverinen 2013) are also present in smooth dogfish, sandbar shark, and clearnose skate. These species include representatives of the two subdivisions of the subclass Elasmobranchii (Selachii and Batoidea) that separated during the Mesozoic era (~200 × 10^6 years ago) (Klimley 2013); and smooth dogfish and sandbar shark are representatives of the two subdivisions of the Selachii (superorders Squalamorphi and Galeomorphi). Our results, therefore, provide at least an initial indication if the functional differences of atrial and ventricular myocardium are wide spread within elasmobranch fishes (and are therefore phylogenetically ancient); and whether ventricular filling, stroke volume, and regulation of cardiac output is (at least in part) likely to be dependent on atrial function throughout this group. Our data also provide at least an initial indication as to whether atrial-ventricular functional differences are homogenous within the Elasmobranchii, or whether chamber-specific functional properties are as diverse in elasmobranch fishes as are the morphologies, ecologies, energetics, behaviors, etc. of this group (Compagno 1990; Klimley 2013). We note, however, that representatives of the elasmobranch subclass Holocephali, as well as representatives of the many orders within class Chondrichthyes, remain unstudied.

Our data show significant differences in $+dF/dt_{max}$, $-dF/dt_{min}$, times to peak force, and times to half relaxation in atrial versus ventricular myocardium in all three elasmobranch species (Fig. 2). These results indicate generally faster rates of increase and decrease in cytoplasmic Ca^{2+} during EC coupling in atrial tissue. The fact that these differences are observed across phylogenetically distant species are, moreover, supportive of the general conclusion that atrial contraction is important to ventricular filling in elasmobranch fishes (Brill and Lai 2016).

In contrast, significant atrial-ventricular differences in net force are apparent only in myocardium from smooth dogfish (Fig. 3). The net force produced by muscle fibers depends on the myosin heavy chains (MHC), and the concentration of MHC proteins has been shown to be higher in atrial than in the ventricular myocardium of other vertebrates (Karasinski 1993; Bottinelli et al. 1995b; Karasinski et al. 2001). Atrial myocardium may, therefore, develop higher net force than the latter simply due to higher amount of MHC (Genge et al. 2012). The specific paralog (i.e., isoform) of MHC protein expressed is also chamber-specific in fishes (Yelon et al. 1999); this may be correlated with chamber-specific differences in myofibrillar ATPase activity (Vornanen 1994). These differences could also account for the atrial-ventricular differences in net force (Genge et al. 2012, 2013). We also note that elasmobranch hearts from all three species were too small to use isolated trabeculae from the ventricle, and that atria do not contain these structures. Given the complex trajectories of muscle fibers in fish hearts (Sanchez-Quintana and Hurle 1987; Farrell and Jones 1992), it is likely that muscle fiber orientations relative to the force transducers were not uniform. Although this would not affect the absolute values of parameters such as time to peak tension and time to half relaxation, it unquestionably added to the scatter in the data on net force, $+dF/dt_{max}$ and $-dF/dt_{min}$, and could (at least in part) explain the differences in net force produced by atrial and ventricular myocardium from smooth dogfish (Fig. 2). For example, fiber orientation in atrial

Table 1 Pairwise comparisons of functional differences between species within tissue types and temperature treatments tested using Kruskal–Wallis One Way Analysis of Variance on Ranks procedure, with all possible pairwise multiple comparisons done using Dunn's Method

Net force 20 °C			Net force 30 °C		
	SB atria	SK atria		SB atria	SK atria
SD atria	X	X	SD atria	X	X
SB atria			SB atria		
	SB vent	SK vent		SB vent	SK vent
SD vent			SD vent		
SB vent		X	SB vent		
Time to peak force 20 °C			Time to peak force 30 °C		
	SB atria	SK atria		SB atria	SK atria
SD atria	X		SD atria		
SB atria			SB atria		
	SB vent	SK vent		SB vent	SK vent
SD vent	X	X	SD vent	X	X
SB vent			SB vent		
Time to 50 % relax. 20 °C			Time to 50 % relax. 30 °C		
	SB atria	SK atria		SB atria	SK atria
SD atria			SD atria		
SB atria			SB atria		
	SB vent	SK vent		SB vent	SK vent
SD vent			SD vent		
SB vent			SB vent		
$+dF/dt_{max}$ 20 °C			$+dF/dt_{max}$ 30 °C		
	SB atria	SK atria		SB atria	SK atria
SD atria	X	X	SD atria		X
SB atria			SB atria		
	SB vent	SK vent		SB vent	SK vent
SD vent		X	SD vent		
SB vent		X	SB vent		
$-dF/dt_{min}$ 20 °C			$-dF/dt_{min}$ 30 °C		
	SB atria	SK atria		SB atria	SK atria
SD atria			SD atria		X
SB atria			SB atria		
	SB vent	SK vent		SB vent	SK vent
SD vent		X	SD vent	X	X
SB vent			SB vent		

Pairwise comparisons with significant differences ($P < 0.05$) are shown with an "X" symbol

SD smooth dogfish, *SB* sandbar shark, *SK* clearnose skate

myocardium may be more uniform than in ventricular tissue; plus the former most likely lack trabeculae. Our data provide no basis upon which to choose among these possibilities. Our results show, however, that whatever the mechanistic difference(s) it (they) is (are) not universal in elasmobranch fishes.

Effect of temperature on atrial-ventricular functional differences within species

Although the holding tank temperatures reflected environmental temperatures from which the animals were caught, different times in captivity undoubtedly induced additional

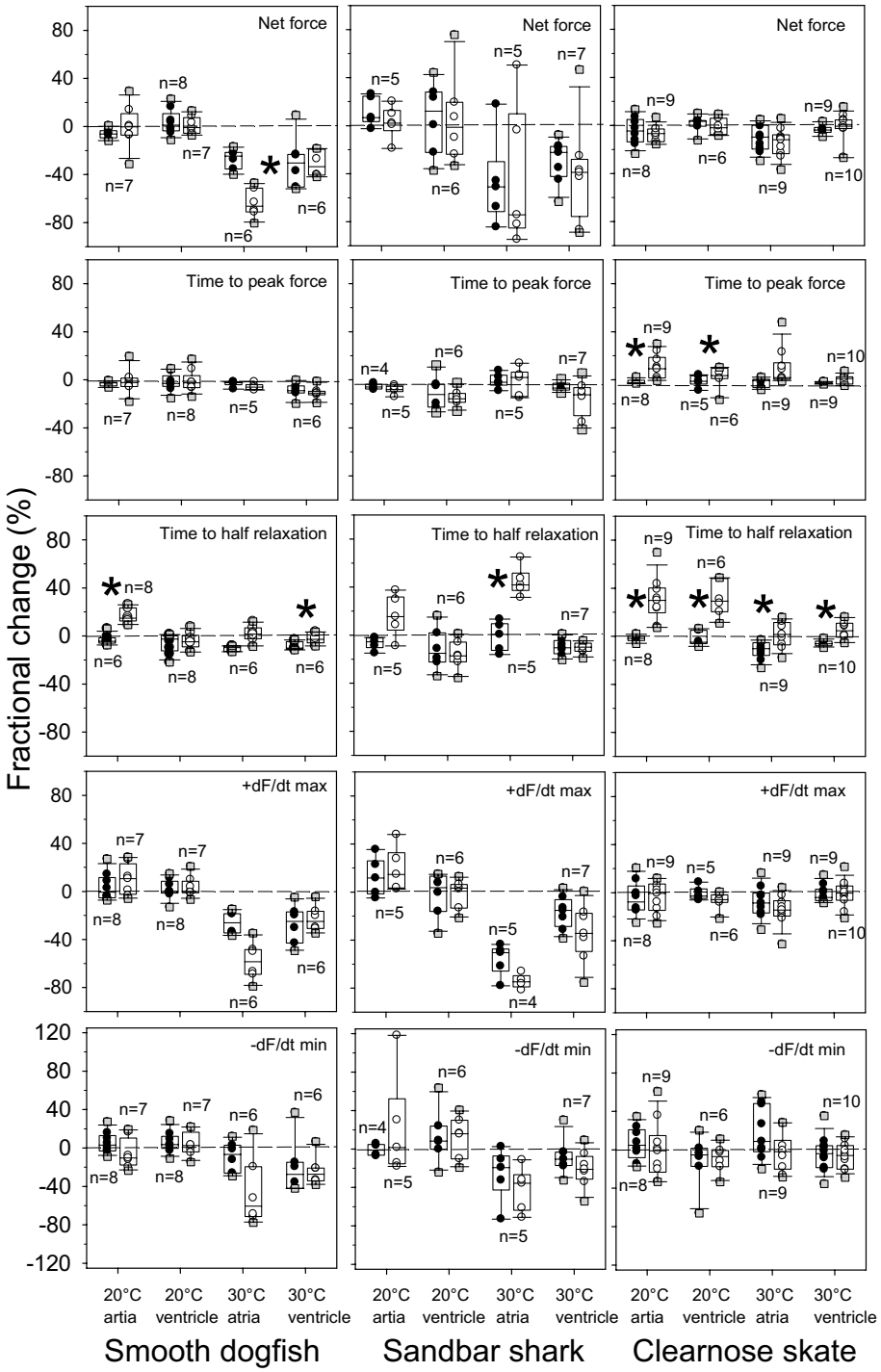


Fig. 4 The fractional (i.e., %) change in contraction–relaxation parameters measured in atrial and ventricular myocardium from smooth dogfish, sandbar shark, and clearnose skate resulting from exposure to 10 μM ryanodine and 2.5 μM thapsigargin (indicated by *open circles*), or receiving equivalent volumes 0.9 % saline and DMSO (indicated by *filled circles*) which were the solvents for ryanodine and thapsigargin, respectively. Experiments were conducted at both 20 and 30 °C, and stimulus frequency was 0.2 Hz. The *solid line* within the *box* marks the median value, the boundaries of the box the 25th and 75th percentiles, and the *whiskers* (*error bars*) above and below the box the 90th and 10th percentiles. Data points between the 90th and 10th percentiles are shown as *circles* and those outside this interval as *squares*. Significant differences between median values for atrial and ventricular myocardium are indicated by *asterisk* symbol. The number of individuals from which atrial or ventricular data on various parameters were obtained ("*n*") are shown above or below each *bar*; where only one value is listed, atrial and ventricular data were obtained from the same number of individuals

variability in our results. The functional differences between atrial and ventricular myocardium we measured were nonetheless affected by experimental temperature in a species-specific manner. Atrial and ventricular myocardium from smooth dogfish showed significant differences in all measured functional characteristics both at 20 and 30 °C; as did atrial and ventricular myocardium from sandbar shark at 25 °C (Fig. 2). In contrast, atrial and ventricular myocardium from sandbar shark and clearnose skate demonstrated fewer differences at 30 °C. Most notable is a lack of difference in net force production at both temperatures. Only time to half relaxation at 20 °C and $+dF/dt_{max}$ at 30 °C differed between atria and ventricle tissue from sandbar shark. The number of functional differences between atrial and ventricular myocardium was intermediate in clearnose skate. These included shorter times to peak force and half relaxation

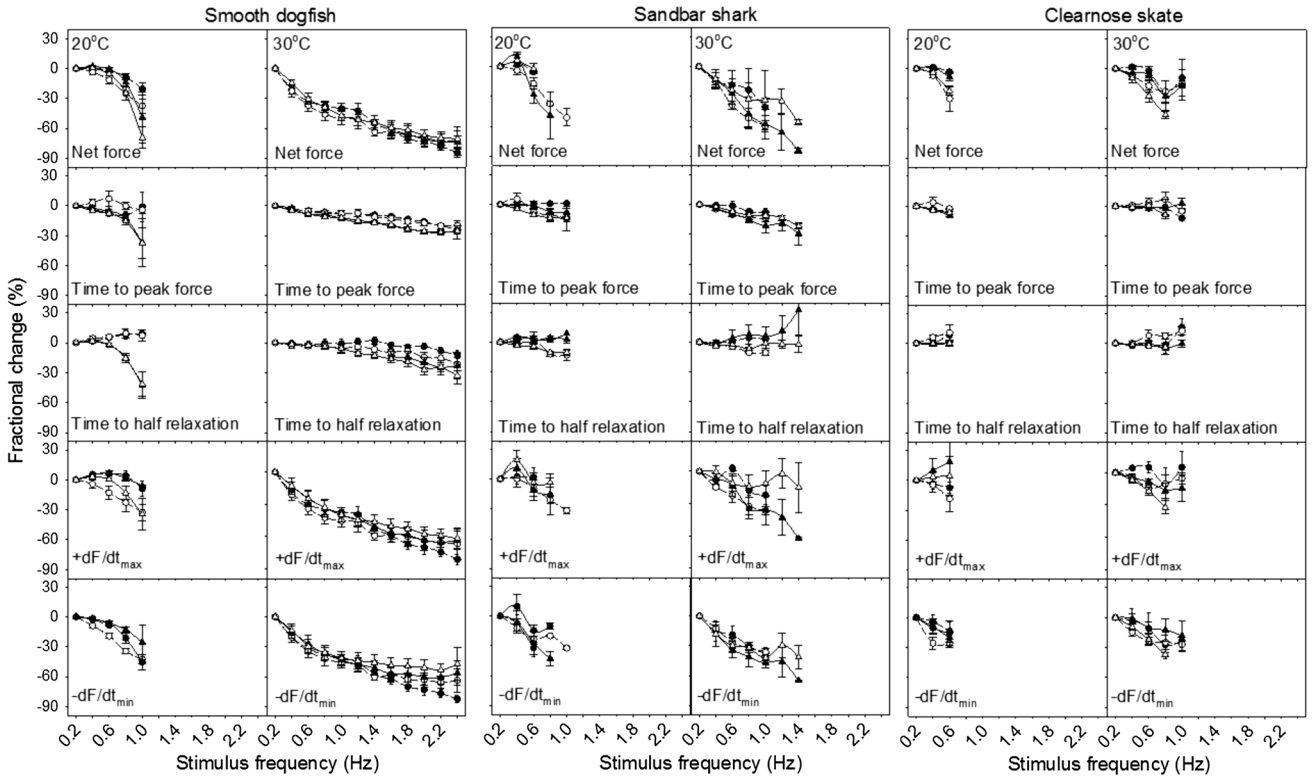


Fig. 5 The mean fractional (%) change (±SEM) in contraction–relaxation parameters measured in atrial (*circular symbols*) and ventricular (*triangular symbols*) myocardium from smooth dogfish, sandbar shark, and clearnose skate resulting from increasing stimulus frequency at 20 and 30 °C. Myocardium exposed to 10 μM ryanodine and 2.5 μM thapsigargin are indicated by *open symbols*. Tissues exposed to 0.9 % saline and DMSO (which were the solvents for ryanodine and thapsigargin, respectively) are shown by *filled symbols*

at both temperatures, as well as measures of contractility ($+dF/dt_{max}$ and $-dF/dt_{min}$) at 30 °C. Ca^{2+} influx through voltage-gated L-type channels and the NCX is dependent on the duration and height of the action potential, and both are tissue-specific and significantly affected by temperature (Vornanen et al. 2002a; Galli and Shiels 2012; Shiels and Galli 2014). Accordingly, our results (Fig. 2) imply that action potential duration and height (and the resultant sarcolemmal Ca^{2+} influx and efflux in elasmobranch myocardium) vary with temperature in a complex species-specific manner. Intracellular Ca^{2+} buffers and Ca^{2+} affinity of troponin C are also temperature dependent (Gillis et al. 2000; Gillis and Tibbits 2002), but to the best of our knowledge these have not been studied in elasmobranch myocardium; and our results provide no basis upon which to decide among these various hypotheses.

Effects of temperature on atrial-ventricular functional differences between species

Not unexpectedly, the effects of temperature on myocardial function are more numerous when comparisons are made within tissue types, especially the times to peak force and half relaxation (Fig. 2). There was, however, also a small inverse relationship of temperature and net force in ventricular myocardium of smooth dogfish and clearnose skate (i.e., net force was higher at the lower temperature). A similar inverse relationship of temperature and net force has been observed in ventricular myocardium of rainbow trout (when tissue from fish adapted to 12 °C was tested at this temperature and 22 °C) (Shiels and Farrell 1997) and in three species of pelagic fish: yellowfin tuna (*Thunnus albacares*), bigeye tuna (*T. obesus*) and mahimahi (*Coryphaena hippurus*) (Galli et al. 2009). No effects of acute 10 °C changes on net force or power production (i.e., the product of net force and heart rate) were observed in ventricular strips from European eel (*Anguilla anguilla*) (Methling et al. 2012). The myocardium of ectothermic vertebrates is generally considered to have evolved specific mechanisms to maintain overall cardiac function in the face of acute and seasonal temperature changes (Vornanen et al. 2002a; Shiels et al. 2002a; Methling et al. 2012). An inverse relationship of net force and temperature may, therefore, allow fishes (and other ectothermic vertebrates) to maintain cardiac pumping capacity at colder temperatures, when heart rates tend to be lower (Shiels et al. 1999, 2002a). Because

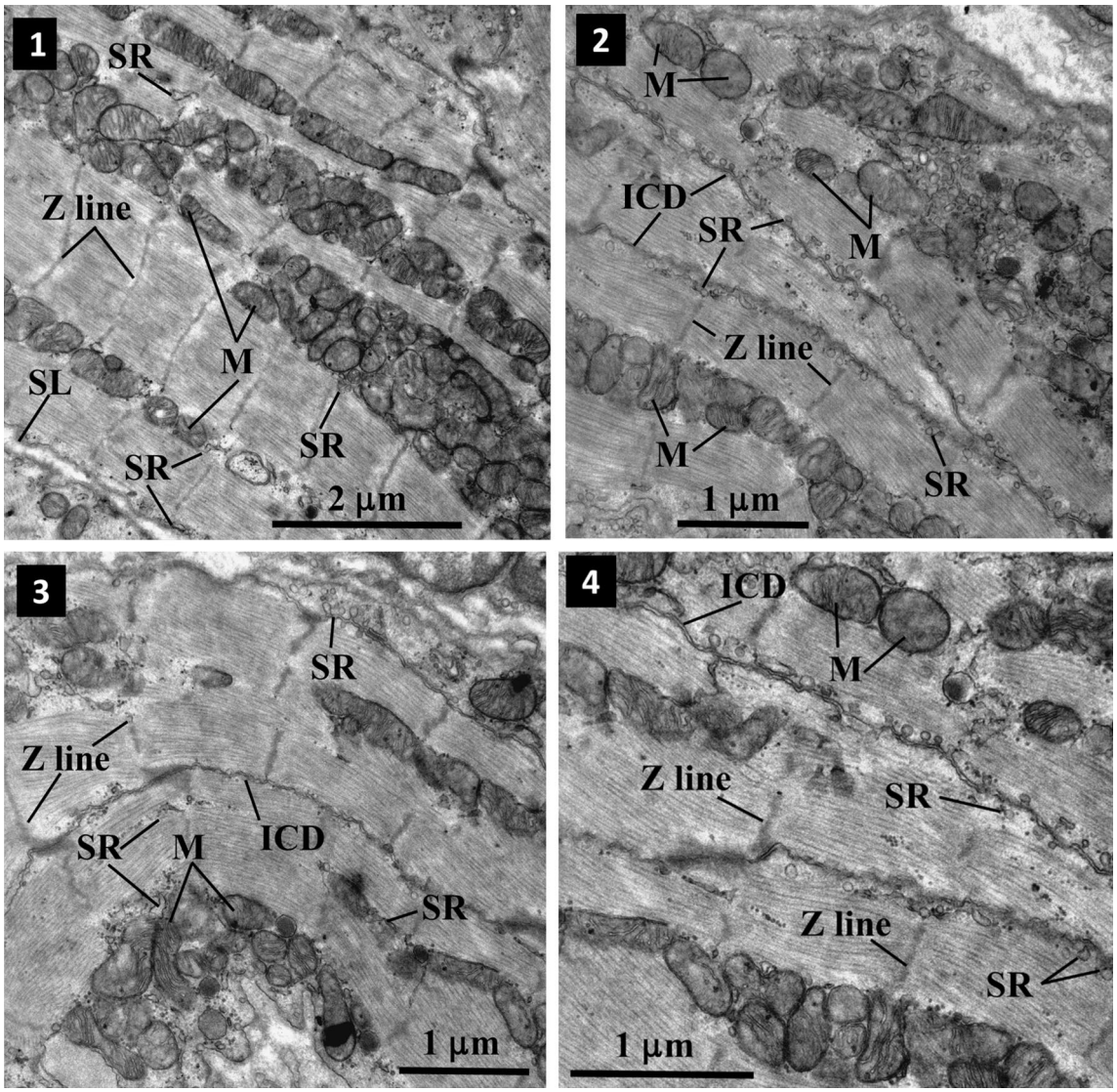


Fig. 6 Electron micrographs of a longitudinal section through atrial myocardium of smooth dogfish. The sarcoplasmic reticulum (SR) is generally located in close proximity to mitochondria (M) (*panels 1* and *3*) and intercalated discs (ICD) (*panel 3*), and to a lesser extent between the myofibrils (*panels 3* and *4*). Z lines and sarcolemma (SL) (*panel 1*) are also visible

of our inability to control water temperature in their holding tank, however, sandbar shark were maintained at temperatures up to 26 °C, whereas smooth dogfish and clearnose skate were maintained at 20 °C, which may at least in some part explain the observed differences.

Functional differences between species

The myocardium from smooth dogfish stands out as demonstrating the most numerous significant differences in functional properties when comparisons are made between species within tissue types and temperature treatments (Table 1). Our data do not, however, provide a definitive explanation for these differences, although one plausible explanation is a greater functional role of SR Ca^{2+} in EC coupling in smooth dogfish atrial myocardium.

One of our primary objectives was to test if atrial-ventricular functional differences in elasmobranch fishes result from differences in the relative importance of SR Ca^{2+} to EC coupling. This aspect of cardiac function is relatively unstudied in elasmobranch fishes, compared to teleost fishes and other vertebrate groups (Bootman et al. 2006; Genge et al. 2012; Korajoki and Vornanen 2009, 2012, 2014). Control of cytoplasmic free Ca^{2+} levels is the primary basis for the control of myocyte contraction and relaxation in vertebrate myocardium (Bers 2002; Bootman et al. 2006; Genge et al. 2012). Ca^{2+} delivery to, and removal from, cardiac troponin C (the thin filament protein

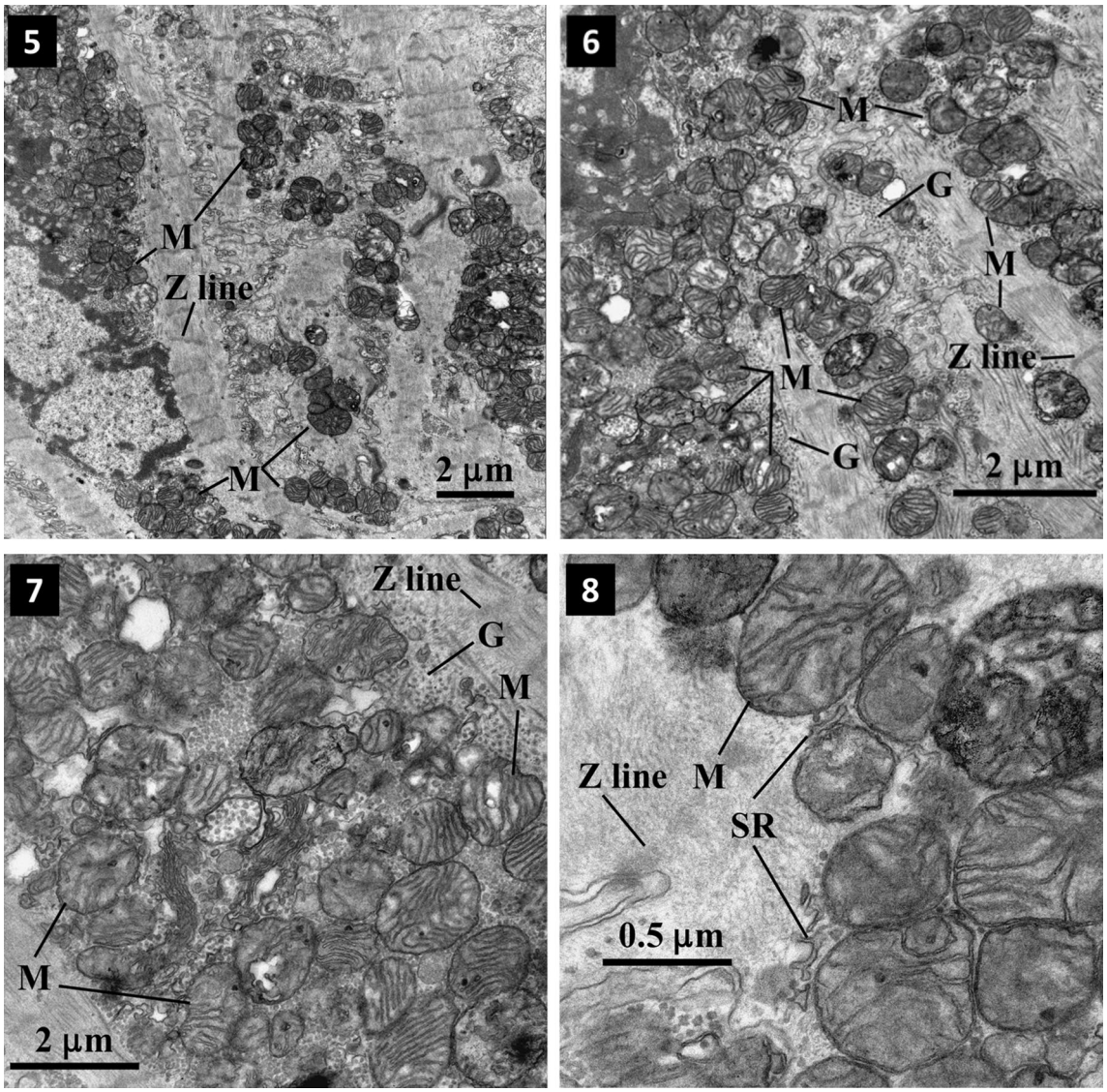


Fig. 7 Electron micrographs of a longitudinal section through ventricular myocardium of smooth dogfish. Mitochondria (M) are more numerous than in atrial myocardium, and glycogen storage granules (G) are seen in close proximity to the mitochondria (*panels 5, 6* and *7*). The sarcoplasmic reticulum (SR) is limited, and generally present between the mitochondria (*panel 8*). Z lines are also visible

which interacts with changes in cytosolic Ca^{2+}) controls the contractile process, modulates net force production and the kinetics of contraction–relaxation, and determines the force–frequency relationship (Shiels et al. 2002b; Bootman et al. 2006; Galli and Shiels 2012; Shiels and Galli 2014). The general consensus is that the myocardium in most ectothermic vertebrates is not dependent on SR Ca^{2+} for EC coupling under routine circumstances, but utilize SR Ca^{2+} to augment trans-sarcolemmal Ca^{2+} (i.e., to increase myocardial performance) under conditions requiring higher rates of oxygen delivery by the cardio-respiratory system (i.e., higher rates of cardiac output) (Shiels and Galli 2014; Shiels and Sitsapesan 2015). We do admit, however, that chamber-specific differences in Ca^{2+} sensitivity of cardiac troponin C may also explain differences between contractility of atrial and ventricular myocardium (Genge et al. 2012), but we are aware of no published data on this topic for elasmobranch fishes.

The greater reliance on SR Ca^{2+} release and reuptake (via sarco-endoplasmic reticulum Ca^{2+}-ATPase, SERCA) in atrial than in ventricular myocardium has been demonstrated in several teleost species (e.g., rainbow trout and burbot) (Aho and Vornanen 1999; Haverinen and Vornanen 2009); and is considered to be the mechanism underlying the higher net force production and more rapid contraction–relaxation kinetics (the latter due to higher SERCA protein content in atrial myocardium) (Walden et al. 2009; Genge et al. 2012; Shiels and Galli 2014). Our observations

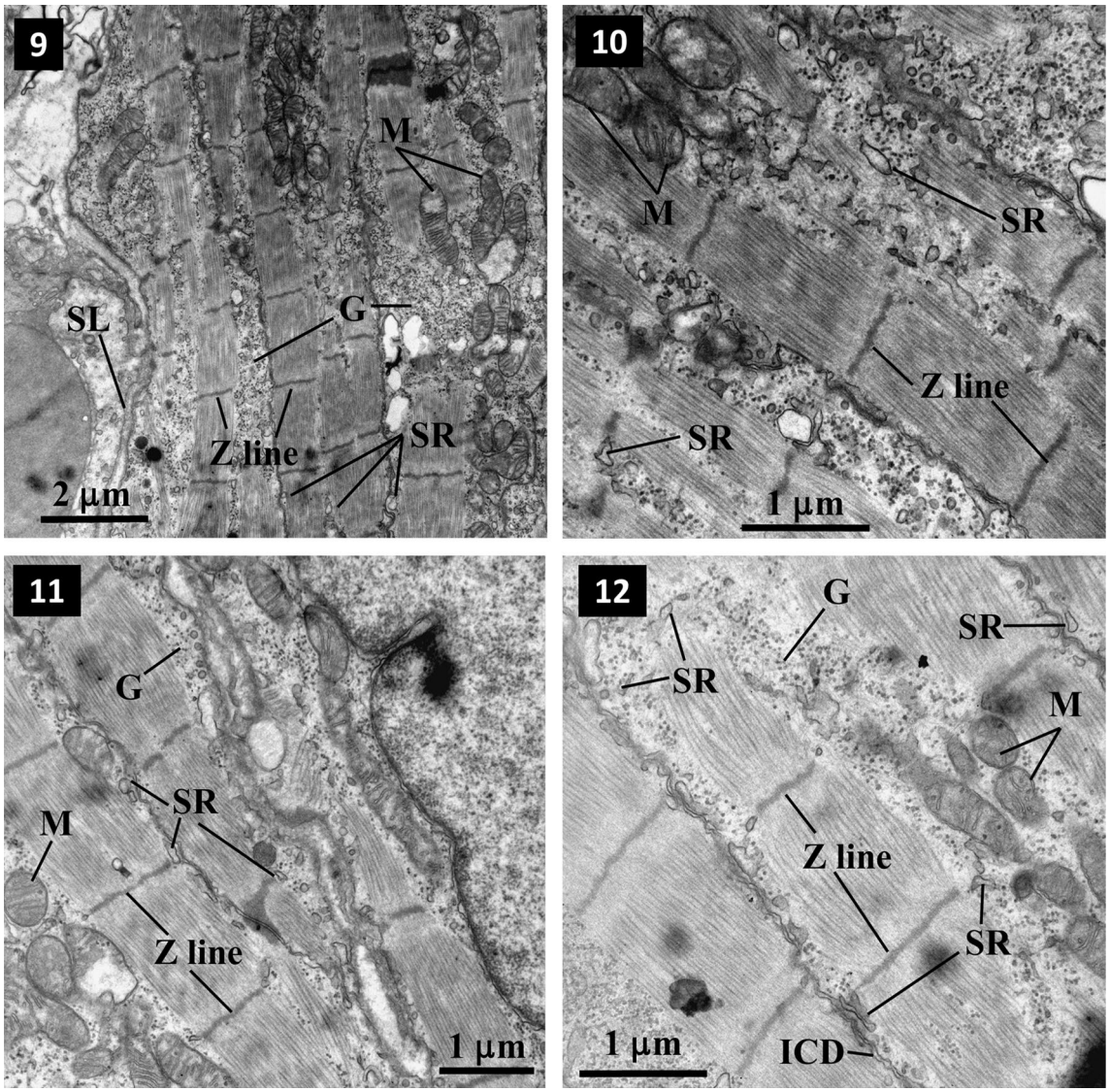


Fig. 8 Electron micrographs of a longitudinal section through atrial myocardium of sandbar shark. The sarcoplasmic reticulum (SR) is present between the myofibrils (*panel 10*), and next to mitochondria (M) (*panel 11*) and intercalated discs (ICD) (*panel 12*). Z lines, glycogen storage granules (G), and sarcolemma (SL) (*panels 9*, *11*, and *12*) are also visible

therefore support the conclusion of a greater reliance on SR Ca^{2+} release and reuptake in atrial than ventricular myocardium of elasmobranch fishes, but the extent of this difference is both species-specific and effected by temperature. Net force production was reduced by the application of ryanodine-thapsigargin (i.e., removal of SR Ca^{2+} release and reuptake) at 30 °C in atrial myocardium from smooth dogfish. Time to half relaxation was likewise significantly influenced by ryanodine-thapsigargin in atrial myocardium at 20 and 30 °C from smooth dogfish, and in atrial myocardium at 30 °C from clearnose skate and sandbar shark (Fig. 4). These results imply that SERCA is involved in myocardial relaxation at least under these instances. Our results also indicate that SERCA activity in atrial myocardium contributes to higher mechanical restitution rates ensuring the contraction–relaxation cycle is completed prior to ventricular systole in elasmobranch fishes, as is the case in other vertebrates.

In teleost fishes, the relative importance of sarcolemmal and SR Ca^{2+} to EC coupling is known to be species-specific, and to depend on tissue type, test temperature, acclimation temperature, and pacing frequency (Shiels et al. 2002a; Galli and Shiels 2012; Shiels and Sitsapesan 2015). More specifically, myocardium from a range of teleost species acclimated to low temperatures have an increased involvement of SR Ca^{2+} in EC coupling (Hove-Madsen 1992; Keen et al. 1994; Aho and Vornanen 1999; Korajoki and Vornanen 2009; Shiels et al. 2011). In contrast,

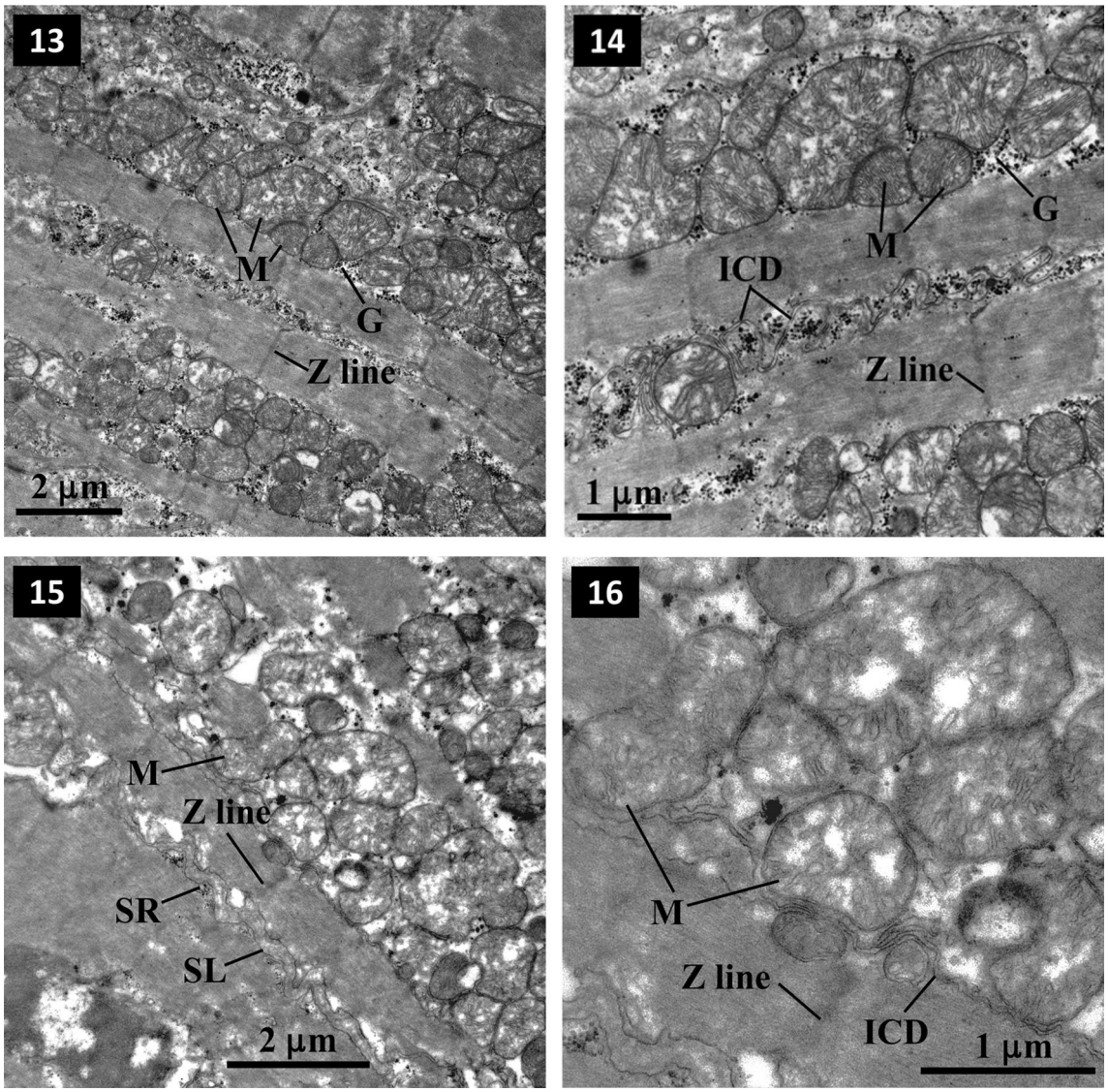


Fig. 9 Electron micrographs of a longitudinal section through ventricular myocardium of sandbar shark. The sarcoplasmic reticulum (SR) is sparse (*panel 15*) and mitochondria (M) are numerous (*panels 13* and *14*). Glycogen storage granules (G) are generally located in proximity to the mitochondria (*panels 13* and *14*), intercalated discs (ICD) (*panel 16*), Z lines, and sarcolemma (SL) are also visible (*panel 15*)

Hove-Madsen (1992) and Hove-Madsen et al. (1998) have shown an increased SR Ca^{2+} involvement in EC coupling in teleost ventricular myocardium when temperature is acutely increased. Aho and Vornanen (1999) reported that the effect of temperature on the importance of SR Ca^{2} to EC coupling is greater in atrial than ventricular myocytes from rainbow trout; whereas Hove-Madsen et al. (2001) reported that the involvement of the SR in EC coupling in atrial myocytes from rainbow trout is the same at 7 and 21 °C.

Our results suggest that the relative importance of sarcolemmal and SR Ca^{2+} cycling in the myocardium of elasmobranch fishes is similarly dependent on species, tissue type, and temperature. For example, exposure of smooth dogfish atrial myocardium to ryanodine and thapsigargin decreased net force and $+dF/dt_{max}$ at 30 °C, and increased times to peak force and half relaxation at 20 and 30 °C. In contrast, exposure of sandbar shark atrial myocardium to ryanodine and thapsigargin had no effect on net force nor $+dF/dt_{max}$ but (as observed in smooth dogfish myocardium) increased time to peak force and time to half relaxation at both temperatures; whereas the same treatment increased only time to half relaxation of sandbar shark ventricular myocardium at both temperatures (Fig. 4). Our results therefore indicate that the different dependences on sarcolemmal and SR Ca^{2+} cycling between atrial and ventricular myocardium are temperature and species-specific

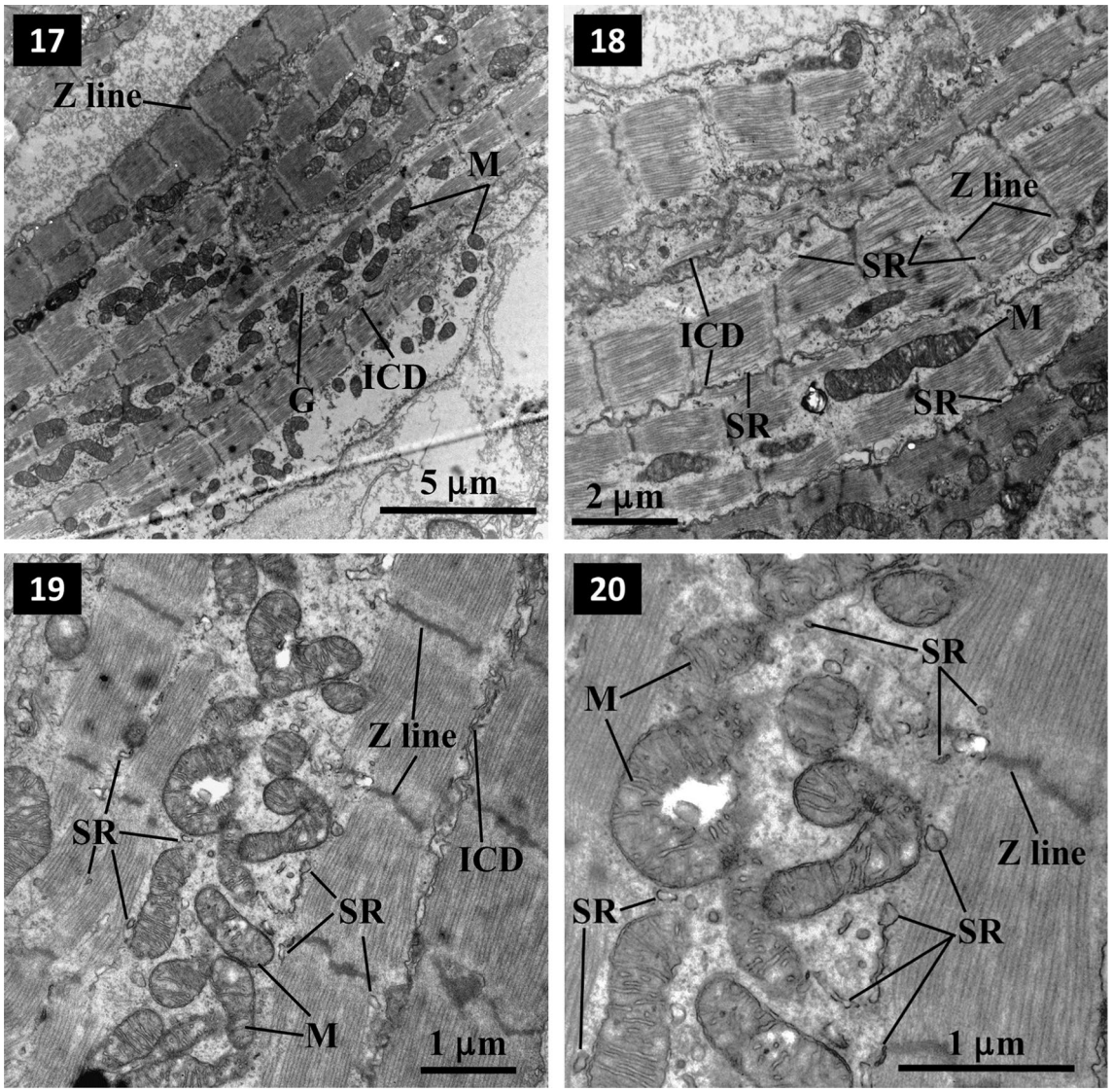


Fig. 10 Electron micrographs of a longitudinal section through atrial myocardium from clearnose skate. The abundance of mitochondria (M) is relatively sparse (*panel 17*); and the sarcoplasmic reticulum (SR) is generally found next to mitochondria (*panels 18* and *19*), intercalated discs (ICD) (*panel 18*), and between the myofibrils (*panels 19* and *20*). Intercalated discs, glycogen storage granules (G) (*panel 17*), and Z lines (*panels 19* and *20*) are also visible

in elasmobranch fishes, as they are in teleost fishes (Genge et al. 2012). Species-specific differences in the involvement of SR Ca^{2+} in EC coupling (from 0 to ~50 %) have, moreover, been demonstrated in teleost fishes (Shiels and Farrell 1997; Driedzic and Gesser 1988; Hove-Madsen et al. 1999; Shiels et al. 1999; Korajoki and Vornanen 2009, 2012). We therefore argue that the differences in the involvement of SR Ca^{2+} in EC coupling between elasmobranch species we measured are plausible, as they are within the range of species-specific differences in the involvement of SR Ca^{2+} in EC coupling observed in teleost fish myocardium.

The force–frequency relationship

To the best of our knowledge, there are no data on in vivo heart rates of clearnose skate, smooth dogfish, or sandbar shark under normal conditions. There are, however, heart rate data on sandbar shark immobilized with neuromuscular blocking drug, which ranged from ~30–40, ~50–60, and ~70–80 beats per minute at 18, 24, and 28 °C, respectively (Dowd et al. 2006). The heart rates measured in sandbar shark in vivo are therefore approximately in the middle of the range of the stimulation frequencies over which atrial and ventricular myocardial strips can operate (Fig. 5).

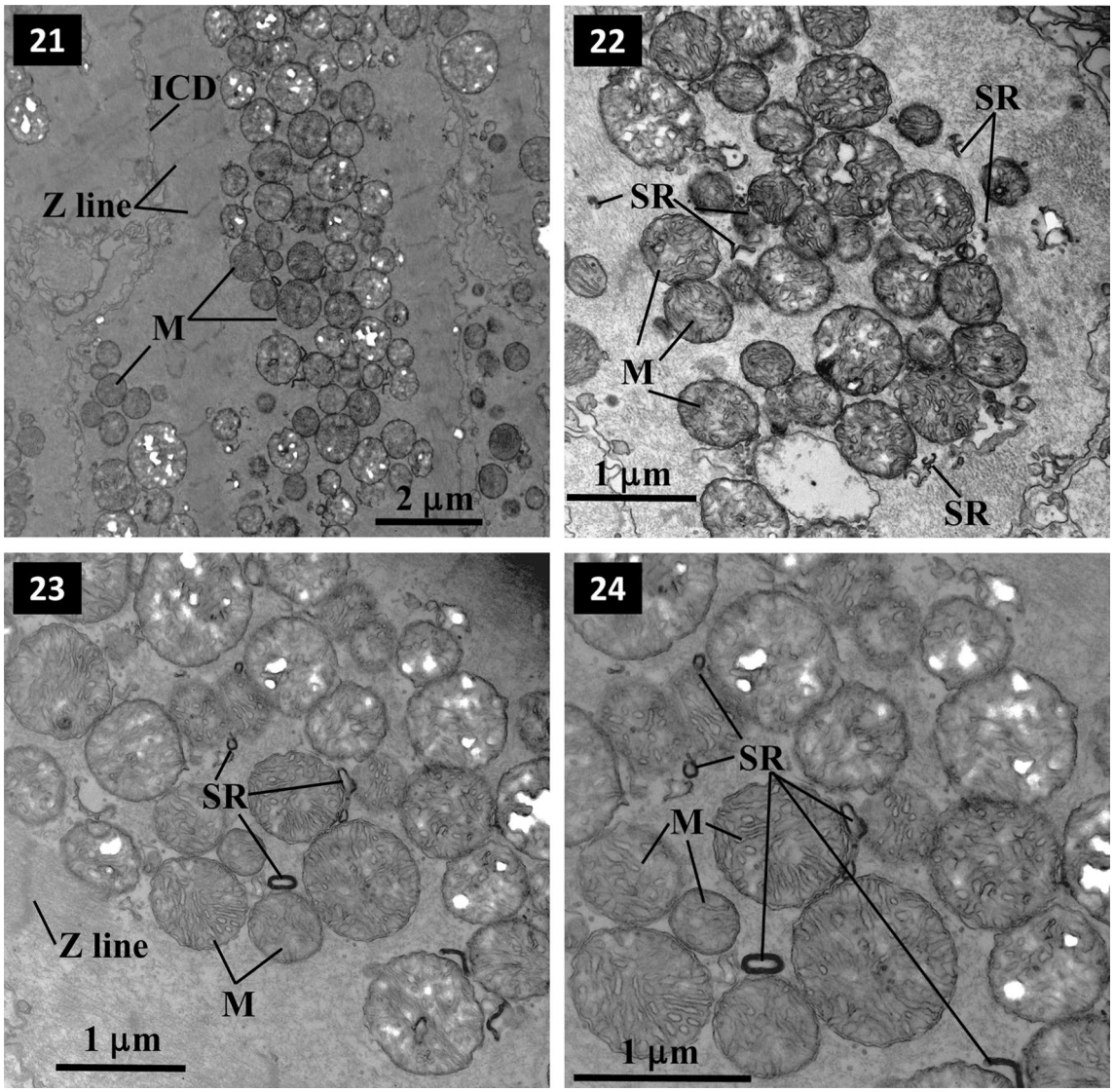


Fig. 11 Electron micrographs of a longitudinal (*panel 21*) and cross-sections (*panels 22*, *23*, and *24*) through ventricular myocardium from clearnose skate. Mitochondria (M) are relatively abundant (*panels 21* and *22*), and the sarcoplasmic reticulum (SR) is sparse (*panels 22*, *23*, and *24*). Intercalated discs (ICD) and Z lines are also visible (*panel 21*)

While the force–frequency relationships of myocardium from teleost fishes have been extensively investigated (reviewed by Shiels et al. 2002b), we are aware of only one study on the force–frequency relationship of myocardium involving elasmobranch fishes (Driedzic and Gesser 1988). With a few exceptions, myocardium from both groups exhibit a negative force–frequency relationship (Shiels et al. 2002b), as do the atrial and ventricular myocardium from the three elasmobranch species we investigated (Fig. 5). A negative force–frequency response is due (in part) to a frequency-dependent decline in trans-sarcolemmal Ca^{2+} movement, and thus CICR Ca^{2+} release from the SR, and (in part) to incomplete mechanical restitution resulting from a frequency-dependent decline in trans-sarcolemmal Ca^{2+} movement out of the cell and uptake into the SR (Shiels et al. 2002b; Shiels and Galli 2014).

Ryanodine–thapsigargin treatment did not have any effect on the force–frequency relationship, or the effects of frequency on other contraction–relaxation parameters in any of the three elasmobranch species we studied, with the exception of time to half relaxation in smooth dogfish atrial myocardium at 20 °C (Fig. 5). We note, however, that force production in atrial tissue was reduced by ryanodine–thapsigargin treatment, implying that the entire force–frequency curve was shifted downward, although this is not displayed in our results, as we calculated changes with frequency as relative values. The clearest species-specific difference was

that clearnose skate myocardium was less able to respond to increases in stimulation frequency, compared to myocardium from smooth dogfish and sandbar shark, as indicated by the higher frequencies at which myocardium from the latter two species maintained coordinated contractions (Fig. 5).

Atrial and ventricular morphological differences

We found no evidence of sarcolemmal invaginations forming T-tubules in three elasmobranch species examined, although internal membrane systems (i.e., SR) were frequently observed. Clear differences between atrial and ventricular tissue were evident in the distribution of SR, although we could not compare quantitatively the amounts of SR present in atrial and ventricular myocardium due to the small number of samples we were able to include in our study. It is possible, however, to observe differences even without quantitative data for each tissue.

The SR was generally more pronounced in the atria, and mitochondria were present in higher quantities in the ventricle. The SR cisterns were located between the myofibrils and in close proximity to the mitochondria in the atria, whereas the SR cisterns were observed only in close proximity to the mitochondria in the ventricle. Likewise, it is at least clear qualitatively that the SR is more pronounced in atrial compared to ventricle myocardium in the three elasmobranch species we investigated. The tissue-specific differences in distribution of SR were most obvious in atrial and ventricular myocardium from smooth dogfish (Fig. 6), which is congruent with the differences in the functional properties of these tissues (Fig. 2), and with the effects of exposure to ryanodine and thapsigargin (i.e., the reduction in net force and $+dF/dt_{max}$ and the increase in time to half relaxation observed in the atrial tissue of this species, Fig. 4). Our observations therefore match those from teleost fishes where atrial myocardium also contains a better developed SR than ventricular tissue (Sætersdal et al. 1974; Santer 1974; Genge et al. 2012).

Some fish species have significant SR Ca^{2+} stores in their cardiac myocytes (Korajoki and Vornanen 2009). These SR Ca^{2+} stores exceed those in mammalian myocardium even though (albeit with some exceptions) fish generally do not normally utilize SR Ca^{2+} in myocardial EC coupling (Galli and Shiels 2012; Shiels and Galli 2014). For example, rainbow trout cardiac myocytes contain an order of magnitude more SR Ca^{2+} (approximately 1250 mmol l^{-1}) compared to mammals (100–150 mmol l^{-1}), even though SR Ca^{2+} release dominates Ca^{2+} delivery during EC coupling in the latter (Shiels et al. 2002b; Galli and Shiels 2012; Shiels and Galli 2014). Fish cardiomyocytes actually contain ten times more Ca^{2+} than the amount being released on each heartbeat, which provides an intracellular Ca^{2+} reservoir that appears to be employed during periods requiring high cardiac performance, such as during stress or exercise (Genge et al. 2012; Cros et al. 2014; Shiels and Sitsapesan 2015). From our observations of SR in atrial and ventricular myocardium, it seems likely that elasmobranch fishes likewise have large stores of SR Ca^{2+} which may be employed under similar circumstances, although to the best of our knowledge this has not yet be directly demonstrated.

Acknowledgments The authors gratefully acknowledge Holly Shiels and Gina Galli (University of Manchester, U.K.) for their critical reviews of earlier versions of this manuscript, although any errors of omission or commission are solely ours. We also greatly recognized the entire staff of the VIMS Eastern Shore Laboratory for their ongoing hospitality and help in running the experiments. Support was provided by the Core Facility for Integrated Microscopy, University of Copenhagen; the University of Indiana South Bend; and the Northeast Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration (NOAA). This is contribution 3582 from the Virginia Institute of Marine Science, College of William and Mary. The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Department of Commerce, National Oceanic and Atmospheric Administration (NOAA), or any of their sub-agencies. Likewise, mention of trade names, products, or commercial companies is for identification purposes only and does not imply endorsement by NOAA or any of its sub-agencies. The authors declare that they have no conflicts of interest.

References

Able KW, Fahay MP (2010) Ecology of the estuarine fishes: temperate waters of the western North Atlantic. The John Hopkins University Press, Baltimore

Aho E, Vornanen M (1998) Ca^{2+}-ATPase activity and Ca^{2+} uptake by sarcoplasmic reticulum in fish heart: effects of thermal acclimation. J Exp Biol 201:525–532

Aho E, Vornanen M (1999) Contractile properties of atrial and ventricular myocardium of the heart of rainbow trout *Oncorhynchus mykiss*: effects of thermal acclimation. J Exp Biol 202:2663–2677

Asgrimsson H, Johansson M, Arnardottir SA (1995) Excitation and contraction in atrial and ventricular myocardium of the guinea-pig. Acta Physiol Scand 153:133–141

Baumann H, Wallace RB, Tagliaferri T, Gobler CJ (2015) Large natural pH, CO_2 and O_2 fluctuations in a temperate tidal salt marsh on diel, seasonal, and interannual time scales. Estuaries Coasts 38:220–231

Bers DM (2002) Cardiac excitation–contraction coupling. Nature 415:198–205

Bootman MD, Higazi DR, Coombes S, Roderick HL (2006) Calcium signaling during excitation–contraction coupling in mammalian atrial myocytes. J Cell Sci 119:3915–3925

Bottinelli R, Canepari M, Cappelli V, Reggiani C (1995a) Maximum speed of shortening and ATPase activity in atrial and ventricular myocardia of hyperthyroid rats. Am J Physiol 269:C785–C790

Bottinelli R, Canepari M, Pellegrino M, Reggiani C (1995b) Force–velocity properties of human skeletal muscle fibres: myosin heavy chain isoform and temperature dependence. J Physiol 495:573–586

Breitburg DL, Hondorp D, Audemard C, Carnegie RB, Burrell RB, Trice M, Clark V (2015a) Landscape-level variation in

disease susceptibility related to shallow-water hypoxia. PLoS One 10:1–27

Breitburg DL, Salisbury J, Bernhard JM, Cai W-J, Dupont S, Doney SC, Kroeker KJ, Levin LA, Long WC, Milke LM, Miller SH, Phelan B, Passow U, Seibel BA, Todgham AR, Tarrant AM (2015b) And on top of all that… Coping with ocean acidification in the midst of many stressors. Oceanography 28:48–461

Brill RW, Lai NC (2016) Elasmobranch cardiovascular system. In: Shadwick RE, Farrell AP, Brauner CJ (eds) Fish physiology, vol 34B., Physiology of elasmobranch fishes—internal processesAcademic Press, San Diego, pp 2–83

Collette BB, Klein-MacPhee G (2002) Bigelow and Schroeder's fishes of the Gulf of Maine, 3rd edn. Smithsonian Institution Press, Washington, DC

Compagno LJV (1984) FAO species catalogue. Sharks of the world. An annotated and illustrated catalogue of shark species known to date, vol 4, part 2—Carcharhiniformes, FAO Fish Synop 125(4/2). FAO, Rome, pp 251–655

Compagno LJV (1990) Alternative life-history styles of cartilaginous fishes in time and space. Environ Biol Fish 28:33–75

Conrath CL, Musick JA (2008) Investigations into depth and temperature habitat utilization and overwintering grounds of juvenile sandbar sharks, *Carcharhinus plumbeus*: the importance of near shore North Carolina waters. Environ Biol Fish 82:123–131

Cooper AR, Morris S (2004) Haemoglobin function and respiratory status of the Port Jackson shark, *Heterodontus portusjacksoni*, in response to lowered salinity. J Comp Physiol B 174:223–236

Cotter PA, Han AJ, Everson JJ, Rodnick KJ (2008) Cardiac hemodynamics of the rainbow trout (*Oncorhynchus mykiss*) using simultaneous Doppler echocardiography and electrocardiography. J Exp Zool 309A:243–254

Cros C, Sallé L, Warren DE, Shiels HA, Brette F (2014) The calcium stored in the sarcoplasmic reticulum acts as a safety mechanism in rainbow trout heart. Am J Physiol 307:R1493–R1501

Davie PS, Farrell AP (1991) Cardiac performance of an isolated heart preparation from the dogfish (*Squalus acanthias*): the effects of hypoxia and coronary artery perfusion. Can J Zool 69:1822–1828

Davie PS, Franklin CE (1992) Myocardial oxygen consumption and mechanical efficiency of a perfused dogfish heart preparation. J Comp Physiol 162:256–262

Dowd W, Brill R, Bushnell P, Musick J (2006) Standard and routine metabolic rates of juvenile sandbar sharks (*Carcharhinus plumbeus* Nardo), including the effects of body mass and acute temperature change. Fish Bull 104:323–331

Driedzic WR, Gesser H (1988) Differences in force–frequency relationships and calcium dependency between elasmobranch and teleost hearts. J Exp Biol 140:227–241

Farrell AP (1991) From hagfish to tuna: a perspective on cardiac-function in fish. Physiol Zool 64:1137–1164

Farrell AP, Jones DR (1992) The heart. In: Hoar WS, Randall DJ, Farrell AP (eds) Fish physiology, vol 12A., The cardiovascular systemAcademic Press, San Diego, pp 1–87

Galli GLJ (2006) The role of the sarcoplasmic reticulum in the generation of high heart rates and blood pressures in reptiles. J Exp Biol 209:1956–1963

Galli GJ, Shiels H (2012) The sarcoplasmic reticulum in the vertebrate heart. In: Sedmera D, Wang T (eds) Ontogeny and phylogeny of the vertebrate heart. Springer, New York, pp 103–124

Galli GJ, Gesser H, Taylor EW, Shiels HA, Wang T (2006) The role of the sarcoplasmic reticulum in the generation of high heart rates and blood pressures in reptiles. J Exp Biol 209:1956–1963

Galli G, Shiels H, Brill R (2009) Cardiac temperature sensitivity in yellowfin tuna (*Thunnus albacares*), bigeye tuna (*T. obesus*), mahimahi (*Coryphaena hippurus*) and swordfish (*Xiphias gladius*). Physiol Biochem Zool 82:280–290

Gamperl AK, Shiels HA (2014) Cardiovascular system. In: Evans DH, Claiborne JB, Currie S (eds) The physiology of fishes, 4th edn. CRC Press, Boca Raton, pp 33–79

Genge C, Hove-Madsen L, Tibbits GF (2012) Functional and structural differences in atria versus ventricles in teleost hearts. In: Turker H (ed) New advances and contributions to fish biology. INTECH Open Access Publisher, pp 221–245

Genge CE, Davidson WS, Tibbits GF (2013) Adult teleost heart expresses two distinct troponin C paralogs: cardiac TnC and a novel and teleost-specific ssTnC in a chamber- and temperature-dependent manner. Physiol Genomics 45:866–875

Gillis TE, Klaiman JM (2011) The influence of PKA treatment on the Ca^{2+} activation of force generation by trout cardiac muscle. J Exp Biol 214:1989–1996

Gillis TE, Tibbits GF (2002) Beating the cold, the functional evolution of cardiac troponin C in teleost fish. Comp Biochem Physiol 132:763–772

Gillis TE, Marshall CR, Xue X-H, Borgford TJ, Tibbits GF (2000) Ca^{2+} binding to cardiac troponin C: effects of temperature and pH on mammalian and salmonid isoforms. Am J Physiol 279:R1707–R1715

Grubbs RD, Musick JA (2007) Spatial delineation of summer nursery areas for juvenile sandbar sharks in Chesapeake Bay, Virginia. Am Fish Soc Symp 50:63–86

Grubbs RD, Musick JA, Conrath CL, Romaine JG (2007) Long-term movements, migration, and temporal delineation of summer nurseries for juvenile sandbar sharks in the Chesapeake Bay region. Am Fish Soc Symp 50:87–108

Haverinen J, Vornanen M (2009) Comparison of sarcoplasmic reticulum calcium content in arterial and ventricular myocytes of three fish species. Am J Physiol 297:R1180–R1187

Hofmann GE, Smith JE, Johnson KS, Send U, Levin LA, Micheli F, Paytan A, Price NN, Peterson B, Takeshita Y, Matson PG, Derse Crook E, Kroeker KJ, Cristina Gambi M, Rivest EB, Frieder CA, Yu PC, Martz TR (2011) High-frequency dynamics of ocean pH: a multi-ecosystem comparison. PLoS One 6:1–11

Hove-Madsen L (1992) The influence of temperature on ryanodine sensitivity and the force–frequency relationship in the myocardium of the rainbow trout. J Exp Biol 167:47–60

Hove-Madsen L, Llach L, Tort L (1998) Quantification of Ca^{2+} uptake in the sarcoplasmic reticulum of trout ventricular myocytes. Am J Physiol 275:R2070–R2080

Hove-Madsen L, Llach A, Tort L (1999) Quantification of calcium release from the sarcoplasmic reticulum in rainbow trout atrial myocytes. Pflügers Arch 438:545–552

Hove-Madsen L, Llach L, Tort L (2001) The function of the sarcoplasmic reticulum is not inhibited by low temperatures in trout atrial myocytes. Am J Physiol 281:R1902–R1906

Karasinski J (1993) Diversity of native myosin and myosin heavy chain in fish skeletal muscles. Comp Biochem Physiol 106B:1041–1047

Karasinski J, Sokalski A, Kilarski W (2001) Correlation of myofibrillar ATPase activity and myosin heavy chain content in ventricular and atrial myocardium of fish heart. Folia Histochem Cytobiol 39:23–28

Keen JE, Farrell AP, Tibbits GF, Brill RW (1992) Cardiac dynamics in tunas. II. Effect of ryanodine, calcium, and adrenaline on force–frequency relationships in atrial strips from skipjack tuna, *Katsuwonus pelamis*. Can J Zool 70:1211–1217

Keen JE, Vianzon D-M, Farrell AP, Tibbits GF (1994) Effect of temperature and temperature acclimation on the ryanodine sensitivity of the trout myocardium. J Comp Physiol 164B:438–443

Kiraly SJ, Moore JA, Jasinski PA (2003) Deepwater and other sharks of the U.S. Atlantic Ocean exclusive economic zone. Mar Fish Rev 65:1–20

Klimley AP (2013) The Biology of Sharks and Rays. The University of Chicago Press, Chicago

Korajoki H, Vornanen M (2009) Expression of calsequestrin in atrial and ventricular muscle of thermally acclimated rainbow trout. J Exp Biol 212:3403–3414

Korajoki H, Vornanen M (2012) Expression of SERCA and phospholamban in rainbow trout (*Oncorhynchus mykiss*) heart: comparison of atrial and ventricular tissue and effects of thermal acclimation. J Exp Biol 215:1162–1169

Korajoki H, Vornanen M (2014) Species- and chamber-specific responses of 12 kDa FK506-binding protein to temperature in fish heart. Fish Physiol Biochem 40:539–549

Lai NC, Graham JB, Bhargava V, Shabetai R (1996) Mechanisms of venous return and ventricular filling in elasmobranch fish. Am J Physiol 270:H1766–H1771

Lai NC, Graham JB, Dalton N, Shabetai R, Bhargava V (1998) Echocardiographic and hemodynamic determinations of the ventricular filling pattern in some teleost fishes. Physiol Zool 71:157–167

Lai NC, Dalton N, Lai YY, Kwong C, Rasmussen R, Holts D, Graham JB (2004) A comparative echocardiographic assessment of ventricular function in five species of sharks. Comp Biochem Physiol 137A:505–521

Lo HM, Lin FY, Lin JL, Hsu KL, Chiang FT, Tseng CD, Tseng YZ (1999) Impaired cardiac performance relating to delayed left atrial activation after atrial compartment operation for chronic atrial fibrillation. Pacing Clin Electrophysiol 22:379–381

Luft JH (1977) Improvements in epoxy resin embedding methods. J Biophys Biochem Cy 9:409–414

Marshall H, Field L, Afiadata A, Sepulveda C, Skomal G, Bernal D (2012) Hematological indicators of stress in longline captured sharks. Comp Biochem Physiol 162:121–129

Maylie J, Morad M (1979) Inotropic effects of adrenaline in dogfish heart (*Squalus acanthias*). Bull Mt Desert Isl Biol Lab 19:87–92

Maylie J, Morad M (1981) Ionic and pharmacological characterization of excitation and contraction in dogfish (*Squalus acanthias*). Bull Mt Desert Isl Biol Lab 20:122–126

Maylie J, Morad M (1995) Evaluation of T- and L-type Ca^{2+} currents in shark ventricular myocytes. Am J Physiol 269:H1695–H1703

Maylie J, Nunzi MG, Morad M (1979) Excitation–contraction coupling in ventricular muscle of dogfish (*Squalus acanthias*). Bull Mt Desert Isl Biol Lab 19:84–87

Maylie J, Varnum M, Morad M (1994) Characterization of a voltage-gated cardiac potassium channel in *Squalus acanthias* and its expression in *Xenopus* oocytes injected with mRNA isolated from hearts of *Squalus acanthias*. Bull Mt Desert Isl Biol Lab 33:29–30

McCandless CT, Pratt HL, Kohler NE, Merson RR, Recksiek CW (2007) Distributions, localized abundance, movements and migrations of juvenile sandbar sharks tagged in Delaware Bay. Am Fish Soc Symp 50:45–62

McEachran JD, Musick JA (1975) Distribution and relative abundance of seven species of skates (Pisces: Rajidae) which occur between Nova Scotia and Cape Hatteras. Fish Bull 73:110–136

Medved RJ, Marshall JA (1981) Feeding behavior and biology of young sandbar sharks, *Carcharhinus plumbeus* (Pisces, Carcharhinidae), in Chincoteague Bay, Virginia. Fish Bull 79:441–447

Medved RJ, Stillwell CE, Casey JG (1985) Stomach contents of young sandbar sharks, *Carcharhinus plumbeus*, in Chincoteague Bay, Virginia. Fish Bull 83:395–402

Methling C, Steffensen JF, Skov PV (2012) The temperature challenges on cardiac performance in winter-quiescent and migration-stage eels *Anguilla anguilla*. Comp Biochem Physiol 163A:66–73

Näbauer M, Morad M (1992) Modulation of contraction by intracellular Na^+ via Na^+–Ca^{2+} exchange in single shark (*Squalus acanthias*) ventricular myocytes. J Physiol 457:627–637

Olson KR, Farrell AP (2006) The Cardiovascular System. In: Evans DH, Claiborne JB (eds) The physiology of fishes. CRC Press, Boca Raton, pp 119–152

Prosser CL (1973) Chapter 9, temperature. In: Prosser CL (ed) Comparative animal physiology. W.B. Saunders Company, Philadelphia, pp 317–361

Reum JCP, Alin SR, Feely RA, Newton J, Warner M, McElhany P (2014) Seasonal carbonate chemistry covariation with temperature, oxygen, and salinity in a fjord estuary: implications for the design of ocean acidification experiments. PLoS One 9:1–12

Rousseau E, Smith JS, Meissner G (1987) Ryanodine modifies conductance and gating behavior of single Ca^{2+} release channel. Am J Physiol 253:C364–C368

Sætersdal TS, Justesen N-P, Krohnstad AW (1974) Ultrastructure and innervation of the teleostean atrium. J Mol Cell Cardiol 6:415–437

Sagara Y, Inesi G (1991) Inhibition of the sarcoplasmic reticulum Ca^{2+} transport ATPase by thapsigargin at subnanomolar concentrations. J Biol Chem 266:13503–13506

Sanchez-Quintana D, Hurle J (1987) Ventricular myocardial architecture in marine fishes. Anat Rec 217:263–273

Santer RM (1974) The organization of the sarcoplasmic reticulum in teleost ventricular myocardial cells. Cell Tissue Res 151:395–402

Schwartz FJ (1996) Biology of the clearnose skate, *Raja eglanteria*, from North Carolina. Fla Sci 59:82–95

Shiels HA, Farrell AP (1997) The effect of temperature and adrenaline on the relative importance of the sarcoplasmic reticulum in contributing Ca^{2+} to force development in isolated ventricular trabeculae from rainbow trout. J Exp Biol 200:1607–1621

Shiels HA, Galli GL (2014) The sarcoplasmic reticulum and the evolution of the vertebrate heart. Physiology 29:456–469

Shiels HA, Sitsapesan R (2015) Is there something fishy about the regulation of the ryanodine receptor in the fish heart? Exp Physiol 100:1412–1420

Shiels HA, White E (2005) Temporal and spatial properties of cellular Ca^{2+} flux in trout ventricular myocytes. Am J Physiol 288:R1756–R1766

Shiels HA, Freund EV, Farrell AP, Block BA (1999) The sarcoplasmic reticulum plays a major role in isometric contraction in atrial muscle of yellowfin tuna. J Exp Biol 202:881–890

Shiels HA, Vornanen M, Farrell AP (2002a) Temperature dependence of cardiac sarcoplasmic reticulum function in rainbow trout myocytes. J Exp Biol 205:3631–3639

Shiels HA, Vornanen M, Farrell AP (2002b) The force–frequency relationship in fish hearts—a review. Comp Biochem Physiol 132A:811–826

Shiels HA, Di Maio A, Thompson S, Block BA (2011) Warm fish with cold hearts: thermal plasticity of excitation–contraction coupling in bluefin tuna. Proc Biol Sci 278:18–27

Stillwell CE, Kohler NE (1993) Food habits of the sandbar shark *Carcharhinus plumbeus* off the U.S. northeast coast, with estimates of daily ration. Fish Bull 91:138–150

Thomas MJ, Hamman BN, Tibbits GF (1996) Dihydropyridine and ryanodine binding in ventricles from rat, trout, dogfish, and hagfish. J Exp Biol 199:1999–2009

Thompson AP, O'Shea JE (1997) The unusual adrenergic-like excitatory action of acetylcholine on the ventricular cardiac muscle of the horned shark, *Heterodontus portusjacksoni*. Physiol Zool 70:135–142

Tibbits GF, Hove-Madsen L, Bers DM (1991) Calcium-transport and the regulation of cardiac contractility in teleosts: a comparison with higher vertebrates. Can J Zool 69:2014–2019

Tibbits GF, Moyes CD, Hove-Madsen L (1992) Excitation–contraction coupling in the teleost heart. In: Hoar WS, Randall DJ, Farrell AP (eds) Fish physiology, vol 12., The cardiovascular systemAcademic Press, San Diego, pp 267–304

Vornanen M (1994) Seasonal adaptation of crucian carp (*Carassius carassius* L.) heart: glycogen stores and lactate dehydrogenase activity. Can J Zool 72:433–442

Vornanen M, Haverinen J (2013) A significant role of sarcoplasmic reticulum in cardiac contraction of a basal vertebrate, the river lamprey (*Lampetra fluviatilis*). Acta Physiol 207:269–279

Vornanen M, Tuomennoro J (1999) Effects of acute anoxia on heart function in crucian carp (*Carassius carassius* L.) heart: importance of cholinergic and purinergic control. Am J Physiol 277:R465–R475

Vornanen M, Ryökkynen A, Nurmi A (2002a) Temperature dependent expression of sarcolemmal K^+ currents in rainbow trout atrial and ventricular myocytes. Am J Physiol 282:R1191–R1199

Vornanen M, Shiels HA, Farrell AP (2002b) Plasticity of excitation–contraction coupling in fish cardiac myocytes. Comp Biochem Physiol 132A:827–846

Walden AP, Dibb KM, Trafford AW (2009) Differences in intracellular calcium homeostasis between atrial and ventricular myocytes. J Mol Cell Cardiol 46:463–473

Weissgerber TL, Milic NM, Winham SJ, Garovic VN (2015) Beyond bar and line graphs: time for a new data presentation paradigm. PLoS Biol 13:1–10

Yelon D, Horne S, Stainier D (1999) Restricted expression of cardiac myosin genes reveals regulated aspects of heart tube assembly in zebrafish. Dev Biol 214:23–37