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THE SARCOPLASMIC RETICULUM PLAYS A MAJOR ROLE IN ISOMETRIC CONTRACTION IN ATRIAL MUSCLE OF YELLOWFIN TUNA

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Summary

We used an isometric muscle preparation to test the hypothesis that yellowfin tuna *Thunnus albacares* utilize the intracellular Ca^{2+} storage sites of the sarcoplasmic reticulum (SR) during routine contractions. Ryanodine (a blocker of SR Ca^{2+} release) reduced the force of contraction by approximately 50 % and the rates of contraction and relaxation by 60 % in yellowfin tuna atrium. High levels of adrenaline were unable to ameliorate the effects of ryanodine. We conclude that the SR is active in contributing Ca^{2+} to force development at physiological contraction frequencies. Further, we suggest that, by using intracellular Ca^{2+} cycling, the yellowfin tuna is able to increase the maximum contraction frequency of its cardiac muscle beyond that of most other fishes.

Key words: yellowfin tuna, *Thunnus albacares*, atrium, muscle, adrenaline, ryanodine, force–frequency, temperature, heart.

Introduction

Maximal heart rates of 130–140 beats min^{-1} have been measured in yellowfin tuna (*Thunnus albacares*) (Brill, 1987; Farrell et al., 1992; Keen et al., 1995). These heart rates slightly exceed the suggested upper limit to contraction frequency of 120 beats min^{-1} observed for most fish (Farrell, 1991). Likewise, skipjack tuna (*Katsuwonus pelamis*) have maximum heart rates of 154–191 beats min^{-1} (Brill, 1987; Farrell et al., 1992; Keen et al., 1995). These findings suggest differences between cardiac excitation–contraction coupling in tuna and other fishes.

One possible explanation for the exceptional maximum heart rates in tuna is the role of intracellular Ca^{2+} cycling during excitation–contraction coupling. In cardiac cells, mammals cycle Ca^{2+} between the intracellular Ca^{2+} stores of the sarcoplasmic reticulum (SR) and the myofilaments. However, despite the central role of the SR in mammalian excitation–contraction coupling, there exists a graded dependence on SR Ca^{2+} release which changes with species, developmental stage and regionally within the heart. For example, adult rabbit ventricle is less dependent on SR Ca^{2+} release than that of adult rat (Bers, 1991); within a species, neonate hearts are less dependent on SR Ca^{2+} than adult hearts, and the atrium is more SR-dependent than the ventricle (Bers, 1991). Unlike mammals, most fish cycle extracellular Ca^{2+} back and forth across the cell membrane (sarcolemma) between the extracellular space and the myofilaments with each beat. Therefore, the mode of excitation–contraction coupling in fish may result in longer diffusional distances for Ca^{2+} movement which, in turn, may compromise maximum contraction frequency. Skipjack tuna, which have relatively high maximum contraction frequencies compared with those of other fish, utilize both intra- and extracellular Ca^{2+} cycling to satisfy the Ca^{2+} requirements of the myofilaments (at 25 °C; Keen et al., 1992). Thus, Ca^{2+} cycling during excitation–contraction coupling in skipjack tuna appears to be intermediate between traditional ‘fish’ and ‘mammal’ models.

Although most teleost hearts do not routinely use SR Ca^{2+} (Driedzic and Gesser, 1988), rainbow trout (*Oncorhynchus mykiss*) ventricles demonstrate increased SR involvement at temperatures near their upper incipient lethal temperature (23–25 °C; Black, 1953) and at subphysiological contraction frequencies (Hove-Madsen, 1992; Keen et al., 1994; Shiels and Farrell, 1997). Thus, the ability to utilize SR Ca^{2+} appears to be both frequency- and temperature-dependent. Indeed, the temperature-dependency of SR utilization in fish may stem from the temperature-dependency of the SR Ca^{2+}-release channel itself. In mammalian cardiac muscle, an acute decrease in temperature increases the open probability of the SR Ca^{2+}-release channel (Bers, 1987, 1989; Sitsapesan et al., 1991), thus rendering the SR ineffective in sequestering and releasing Ca^{2+} during contraction. The lack of SR involvement during excitation–contraction coupling in most fish hearts may therefore reflect the fact that most studies are conducted between 5 and 15 °C where there may be cold-induced opening of the SR Ca^{2+}-release channel (Tibbits et al., 1992). Conversely, the involvement of SR Ca^{2+} during force development in skipjack tuna may simply reflect the

experimental temperature of 25 °C. However, the effect of temperature on SR Ca^{2+} release during excitation–contraction coupling has not been examined in tuna hearts. Therefore, the purpose of the present study was to investigate how acute, physiological temperature change affects the contribution of SR Ca^{2+} during force development in the atrium of yellowfin tuna under physiological levels of adrenergic stimulation. Since Ca^{2+} cycling has been implicated in contributing to the elevated maximal heart rates in tuna, the experiments were conducted over a range of pacing frequencies from sub- (0.2 Hz) to superphysiological (3.5 Hz).

Materials and methods

Fish origin and care

Yellowfin tuna *Thunnus albacares* (Bonaterre) were caught off the coast of California in September 1996 and transferred to the Tuna Research and Conservation Center, Pacific Grove, CA, USA, as described by Altringham and Block (1997). Fish were maintained for more than 1 year in tanks 10 m in diameter and 2 m deep. Sea water was recirculated, filtered, aerated and maintained at 20±0.5 °C. Fish were fed a diet of fish and squid three times per week, at approximately 115 kJ kg^{-1} day^{-1}.

Tissue preparation

Fish (4.99±0.13 kg) were netted from the holding tanks and killed by cervical dislocation. The heart was quickly excised and placed in ice-cooled, oxygenated physiological saline (containing, in mol l^{-1}, NaCl, 175.7; KCl, 7; $CaCl_2$, 1.9; $MgCl_2$, 1.1; sodium pyruvate, 10; Hepes, 10; pH 7.8 at 25 °C). The atrium (1.89±0.11 g) was isolated and cut lengthwise to expose the lumen. Four individual trabeculae were dissected out with the aid of a dissecting microscope and hung between an isometric force transducer (Kulite Semiconductors, Leonia, NJ, USA) and a fixed post, in an oxygenated, water-jacketed organ bath (described in detail by Shiels and Farrell, 1997). The organ bath was maintained at 15 °C, 18 °C or 25 °C. The preparation was lengthened to remove slack and left for 30 min before stimulation. The muscle was then stimulated at 1.0 Hz, and muscle length L was adjusted to maximize force production (i.e. such that the preparation was operating on the plateau of the length–tension curve), defined as L_{max}. Trabeculae were left to stabilize at L_{max} for a minimum of 30 min. Stimulation was achieved using a Harvard Student Stimulator (Harvard Apparatus Ltd, Edenbridge, Kent, UK) delivering current pulses (20 V, 10 ms duration) *via* platinum plate electrodes positioned on either side of the muscle. Signals from the transducers were displayed on a Windograf digital oscilloscope (Gould, Cleveland, OH, USA) and stored to disk for later analysis.

Experimental protocol

The experiments were designed to test the acute effects of temperature and adrenaline on the contribution of SR Ca^{2+} during force development in yellowfin atrial muscle. The degree of involvement of the SR in force production was assessed using ryanodine, a specific and irreversible ligand for the Ca^{2+}-release channel of the SR. When ryanodine is applied to muscle in the micromolar range (10 μmol l^{-1}), as in the present study, it locks the SR Ca^{2+}-release channel closed, rendering it ineffective in contributing Ca^{2+} to force production (Rousseau et al., 1987). Tissue sensitivity to ryanodine is therefore considered to reflect the dependence of contractility on Ca^{2+} released from intracellular stores. Experiments were conducted at 15 °C, 18 °C or 25 °C to investigate the temperature-dependency of the ryanodine response. Because adrenaline is present at nanomolar concentrations in the circulation of resting fish (Milligan et al., 1989), and because it has been shown to preserve cardiac tonus *in situ* (Farrell et al., 1986; Graham and Farrell, 1989; Davie and Farrell, 1991) and *in vitro* (Shiels and Farrell, 1997), we utilized a tonic level (1 nmol l^{-1}) of adrenergic stimulation in all experiments. In rainbow trout, maximal (10 μmol l^{-1}) adrenergic stimulation overwhelms the relative importance of the intracellular (SR) Ca^{2+} contribution to force production by dramatically increasing the peak force production of isolated ventricular muscle, even after it has been treated with ryanodine and irrespective of temperature (Shiels and Farrell, 1997). We therefore also employed a maximal level of adrenergic stimulation (1 μmol l^{-1}) to observe its effect on the relative importance of SR and sarcolemma Ca^{2+} flux during force development in yellowfin tuna atrium.

Each experiment consisted of three force–frequency trials performed sequentially on the same trabeculae: (1) with a low adrenaline concentration, (2) with low adrenaline and ryanodine concentrations, and (3) with high adrenaline and ryanodine concentrations. In each force–frequency trial, stimulation frequency was increased from 0.2 to 0.5 Hz and then to 3.5 Hz in 0.5 Hz increments. Force, time to peak tension, time to 50 % relaxation and the rates (df/dt) of contraction and relaxation were measured at each frequency. After each change in stimulation frequency, the muscle was allowed to stabilize for 5 min before new force measurements were made. In the force–frequency trials involving ryanodine, the muscle was equilibrated with 10 μmol l^{-1} ryanodine for 45 min prior to the onset of the trial. Muscles were allowed to recover at 1.0 Hz for 45 min between trials.

After the first experiments at 15 °C and 25 °C, we noticed that the positive inotropic effect of adrenaline was deteriorating by the time a stimulation frequency of 1.0 Hz had been reached. This suggested either (1) a time-dependent desensitization with high adrenaline concentration, or (2) a frequency-dependent response to high adrenaline concentration. To test these possibilities, the order of application of stimulation frequency was changed for the 18 °C group and for half the 25 °C group, such that frequency was increased from 1.0 to 3.5 Hz and then reduced to 0.2 and 0.5 Hz.

To account for the slow deterioration of the preparation over the duration of the experiment, control and test trabeculae were dissected from the same heart, and control trabeculae were monitored in parallel with the test trabeculae in a separate organ bath. Control trabeculae were exposed to the same

conditions of temperature, stimulation frequency and voltage as the test muscle but were only exposed to the low adrenaline concentrations throughout the force–frequency trials. Thus, changes in force development due to muscle fatigue were accounted for by subtracting the changes in force (as percentages of the initial tension generated) in controls from those for the experimental atria.

Chemicals, calculations and statistical analyses

All chemicals were purchased from Sigma (St Louis, MO, USA) with the exception of ryanodine, which was purchased from Calbiochem (San Diego, CA, USA). Force is expressed as $mN\,mm^{-2}$. Mean cross-sectional area was calculated using wet muscle mass, trabecular length and an assumed muscle density of $1.06\,g\,cm^{-3}$ (Layland et al., 1995). Measurements of force, time to peak tension, time to 50 % relaxation and rates (df/dt) of contraction and relaxation were achieved using the View II data analysis system (Gould, Cleveland, OH, USA). Significant increases after adrenaline stimulation and significant decreases after ryanodine treatment were assessed using one-way Student's *t*-tests ($P \leqslant 0.05$).

Results

Force–frequency effects

Temperature and frequency effects

The yellowfin tuna atrium exhibits a biphasic force–frequency response similar to that observed in skipjack tuna atrium (Keen et al., 1992), with a positive staircase at low frequencies (0.2–0.5 Hz) and a negative staircase within the more physiologically relevant frequency range (greater than 1.0 Hz) (Fig. 1).

At 15 °C and 18 °C, trabecular contractions often became irregular at frequencies greater than 2.0 Hz and 3.0 Hz, respectively (denoted by the unconnected data points in Fig. 1). At 25 °C, however, the muscle could maintain regular contractions above 3.5 Hz.

For most parameters measured, the individual variability at 18 °C was less than that at both 15 °C and 25 °C, which may be related to the fact that 18 °C is the test temperature closest to the acclimation temperature of 20 °C.

Effects of ryanodine

Ryanodine significantly decreased force at all temperatures and at all pacing frequencies (Fig. 1). This indicates an active role for SR Ca^{2+} release during excitation–contraction coupling in yellowfin tuna atrium that is to some extent independent of the contraction frequency and the temperature. Indeed, after ryanodine treatment, an equivalent level of force production to that obtained under control conditions could only be achieved at a lower pacing frequency. For example, force development under control conditions at 2.5 Hz and 18 °C (left-hand panel in Fig. 1) was approximately $7\,mN\,mm^2$; after ryanodine treatment, the same degree of force production could not be maintained at frequencies greater than 1.5 Hz.

The degree of SR Ca^{2+} utilization also had a component that was both temperature- and frequency-dependent. The temperature-dependence of SR Ca^{2+} utilization was most evident at low pacing frequencies (0.2–1.0 Hz), where colder muscles were found to use less SR Ca^{2+} than warmer muscles (Fig. 2). No clear temperature-threshold for SR Ca^{2+}-release channel function was observed over the range of temperatures tested. The response to ryanodine was frequency-dependent (Fig. 2) at 15 °C and 18 °C, where SR Ca^{2+} involvement increased as contraction frequency increased (non-parametric ANOVA, Kruskal–Wallis). At 25 °C, SR utilization was more or less constant over the physiological range of frequencies, but increased significantly at the highest frequencies. The greatest percentage decrease in force after ryanodine treatment occurred at the highest frequencies at all temperatures. This supports the suggestion of increased dependence on SR Ca^{2+} cycling during high-frequency contractions in yellowfin tuna heart muscle. In fact, at high frequencies, the temperature-dependency of the ryanodine response was almost abolished since the SR contribution approached or exceeded approximately 60 % at all temperatures (Fig. 2).

Effects of adrenaline

No significant positive inotropic effect was observed at physiologically relevant frequencies when $1\,\mu mol\,l^{-1}$ adrenaline was applied to muscle pre-treated with ryanodine (Fig. 1). When the order of imposed frequency of stimulation was changed, such that frequency was increased from 1.0 to 3.5 Hz and then back to 0.2 and 0.5 Hz in the 18 °C group, there were again no significant effects of adrenaline at any frequency. In the 25 °C group, where the order of stimulation frequency was changed half-way through the experiments, there was a significant effect of adrenaline at only 0.2 and 0.5 Hz in the pooled groups. Thus, adrenergic stimulation did not overcome the negative effect of SR inhibition at physiological contraction frequencies in the atria of yellowfin tuna.

Contraction kinetics

Temperature and frequency effects

Contraction kinetics were temperature- and frequency-dependent. The time to peak force and the time to 50 % relaxation decreased as temperature and frequency increased (Fig. 3). The rates of contraction (Fig. 4) were not changed significantly by temperature, primarily because peak force itself decreased while the absolute time to peak force decreased with increasing temperature. The data suggest a slower rate of contraction at 18 °C that at 15 °C; however, this was found not to be significant (Student's *t*-test, $P>0.05$), possibly because of the small sample size. We have no explanation for this trend. Rates of relaxation were also similar among temperatures (Fig. 4).

Effects of ryanodine

Ryanodine increased the absolute time required to reach peak tension (Fig. 3) and decreased the rate of contraction (Fig. 4), suggesting slower activation of the myofilaments in the absence of SR Ca^{2+} release. Similarly, the time to 50 % relaxation increased (Fig. 3) and the rate of 50 % relaxation

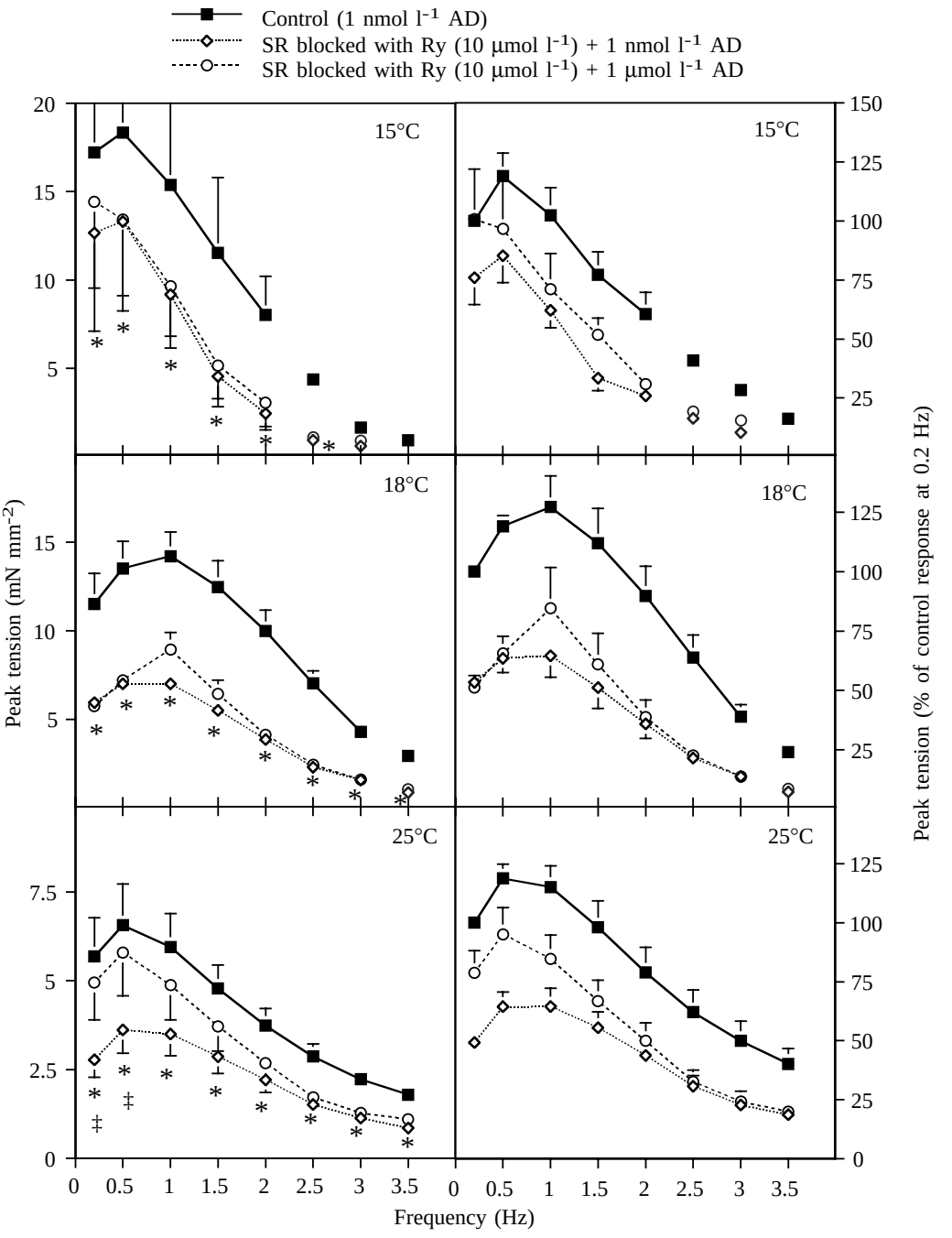

Fig. 1. Force–frequency responses from yellowfin tuna atrial trabeculae at 15 °C, 18 °C and 25 °C as indicated in the upper right-hand corner of each graph. The left-hand panel shows absolute values in $mN\,m^{-2}$ tissue. The right-hand panel shows data normalized to the control condition at 0.2 Hz. Force–frequency responses were obtained under different drug treatments, as indicated by the legend. Values are means ± S.E.M.; *N*=4 at 15 °C, *N*=4 at 18 °C and *N*=8 at 25 °C. Solitary points (not connected by lines) are values where *N* is less than that stated above. *Significant ($P<0.05$) decreases with ryanodine (Ry); ‡significant increases with adrenaline (AD), determined using paired one-way Student's *t*-tests.

(Fig. 4) decreased after ryanodine treatment. This suggests that SR Ca^{2+} uptake, as well as release, is impaired in ryanodine-treated muscle. This would be expected if SR loading were compromised by the inability of the SR to release Ca^{2+} in the presence of a high (10 μmol l^{-1}) ryanodine concentration. In all cases, the rates of contraction and relaxation never reach the pre-ryanodine-treated level at any contraction frequency.

Discussion

This is the first study to examine the relative importance of SR Ca^{2+} cycling during force development in the atria of yellowfin tuna. Our results indicate that the SR can contribute up to 60 % of the activator Ca^{2+} during force development at maximal contraction frequencies in the cardiac muscle of yellowfin tuna. This is the highest level of SR Ca^{2+} involvement in force production reported for a teleost and is the first evidence directly linking the high heart rates of tuna with increased dependence on intracellular Ca^{2+} cycling. Freund and Block (1998 and in preparation) measured very large post-rest potentiations (a doubling of peak tension after a 20 s rest period) in yellowfin tuna ventricle, indicating that the SR contributes a significant amount of Ca^{2+} to both atrial and ventricular tissue. Further, their work revealed that Pacific

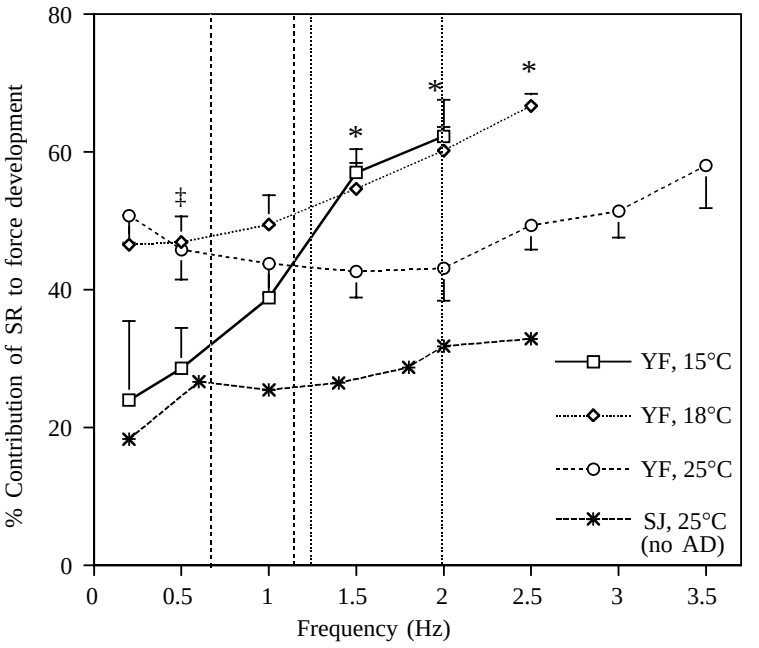

Fig. 2. Percentage contribution of sarcolasmic reticulum (SR) Ca^{2+} to force development in atrial trabeculae from yellowfin tuna (YF) at 15 °C, 18 °C and 25 °C. Results were calculated as the percentage decrease in force from the control condition after ryanodine (10 μmol l^{-1}) treatment at each frequency. Data for skipjack tuna (SJ) at 25 °C (in the absence of adrenaline, AD) were calculated from the results of Keen et al. (1992). The two vertical dashed lines indicate the range of *in vivo* heart rates for free-swimming yellowfin tuna at 18 °C, and the two vertical dotted lines indicate the range of *in vivo* heart rates at 24 °C (heart rate range taken from Korsmeyer et al., 1997). *Significant difference between the 25 °C group and the other groups (18 and 15 °C), ‡significant difference between the 15 °C group and 18 and 25 °C groups calculated using a non-parametric ANOVA (Kruskal–Wallis). Skipjack tuna data were not statistically compared with yellowfin tuna data. Values are means ± S.E.M. (values of *N* are as in Fig. 1).

mackerel (*Scomber japonicus*), a close phylogenetic relative of the yellowfin tuna, also demonstrates large ventricular post-rest potentiations, indicating that the large degree of SR utilization may not be limited to tuna, but rather may be a common trait of the Scombridae family.

Significant levels of SR Ca^{2+} involvement during force development have been demonstrated previously by Keen et al. (1992) for the skipjack tuna atrium. Their results indicate a maximum 30 % decrease in peak force after ryanodine treatment at 2.5 Hz (these data are shown in Fig. 2 for comparison). A possible explanation for the greater response to ryanodine treatment in yellowfin compared with skipjack tuna may be related to tonic adrenergic stimulation. Fish hearts, including those of skipjack and yellowfin tuna (Keen et al., 1995), always have humoral adrenergic tonus *in vivo*, but most studies of cardiac tissue performance *in vitro* use saline without adrenaline as the control situation. Although we have no direct evidence, it is possible that, as a result of the absence of tonic adrenaline levels in the skipjack tuna study, sarcolemma Ca^{2+} influx was understimulated relative to the routine *in vivo* condition, which may alter SR Ca^{2+} loading and affect the response to ryanodine. In addition, the skipjack tuna were held and tested at 25 °C, but contraction frequency was not increased above 2.5 Hz. On the basis of our results, if contraction frequency had been increased further, it might have resulted in greater SR involvement. However, the yellowfin tuna may simply have more SR Ca^{2+} or utilize more SR Ca^{2+} than the skipjack tuna. The yellowfin tuna (*Thunnus*) evolved more recently than the skipjack tuna (*Katsuwonus*) (Block et al., 1993), which suggests that SR utilization may be a phylogenetic correlate.

In most fish, SR Ca^{2+} utilization is low or absent (Driedzic and Gesser, 1988) and is only revealed under particular experimental conditions. For example, ryanodine abolishes post-rest potentiations in trout ventricle (El-Sayed and Gesser, 1989; Møller-Nielsen and Gesser, 1992; Hove-Madsen, 1992). Furthermore, peak force in trout can be reduced by ryanodine either at subphysiological frequencies (≤0.2 Hz) (Keen et al., 1994; Gesser, 1996) or at temperatures (>20 °C) that exceed the thermal preference of the fish (Hove-Madsen, 1992; Shiels and Farrell, 1997). However, under normal physiological conditions of temperature and heart rate, SR Ca^{2+} cycling in most fish is negligible. Thus, the large degree of SR Ca^{2+} utilization in the yellowfin tuna atrium (present study), the yellowfin tuna ventricle (Freund and Block, 1998), the skipjack tuna atrium (Keen et al., 1992) and the pacific mackerel ventricle (E. V. Freund and B. A. Block, in preparation) differs from other fish studied to date. Studies of SR utilization in other members of the Scombridae family will be necessary to establish whether this trait is conserved throughout the scombrid phylogeny.

At low pacing frequencies, the temperature-dependency of SR Ca^{2+} utilization in yellowfin tuna atrium is similar to that observed for trout ventricle, with a greater ryanodine response at warmer temperatures than at cooler temperatures (Figs 1, 2). At low frequencies, muscles tested at 18 °C and 25 °C utilized more SR Ca^{2+} than those tested at 15 °C. However, within the physiologically relevant range of pacing frequencies (indicated by the vertical lines on Fig. 2), percentage SR Ca^{2+} utilization was greater at colder temperatures. This observation may have important ecophysiological implications. Tracking records of yellowfin tuna show that the fish spend the majority of their time within 8 °C of the temperature of the surface water (R. W. Brill, B. A. Block, C. H. Boggs, K. A. Bigelow, E. V. Freund and D. J. Marcinek, in preparation; Holland et al., 1990; Block et al., 1997) (which is approximately 20 °C off the coast of California). Thus, our acute temperature changes from the acclimation temperature of 20 °C to either 15 ° or 18 °C

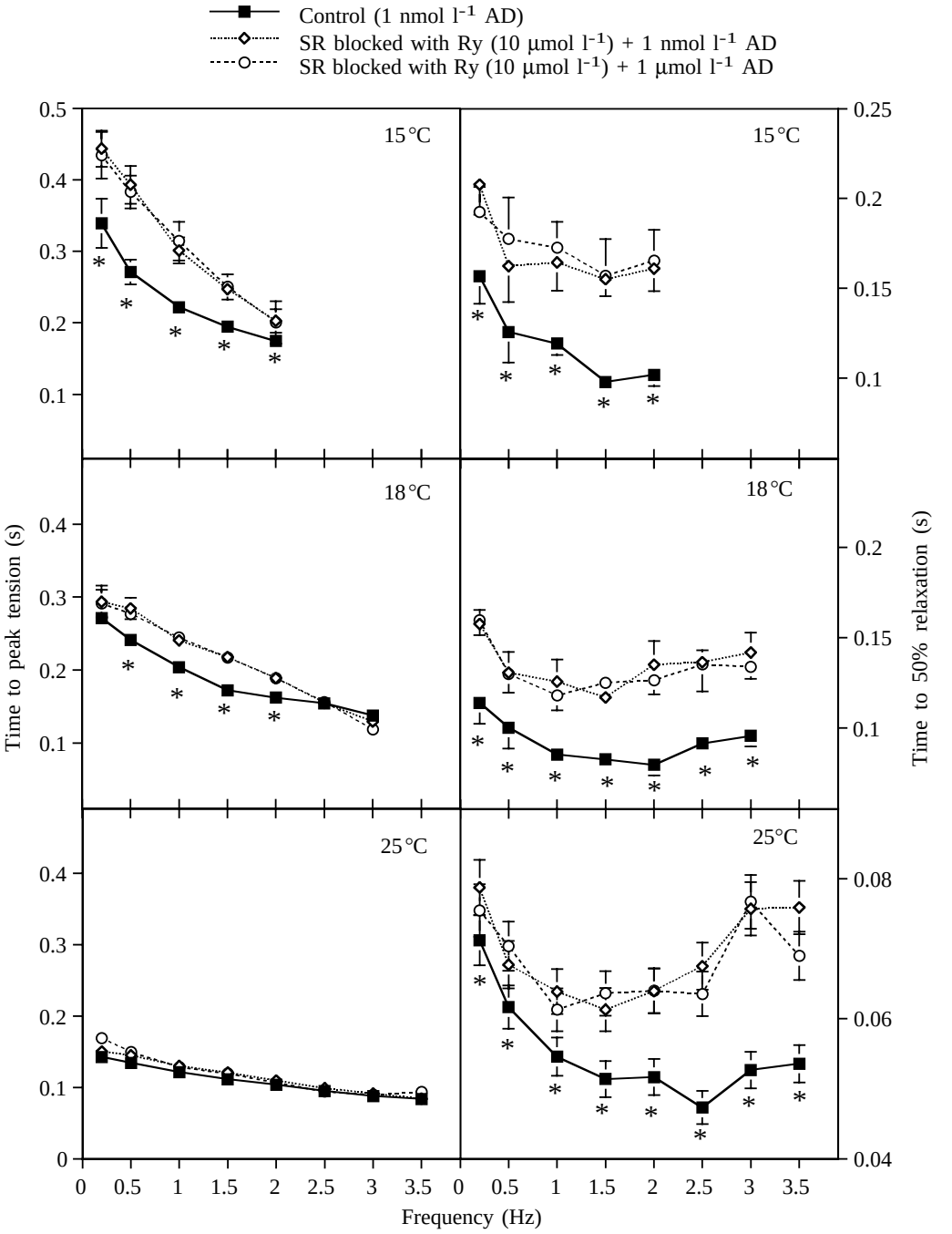

Fig. 3. Time (s) required to reach peak tension (left-hand panel) and time (s) required to reach 50 % relaxation (right-hand panel) in paced atrial trabeculae from yellowfin tuna at three different temperatures. Note the change of scale in the bottom right-hand panel. Values are means ± S.E.M. (values of *N* are as in Fig. 1). All other details are as in Fig. 1.

represent physiologically realistic temperature fluctuations that may be experienced when tuna dive in nature. The results of the present study show that SR Ca^{2+} utilization for a given frequency within the physiological range is greater at 15 or 18 °C than at 25 °C. This suggests that when yellowfin dive from 20 °C into water at 18 or 15 °C to forage (Holland et al., 1990; Block et al., 1997) they must increase their dependence on SR Ca^{2+} cycling to maintain a level of cardiac output similar to that at 25 °C. This may help to explain why the dive duration to cooler waters is usually short (<30 min) (R. W. Brill, personal communication). It is important to note that ryanodine binding is temperature-dependent in skeletal muscle, with lower binding affinity at colder temperatures (Ogawa and Harafuji, 1990). The temperature-dependence of ryanodine binding has not been examined in fish cardiac muscle; however, if the binding characteristics are similar to those of skeletal muscle, our results could underestimate SR Ca^{2+} utilization at the colder temperatures.

As pacing frequency was increased to a maximum level, the temperature-dependency of the ryanodine response was reduced, and SR Ca^{2+} utilization reached approximately 60 % at all temperatures. Further, no clear temperature threshold for SR Ca^{2+}-release channel function was observed over the range of temperatures used (15, 18 and 25 °C). Over a similar

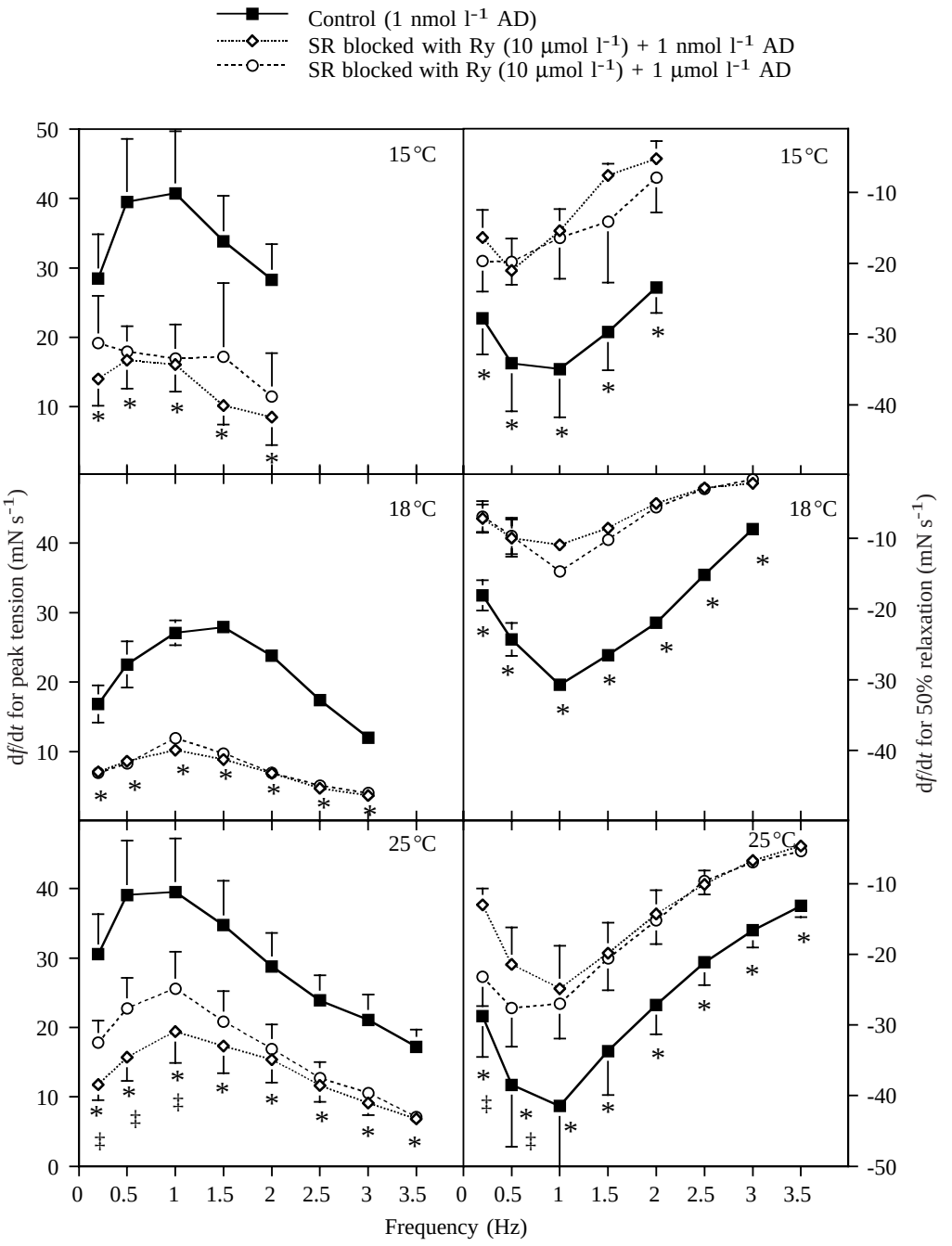

Fig. 4. Rates (d*f*/d*t*) of contraction (left-hand panel) and of 50 % relaxation (right-hand panel) in paced atrial trabeculae from yellowfin tuna at three different temperatures. Values are means ± S.E.M. (values of *N* are as in Fig. 1). All other details are as in Fig. 1.

range of temperatures in rainbow trout ventricle, SR utilization varied greatly. Significant SR Ca^{2+} utilization occurred at 22 °C at physiologically relevant contraction frequencies (Shiels and Farrell, 1997), but at 18 °C, SR Ca^{2+} utilization was limited to low-frequency contractions (Keen et al., 1994); below 15 °C, SR Ca^{2+} utilization was non-existent (El-Sayed and Gesser, 1998; Møller Nielsen and Gesser, 1992; Keen et al., 1994). Despite the possible differences in the degree of SR development in fish atrium *versus* fish ventricle, the degree of SR Ca^{2+} utilization reported here for tuna atrial muscle is the highest ever reported for a fish. Thus, yellowfin tuna differ from most fish studied to date in both the temperature- and frequency-dependent responses to ryanodine.

To obtain a more integrative perspective on the effects of ryanodine on heart function in yellowfin tuna, we calculated the pumping capacity of the muscle at each temperature (Fig. 5). Cardiac pumping capacity, the product of pacing rate and peak force (Matikainen and Vornanen, 1992; Shiels and Farrell, 1997), can be used as an index of power output for isolated muscle preparations since it integrates the effects of changes in force and changes in stimulation frequency. Moreover, the peak of the pumping capacity *versus* frequency curve suggests an optimum frequency for pumping capacity or

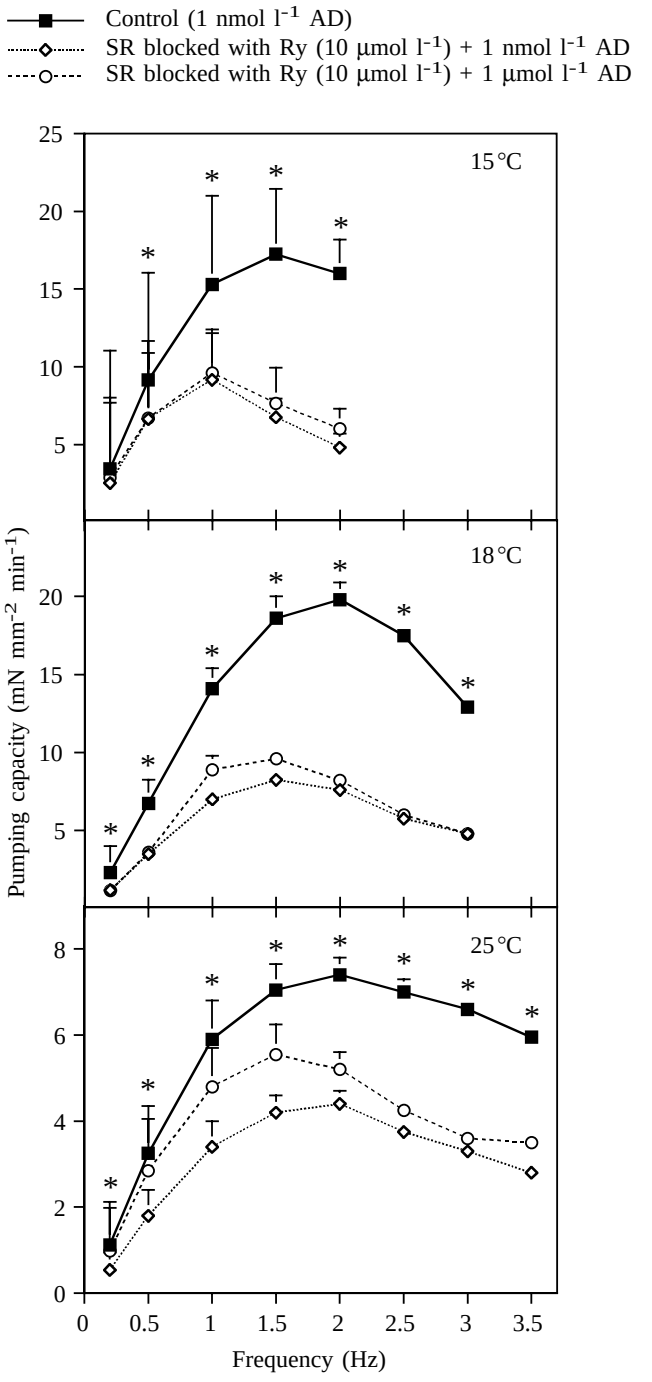

Fig. 5. Pumping capacity (the product of frequency and peak force) calculated for isolated atrial trabeculae from yellowfin tuna at three different temperatures. Note that the scale at 25 °C is different from than that at 15 and 18 °C. Values are means ± S.E.M. (values of *N* are as in Fig. 1). *Significant decrease with ryanodine application, using one-tail Student's *t*-test. All other details are as in Fig. 1.

'power output'. The optimum frequency for pumping capacity in yellowfin tuna atrium was approximately 2 Hz at 18 and 25 °C, and approximately 1.5 Hz at 15 °C (Fig. 5). *In vivo* heart rate measurements from yellowfin tuna range from approximately 0.6 to 1.3 Hz at 18 °C and approximately 1.3 to 2.0 Hz at 24 °C (measured while the fish swam in a swim tunnel; Korsmeyer et al., 1997) to approximately 1.8 Hz at 25 °C (measured in free-swimming fish; Jones et al., 1993) (no data are available for 15 °C). Thus, the optimum frequency for pumping capacity calculated from our study falls within the upper range of actual *in vivo* contraction frequencies.

Ryanodine decreases the pumping ability of the yellowfin tuna atrium at all temperatures and pacing frequencies, as anticipated from the peak force data. Clearly, this is related to the slower rates of tension development and relaxation in the presence of ryanodine. Moreover, ryanodine shifts the optimum frequency for 'power output' downwards and to the left. This is especially evident at 15 and 18 °C, where the frequency-dependence of the ryanodine response is accentuated. The reduction in optimal stimulation frequency after ryanodine treatment in yellowfin tuna atrium is similar, but more pronounced, than that observed in isolated rainbow trout ventricular trabeculae at 22 °C both contracting isometrically (Shiels and Farrell, 1997) and performing work-loops (Shiels et al., 1998). Thus, the inotropic and chronotropic pumping abilities of the yellowfin atrium are impaired when SR Ca^{2+} release is inhibited with ryanodine, providing further evidence that SR Ca^{2+} delivery is necessary during high-frequency contractions in yellowfin tuna. This will have considerable physiological importance since tuna modulate their cardiac output (the volume of blood pumped per unit time) primarily through changes in heart rate (Farrell, 1996; Korsmeyer et al., 1997), whereas most teleosts regulate their cardiac output *via* changes in stroke volume (the volume of blood pumped per beat).

In fish, activator Ca^{2+} can arrive at the myofilaments *via* both the SR and the sarcolemma, with the proportion of Ca^{2+} from each source varying with species, temperature and cardiac frequency. Sarcolemma Ca^{2+} flux is normally modulated to a significant extent by adrenergic stimulation. In fact, studies with rainbow trout show that, even under conditions where the SR is significantly involved in excitation–contraction coupling, increasing sarcolemma Ca^{2+} influx in response to adrenergic stimulation can ameliorate any negative effect of ryanodine on force production (Shiels and Farrell, 1997). This suggests that the relative importance of the contribution of the SR is easily overshadowed by increased sarcolemma Ca^{2+} flux in situations where cardiac tissue is stimulated by adrenaline. This was not the case in yellowfin tuna. High doses of adrenaline did not ameliorate the negative inotropic effects of ryanodine. This was surprising because preliminary studies had suggested that yellowfin tuna atria are responsive to adrenergic stimulation and because isolated trabeculae from skipjack tuna show dose-dependent inotropic responses to adrenaline in the absence of ryanodine (Keen et al., 1992). However, as for yellowfin tuna, ryanodine-treated atrial trabeculae from skipjack tuna were also unresponsive to adrenergic stimulation (H. A. Shiels, unpublished observations). The lack of a positive inotropic response to adrenaline after ryanodine treatment in the yellowfin and skipjack tuna may be related to the mechanism of action of adrenaline. In general,

β-adrenoceptor agonists increase sarcolemma Ca^{2+} flux by initiating the cAMP second messenger cascade (Lefkowitz et al., 1983), which culminates in phosphorylation of the L-type Ca^{2+} channel, increasing its open probability. Recently, increased Ca^{2+} influx through L-type Ca^{2+} channels in response to adrenergic stimulation has been demonstrated in patch-clamped trout and carp *Cyprinus carpio* cardiac myocytes (Vornanen, 1997, 1998). In mammals, however, an additional positive inotropic (and chronotropic) adrenergic mechanism involves the cAMP-dependent and Ca^{2+}-calmodulin-dependent protein-kinase-mediated phosphorylation of phospholamban, an integral membrane protein of cardiac SR (Koss and Kranias, 1996). It has been suggested that phospholamban is involved in mediating the relaxant and inotropic effects of β-agonists in the heart by increasing the efficacy of the SR Ca^{2+}-ATPase (Talosi et al., 1993). An increase in the activity of the SR Ca^{2+}-ATPase would lead to increased SR Ca^{2+} loading during the diastolic interval, which makes more Ca^{2+} available for subsequent contractions and leads to increased contractile force (i.e. positive inotropy) (Koss and Kranias, 1996). To our knowledge, the presence or absence of phospholamban has yet to be established for fish. However, if phospholamban is present in the tuna heart, the possibility exists that the positive inotropic response to adrenergic stimulation in tuna is mediated, at least in part, *via* phospholamban at the level of the SR. This mechanism would then explain the lack of an adrenergic effect after ryanodine incubation in yellowfin atrium.

Adrenergic desensitization could also account for the inability of adrenaline to offset the effects ryanodine. To test this possibility, we tested low frequencies (0.2 and 0.5 Hz) at the end of the force–frequency trial in the 18 °C group and in half the 25 °C group. There was no significant increase in tension at 1.0 Hz at 18 and 25 °C, suggesting that adrenergic desensitization over the course of the experiment is minor. Rather, it appears that, in the yellowfin atrial muscle, adrenaline is less able to overcome the effects of ryanodine, especially when compared with trout. Indeed, Keen et al. (1995) suggest that the yellowfin tuna myocardium is less responsive to adrenergic antagonists than that of most other fish studied to date. In agreement with this suggestion, high levels of extracellular Ca^{2+} (which could mimic the effects of adrenergic stimulation on the sarcolemma L-type Ca^{2+} channel) do not increase force in the ventricular trabeculae of yellowfin tuna at high frequencies (Freund et al., 1996). In contrast, Driedzic and Gesser (1985) have demonstrated that high extracellular [Ca^{2+}] ameliorated the negative effects of high pacing frequency in isolated cod *Gadus morhua* hearts. Again, this suggests a fundamental difference in Ca^{2+} cycling between tuna and other teleosts.

Studies of tuna physiology have revealed differences between tuna and other teleosts in such characteristics as standard metabolic rate, gill morphology and cardiac performance (Brill, 1996; Farrell, 1996). We have shown that the yellowfin tuna atrium routinely utilizes SR Ca^{2+} during force development, with the degree of SR involvement being both temperature- and frequency-dependent. Further, we find that the percentage of SR Ca^{2+} utilization increases with increased cardiac frequency, and suggest that this ability to utilize SR Ca^{2+} cycling to deliver and remove Ca^{2+} from the myofilaments allows yellowfin tuna to increase the maximum contraction frequency of their cardiac muscle beyond those of most other fish. These results, in conjunction with those of Keen et al. (1992) and Freund and Block (1998), again suggest that tuna differ from other fishes in their ability routinely to utilize SR Ca^{2+}. Indeed, that Pacific mackerel also demonstrate routine SR Ca^{2+} utilization (Freund and Block, 1998) may indicate that it is not tuna *per se*, but rather the family Scombridae, that are unique in their ability to utilize SR Ca^{2+}. Further studies with other scombrid fishes would be necessary to test this hypothesis.

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