Increased ventricular stiffness and decreased cardiac function in Atlantic cod (Gadus morhua) at high temperatures

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Syme DA, Gamperl AK, Nash GW, Rodnick KJ. Increased ventricular stiffness and decreased cardiac function in Atlantic cod (Gadus morhua) at high temperatures. Am J Physiol Regul Integr Comp Physiol 305: R864–R876, 2013. First published July 24, 2013; doi:10.1152/ajpregu.00055.2013.—We employed the work loop method to study the ability of ventricular and atrial trabeculae from Atlantic cod to sustain power production during repeated contractions at acclimation temperatures (10°C) and when acutely warmed (20°C). Oxygen tension (PO2) was lowered from 450 to 34% air saturation to augment the thermal stress. Preparations worked under conditions simulating either a large stroke volume (35 contractions/min rate, 8–12% muscle strain) or a high heart rate (70 contractions/min, 2–4% strain), with power initially equal under both conditions. The effect of declining PO2 on power was similar under both conditions but was temperature and tissue dependent. In ventricular trabeculae at 10°C (and atria at 20°C), shortening power declined across the full range of PO2 studied, whereas the power required to lengthen the muscle was unaffected. Conversely, in ventricular trabeculae at 20°C, there was no decline in shortening power but an increase in lengthening power when PO2 fell below 100% air saturation. Finally, when ventricular trabeculae were paced at rates of up to 115 contractions/min at 20°C (vs. the maximum of 70 contractions/min in vivo), they showed marked increases in both shortening and lengthening power. Our results suggest that although elevated heart rates may not impair ventricular power as they commonly do isometric force, limited atrial power and the increased work required to expand the ventricle during diastole may compromise ventricular filling and hence, stroke volume in Atlantic cod at warm temperatures. Neither large strains nor high contraction rates convey an apparent advantage in circumventing this.

Current evidence suggests that the failure of the heart to maintain tissue perfusion and adequate oxygenation primarily determines the upper limit of temperature tolerance of fishes (11, 18) and ultimately their distribution and adaptability to changing thermal conditions (5, 8, 56). Thus, it is critical that we understand what limits cardiac function and power output at elevated temperatures. Cardiac output is the product of heart rate and stroke volume and can be enhanced by increasing either or both of these parameters. For example, cardiac output increases when fish are exercised (swum) due to increases in both heart rate and stroke volume (28, 39, 50). Whereas with increased water temperature there is a corresponding increase in heart rate, while stroke volume remains relatively constant or increases only slightly just prior to the fish’s upper temperature limit (6, 11, 18, 19, 22, 50). However, it is still not clear why fish increase cardiac output solely through increases in heart rate when exposed to elevated temperatures, particularly in light of an apparent ability to increase stroke volume. Adult sockeye salmon (Oncorhynchus nerka) acclimated to 13°C can maintain stroke volume at twice resting levels when acutely exposed to 24°C and simultaneously swum at 75% of their critical swimming speed (50). Gamperl et al. (19) and Keen and Gamperl (30) have shown that rainbow trout (O. mykiss) are able to achieve equivalent maximum values of cardiac output by elevating stroke volume exclusively when temperature is raised to the fish’s critical thermal maximum following pretreatment with the bradycardic agent zatebradine to limit increases in heart rate. Finally, increasing stroke volume, as opposed to heart rate, when exposed to elevated temperatures could have several benefits for the heart. These include improved oxygen delivery to the myocardium, reduced cardiac oxygen consumption, and enhanced myocardial function via the Frank-Starling mechanism at a time when oxygen levels in the venous blood returning to the heart may be limiting (12).

Why only heart rate increases with temperature may be due in part to the interactive effects of temperature, venous oxygen tension, heart rate, and muscle strain on the ability of the heart to produce mechanical power while shortening (i.e., stroke work) and the capacity of the myocardium to extend with little resistance (i.e., accommodate filling), both of which are factors that will significantly influence cardiac output and performance. Thus, this research had three main objectives. The first was to examine whether increases in heart rate (contraction frequency) at warm temperatures, as seen in vivo in Atlantic cod (Gadus morhua), can sustain cardiac power output better than if stroke volume (muscle strain) was increased. We hypothesized that if elevating cardiac output (i.e., muscle power) through increased heart rate is energetically favorable to increasing stroke volume, myocardial power would be better sustained by increasing heart rate. The second objective was to understand how the ability to sustain power output at warm temperatures is impacted when concomitantly faced with falling oxygen levels, as is often observed in vivo (6, 26). Recent studies show that acute environmental hypoxia (PO2 8–9 kPa) impairs maximum exercise-induced heart rate and cardiac output in Atlantic cod (40). Thus, we hypothesized that reducing oxygen levels while the heart was exposed to elevated temperature would further impair cardiac power output primarily through a failure to produce force but also possibly by increasing the work required to lengthen the myocardium. The third objective was to test the hypothesis that heart rates higher than observed in vivo, while having the potential to increase...
cardiac power during muscle shortening, may not enhance cardiac function due to a failure of ventricular muscle to relax during diastole (i.e., increased lengthening power). An inability to fill the ventricle at the high heart rates concomitant with elevated temperatures may ultimately limit stroke volume and thus cardiac output and power in fishes (10).

These hypotheses were tested using isolated segments of atrial and ventricular muscle, where the rate of contraction, muscle strain amplitude, PO2, and temperature are controlled precisely, and their impact on mechanical work and power output are measured directly (25, 33, 48, 52, 54). The approach, in brief, was to measure the work and power of isolated trabeculae at the acclimation temperature (10°C) of the cod and after acute warming (20°C). The latter temperature is ~2°C below the thermal maximum of this species and slightly above the temperature at which cardiac function begins to become compromised (22). Muscle preparations were made to perform repeated contractions under conditions of either a high rate and small muscle strain (simulating fast heart rates and small stroke volumes) or low rate and large muscle strain (simulating slow heart rates and large stroke volumes). Preparations were also tested across a range of oxygen levels to allow for comparison of results with studies using high PO2 and to assess cardiac function at values of PO2 that approach those in vivo.

METHODS

All procedures were approved by the Memorial University of Newfoundland’s and University of Calgary’s animal care committees. Atlantic cod (n = 15, 0.32 ± 0.02 kg mass, means ± SE, 30.5 ± 0.41 cm fork length, ~20 mo of age) of mixed sex were spawned from wild parents and reared at the Dr. Joe Brown Aquatic Research Building at the Ocean Sciences Centre, Memorial University of Newfoundland.

Fish were housed in 3,000-liter tanks supplied with aerated/oxygenated seawater at 10°C and a 12:12-h light-dark photoperiod and fed commercial pelleted cod feed (Ewos Canada, Surrey, BC, Canada) at 1.5% body mass/day.

Estimation of Muscle Strain

To obtain an estimate of muscle strain in ventricular trabeculae as would occur in vivo for use during measurements of work from the isolated muscle preparations, the movement of discrete markers on the surface of a beating heart were measured in one fish, similar to Harwood et al. (25). The fish was anesthetized in 0.15 g/l tricaine methanesulfonate, with the gills continuously irrigated with chilled seawater containing anesthetic so that the heart remained at ~9°C. A ventral incision was made to expose the heart, and four markers (small, ~0.5-mm2 pieces of filter paper) were placed on the lateral surface of the ventricle, one centrally, one near the apex, one near the ventricular outflow, and one at the opposite edge from the outflow. A digital video camera (VDC-297; Sanyo) mounted onto a dissection microscope (MZ 9.5; Leica Microsystems, Concord, ON, Canada) captured images of the beating heart at 60 Hz. Seven cardiac cycles were then analyzed (Infinity Capture V 6.1; Luminaer, Ottawa, ON, Canada) to measure the minimum (end-systole) and maximum (end-diastole) distance between the central marker and each of the peripheral markers. Muscle strain for each pair of markers was calculated as the maximum distance minus the minimum distance divided by the average distance.

Preparation of Isolated Muscle Bundles From Atria and Ventricles

Individual fish were captured by dip net, were euthanized by a blow to the head, and had their hearts removed, cut in half lengthwise, and rinsed in ice-cold physiological saline for marine teleosts (40), pH 7.6, at 20°C. Pyruvate (5 mM) was added to the saline as an additional aerobic energy substrate, and the calcium concentration used (2.3 mM) was similar to that reported in plasma of Atlantic cod (1.8–2.5 mM) (2, 51) but greater than that used (1.25 mM) in a previous study on cardiac muscle function in Atlantic cod (7). A small segment of spongy trabecular muscle [Atlantic cod do not possess compact myocardium (14)] was isolated from the inner surface of the atrium or atria on the chilled (5°C) stage of the dissecting microscope. Muscle segments for both atrial and ventricular preparations were selected so that the majority of fibers ran parallel to the long axis of the preparation with minimal branching along their length. Ventricular preparations averaged 5.48 ± 0.77 mg wet mass and 5.83 ± 0.04 mm resting length. Atrial preparations averaged 3.72 ± 0.69 mg wet mass and 6.23 ± 0.82 mm resting length. The tips of each preparation were inserted into short segments of silicone tubing (1.6 mm outer diameter), and a ~10-cm silture was tied around the tube so that the suture did not contact the muscle directly. A short tab on the end of each piece of tubing was used to attach the tubing and muscle to stainless-steel pins on the arm of a servomotor (300C-LR; Aurora Scientific, Aurora, ON, Canada) and a force transducer (404A; Aurora Scientific). There was no detectable stretch of the tubing even during the most forceful isometric contractions.

The muscle segment was bathed in physiological saline maintained at 10 ± 0.2°C with Peltrier thermoelectric modules and a temperature controller (TC-24-12; TE Technology, Traverse City, MI). For those experiments conducted at 20°C, the temperature of the saline was increased over ~5 min, and preparations were maintained at 20°C for ~15 min before measurements commenced. Platinum-plate stimulating electrodes, used to activate the muscle, were positioned on both sides of the preparation and connected to a Darlington transistor follower circuit powered by 36-volt wet cells gated by a stimulator (Isostim A320; World Precision Instruments, Sarasota, FL) controlled by a computer. Custom software written using LabView software (National Instruments, Austin, TX) controlled a 12-bit analog/digital converter card (PCI MIO 16E-4; National Instruments) that controlled the stimulator and servomotor (5 kHz D/A output) and collected force, position, and stimulus signals (500 Hz A/D input).

The length of each preparation was increased systematically until isometric twitch force, elicited by a 1-ms supra-maximal shock, was maximal. Muscle length was then reduced to 95% of this value, resulting in the muscle operating on the ascending limb of its length tension relationship and producing ~90% of maximal isometric twitch force. In cases where passive force rose steeply when strain was applied to the muscle during work loop experiments, muscle length was decreased slightly to maximize net work output. The preparation was then allowed to rest for ~15 min.

PO2 was maintained by bubbling a mixture of O2 and N2 gas controlled with flow meters and monitored using a calibrated, fiber-optic dipping oxygen probe (PS13; PreSens, Regensburg, Germany) and oxygen meter ( Fibox 3; PreSens) into a reservoir of saline that was then drained via stainless-steel tubing into the muscle chamber. Complete turnover of the saline in the chamber occurred approximately once/min. The PO2 of the chamber was set at 453 ± 0.47, 306 ± 1.3, 204 ± 0.51, 106 ± 0.23, 76 ± 0.55, 52 ± 0.17, and 34 ± 0.94% of air saturation for ventricular trabeculae and 441 ± 2.7, 107 ± 0.49, and 53 ± 0.37% of air saturation for atrial trabeculae. The lowest PO2 values tested were selected on the basis of preliminary trials to cause a substantial depression of performance relative to what occurred at high PO2 without resulting in irreversible damage when the muscles worked for long periods at each PO2 and approached what the heart would encounter in vivo (see DISCUSSION).

Measuring Muscle Work and Power

The work loop method (25, 27, 29, 48, 52, 53), whereby cyclic changes in length and activation simulate muscle movement and contractions in a beating heart, was used to assess the ability of
trabeculae to produce work and power. Sinusoidal strain was imposed on the muscle by the servomotor, with the amplitude of strain (analogous to stroke volume) and the rate of cycling and activation (analogous to heart rate) selected so that the preparations worked under conditions of either a high rate and small strain (hereafter referred to as Rs) or a low rate and large strain (hereafter referred to as rS). The strain amplitude measured in the intact beating ventricle (described above) averaged 9.2 $\pm$ 0.52%, and pilot trials on two isolated ventricular preparations revealed that the muscle produced $\sim$95% of its maximal work output at strains of 8–9% (work was maximal at 10% strain). Hence, 8% strain was selected as the value of large strain (S) for ventricular preparations, close to that measured in the ventricle and which produced near-maximal work. Measures of atrial strain in the working heart were not attempted; however, preliminary trials on two atrial trabecular preparations revealed that a strain of 12% resulted in $\sim$90% of maximal work output, and so 12% strain was used as the value of large strain (S) for atrial preparations. The values of small strain ($s$) for atrial (4.24 $\pm$ 0.14%) and ventricular trabeculae (2.17 $\pm$ 0.06%) were selected independently for each preparation so that power under the Rs condition matched power under the rS condition. This allowed for direct comparisons of the ability of the muscles to sustain power output under the two conditions. The contraction rates used were selected on the basis of values measured in vivo. The slow rate ($r$) was 35 contractions/min, the resting heart rate of Atlantic cod at 10°C. The high rate ($R$) was 70 contractions/min, the maximum resting heart rate for this species at 20°C (22, 39).

The strain trajectory imposed on ventricular preparations was symmetrical in the time base (i.e., muscle shortening and muscle lengthening each comprised 50% of the cycle period), as has been used in other studies on fish ventricular myocardium (25, 48). Under these conditions and at the contraction rates employed, the muscle generated force over the majority of the shortening portion of the strain cycle, as would occur during a typical heart beat (see RESULTS). The twitch duration of atrial preparations was shorter than for ventricular preparations (see RESULTS). Thus, a length trajectory where muscle shortening comprised 20% of the cycle period and muscle lengthening 80% was used. This matched the duration of the twitch to the period of atrial muscle shortening and enabled the trabeculae to achieve maximal work output.

A single, supramaximal (typically 5–10 V), 1-ms shock was applied to the muscle during each cycle at the point during the strain cycle that resulted in maximal net work output. Work ($W$) provides a measure of the mechanical energy produced during each beat of the heart and was calculated as the integral of muscle force with respect to length change. The work required to lengthen the muscle in each cycle (analogous to filling work in an intact heart), the work done by the muscle when it shortened in each cycle (analogous to stroke work), and net work (the difference between shortening work and lengthening work) were measured. Power ($P$) provides a measure of the sustained rate of mechanical energy output and was calculated as the product of the work done per cycle (shortening, lengthening, or net) and contraction frequency (Hz).

**Effects of Temperature, Strain Versus Contraction Rate, and PO$_2$ on Muscle Work**

To test the effects of elevated heart rate versus increased stroke volume on sustained performance at warm temperatures (20°C), ventricular and atrial preparations were made to perform repeated contractions under either the Rs or rS conditions (ventricular: $n = 7$ Rs and 9 rS; atrial: $n = 4$ Rs and 5 rS). Preparations performed contractions for 5 min at each of a series of successively lower values of PO$_2$, as noted above. Muscle preparations were allowed to rest (not stimulated or contracting spontaneously) for $\sim$15 min between each series of induced contractions while the PO$_2$ was adjusted to a new level. PO$_2$ was then returned to the highest level at the end of the experiment to assess recovery. Because of the large number of contractions (350 for Rs, 175 for rS), it was required to complete a series of measurements at each of the PO$_2$s tested, each preparation was tested under only the Rs or rS conditions to avoid fatigue.

To assess the effects of temperature on work output, measurements were also made at 10°C under the rS condition and the highest PO$_2$ in ventricular ($n = 16$) and atrial ($n = 9$) preparations. Furthermore, preliminary data from two additional ventricular preparations tested at 10°C under the rS condition, but across the full range of PO$_2$s, are shown to compare with effects at 20°C. Because of the low number of observations, statistical comparisons between these results and those at 20°C were not performed. Finally, isometric twitch force and kinetics were measured from ventricular ($n = 16$) and atrial ($n = 9$) preparations during single twitches at both 10 and 20°C to assess their potential impact on work and power.

**Effects of High Contraction Rates on Ventricular Muscle Force and Power When Warm**

Ventricular preparations ($n = 5$) at 20°C were subjected to a series of 20 isometric twitches and then 20 cyclic contractions at rates ranging from 55 to 115 contractions/min, with strain fixed at 8% and PO$_2$ at 453% air saturation. Power output (shortening, lengthening, and net) and twitch force (peak, minimal, and developed) were assessed from a contraction near the end of each series, where performance had stabilized.

**Analysis**

At the conclusion of each experiment, the muscle preparations were removed from the apparatus, trimmed of any obviously nonviable tissue, blotted on filter paper, and weighed on a microbalance (Mettler UMT2; Mettler-Toledo, Columbus, OH). Vital staining to more accurately assess the viable tissue mass was not performed. Thus, muscle mass likely overestimates viable tissue mass and measurements of area-specific force and mass-specific work are underestimates of viable tissue performance. To avoid variability associated with imprecise measurements of viable tissue mass, measurements of force and work within each preparation were expressed relative to values obtained under a given set of conditions (see figure legends for details). This does not constrain the data for purposes of statistical analysis, and absolute measurements of power (W/kg) and force (kN/m$^2$) are also provided for comparison with other studies where relevant. Cross-sectional area of preparations was calculated, assuming a muscle density of 1.05 g/cm$^3$.

All records of muscle force and length were subjected to a 20-point median filter and finite impulse response filter (20 Hz high cutoff, 0.01 Hz low cutoff, 100 pole) before analyses was performed to remove any noise artefacts and checked to ensure they were not inappropriately distorted by the filtering process. Performance of the working preparations was assessed in two ways, as the average work done per cycle during each 5-min series of contractions and as the maximal work recorded in any cycle during the series (see RESULTS). However, there were either no or only very small differences in the pattern or absolute level of performance between the two methods of assessment. Hence, although both sets of data are presented, we do not distinguish between them, and statistical analysis was performed only on the average data.

The replicates ($n$) indicated for each experiment refer to the number of trabeculae tested, with each trabeculae coming from a different fish. The numbers of males and females in each experiment were not controlled, and this generally resulted in there being relatively few ($n = 1$–3) of one sex or the other in each experiment. This precluded statistical analysis that took sex into account. Nonetheless, preliminary analysis of the effects of sex on ventricular power under both Rs and rS conditions, where the numbers of replicates were highest, revealed no significant effects ($P$ value ranged from 0.09 to 0.99). Thus, results from males and females were pooled for all analyses.
Comparisons of work output across PO2 levels were made using one-way repeated-measures ANOVA, followed by Holm-Sidak pairwise comparisons where significance (P < 0.05) was found. Comparisons between initial and recovery work and between twitch force and kinetics at 10 and 20°C were made using paired t-tests. Comparisons between work under Rs and rS conditions were made using t-tests. Values of isometric force and power at 55 contractions/min were compared with values at higher frequencies using one-way repeated-measures ANOVAs, followed by Dunnett’s tests. All statistical analyses were performed using SigmaStat 3.1.1 statistical software (Systat Software, Chicago, IL). All data in the text and figures are expressed as means ± SE.

RESULTS

Effects of Temperature on Cardiac Muscle Force and Work

Isometric twitch force at 10°C averaged 6.23 ± 0.65 kN/m² in ventricular muscle (n = 16) and 2.48 ± 0.43 kN/m² in atrial muscle (n = 9) and at 20°C averaged 6.39 ± 0.59 kN/m² in ventricular muscle (n = 16) and 3.09 ± 0.52 kN/m² in atrial muscle (n = 9). There was no significant effect of the acute temperature change on twitch force in ventricular muscle (P = 0.81) and a marginally significant increase in twitch force with increasing temperature in atrial muscle (P = 0.047). The twitch duration of atrial muscle was approximately one-half that of ventricular muscle when assessed as either the period of twitch contraction (10–90% force) or the twitch duration at half-maximal amplitude (50–50% force) but was ~70% of that measured in ventricular muscle during the period of twitch relaxation (90–10% force) (Fig. 1). These measurements of isometric twitch duration approximately doubled when preparations were tested at 10 vs. 20°C (Fig. 1). The Q10 for the duration of twitch contraction was 2.28 ± 0.05 in ventricular muscle and 2.11 ± 0.12 in atrial muscle, the Q10 for twitch duration at half-maximal amplitude was 2.10 ± 0.03 in ventricular muscle and 2.02 ± 0.08 in atrial muscle, and the Q10 for twitch relaxation was 2.04 ± 0.09 in ventricular muscle and 1.95 ± 0.14 in atrial muscle.

Similar to twitch duration, but unlike isometric twitch force, ventricular work obtained using the rS protocol and high O2 approximately doubled at 10 vs. 20°C; values at 10°C averaged 1.72 ± 0.074 of those at 20°C for shortening work, 2.13 ± 0.026 for lengthening work, and 1.66 ± 0.100 for net work. Unlike for the ventricle, atrial work obtained using the rS protocol and high O2 at 10°C was not different from that at 20°C; values at 10°C averaged 1.02 ± 0.105 of those at 20°C for shortening work, 1.01 ± 0.077 for lengthening work, and 1.00 ± 0.127 for net work.

Effects of PO2 and Strain Versus Contraction Rate on Work

Ventricular muscle. At 20°C and 453% air saturation, net power at the start of the 5-min series of contractions averaged 83 ± 15 mW/kg under the Rs condition and 85 ± 16 mW/kg under the rS condition, confirming a close match in power output between the two conditions. The peak force and work produced by the muscles during each contraction tended to increase over the first 2–3 min of each series of contractions and reached or closely approached a plateau toward the end of the series, and none of the preparations showed a decline in force over any given series of contractions, even at 34% air saturation (Fig. 2). The extent of the increase in performance over the 5 min of contractions, its time course, and the pattern of change with PO2 appeared similar between preparations working at either Rs or rS (Fig. 2).

Both net and shortening work tended to decline with decreasing PO2 until ~100% air saturation, and muscles working under rS conditions maintained values of net and shortening work 20–30% higher compared with those working under Rs conditions over this range of PO2s (Fig. 3). Between 100 and 34% air saturation, shortening work remained at ~50% of its maximal value, net work continued to decline toward 20% of its maximum value, and lengthening work doubled (Fig. 3). These effects were similar for both rS and Rs conditions, although the increase in lengthening work was only statistically significant for the Rs preparations. After returning the trabeculae to fully oxygenated conditions (453% air saturation; Fig. 3, open symbols), shortening work recovered to values not significantly different from the initial values (P = 0.16 for rS, P = 0.17 for Rs), but lengthening work either did not recover fully (P = 0.013 for Rs) or recovered (P = 0.29 for rS) on average less than shortening work. This resulted in net work being reduced significantly after recovery (P = 0.004 for rS, P = 0.002 for Rs).

The two ventricular preparations tested at 10°C across the full range of PO2s showed several notable differences compared with the results obtained at 20°C (Fig. 4). Shortening and net work did not decline with reduced PO2 until ~100% air saturation, both shortening and net work continued to fall when...
PO2 was reduced below 100% air saturation, lengthening work did not increase with reduced PO2 but instead showed a nonsignificant decreasing trend, and all of the measurements of work upon recovery were comparable with initial values.

Atrial muscle. At 20°C and 441% air saturation, net power at the start of the 5-min series of contractions averaged 61 ± 15 mW/kg under the Rs condition and 62 ± 16 mW/kg under the rS condition, confirming a close match in power output between the two conditions. Both shortening and net work declined with reduced PO2, with the rate of decline appearing to be greater when PO2 was reduced below 100% air saturation (Fig. 5). In contrast, lengthening work was independent of PO2 across the full range of values tested (Fig. 5). After returning to the highest PO2 (Fig. 5, open symbols), shortening work recovered fully under the rS condition (P = 0.13) but was marginally reduced (P = 0.049) under the Rs condition, lengthening work recovered fully under both conditions (P = 0.97 for rS, P = 0.90 for Rs), and net work was reduced under the rS
condition (P = 0.02) but recovered fully under the Rs condition (P = 0.09).

Effects of High Contraction Rates on Force and Power at 20°C

At 20°C, peak isometric force rose by ~30% with increasing contraction frequency (55–115 contractions/min), whereas the resting (minimal) force rose by ~75% (Figs. 6 and 7). As a result, developed isometric twitch force was maximal at frequencies between 75 and 95 contractions/min but was not significantly different at high vs. low frequencies (Figs. 6 and 7). In contrast, when force was multiplied by contraction rate to allow for a conceptually equivalent comparison with power output, the peak, rest, and developed force all increased with contraction rate and were all markedly elevated at higher frequencies (Fig. 7). The patterns of change in power output were very similar to the patterns of change in the product of force and contraction rate, with shortening power tripling over the range of contraction rates studied, lengthening power increasing more than sixfold, and net power approximately doubling before reaching a plateau of nearly 100 contractions/min and subsequently decreasing (Figs. 6 and 7).

DISCUSSION

Myocardial Force and Power Output

The isometric force developed by Atlantic cod ventricular trabeculae (~6 kN/m² at both 10 and 20°C) is within the range of values reported for trabeculae from other fish species at comparable temperatures, including armored catfish [6 kN/m² at 25°C (47)] and rainbow trout [1.6 kN/m² at 12°C (44), 4 kN/m² at 10 and 22°C (21, 48), and 22 kN/m² for compact myocardium at 15°C (25)], although there is considerable variability in the rainbow trout data between studies and heart muscle type. Isometric force developed by Atlantic cod atrial trabeculae (~2.5 kN/m² at 10°C and 3 kN/m² at 20°C) is less than the values reported for yellowfin tuna (Thunnus albacares) at cooler temperatures (18 kN/m² at 15°C and 15 kN/m² at 18°C) but closer to values at
warm temperatures (7 kN/m² at 25°C) (46). Reports of atrial force from other fish species are not available.

Atrial contractions were approximately twice as fast as those of the ventricular myocardium (Fig. 1). This is consistent with twitch kinetics reported for rainbow trout myocardium (1) and is purported to allow for rapid and precisely timed atrial contractions to enhance ventricular function (1, 31) and to be associated with a greater contribution of the sarcoplasmic reticulum to cytosolic calcium cycling in atrial versus ventricular muscle (1). Because of the relatively brief atrial contraction, the shortening portion of the imposed strain cycle was set to comprise only 20% of the cycle period versus 50% in ventricular muscle to maximize work output. Under these conditions, net power at 20°C, averaged over the 5 min of contraction, was ~127 mW/kg for ventricular myocardium and 100 mW/kg for atrial muscle. The value for ventricular muscle is similar to other reports of power produced by isolated segments of myocardium, including rainbow trout myocardium from the lumen of the ventricle (100 mW/kg at 12°C and 20 mW/kg at 22°C) (48). However, it is considerably less than that measured for rainbow trout compact ventricular myocardium (1.3 W/kg at 15°C) (25). This discrepancy may be due to species and tissue layer differences in myocardial performance/function and because attempts were not made to maximize power in the present study.

Effects of Acute Warming on Cardiac Muscle Force Production

Cardiac inotropy in fish has generally been reported to decline with increasing temperature, especially at temperatures near the fish’s upper thermal limits (e.g., see Refs. 10, 34, 36, 48, and 49). However, it is unclear whether this temperature-dependent decrease in contractility is a direct effect of temperature on inotropy or the result of the negative staircase effect (reduced force) that occurs at the higher heart rates accompanying these temperatures. Few studies have controlled for the effects of heart rate when measuring the effects of temperature on myocardial performance. In the present study, the developed twitch force measured in Atlantic cod ventricular trabeculae was independent of temperature between 10 and 20°C, and in atrial muscle there was a small (~25%) increase in force with elevated temperature. These findings are similar to those reported for other teleosts. Shielis et al. (48) reported that the isometric force developed by rainbow trout ventricular trabeculae paced at either 24, 60, or 84 contractions/min was largely independent of temperature between 12 and 22°C. Matikainen and Vornanen (36) reported an ~50% drop in force between 5 and 20°C but an ~50% rise in force between 20 and 35°C in isolated Crucian carp (Carassius carassius) hearts paced at 12 contractions/min. Likewise, although hyperthermia during exercise in humans is associated with an increase in heart rate and decrease in stroke volume, there is an increase in stroke volume when the hyperthermic effect on heart rate is blocked (55). Furthermore, there is evidence for increased atrial and systolic contractile function with heat stress in human hearts (4). Collectively, these results suggest that inotropy in fish cardiac tissue or hearts is not necessarily impaired when acutely exposed to warm temperatures.

**Effects of Acute Warming on Cardiac Muscle Work**

The 10°C increase in temperature resulted in an ~50% reduction in net work per cycle for Atlantic cod ventricular tissue. However, this effect was likely not due to a decline in inotropy per se, as evidenced by the minimal effect of temperature on isometric force. Rather, it was likely due to an approximate halving of the twitch duration as temperature was increased acutely (Fig. 1), which resulted in force being maintained over a smaller portion of the imposed strain cycle (compare Fig. 2, E and F). A similar reduction in work at increased temperatures in ventricular trabeculae isolated from rainbow trout was noted by Shielis et al. (see the 4th figure in Ref. 48), and likely for the same reason; the strain trajectory was not adjusted to match the changing twitch kinetics with increased temperature. The work output of cod atrial muscle was independent of temperature despite a similar relative effect of temperature on twitch duration as for ventricular muscle (Fig. 1). Perhaps the briefer atrial twitch, being less affected by temperature in an absolute sense, resulted in less impact on work production. Clearly, more information about the effects of temperature on the time course of the strain trajectory of muscle in the beating heart is required before experiments on isolated trabeculae can accurately mimic conditions in vivo to fully understand temperature-dependent effects on cardiac function.

**Effects of Strain Versus Contraction Rate on Work**

Our data strongly suggest that there was no notable mechanical advantage to working with increased heart rate (Rs) vs. increased strain (rS) at high temperatures and physiological levels of PO2 (Figs. 2–5). Work was equally well maintained for the 5-min series of contractions and was equally impacted by PO2 under both conditions. Thus, it is still not clear why fish exclusively increase heart rate in vivo when faced with rising
temperatures. The only exception to the similarity in performance of muscle working under rS versus Rs conditions occurred at supraphysiological PO2s (i.e., between 100 and 300% air saturation), where ventricular muscle under the rS condition maintained shortening and net work at levels 20–30% higher than muscle working under the Rs condition (Fig. 3). The enhanced stretch sensitivity of fish cardiac myofilaments to calcium (38, 45), as discussed below, may contribute to the increased work produced by muscles under the rS condition, where the muscle would be subjected to greater stretch. Why this pattern did not persist below 100% air saturation, where work was not different between the Rs and rS conditions, is unclear.

Effects of High Contraction Rates on Force and Work When Warm

Isometric twitch force tends to decrease with increased heart rates in most teleost fishes, which is due in part to reduced transsarcolemmal calcium influx, action potential duration, and amplitude at higher heart rates (24, 49). However, our data suggest that the ventricular muscle of Atlantic cod does not follow this pattern, as has also been observed for some other species (47). Developed isometric twitch force at 20°C increased with contraction frequency up to about 90 contractions/min, and the product of force and frequency was maximal at more than 110 contractions/min, being about double that measured at slower heart rates (Figs. 6 and 7). Similarly, Driedzic and Gesser (7) showed that isometric force developed by Atlantic cod ventricular muscle incubated at 10°C with either 1.25 or 5 mM Ca²⁺ increased or was maintained, respectively, up to contraction frequencies of ~40 contractions/min before falling. Increased force with increased heart rate in active species such as scombrids, in cold-acclimated species like cod, and after acute warming (44, 49) may be due to an increased contribution of sarcoplasmic Ca²⁺ to activation and hence, the ability to sustain force at high heart rates. Indeed, force produced by ventricular strips from Atlantic cod is reduced when treated with ryanodine (7), and the contribution of

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Fig. 5. Shortening work (A and C), lengthening work (B and D), and net work (E and F) done by Atlantic cod atrial preparations at 20°C and different oxygen pressures during 5 min of repeated contractions. Lengthening work is shown as a negative value to reflect the mathematically negative length change. A and B: work done by muscle contracting under the Rs condition (70 contractions/min, average 4.2% strain). C and D: work done by muscle contracting under the rS condition (35 contractions/min, 12% strain). A and C: average work done per cycle during the 5-min series of contractions. B and D: maximum work done per cycle during the 5-min series of contractions. Data were normalized within each preparation to the net work done at the highest Po2 (inside square boxes). Open symbols show work when the muscle was returned to the highest Po2 at the end of the experiment to assess recovery. Horizontal bars span data points that were not significantly different (P > 0.05) from one another. #Significant difference (P < 0.05) in recovery work (open symbols) from initial values at high Po2. Values are means ± SE. n = 5 rS, n = 4 Rs.
sarcoplasmic calcium to force production appears most evident at contraction frequencies of 40 contractions/min and higher, although those authors contend the contribution of sarcoplasmic Ca\(^{2+}\) was minimal and remained insufficient to prevent a fall in force at very high heart rates. Further, Lurman et al. (35) recently reported that the response of the in situ Atlantic cod heart to an acute temperature increase from 4 to 10\(^{\circ}\)C was also atypical for fishes and suggested that a prolonged action potential, enhancement of sarcolemmal Na\(^{+}\)/H\(^{+}\) current (\(I_{Na}\)) that augments Ca\(^{2+}\) influx through Na\(^{+}\)/Ca\(^{2+}\) exchange, or unique aspects of SR Ca\(^{2+}\) cycling may be responsible.

A higher frequency for maximal force when warm and an increase in force with increased heart rate in the Atlantic cod...
and some other species suggest that increasing heart rate at elevated temperatures may be an effective strategy to increase cardiac output in these species. In support of this hypothesis, net power of cod ventricular myocardium approximately doubled between 55 and 105 contractions/min, whereas shortening power approximately tripled (Figs. 6 and 7), which is more than would be expected based on the doubling of heart rate alone. A rise in myocardial power output with increasing contractile frequency has also been reported in studies using isolated working rainbow trout ventricular preparations, where net power peaked at 60–70 contractions/min at 12–15°C, and interestingly, this occurred despite a steady decrease in isometric twitch force with increasing frequency (48). The increase in power with increased heart rate observed in Atlantic cod and rainbow trout may reflect an increase in the rate of contraction that is disproportionate to any changes in isometric force. This idea is supported by data showing that the product of force and frequency increased with the rate of contraction in trout (48) and cod myocardium (Fig. 7). It may also reflect an effect of stretch and shortening in working muscle that results in a different relationship with frequency from that seen during isometric contractions (25). Two mechanisms may account for this. First, the fish myocardium is capable of generating high levels of force over a wider range of sarcomere lengths than is mammalian myocardium (45), and recent evidence suggests that this results from greater length-dependent sensitivity to activator calcium and titin-based passive tension in fish vs. mammalian ventricular cardiomyocytes (38). This increase in calcium sensitivity associated with muscle stretch during cyclic contractions might fully or partially compensate for reduced transsarcolemmal calcium influx observed at increased heart rates (49), to the extent that work does not fall and may actually increase at higher heart rates. Second, both peak and resting force increased monotonically and by severalfold with increased heart rate when expressed as the product of force and frequency and showed no evidence of reaching a plateau (Fig. 7). This finding is consistent with that observed for the rainbow trout myocardium and isolated myocytes and likely reflects a failure of complete mechanical restitution (relaxation, lusitropy) between beats at high heart rates (24, 44, 49). Increased resting tension would lead to an increase in work required to lengthen the muscle and would contribute to an increase in the force generated and work done during subsequent shortening (Fig. 7), and the magnitude of the effect will likely depend on heart rate, even if strain is maintained constant. This is in agreement with observations for ventricular muscle of rainbow trout by Harwood et al. (25), who suggested that the eventual fall in net work output at high heart rates is due to failure to relax fully. Thus, although developed isometric force may predict increased, unchanging, or even reduced performance with increased heart rate, there is growing evidence suggesting that increased heart rates lead to increased performance in the working myocardium. Based on the above observations and the work of others (10, 48), it is evident that developed isometric force may by itself be a misleading index of cardiac performance, particularly at warmer temperatures and higher heart rates.

**Effects of Reduced P\textsubscript{O}2**

**Ventricular stiffness and atrial work.** Although ventricular shortening work in Atlantic cod remained relatively constant as oxygen pressure was reduced below 100% air saturation, the work required to lengthen the muscle increased at 20°C (Fig. 3), but not at 10°C (Fig. 4); in absolute terms, work required to lengthen ventricular trabeculae at 34% air saturation was 0.054 ± 0.008 J/kg at 10°C (n = 2) and 0.092 ± 0.023 J/kg at 20°C (n = 9). These data suggest, for the first time, that elevated temperatures increase the resting tension of ventricular muscle at physiological levels of P\textsubscript{O}2 and that this could impair cardiac filling (i.e., limit end-diastolic volume). Measurements of ventricular muscle resting force support this contention. At 10°C, resting force at 34% air saturation averaged 36% less than at 100% air saturation (n = 2), whereas at 20°C it averaged 84% greater (n = 10, P = 0.02, repeated-measures ANOVA).

The hypothesis that ventricular filling may be impaired in Atlantic cod at high temperatures and low P\textsubscript{O}2 is further supported by the corresponding data for atrial muscle. Atrial shortening work at 20°C decreased abruptly as P\textsubscript{O}2 fell below 100% air saturation, being ~40% of maximal at 34% P\textsubscript{O}2 saturation (Fig. 5). The majority of ventricular filling in fish hearts is attributed to atrial contraction (14), and only during the latter stages of ventricular filling in some fishes (31). Thus, the combination of decreased atrial shortening work and a stiffened resting ventricle that is more difficult to fill may contribute to the apparent inability of the fish heart to increase or maintain stroke volume as temperature and heart rates rise (10, 13, 19, 22). The apparent lack of an increase, and perhaps a decrease, in lengthening work and resting force of ventricular muscle of Atlantic cod at low P\textsubscript{O}2 at 10°C (Fig. 4) is noteworthy and suggests that the ventricles of these fish may not suffer from increased stiffness at lower oxygen levels when cool.

The lack of a rise in atrial lengthening work under the combined conditions of high temperature and reduced P\textsubscript{O}2 (Fig. 5) is in contrast to that observed in ventricular preparations and may be related to the relatively short twitch duration (systole) of atrial muscle (Fig. 1). A brief twitch would afford an extended period of atrial diastole and time for muscle relaxation, reducing the likelihood of increased lengthening work at high heart rates and temperatures. As a result, venous filling pressure may continue to be adequate to fill the atrium even at elevated temperatures and high heart rates, and there is evidence that a rise in venous filling pressure under these conditions may help sustain venous return in rainbow trout (41).

**Basis of cardiac sensitivity to reduced oxygen availability.** The lowest P\textsubscript{O}2 used in this study was approximately double that measured in fish in vivo at high temperatures; venous P\textsubscript{O}2, as the spongy myocardium would experience, is ~25–40 mmHg (16–25% air saturation) (6, 32, 50). Furthermore, there was no apparent plateau to the rise in ventricular lengthening work (Fig. 3) or the fall in atrial shortening work (Fig. 5) at the lowest P\textsubscript{O}2 tested. Thus, we expect that even greater increases in ventricular resting stiffness and losses in atrial contractility might have occurred at venous P\textsubscript{O}2s. This hypothesis is supported by data from in situ sea raven (*Hemitripterus americanus*) (16) and rainbow trout hearts (15).

However, it is far from clear what the physiological basis was for these decrements in teleost myocardial function at
Perspectives and Significance

These studies examined the mechanical performance of cod atrial and ventricular cardiac muscle in vitro and provide several novel findings with respect to fish heart function under stressful environmental conditions. First, there was an increase in both isometric twitch force and shortening power as contraction frequency was increased at high temperatures, even at rates 50% greater than the cod heart experiences in vivo. This would occur especially toward the later stages of the experiments at 34% air saturation, where the preparations had been working in reduced PO2 for more than 1 h. However, none of these effects were observed in the present study. Force tended to rise and establish a plateau within each of the 5-min series of contractions, the level of which was dependent on the PO2 tested (Fig. 2), and although not quantified, twitch kinetics did not appear to be notably impacted by reduced PO2. Furthermore, recovery in high PO2 at the conclusion of the experiment was normally complete within minutes, which would not be expected from a muscle that was oxygen deprived and working in an oxygen-limited state for almost 2 h. Of note, we also observed PO2-related decreases in myocardial work and power even when oxygen levels were well above physiological. Overall, these results suggest that a mechanism other than reduced energy supply was responsible for the downregulation of force production with reduced PO2. The presence of oxygen-sensing mechanisms in both mammals and fish is well established. Oxygen-reporting neuroepithelial cells are present in the gill arch of fishes and initiate reflex bradycardia and ventilatory responses during hypoxia, and vasoactive oxygen responders in vascular smooth muscle and oxygen-sensing cells in the myocardium have been identified (reviewed in Refs. 9 and 37). H2S is an established oxygen-sensing molecule that accumulates in trout ventricular myocardium when oxygen levels are low (57), has negative inotropic effects on the rat myocardium (20, 58), and has several other direct effects on the myocardium, including cardioprotective effects against ischemia (reviewed in Ref. 9). Finally, nitric oxide is associated with short-term regulation of contractility and excitation-contraction coupling (3, 43) [also see a review by Fago et al. (9)], and elevated levels of nitrites and nitric oxide have been recorded in the hearts of Crucian carp exposed to anoxia, where they are thought to play a cardioprotective role (42). Thus, a precedent exists for oxygen sensing in the fish myocardium and for this mechanism to potentially regulate muscle performance directly. However, the nature of the sensing mechanism(s) and its effectors in the myocardium of Atlantic cod are unknown and will require further investigation.

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WARM TEMPERATURE AND PERFORMANCE OF COD CARDIAC MUSCLE

R875

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

D.A.S., A.K.G., and K.J.R. contributed to the conception and design of the research; D.A.S., A.K.G., and G.W.N. performed the experiments; D.A.S. and G.W.N. analyzed the data; D.A.S., A.K.G., and K.J.R. interpreted the results of the experiments; D.A.S. and G.W.N. prepared the figures; D.A.S., A.K.G., and K.J.R. drafted the manuscript; D.A.S., A.K.G., and K.J.R. edited and revised the manuscript; D.A.S., A.K.G., and K.J.R. approved the final version of the manuscript.

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