Thermal acclimation leads to variable muscle responses in two temperate labrid fishes.

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SUMMARY STATEMENT

Two fish species from the Northwest Atlantic demonstrate variable acclimation responses when presented with temperatures which naturally induce winter quiescence.

ABSTRACT

Temperature can be a key abiotic factor in fish distribution, as it impacts most physiological processes. Specifically, temperature can affect locomotor capabilities especially as species are exposed to temperatures nearing their thermal limits. In this study we aimed to understand the effects of temperature on muscle in two labrids that occupy the Northwest Atlantic Ocean. When exposed to cold temperatures in autumn, cunner (*Tautogolabrus adspersus*) and tautog (*Tautoga onitis*) go into a state of winter dormancy. Transitions into dormancy vary slightly where tautog will make short migrations to overwintering habitats while cunner overwinter in year-round habitats. To understand how muscle function changes with temperature, we held fish for four weeks at either 5 or 20°C then ran muscle kinetic and workloop experiments at 5, 10 and 20°C. Following experiments, we used immunohistochemistry staining to identify acclimation effects on myosin isoform expression. Muscle taken from warm acclimated cunner performed the best, whereas there were relatively few differences among the other three groups. Cunner acclimated at both temperatures downregulated the myosin heavy chain, suggesting a transition in fiber type from slow-oxidative to fast-glycolytic. This change did not amount to a detectable difference in muscle power production and kinetics. However, overall poor performance at cold temperatures could force these fishes into torpor to overwinter. Tautog, alternatively, retained myosin heavy chains which likely increases locomotor capabilities when making short migrations to overwintering habitats.

INTRODUCTION

Temperature is a key abiotic factor that impacts most physiological processes and behavior in ectotherms. As a result, water temperature is important in limiting the distribution of marine fishes around the world. Recent projections on climate change predict Northern hemisphere temperate species will expand their ranges poleward in response to warming waters on the southern edge of their distributions (Hickling et al., 2006). In an analysis of commercially important species from the Northwest Atlantic Ocean, Nye et al. (2009) found that many of the 36 species tested have already experienced a range shift toward the poles. Specifically, southern ranges of the examined species showed the greatest displacement. These range shifts create physiological problems when a species encounters temperatures below their thermal limits in new, poleward shifted, environments (Pörtner and Peck, 2010).

In fishes, temperatures on the low end of a species' thermal limits can dramatically impact physiological processes. For example, Crawshaw (1984) found largemouth bass (*Micropterus salmoides*) and brown bullhead (*Ictalurus nebulosus,*) had a drop in routine metabolic rate which equated to a Q_{10} (change in rate over a 10°C change in temperature) of 2-3 when temperatures were decreased from 17 °C to 7°C. Costa et al. (2013) found that cunner (*Tautogolabrus adspersus),* which undergo winter dormancy, experienced a decrease in routine metabolic rate which equated to Q_{10} =10.4 when temperature was dropped from 5°C to 0°C. Additionally, swimming performance can be impacted by decreasing temperatures. As temperatures drop below a thermal optimum the viscosity of water increases and muscle performance is reduced which lowers locomotor capabilities (Johnson et al., 1996; Beddow et al., 1995; Herbing 2002). As a class that is made up of primarily ectothermic organisms, the muscles of fishes are subject to the surrounding water temperature. Thus, as temperature approaches the low end of their thermal limits so does overall muscle performance (for review see Guderley 2004). In summary, contraction kinetics slow with decreasing temperature which causes a decrease in power and slower contraction velocity (Coughlin et al., 1996; Altringham and Block 1997; Rome et al., 2000; Moran et al., 2019). However, thermal compensation is possible through physiological modifications of muscle fibers.

Fish muscle tissue has variable responses to altered temperatures. Phenotypic changes to fish muscle in response to temperature have been explored in a variety of fish lineages (for reviews see: Guderley and Blier, 1988; Guderley, 1990; Guderley, 2004). During locomotion, fishes have been shown to recruit fast glycolytic fibers at lower speeds as temperature decreases. The recruitment of fast glycolytic muscle fibers indicates a transition from aerobic (sustained) locomotion to anaerobic (short burst) locomotion (Rome et al., 1985). Compensation for cold temperatures has been shown to manifest itself as hyperplasia of slow, oxidative, red muscle fibers. This has been demonstrated in goldfish (*Carassius auratus*) (Smith et al., 1973, Johnston and Lucking, 1978, Sidell 1980, Johnston et al., 1990), striped bass (*Morone saxatilis*) (Jones and Sidell 1982), and trout (*Oncorhynchus mykiss)* (Egginton et al., 2000). Jones and Sidell (1982) found that compensatory mechanisms increased the proportion of oxidative myotomal muscle in response to cold temperatures which equated to greater speeds at which aerobic locomotion was maintained. While adaptation for performance compensation at cold temperatures is common in ectotherms it is not ubiquitous across all fish lineages. Specifically, fishes that enter into winter dormancy express less thermal compensation than those that do not (Guderley, 1990).

Despite being rich with rocky reef habitat, cunner and tautog (*Tautoga onitis*) are the only species of labrid that can be found in the Northwestern Atlantic Ocean. While having significant overlap, the ranges of these species varies slightly. Cunner range from the mouth of the Chesapeake (Maryland, USA) to Newfoundland, Canada. Tautog range from South Carolina, USA to Massachusetts, USA (Bigelow et al., 2002). When these species encounter temperatures between 3 and 10°C they enter into a winter quiescence or torpor. Prior to entering into torpor these species take cover in the substrate to avoid predation (Bradbury et al., 1995, Olla et al., 1980). They stay quiescent until temperatures reach ~10°C, at which point, they return to or emerge from inshore habitats. Energy savings in this quiescent period has been linked to inactivity and not metabolic depression (Speers-Roesch et al., 2018). Previous work by Moran et al. (2019) has demonstrated that cunner show reduced aerobic swimming performance with decreasing temperature which could be attributed to reduced muscle kinetic performance at 5 °C and 10 °C .

Given the taxonomic similarities but differing ranges between cunner and tautog, these two species are good candidates for comparative ecophysiological studies. We aimed to answer the question: how does the muscle physiology of cunner and tautog change in response to temperature? Because cunner occupy colder habitats we predict that they will have greater muscle performance at cold temperatures as indicated by faster twitch kinetics and greater relative power production. Conversely, we predict that tautog will have greater muscle performance at warm temperatures. Further we suggest that shifts in muscle performance will be associated with changes in myosin expression in the muscle used to power labriform swimming in these fishes. By addressing these predictions, we will make inferences to the connection between physiology and biogeography while relating this to the physiological stress imposed by climate warming.

METHODS

Fish Specimens

All fishes were collected via hook and line from two locations in Long Island Sound (41° 9.402' N 73° 10.799' W and 41° 8.579' N 73° 13.355' W) in Bridgeport CT, USA between May 2018 and October 2018 under collecting permit SC-18005b. Once captured, fishes were transported to Fairfield University where they were kept in 340 l and 1514 l holding tanks of artificial seawater under IACUC protocol #15-002. For immunohistochemistry controls separate fish ("Natural") were collected and sacrificed within 24 hours to mitigate the impacts of thermal acclimation. Holding tank temperatures were

controlled by chillers (JBJ Artica) according to their respective temperature treatments. All fishes were held at either 5°C (cold cunner/cold tautog) or 20°C (warm cunner/warm tautog) for a minimum of four weeks. This acclimation period was determined to be appropriate because acclimation can occur over periods much shorter than four weeks. Costa et al., (2013) found that the metabolic rates of fish exposed to acute drops in temperature were not different than seasonal temperature drops. Additionally, enzyme acclimation in fishes has been shown to occur over the course of three weeks (Sidell et al., 1973). Experimental cunner were 16.5±1.8 cm (mean±SD) standard length (L_s) whereas tautog were 28±5.6 cm L_s. Fish were fed tri-weekly using live Asian shore crabs, sandworms and cut squid.

Muscle Performance

Tautog and cunner were sacrificed by a blow to the head and double pithing. The pectoral fin was skinned revealing the abductor superficialis (Fig 1). This muscle was chosen as it is the primary locomotor muscle for labrid fishes (Fulton et al., 2001). The cleithrum was cut dorsally and ventrally to the pectoral fin using bone cutters. Following the separation of any connective tissue the entire pectoral fin complex was removed from the body and placed in ~5°C physiological saline (Composition in gl^{-1}: 7.8 NaCl, 0.18 KCl, 0.166 $CaCl_2$, 0.095 $MgSO_4$, 0.084 $NaHCO_3$, 0.06 NaH_2PO_4). All muscle was removed from the pectoral fin complex except ~3 fascicles of the abductor superficialis. Using 4-0 suture thread, a hook was fixed to the middle fin ray relative to the existing portion of the muscle. A hole was pierced through the cleithrum using a 25G disposable hypodermic needle and a hook placed through this hole. Using both hooks the existing pectoral fin fascicles were submerged in oxygenated physiological saline and fixed to a dual mode muscle ergometer (Aurora Scientific Inc., Aurora, Ontario). Surrounding the bath was a recirculating water bath which drew from and returned to a temperature controlled 19l aquarium. The length of the muscle was adjusted to register 10 mN of passive tension. Following a 0.5 hr acclimation period, the muscle was tested at 5, 10 and 20°C in random order. Following testing at each experimental temperature the saline bath was replaced and temperature changed using a chiller. A 0.5 hr acclimation period was allowed for each change in temperature.

Following the acclimation period each muscle underwent multiple tetanic contractions to find optimal length. Tetanic contractions were elicited using a pulse frequency of 125Hz and a stimulus strength of 300 mA. A stimulus duration of 400 ms was used as this allowed all treatment temperatures to reach a maximum force plateau (Fig. 2). After optimal length (L_0) was found a twitch was run to determine time to maximum isometric contraction, time to half-relax (relaxation time) and maximum twitch force for each muscle. Twitch force was

standardized to physiological cross-sectional area (PCSA) of the muscle. Following twitch tests, ramp protocols were conducted to determine muscle power production.

To determine maximum contraction velocity (V_{max}) of the muscle, standard ramp protocols were conducted following Johnston et al. (1985). Using a constant stimulus with a pulse frequency of 125 Hz the muscle was allowed to reach maximum tetanic contraction before the force was dropped and held constant at 10%, 20%, 30%, 40%, 50%, 60%, 70% 80% or 90% of maximum tetanic force. The muscle was allowed to rest for three minutes between contractions. While at a percentage of maximum force the contraction velocity was recorded and standardized to muscle length. Following all ramps, force-velocity curves were fitted to the data using the modified hyperbolic equation from Marsh and Bennett (1986) in Igor Pro (WaveMetrics, Inc.):

$$V = \frac{B\left(1 - (\frac{P}{Po})\right)}{A + \left(\frac{P}{Po}\right)} + C(1 - \frac{P}{Po})$$

Where V is velocity in $L_M\,s^{-1}$; P/Po is force as a proportion of maximum isometric force; A, B and C are all constants. Following the last test, muscle fiber length was measured while held at L_0.

Standard workloop protocols were utilized to investigate power production following Coughlin and Rome (1996). At every temperature, each muscle was tested at a cycle frequency of 1, 2, 3, 4, 5, 6 Hz for each temperature. Cycle frequency was tested randomly while using a stimulus frequency of 125 Hz and a stimulus strength of 300 mA. Muscle length change during the workloops was ± 3% L_M. Five consecutive workloops were conducted at a given frequency, however, only the fourth loop was used for measurements. Each muscle was allowed to rest for 5 minutes between cycles of five workloops. Using the Aurora Scientific Inc. software, loop integrals were calculated and multiplied by frequency to yield power values. These values were standardized to the mass of the muscle. Following experimental testing all muscles were removed from their origin and insertion, patted dry and weighed.

Muscle Immunohistochemistry

Following muscle kinetic experimentation, the abductor superficialis was removed from the pectoral fin complex on the opposite side of the fish to examine myosin heavy chain (MyHC) expression. The protocols followed Coughlin and Akhtar (2015), which were modified from Campion et al. (2012).

Tissue samples were fixed in Carnoy's buffer for one week then dehydrated in 95% ethanol in buffered saline and rehydrated in buffered saline. The tissue was then embedded in 1% agar solution in 30% sucrose for five hours. After the agar hardened the tissue was frozen using liquid nitrogen and stored at -80°C until shipped overnight on dry ice to Widener University.

The muscle blocks were sectioned at 12 μM using a Microm HM505M cryostat and set on Fisher Superfrost Plus slides. The slides were dried for 2 hr on a slide warmer and stored at -20°C. For a positive control, slides were labelled using the anti-myosin antibody MF20. MF20 binds to all MyHC (Coughlin and Akhtar 2015). Slow myosin content of muscle was labelled using the anti-slow MyHC antibody S58. Primary antibodies were obtained from Developmental Studies Hybridoma Bank (Iowa City, IA). The sections were rehydrated with PBT (Phosphate Buffered Saline + 0.1% Tween) and then incubated in a blocking solution (PBT + 1%BSA). Blocking solution containing 5% normal goat serum (NGS) was used to dilute (1:5) the primary antibody solution. Slides were incubated for 90 min in primary antibody solution in a humid chamber. Slides were then washed with PBT and blocked with a secondary antibody, Alexa Fluor 488 goat anti-mouse IgG (Thermo Fisher, Z25002). The second antibody was diluted (1:250) in blocking solution with 5% NGS. A humid chamber was used to incubate the slides for 30 min. The slides were then washed and stained with 2g mL^{-1} Following three 5 min. washes with PBT, samples were mounted in 50% glycerol in 1 x PBS. Using clear nail polish the slide covers were fixed to the slides, and slides were stored at -20°C. Slides were imaged using a Nikon DS-RI1 digital camera on a Nikon Eclipse 80i compound microscope with Nikon Plan Fluor objective lenses.

Statistics

To test for differences between species and acclimation treatments a one-way analysis of variance (ANOVA) was used for each treatment temperature/frequency using SPSS v.25 (IBM Corp. Armonk, NY). Data was first inspected for outliers using stem and leaf plots. Homogeneity of variance was confirmed using a Levene's Test of Equality of Variances. Normality plots were used to confirm data was normal. To compare within a species/acclimation treatment and among testing conditions, repeated measures ANOVAs were used. Normality was determined by examining normality plots. The sphericity assumption of repeated measures was addressed within the ANOVA results. In the case where the sphericity assumption was violated the Greenhouse-Geisser metric was used to determine statistical significance. All ANOVAs were followed by LSD post-hoc tests.

RESULTS

Muscle Performance

Abductor superficialis muscle kinetics differed among testing temperatures in different acclimation conditions. Additionally, within a testing temperature treatment groups also differed. Average time to maximum isometric contraction decreased with increasing testing temperature for all treatments except cold tautog ($P<0.05$; Fig. 3A). Acclimation treatment results did not differ within a testing temperature for the 5°C and 20°C testing temperatures. However, the cold cunner treatment yielded a shorter contraction time within the 10°C testing temperature ($P<0.01$). The warm cunner treatment was only different from the cold tautog treatment ($P<0.01$) while the warm tautog treatment was only different from the cold cunner treatment (P<0.01, Fig. 3A). No differences were detected among acclimation treatments within a testing temperature when examining twitch relaxation time. However, aside from the warm tautog ($P<0.001$), half relaxation time decreased with increasing temperature for the rest of the temperature treatments ($P<0.01$, Fig. 3B). No differences were detected within an acclimation treatment and between testing temperatures for average relative force production. Thus, testing temperature had no effect on force production. Given the low sample size it is possible that low statistical power masked the effects of temperature on kinetic variables that were not different. Regardless of testing temperature warm cunner produced more force per unit cross-sectional area than any of the other acclimation groups (Table 1; Fig. 3C).

Average maximum contraction velocity did not differ among acclimation groups when tested at 5 and 10°C. When tested at 20°C the warm acclimated cunner contracted faster than the other three treatment groups. Additionally, average maximum contraction velocity did not differ within an acclimation group between testing temperatures (Table 1; Fig. 4).

Power production among acclimation groups differed only at a frequency of 1Hz or 2Hz when tested at 5°C ($P<0.001$; Table 1; Fig. 5A). Within the 1Hz experimental treatment cold cunner demonstrated greater power than cold tautog but less power than warm cunner. Cold tautog yielded the lowest power, which was not different than warm tautog. Warm cunner produced greater power than all other treatment groups. Within 2Hz, cold cunner did not produce significantly different power than the other three groups ($P<0.01$). Both tautog treatments produced less power than warm acclimated cunner. When tested at 10°C the warm acclimated cunner appeared to drive most of the variability among acclimation groups for 1Hz-5Hz ($P<0.001$; Table 1; Fig 4B). When tested at 1Hz cold cunner produced greater

power than cold tautog but less power than warm cunner. Cold tautog produced the least amount of power, however, this average power was not different than that of the warm tautog acclimation group. Warm cunner produced the greatest power at 1Hz and all other frequencies. The same relationship described at 1Hz was observed at 2Hz. At 3Hz cold acclimated cunner differed from both tautog acclimation groups. At frequencies 4Hz and 5Hz, cold cunner, cold tautog and warm tautog were similar. When tested at 20°C cold acclimated cunner and both tautog acclimated groups were similar across all 6 frequencies. Warm cunner produced significantly greater power than the other three acclimation groups at every frequency ($P\leq0.01$; Table 1; Fig. 5C). The results for all multiple comparisons can be found in Table 1.

Muscle Immunohistochemistry

Fiber type and staining protocols were confirmed by staining myotomal muscle with S58 and MF20. The positive control confirmed that MF20 bound to all myosin in both the red and white myotomal and abductor superficialis muscles. The S58 treatment, however, confirmed the binding to slow MyHC only on the superficial red layer of the myotomal muscle (Fig. 6). Cold tautog appeared to downregulate slow myosin heavy chain expression in the abductor superficialis when compared to warm tautog (Fig. 7). Cunner held in the laboratory under both warm and cold conditions displayed a loss of slow MyHC expression in their abductor superficialis (Fig. 8).

DISCUSSION

Acclimation temperature had relatively little impact on muscle kinetic performance in both the cunner and tautog studied here. Similar to Moran et al. (2019), we saw no indication of a strong acclimation response aside from warm (20°C) acclimated cunner. Additionally, as seen in Moran et al. (2019) we saw slowed contraction kinetics when tested at ≤10°C, however, this did not translate to decreased power production. New insight provided here demonstrates that, while muscle kinetics slow at cold temperatures, this does not equate to reduced power output by the muscle. Given the lack of differences among cold acclimated cunner, cold acclimated tautog and warm acclimated tautog we conclude that muscle performance is similar across acclimation temperatures. This is contrasted by results from warm acclimated cunner muscle which outperformed all other acclimation groups for many of the metrics tested here. This result was surprising given the fiber type composition among acclimation groups (Figs. 5 & 6). It is worth noting that some of the null results reported here may be a consequence of low statistical power as a result of low sample sizes.

Tautog showed the typical down regulation of slow MyHC that is associated with decreasing temperature. Similar results have been shown in rainbow smelt (Woytanowski and Coughlin 2013). Despite testing positive for slow MyHC in the myotomal muscle, both warm acclimated and cold acclimated cunner abductor superficialis muscle appeared to have low slow MyHC expression for all individuals tested here (Fig. 7). One possible explanation could be that cunner simply lack slow MyHC in their abductor superficialis. However, for another study, cunner were tested for slow MyHC after being directly removed from the wild and tested positive for this isoform expression (Figs. 7 & 8). This result appeared to have very little functional implication as the cold acclimated cunner (slow MyHC absent) did not differ in performance when compared to warm and cold acclimated tautog (slow MyHC present). However, though not significant, cunner tended to have a higher V_{max} than tautog from the same acclimation temperature. V_{max} is expected to be the most correlative kinetic variable measured here to indicate effects of MyHC composition of given muscle as seen in Woytanowski and Coughlin (2013). Given the variability in the maximum contraction velocity data, it is likely that a greater sample size would enhance the resolution of this data. Given the greater power production in warm acclimated cunner, it appears as if the downregulation of slow MyHC did not impact contraction dynamics when tested at warmer temperatures. We suggest that other mechanisms control contraction dynamics aside from myosin heavy chain isoforms alone. The impact of warmer water on cunner muscle merits further study to determine the biochemical basis of the muscle acclimation response. Although cunner range to the mouth of the Chesapeake Bay they rarely encounter water temperatures exceeding 20°C. The 5°C and 20°C treatments could pose equal amounts of stress which could explain the downregulation of slow twitch fibers in cunner locomotor musculature. Given the more northern range of cunner, the downregulation of MyHC could be a compensatory mechanism to maintain locomotor performance outside of an optimal thermal range.

Cunner and tautog demonstrated decreased slow MyHC expression and performance when acclimated to and tested at 5°C. In both species this temperature has been shown to induce torpor (Olla et al., 1975). Given the poor muscle performance and down regulation of MyHC the data presented here supports the assertion that torpor is a survival strategy to overwinter in cold waters. While there is little work on the effects of cold acclimation on muscle performance in fishes, when testing the effects of cold acclimation on rainbow smelt, Coughlin et al. (2016) observed very different results from fish that occupy similar thermal niches but do not undergo torpor. They found that cold acclimated (4°C) fish outperformed

warm acclimated (10°C) fish when tested at the same temperature (10°C). The data presented here shows a different relationship, broadly stated, one where warm acclimated muscle outperformed cold acclimated muscle regardless of testing temperature. When tested at warm temperatures, warm acclimated cunner muscle performed exceptionally well, however, when tested at 5°C this same muscle performed only slightly better at low operating frequencies (Fig. 5A). Additionally, the overall poor performance at high frequencies when tested at low temperatures demonstrates thermal constraints on the operating frequencies of muscle at these cold temperatures. These thermal constraints can provide insight to the ecology and behaviors of these fishes.

Given the poor performance of all muscle tested at cold temperatures we can make inferences about the behavior of these species during overwintering. As noted by Moran et al. (2019) swimming performance was reduced at ≤10°C. This work supports that result as muscle power production was relatively low through all frequencies at this temperature. In other words, power production of the primary locomotor muscle is reduced at all operating frequencies at 5°C suggesting that sustained locomotion is significantly hindered at this temperature. Interestingly, warm acclimated cunner demonstrated a shift in optimal frequency similar to that seen with fin beat frequency in Moran et al., (2019). In that work they present an optimum frequency for the 20°C treatment group of 4.6 Hz which is similar to the 5-6 Hz where peak power was observed for warm acclimated cunner tested at 20°C (Fig. 5). Similarly, when tested at 10°C and 5°C, peak power production dropped to 3 and 2 Hz respectively correlating with a drop in optimum fin beat frequency of 2.79 and 2.32 Hz at 10°C and 5°C from Moran et al., (2019). These results further support their behavioral transition into torpor at temperatures approaching 5°C. Torpor allows this species to overwinter near their year-round habitats without having to undergo long migrations to follow a thermal regime. Curiously, however, the warmer water species (tautog) retained slow twitch proteins while the cold water species (cunner) lost all slow twitch proteins at the cold temperature treatment. Cunner have been shown to enter into torpor at cold temperatures whereas tautog usually migrate offshore to deep reefs and wrecks when temperatures drop (Auster, 1989). It is theorized that tautog enter torpor offshore during winter, however, their activity levels in these overwintering habitats remains unclear. It is possible that the retention of a relatively unchanging abductor superficialis MyHC composition is an adaptation to aid this species in aerobic locomotion as they migrate offshore where they may or may not remain active during winter.

While the data presented here has addressed the cold end of these species thermal tolerance, future work will explore the effects of warm temperatures on their locomotor physiology. Given the differences in geographical ranges of these species we expect cunner and tautog to express different performance capabilities when presented with temperatures on the high end of their thermal tolerances. By further exploring this aspect of their physiological capabilities we will be able to predict range shifts based on locomotor performance. With this information management agencies can plan for the arrival/disappearance of these species and how it will affect fisheries and ecosystem processes.

Conflict of interest statement

The authors declare no competing interests.

Data availability

Data is available upon request.

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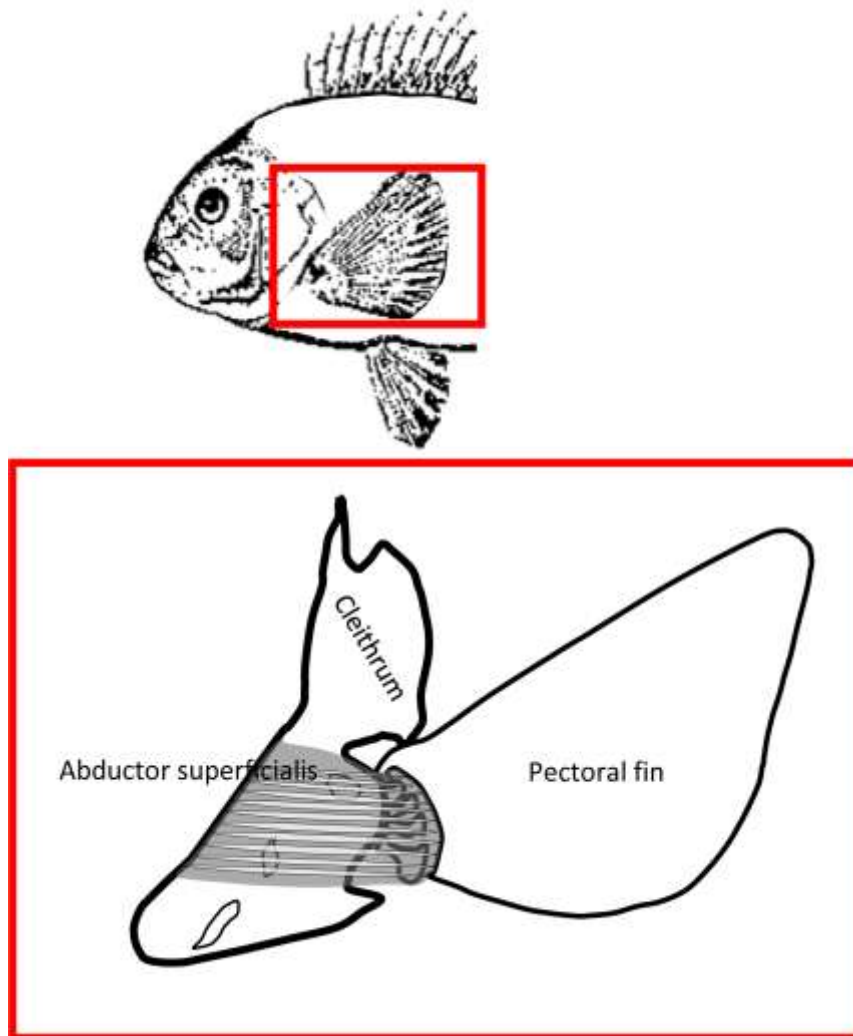
Figures

Fig. 1: A line drawing schematic of the location of the abductor superficialis muscle in tautog (pictured) and cunner. Approximate fiber angle is drawn from the cleithrum to the pectoral fin in elongate white ellipses.
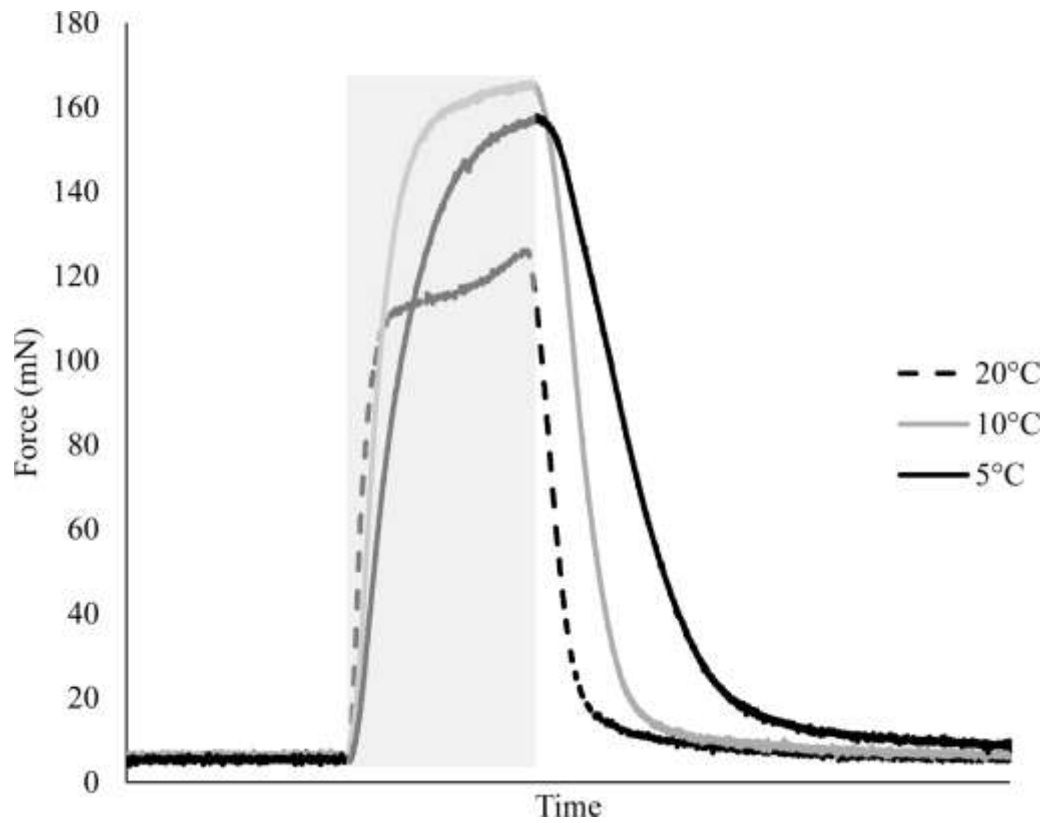
Fig. 2: Representative traces of tetanic contractions demonstrates the force plateau
characteristic of tetanic contractions in abductor superficialis muscle from a warm acclimated
cunner. The gray box represents the duration of applied stimulus. Listed temperatures
indicate testing temperature. Stimuli for all contractions represented here were done with
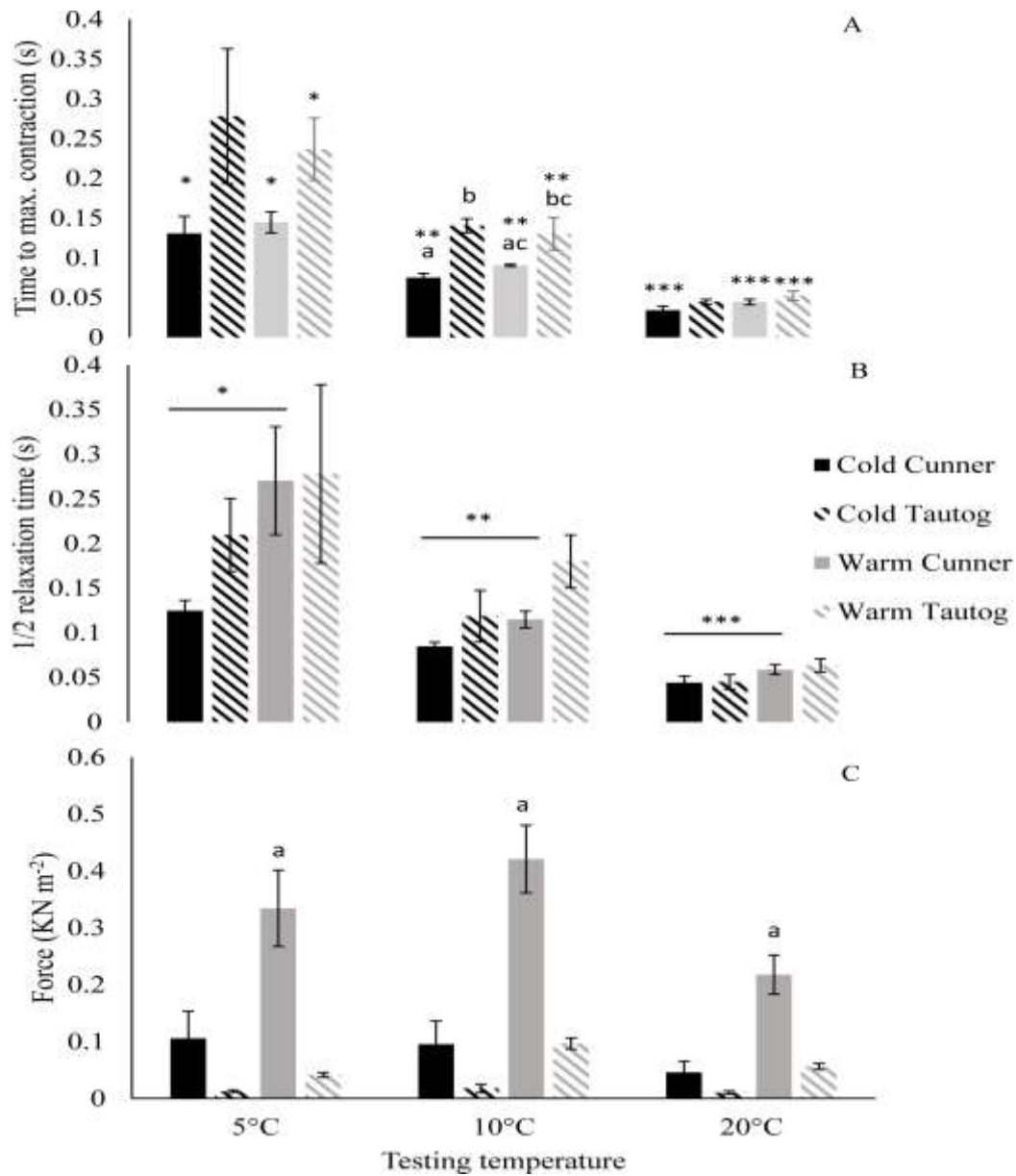200mA of electrical stimulus.

Fig. 3: Mean twitch kinematic comparisons among and between testing temperatures and acclimation treatments for the abductor superficialis. A) Average time to maximum twitch contraction. B) Average time to half relaxation following a twitch. C) Average force (per unit PCSA). Lowercase letters denote differences as determined by ANOVA ($P < 0.05$) within testing temperature and among acclimation groups. Asterisks denote differences ($P < 0.05$) within acclimation group and among testing temperatures. Error bars represent s.e.m. n=5/treatment
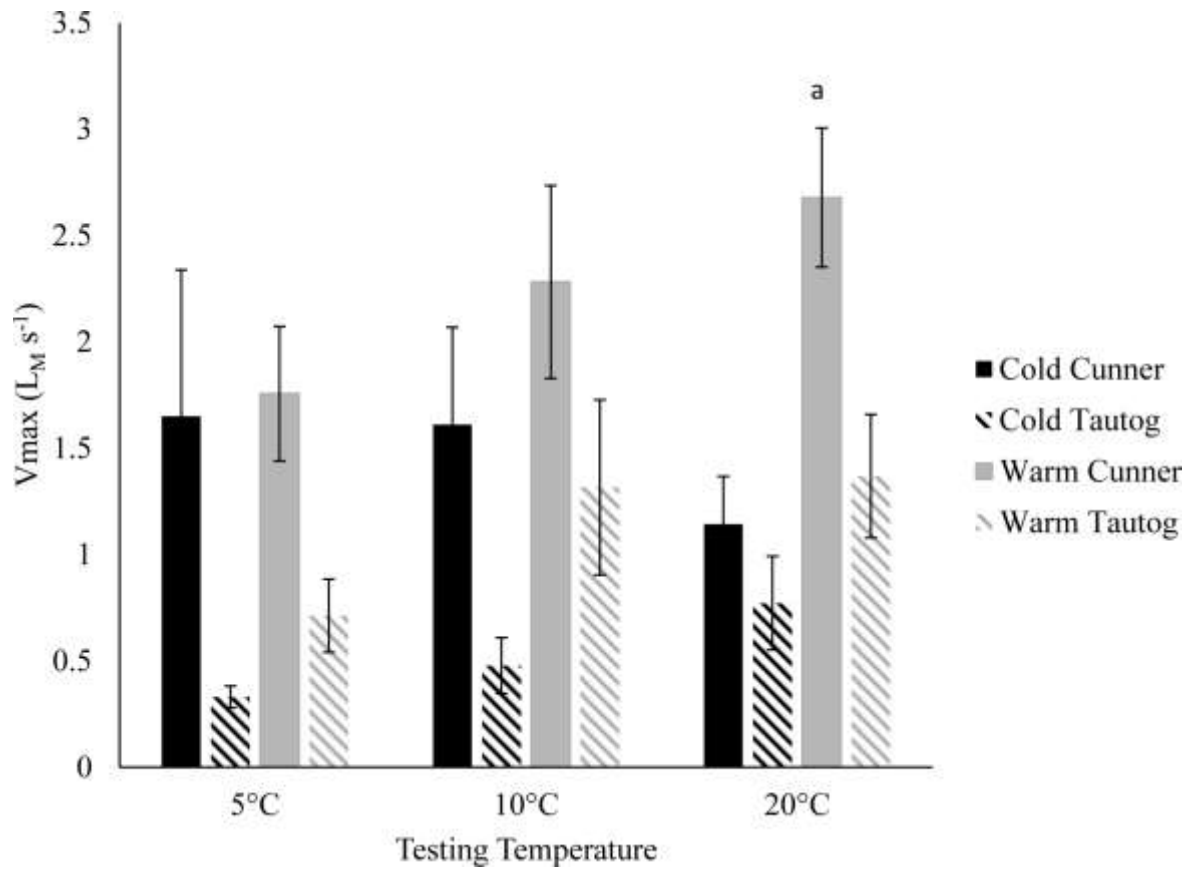
Fig. 4: Comparison of mean maximum contraction velocity (in muscle lengths s^{-1}) between testing temperature and within testing temperature among acclimation groups for the abductor superficialis. Lowercase letters denote within testing temperature differences among acclimation groups as determined by ANOVA ($P< 0.05$). Error bars represent s.e.m. n=5/treatment
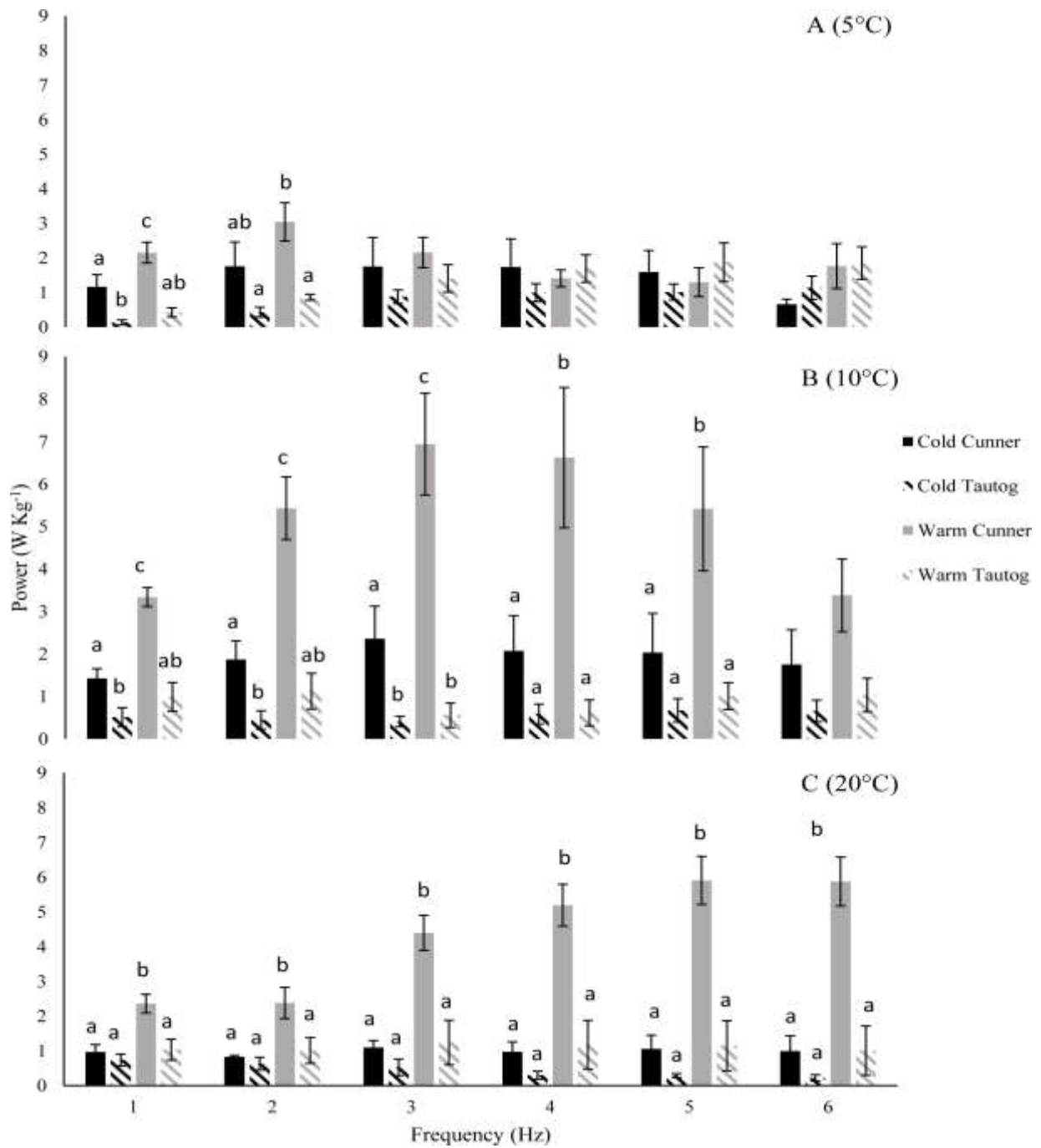
Fig. 5: Mean power production for each testing and treatment temperature for the abductor superficialis. Lowercase letters denote differences among acclimation treatments within a frequency as determined by ANOVA ($P< 0.05$). Error bars represent s.e.m. n=5/treatment
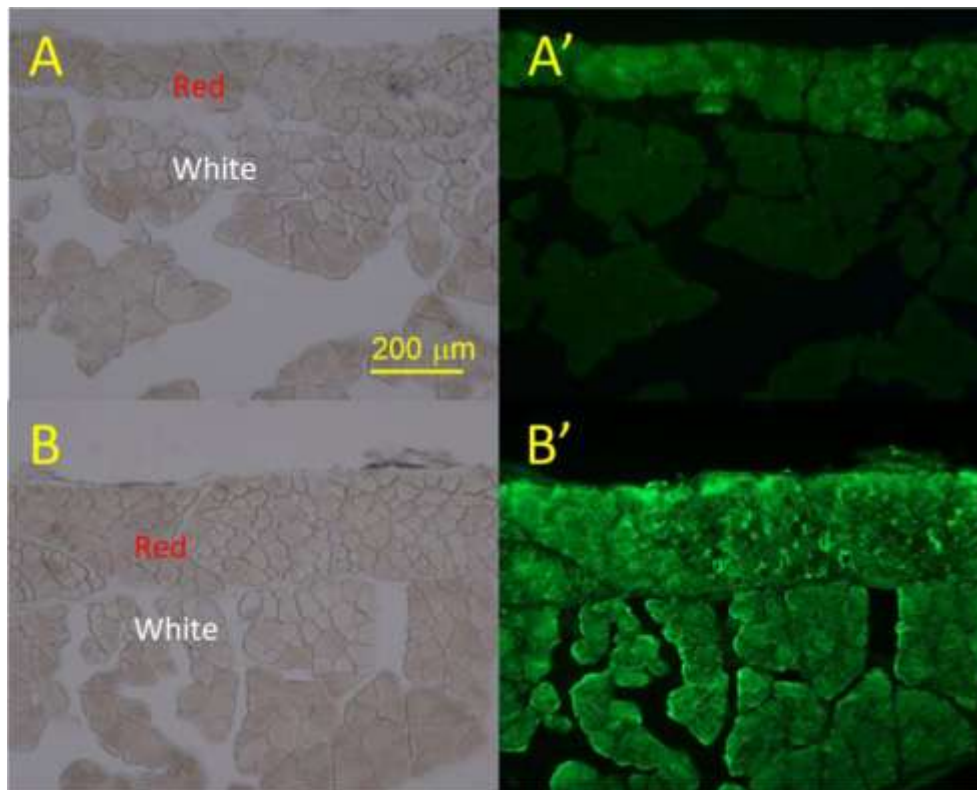
Fig. 6: Anti-myosin heavy chain antibodies allow for determination of fiber type. White light (A, B) and fluorescence (A', B') images are from myotomal muscle from cunner. Section is oriented with the superficial red or slow-twitch muscle above the deeper white or fast-twitch muscle. In A', antibody S58 binds to slow MyHC in the superficial red muscle layer. In B', antibody MF20 serves as a positive control binding to myosin in both red and white muscle.
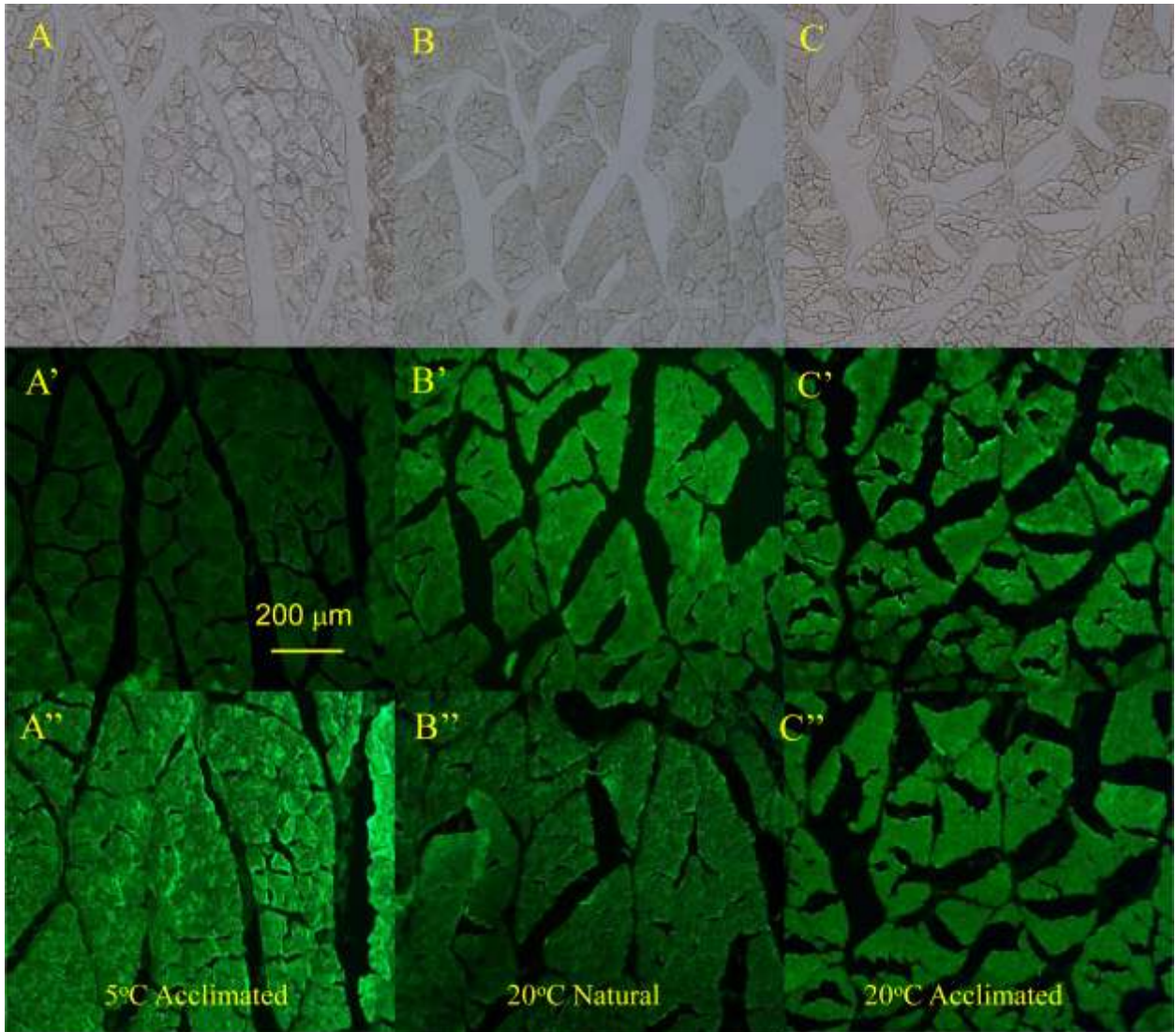
Fig. 7: Representative images of tautog immunohistochemistry reflecting S58 (A'-C') and MF20 (A''-C'') activity. White light images are represented by the top row (A-C). All pictures are of abductor superficialis cross sections. The "natural" sample was taken from fish taken directly from the wild and not acclimated to a controlled thermal regime. These results were consistent with every tautog tested in this study.
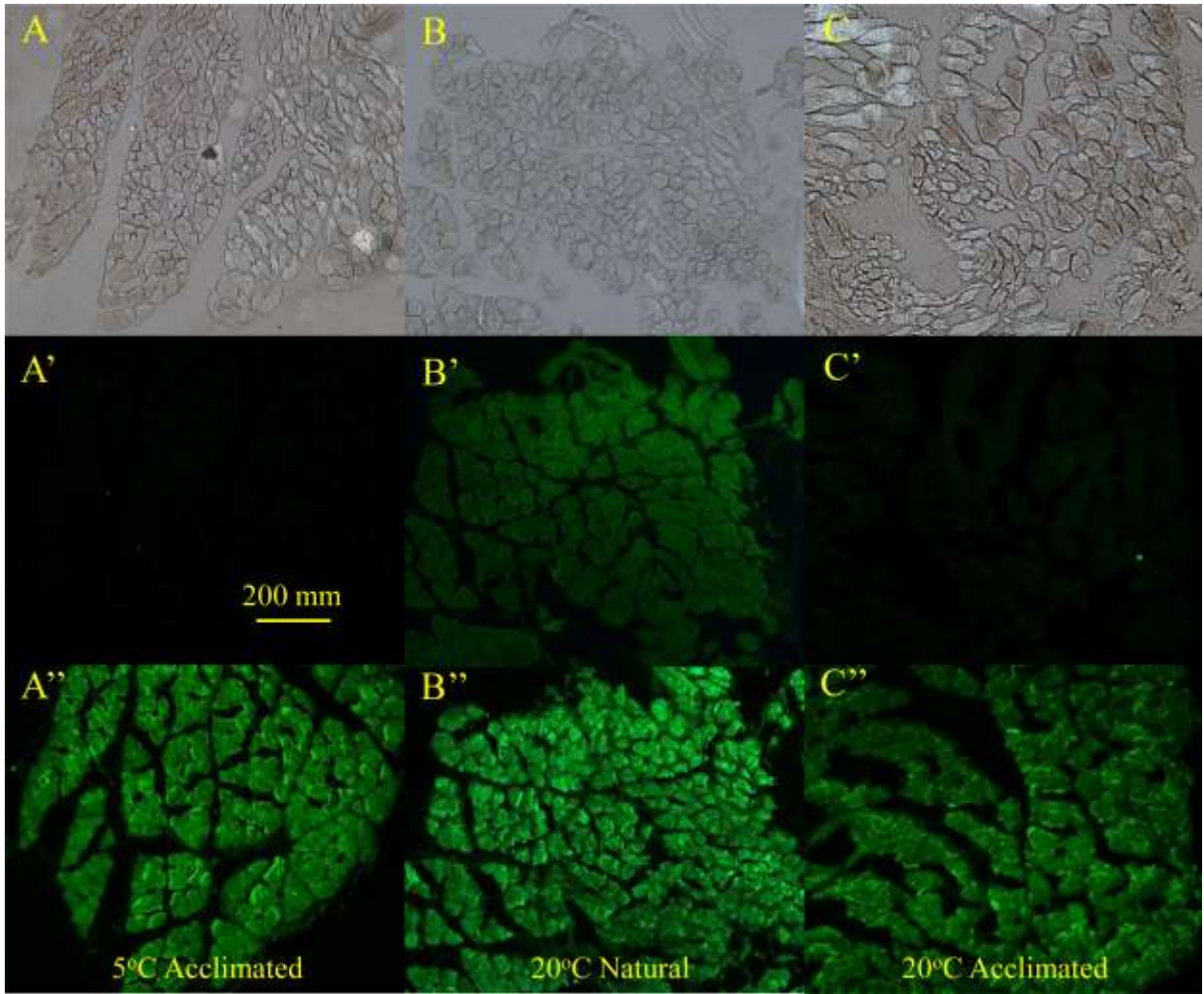
Fig. 8: Representative images of cunner immunohistochemistry reflecting S58 (A'-C') and MF20 (A''-C'') activity. White light images are represented by the top row (A-C). All pictures are of abductor superficialis cross sections. The "natural" sample was taken from fish taken directly from the wild and not acclimated to a controlled thermal regime. These results were consistent with every cunner tested in this study.

Table

Table I: Summary of statistics for all analyses

Average Metric	df	F-value	Sig.	Post-hoc
Twitch force among acclimation groups tested at 5°C	3	11.87	<0.001	WC diff than all (Fig. 3C)
Time to max. twitch among acclimation groups tested at 10°C	3	5.767	0.01	CC diff than all tog, WC diff than CT (Fig. 3A)
Twitch force among acclimation groups tested at 10°C	3	10.66	0.01	WC diff than all (Fig. 3C)
Twitch force among acclimation groups tested at 20°C	3	15.39	<0.01	WC diff than all (Fig. 3C)
F/V among acclimation groups tested at 20°C	3	8.416	0.01	WC diff than all (Fig. 4)
Time to max. twitch for cold cunner among testing temps.	2	25.656	0.01	All different (Fig. 3A)
Time to relax twitch for cold cunner among testing temps.	2	25.351	<0.001	All different (Fig. 3B)
Time to max. twitch for warm cunner among testing temps.	2	60.624	0.01	All different (Fig. 3A)
Time to relax twitch for warm cunner among testing temps.	2	27.121	0.05	All different (Fig. 3B)
Time to max. twitch for warm tautog among testing temps.	2	16.391	0.01	All different (Fig. 3A)
Power among acclimation groups within 5°C testing temp. (Fig. 5A)				
Within 1Hz	3	11.055	<0.001	WC diff than all, CC diff than CT and WC, WT diff than WC only (Fig. 5)
Within 2Hz	3	5.264	0.009	WC diff than CT and WT only
Power among acclimation groups within 10°C testing temp. (Fig. 5B)				
Within 1Hz	3	15.536	<0.001	CC diff than WC and CT, WC diff than all
Within 2Hz	3	18.653	<0.001	CC diff than WC and CT, WC diff than all
Within 3Hz	3	18.325	<0.001	CC and WC diff than all
Within 4Hz	3	10.842	0.01	WC diff than all only
Within 5Hz	3	6.22	0.007	WC diff than all only
Power among acclimation groups within 20°C testing temp. (Fig. 5C)				
Within 1Hz	3	9.019	0.01	WC diff than all
Within 2Hz	3	5.097	0.1	WC diff than all

Within 3Hz		3	15.1 56	<0. 001	WC diff than all
Within 4Hz		3	18.3 31	<0. 001	WC diff than all
Within 5Hz		3	19.4 57	<0. 001	WC diff than all
Within 6Hz		3	19.2 79	<0. 001	WC diff than all

WC= warm cunner treatment, CC= cold cunner treatment, WT= warm tautog treatment, CT= cold tautog treatment. Reported degrees of freedom are from between groups effects.