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RESEARCH ARTICLE

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

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ABSTRACT

Temperature can be a key abiotic factor in fish distribution, as it affects 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 4 weeks at either 5 or 20°C and 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.

KEY WORDS: Kinetics, Wrasse, Pectoral fin, Swimming, Kinetics, Abductor superficialis, Locomotion, Torpor

INTRODUCTION

Temperature is a key abiotic factor that affects 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 that 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 affect physiological processes. For example, Crawshaw (1984) found that 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 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 to 0°C. Additionally, swimming performance can be affected 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, fishes have muscles that 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, 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. Although 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

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the Northwestern Atlantic Ocean. Although having significant overlap, the ranges of these species vary slightly. Cunner range from the mouth of the Chesapeake (Maryland, USA) to Newfoundland, Canada. Tautog range from South Carolina 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 to 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 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 predicted that they would have greater muscle performance at cold temperatures, as indicated by faster twitch kinetics and greater relative power production. Conversely, we predicted that tautog would 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.

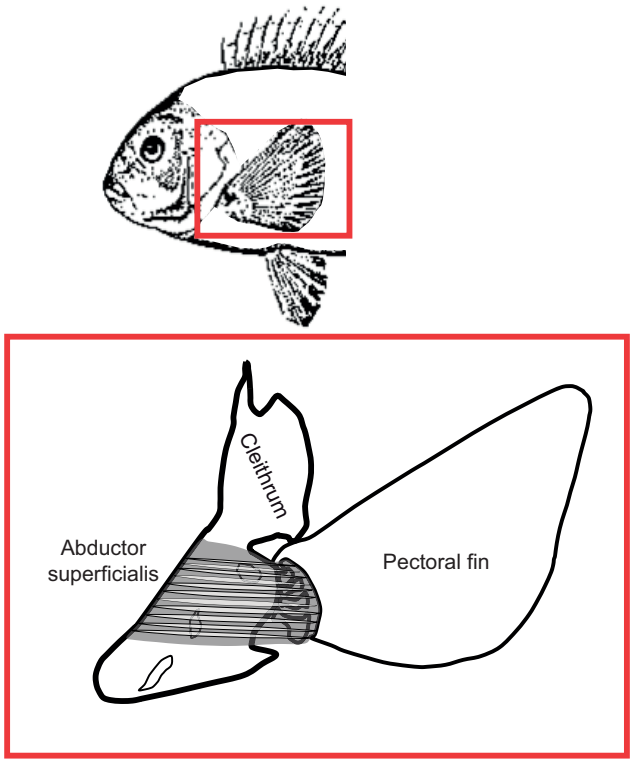

Fig. 1. A line drawing schematic of the location of the abductor superficialis muscle in tautog (pictured) and cunner. Approximate fiber angle is drawn from the cleithrum to the pectoral fin in elongate white ellipses.

MATERIALS AND METHODS

Fish specimens

Cunner [*Tautogolabrus adspersus* (Walbaum 1792)] and tautog [*Tautoga onitis* (Linnaeus 1758)] 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 the Connecticut Department of Energy and Environmental Protection collection permit SC-18005b. Animal care was approved by Fairfield University's IACUC protocol number 15-002. Once captured, fishes were transported to Fairfield University, where they were kept in 34 and 1514 liter holding tanks of artificial seawater. For immunohistochemistry controls, separate fish ('natural') were collected and killed with a blow to the head and double pithing within 24 h to mitigate the affects of thermal acclimation. Holding tank temperatures were controlled by chillers (JBJ Artica) according to the 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 4 weeks. This acclimation period was determined to be appropriate because acclimation can occur over periods much shorter than 4 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 3 weeks (Sidell et al., 1973). Experimental cunner were 16.5±1.8 cm (mean±s.d.) standard length (SL), whereas tautog were 28±5.6 cm SL. Fish were fed tri-weekly using live Asian shore crabs, sandworms and cut squid.

Muscle performance

Tautog and cunner were killed 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 g l^{-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 approximately three 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 25 G disposable hypodermic needle and a hook was 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, Canada). Surrounding the bath was a recirculating water bath, which drew from and returned to a temperature-controlled 19 liter aquarium. The length of the muscle was adjusted to register 10 mN of passive tension. Following a 0.5 h 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 the temperature was changed using a chiller. A 0.5 h acclimation period was allowed for each change in temperature.

Following the acclimation period, each muscle underwent multiple tetanic contractions to find optimal length (L_O). Tetanic contractions were elicited using a pulse frequency of 125 Hz 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 was found, a twitch was run to determine time to maximum isometric contraction, time to

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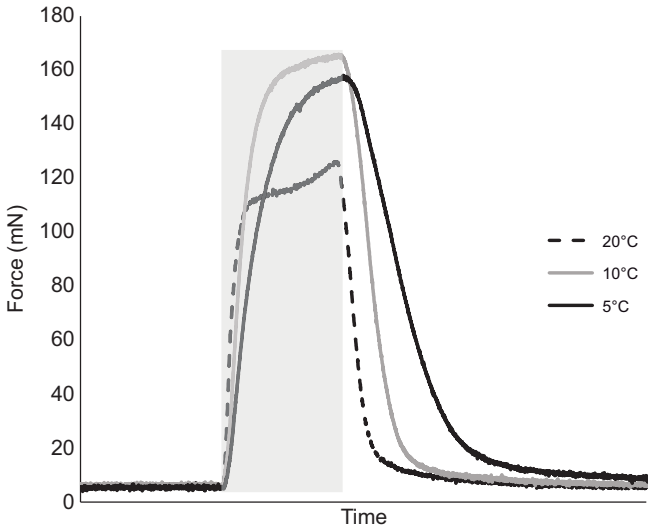

Fig. 2. Representative traces of tetanic contractions demonstrates the force plateau characteristic of tetanic contractions in abductor superficialis muscle from a warm acclimated cunner. The gray box represents the duration of applied stimulus. Listed temperatures indicate testing temperature. Electrical stimuli of 200 mA were used for all contractions represented here.

half relaxation (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 3 min between contractions. Following ramp tests contraction velocity was recorded and standardized to muscle length (L_M). 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.):

$$V = \frac{b\left(1 - \left(\frac{P}{P_o}\right)\right)}{a + \left(\frac{P}{P_o}\right)} + c\left(1 - \frac{P}{P_o}\right), \quad (1)$$

where V is velocity in L_M s^{-1}; P/P_o is force as a proportion of maximum isometric force; and a, b and c are all constants. Following the last test, muscle fiber length was measured while held at L_O.

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 and 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 min between cycles of five workloops. Using the Aurora Scientific 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 1 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 5 h. 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 µmol l^{-1} using a Microm HM505 mol l^{-1} cryostat and set on Fisher Superfrost Plus slides. The slides were dried for 2 h on a slide warmer and stored at −20°C. For a positive control, slides were labeled using the anti-myosin antibody MF20, which binds to all MyHC (Coughlin and Akhtar, 2015). Slow myosin content of muscle was labeled using the anti-slow MyHC antibody S58. Primary antibodies were obtained from the Developmental Studies Hybridoma Bank (Iowa City, IA, USA). 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 Scientific, 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. Following three 5 min washes with PBT, samples were mounted in 50% glycerol in 1×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 ANOVA was used for each treatment temperature/frequency using SPSS v.25 (IBM Corporation, Armonk, NY, USA). Data were 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 that data were normally distributed. 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

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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), whereas 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).

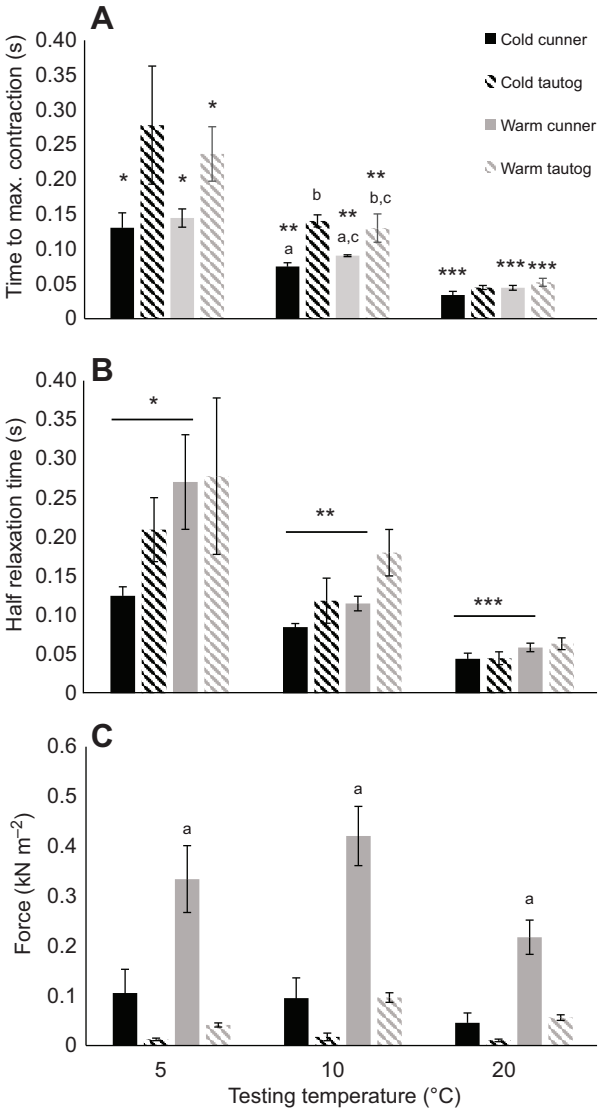

Fig. 3. Mean twitch kinematic comparisons among and between testing temperatures and acclimation treatments for the abductor superficialis. (A) Average time to maximum twitch contraction. (B) Average time to half relaxation following a twitch. (C) Average force (per unit PCSA). Lowercase letters denote 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.

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 1 or 2 Hz when tested at 5°C (P<0.001; Table 1, Fig. 5A). Within the 1 Hz 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 that of warm tautog. Warm cunner produced greater power than all other treatment groups. Within 2 Hz, 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 1–5 Hz (P<0.001; Table 1, Fig. 4B). When tested at 1 Hz, 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 1 Hz and all other frequencies. The same relationship described at 1 Hz was observed at 2 Hz. At 3 Hz, cold-acclimated cunner differed from both tautog acclimation groups. At frequencies of 4 and 5 Hz, 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 six frequencies. Warm cunner produced significantly greater power than the other three acclimation groups at every frequency (P≤0.01; 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 MyHC expression in the abductor superficialis when compared with 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 although muscle kinetics slow at cold temperatures, this does not equate to reduced power output by the

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Table 1. Summary of statistics for all analyses

Average metric	d.f.	*F*-value	*P*	*Post hoc*
Twitch force among acclimation groups tested at 5°C	3	11.887	<0.001	WC different than all (Fig. 3C)
Time to max. twitch among acclimation groups tested at 10°C	3	5.767	0.01	CC different than all together, WC different than CT (Fig. 3A)
Twitch force among acclimation groups tested at 10°C	3	10.666	0.001	WC different than all (Fig. 3C)
Twitch force among acclimation groups tested at 20°C	3	15.39	<0.01	WC different than all (Fig. 3C)
Force–velocity among acclimation groups tested at 20°C	3	8.416	0.001	WC different than all (Fig. 4)
Time to max. twitch for cold cunner among testing temperatures	2	25.656	0.01	All different (Fig. 3A)
Time to relax twitch for cold cunner among testing temperatures	2	25.351	<0.001	All different (Fig. 3B)
Time to max. twitch for warm cunner among testing temperatures	2	60.624	0.001	All different (Fig. 3A)
Time to relax twitch for warm cunner among testing temperatures	2	27.121	0.005	All different (Fig. 3B)
Time to max. twitch for warm tautog among testing temperatures	2	16.391	0.001	All different (Fig. 3A)
Power among acclimation groups within 5°C testing temperature (Fig. 5A)				
Within 1 Hz	3	11.055	<0.001	WC different than all, CC different than CT and WC, WT different than WC only (Fig. 5)
Within 2 Hz	3	5.264	0.009	WC different than CT and WT only
Power among acclimation groups within 10°C testing temperature (Fig. 5B)				
Within 1Hz	3	15.536	<0.001	CC different than WC and CT, WC different than all
Within 2 Hz	3	18.653	<0.001	CC different than WC and CT, WC different than all
Within 3 Hz	3	18.325	<0.001	CC and WC different than all
Within 4 Hz	3	10.842	0.001	WC different than all only
Within 5 Hz	3	6.22	0.007	WC different than all only
Power among acclimation groups within 20°C testing temperature (Fig. 5C)				
Within 1Hz	3	9.019	0.001	WC different than all
Within 2 Hz	3	5.097	0.01	WC different than all
Within 3 Hz	3	15.156	<0.001	WC different than all
Within 4 Hz	3	18.331	<0.001	WC different than all
Within 5 Hz	3	19.457	<0.001	WC different than all
Within 6 Hz	3	19.279	<0.001	WC different 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.

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.

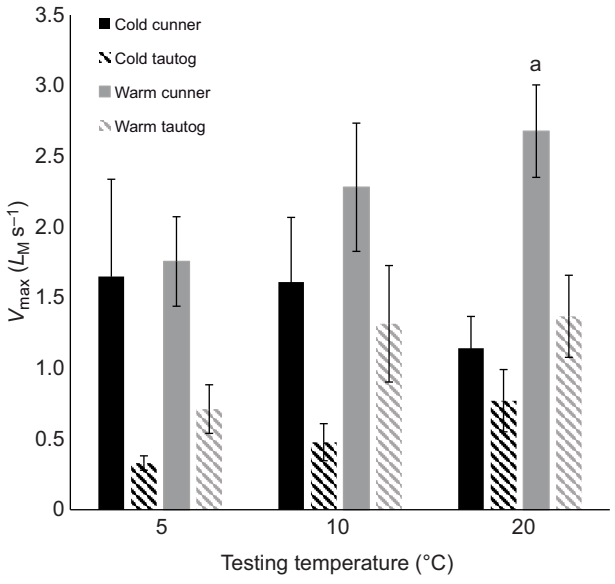

Fig. 4. Comparison of mean maximum contraction velocity (in muscle lengths s^{-1}) between testing temperature and within testing temperature among acclimation groups for the abductor superficialis. Lowercase letters denote within testing temperature differences among acclimation groups as determined by ANOVA (P<0.05). Error bars represent s.e.m. n=5/treatment.

Tautog showed the typical downregulation 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 with 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 a 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

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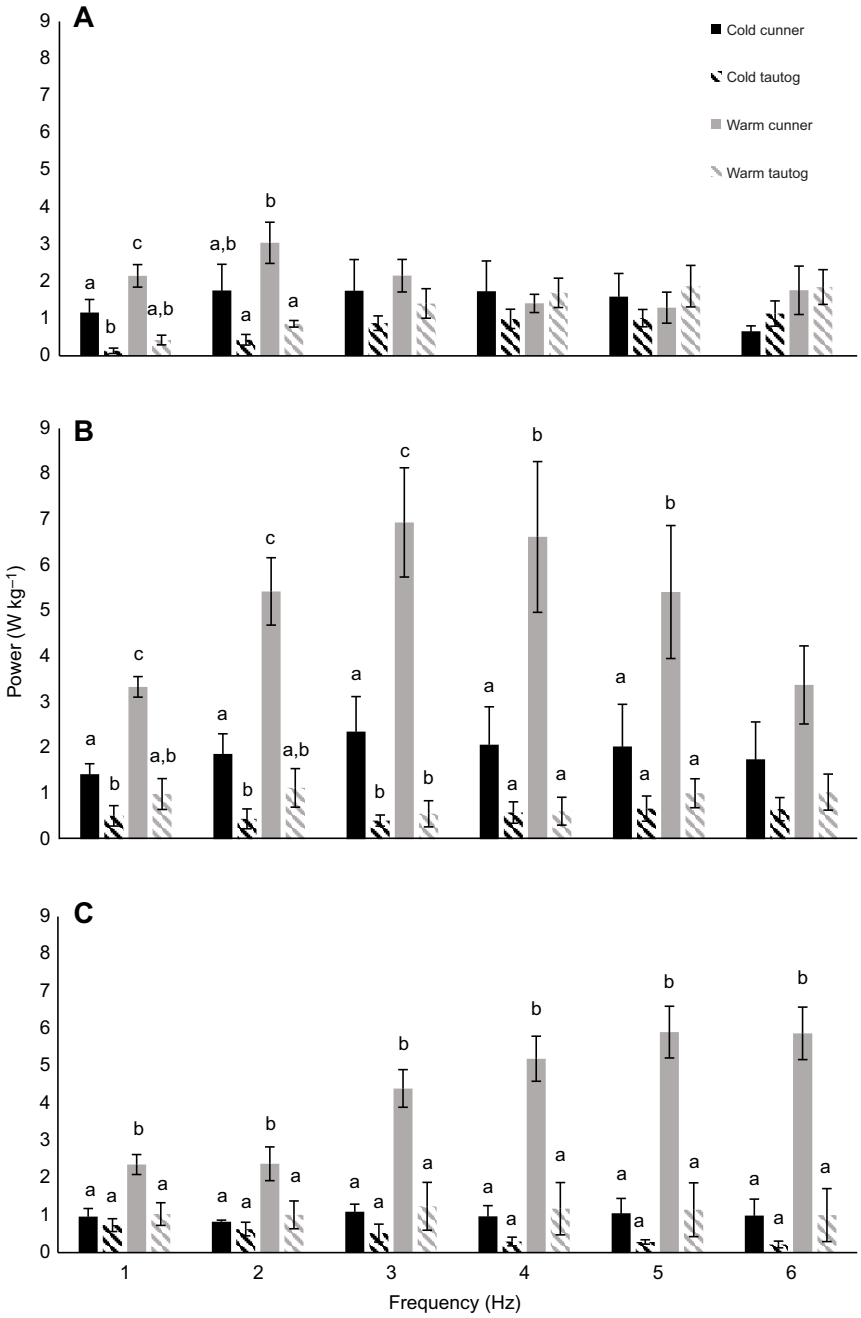

Fig. 5. Mean power production for each testing and treatment temperature for the abductor superficialis. (A) 5°C; (B) 10°C; (C) 20°C. Lowercase letters denote differences among acclimation treatments within a frequency as determined by ANOVA (P<0.05). Error bars represent s.e.m. n=5/treatment.

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 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 downregulation of MyHC, the data presented here support the assertion that torpor is a survival strategy to overwinter in cold waters. Although there has been 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 show 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

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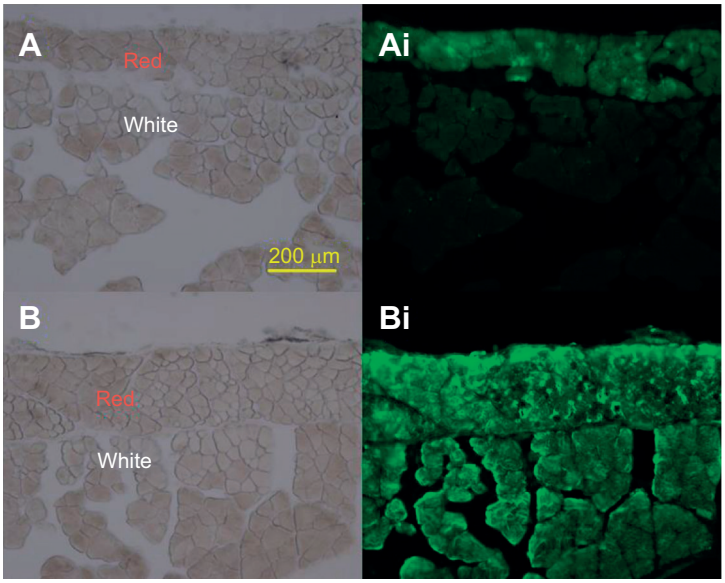

Fig. 6. Anti-myosin heavy chain antibodies allow for determination of fiber type. White light (A,B) and fluorescence (Ai,Bi) images are from myotomal muscle from cunner. Section is oriented with the superficial red or slow-twitch muscle above the deeper white or fast-twitch muscle. In Ai, antibody S58 binds to slow MyHC in the superficial red muscle layer. In Bi, antibody MF20 serves as a positive control binding to myosin in both red and white muscle.

thermal constraints on the operating frequencies of muscle at these cold temperatures. These thermal constraints can provide insight into the ecology and behavior 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 presented an optimum frequency for the 20°C treatment group of 4.6 Hz, which is similar to the 5–6 Hz at which peak power was observed in the present study for warm-acclimated cunner tested at 20°C (Fig. 5). Similarly, when tested at 10 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 and 5°C from Moran et al. (2019). These results further support the behavioral transition of cunner into torpor at temperatures approaching 5°C. Torpor allows this species to overwinter near their year-round habitats without having to undergo

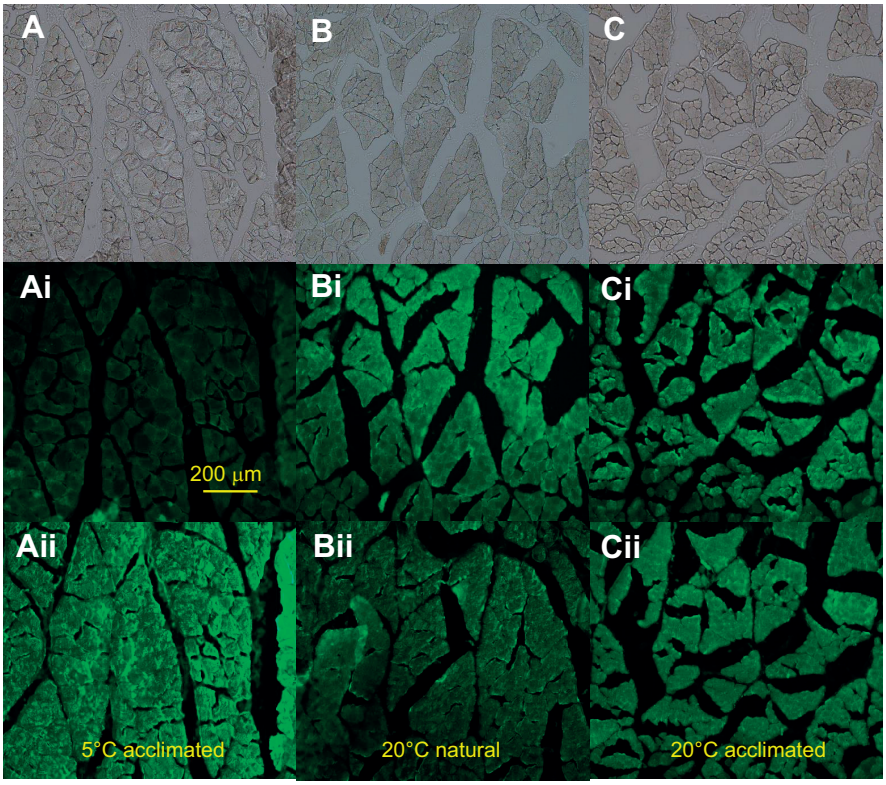

Fig. 7. Representative images of tautog immunohistochemistry reflecting S58 (Ai–Ci) and MF20 (Aii–Cii) activity. White light images are represented by the top row (A–C). All pictures are of abductor superficialis cross sections. The 'natural' sample was taken from fish taken directly from the wild and not acclimated to a controlled thermal regime. These results were consistent with every tautog tested in this study.

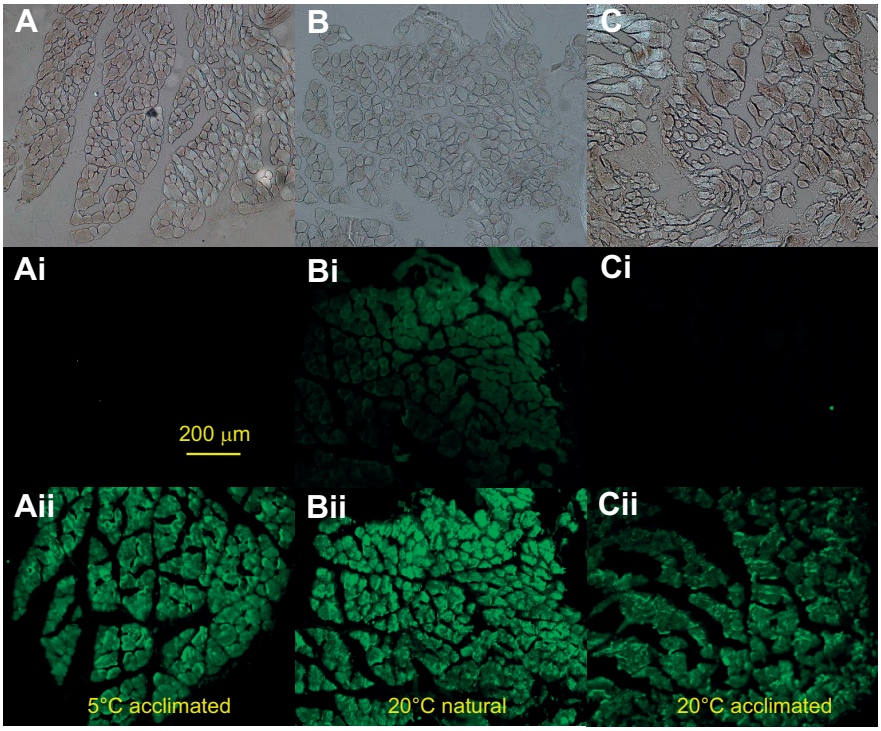

Fig. 8. Representative images of cunner immunohistochemistry reflecting S58 (Ai–Ci) and MF20 (Aii–Cii) activity. White light images are represented by the top row (A–C). All pictures are of abductor superficialis cross sections. The 'natural' sample was taken from fish taken directly from the wild and not acclimated to a controlled thermal regime. These results were consistent with every cunner tested in this study.

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 remain 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.

Although the data presented here have 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 this will affect fisheries and ecosystem processes.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: C.J.M., S.G.; Methodology: C.J.M., D.C., S.G.; Validation: C.J.M., K.J., L.T., D.C., S.G.; Formal analysis: C.J.M., K.J., L.T., D.C., S.G.; Investigation: L.T., D.C.; Resources: K.J.; Data curation: C.J.M., K.J., L.T., D.C.; Writing - original draft: C.J.M.; Writing - review & editing: C.J.M., D.C., S.G.; Visualization: C.J.M., L.T., D.C., S.G.; Supervision: C.J.M.; Project administration: S.G.; Funding acquisition: S.G.

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