

The effects of acute temperature change on swimming performance in bluegill sunfish *Lepomis macrochirus*

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SUMMARY

Many fish change gait within their aerobically supported range of swimming speeds. The effects of acute temperature change on this type of locomotor behavior are poorly understood. Bluegill sunfish swim in the labriform mode at low speeds and switch to undulatory swimming as their swimming speed increases. Maximum aerobic swimming speed (U_{max}), labriform-undulatory gait transition speed (U_{trans}) and the relationships between fin beat frequency and speed were measured at 14, 18, 22, 26 and 30°C in bluegill acclimated to 22°C. At temperatures below the acclimation temperature (T_a), U_{max} , U_{trans} and the caudal and pectoral fin beat frequencies at these speeds were reduced relative to the acclimation level. At temperatures above T_a there was no change in these variables relative to the acclimation level. Supplementation of oxygen levels at 30°C had no effect on swimming performance. The mechanical power output of the abductor superficialis, a pectoral fin abductor muscle, was measured *in vitro* at the same temperatures used for the swimming experiments. At and below T_a , maximal power output was produced at a cycle frequency approximately matching the *in vivo* pectoral fin beat frequency. At temperatures above T_a muscle power output and cycle frequency could be increased above the *in vivo* levels at U_{trans} . Our data suggest that the factors triggering the labriform-undulatory gait transition change with temperature. Muscle mechanical performance limited labriform swimming speed at T_a and below, but other mechanical or energetic factors limited labriform swimming speed at temperatures above T_a .

Key words: gait transition, muscle mechanics, swimming, temperature.

INTRODUCTION

Environmental temperature changes profoundly affect the locomotor performance of ectotherms (e.g. Rome and Alexander, 1970; Casey, 1980; Herreid et al., 1981; Rome, 1982; Hirano and Rome, 1984; Marsh and Bennett, 1986b). This is due to a number of interacting factors. Temperature-related changes in muscle contraction velocity affect mechanical power output (Josephson, 1984; Marsh and Bennett, 1986a; Langfeld et al., 1989; Stevenson and Josephson, 1990; Rome and Swank, 1992; Rome et al., 1992; Altringham and Block, 1997). Temperature changes also impact the performance of the respiratory and cardiovascular systems and their ability to support aerobic metabolism (e.g. Randall and Brauner, 1991; Driedzic and Guesser, 1994; Farrell, 2002; Farrell, 2007; Stecyk et al., 2007). In fish, the effects of seasonal temperature changes on locomotor performance can largely be offset by acclimation of the muscular, respiratory and cardiovascular systems (Rome et al., 1985; Rome and Swank, 1992; Rome et al., 1992; Keen and Farrell, 1994; Beddow and Johnston, 1995; Egginton and Cordiner, 1996; Taylor et al., 1996; Johnston and Temple, 2002; Rome, 2007). Some species, however, must cope with more acute temperature shifts. Fish in temperate freshwater lakes are exposed to a wide range of temperatures, particularly after the establishment of thermoclines in the spring (Shuter et al., 1983; Snucins and Gunn, 1995; Fee et al., 1996). Movement between the warm shallows and cooler, deeper water related to foraging, breeding or predator avoidance can expose fish to rapid temperature changes, which probably affect locomotor performance.

The bluegill sunfish *Lepomis macrochirus* is a temperate, freshwater species potentially exposed to a wide range of water

temperatures. In New England lakes, bluegill breed in shallow water where the temperature can approach 30°C, but even in the summer can also be exposed to temperatures below the thermocline as low as 10°C (P. Zakova and M. V. Moore, personal communication). This species swims in the labriform mode at low speeds, generating lift and thrust by beating the pectoral fins (Gibb et al., 1994; Drucker and Lauder, 1999; Drucker and Lauder, 2000). As swimming speed increases, bluegill change gait from labriform swimming to combined use of their pectoral fins and undulations of the body axis (Gibb et al., 1994; Jones et al., 2007; Kendall et al., 2007). Gait changes may maximize locomotor economy and/or be driven by mechanical factors such as the limits to muscle power or the need to minimize mechanical stresses (Alexander, 1989). When swimming at their acclimation temperature (T_a), the labriform-undulatory gait change in bluegill is primarily driven by the need to recruit additional muscle mass to meet the power requirements of higher speed swimming (Kendall et al., 2007). In ectotherms, environmental temperature is an additional factor determining gait transition speed. An early recruitment of anaerobic muscle at low temperatures has been identified during undulatory fish swimming (Rome et al., 1992), but the effects of temperature on transitions between different styles of aerobically supported swimming have not been quantified. Understanding how temperature affects gait transitions of this type is important because the speeds at or around the gait transition appear to coincide with the minimum total cost of transport in a number of species, including bluegill (Brett and Sutherland, 1965; Korsmeyer et al., 2002; Kendall et al., 2007). Temperature-related changes in gait transition speed are therefore likely to be significant for the energy budgets of gait transitioning species.

Previous work has largely focused on maximal swimming performance, which typically plateaus, and ultimately declines as temperature increases above T_a (Fry and Hart, 1948; Randall and Brauner, 1991; Myrick and Cech, 2000; Ojanguren and Braña, 2000; Lee et al., 2003; Claireaux et al., 2006), likely due to limitations on cardiovascular performance at high temperatures (Randall and Daxboeck, 1982; Jain and Farrell, 2003; Farrell, 2002; Farrell, 2007). Similar constraints are unlikely to apply to the labriform-undulatory gait transition in bluegill as this occurs at less than one third of their maximal aerobic capacity (Kendall et al., 2007). We therefore hypothesized that the relationships of labriform-undulatory gait transition speed (U_{trans}) and maximum aerobic swimming speed (U_{max}) to water temperature would be different from one another, particularly at high temperatures. U_{trans} was expected to increase relative to the acclimation level at temperatures above T_a because of enhanced muscle mechanical performance in the absence of central or external limits to oxygen and substrate delivery. By contrast, U_{max} was not expected to increase at temperatures above the acclimation temperature because of central or external limitations on the capacity of the cardiovascular system to support increased aerobic metabolism. We also hypothesized that as in previous studies carried out at T_a in this species (Kendall et al., 2007), the availability of muscle power from the pectoral girdle muscles would be the primary factor determining U_{trans} at all temperatures. In order to test these hypotheses we determined U_{trans} , U_{max} and pectoral girdle muscle power output at a range of water temperatures.

MATERIALS AND METHODS

Overall experimental approach

We determined U_{trans} , U_{max} and the pectoral and caudal fin beat frequencies at both speeds in bluegill sunfish swimming in air-saturated water at 14, 18, 22, 26 and 30°C. Swimming performance and fin beat frequencies were also measured at 30°C in water with the dissolved oxygen concentration raised above air-saturation levels using 100% oxygen. This allowed us to determine whether external oxygen levels imposed a limit on U_{trans} and U_{max} at high water temperatures. We carried out an additional set of experiments to determine whether pectoral girdle muscle mechanical performance was limiting U_{trans} . The effects of temperature on the mechanical performance of the abductor superficialis, a muscle powering the pectoral fin down stroke, were determined *in vitro*. Power output was measured using the work loop technique (Josephson, 1985). Data were collected at the same temperatures used in the swimming experiments in physiological saline saturated with oxygen.

Animals

Bluegill sunfish (*Lepomis macrochirus* Rafinesque) were collected from Lake Waban, MA, USA using hook and line. Kinematic, maximal swimming performance, and muscle power output data were obtained from six fish [mass 152.3±12.8 g, fork length (L) 19.1±0.5 cm, mean ± s.e.m.]. The fish were kept at 22°C under a 12 h:12 h light:dark cycle and fed chopped earthworms twice a week. All procedures were approved by the Institutional Animal Care and Use Committee.

Swimming flume

Swimming experiments were carried out at 14, 18, 22, 26 and 30°C in a sealable, recirculating flume (Model 90, Loligo Systems, Hobro, Denmark) capable of generating flow velocities ranging from 5 to 150 cm s⁻¹. The flume consisted of an inner chamber 88.6 l in volume with a working section of 20×20×70 cm and an outer tank, which buffered temperature changes and served as a reservoir of

oxygenated water. Ambient temperature in the room housing the flume was 22°C. For experimental temperatures below 22°C, water temperature was reduced using a thermostatically controlled aquarium chiller (Arctica, 1/10 Horsepower, Transworld Aquatics, Inglewood, CA, USA) through which water was circulated with a submersible pump. Two 300 W thermostatically controlled aquarium heaters (Hydor, Vicenza, Italy) were used to raise the temperature above 22°C. Swimming experiments were carried out at all temperatures in air saturated water, and additionally at 30°C with the dissolved oxygen content elevated to 12.0 mg l⁻¹ by bubbling 100% oxygen into the water *via* an air stone. Oxygen concentration was monitored using a polarographic oxygen probe inserted through a port in the lid of the flume (Accumet XL40, Fisher Scientific, Pittsburgh, USA). The order in which fish were exposed to the different experimental conditions was randomized.

The large volume of water in the flume meant that rapid temperature changes could not be applied using the heaters or chiller. Rates of temperature change were limited to approximately 1°C per hour. Consequently fish could not be exposed to an instantaneous temperature change while in the flume. Exposure to slow temperature changes was undesirable, as this had the potential to allow for at least partial acclimation to temperatures other than T_a . Netting fish to allow transfer between the holding tanks and flume can induce escape responses and struggling behaviors leading to the buildup of lactic acid due to intense contractions of the anaerobic myotomal muscle (Lackner et al., 1988). To avoid this, fish were removed from holding tanks by gently scooping them into a 5 l plastic container of water. This was immersed in the working section of the flume and slowly rotated to release the fish. This approach allowed the transfer of fish with a minimum of disturbance, reducing the need for lengthy recovery periods after transfer to the flume, while still enabling exposure to acute temperature changes. If a fish became startled and exhibited escape responses or struggling during transfer it was returned to the holding tank and another individual selected for swimming kinematics or performance measurements.

Swimming kinematics

Video sequences were recorded using a Sony HDR HC-3 camcorder at a frame rate of 120 Hz at speeds ranging from 8.4 to a maximum of 51.1 cm s⁻¹ (0.44–2.68 l s⁻¹), the upper limit varying with water temperature. Under each set of conditions speed was increased from 8.4 cm s⁻¹ (0.44 l s⁻¹) in approximately 4 cm s⁻¹ (0.21 l s⁻¹) increments. At each speed the fish was allowed to swim for 5 min before video recording was started. A mirror mounted above the flume at a 45° angle allowed simultaneous recording of lateral and dorsal views of the fish. Video sequences were captured on a Macintosh iMac computer and analyzed using VideoPoint software (Lenox Softworks, Lenox, MA, USA) to determine pectoral and caudal fin beat frequencies during sequences of steady swimming (mean sequence length 25 fin beats). U_{trans} was defined as the first speed at which there was detectable lateral movement of the caudal fin. The same group of fish was used for measurements of maximal swimming performance.

Maximal swimming performance

A protocol involving incremental changes in swimming speed was used to establish U_{max} using the same group of fish for which kinematic data were obtained. Starting at 8.4 cm s⁻¹, speed was increased in 4 cm s⁻¹ increments and maintained at a given level for 10 min between increments. Speed increases continued until the fish could no longer maintain position in the flume. This coincided with the onset of burst and coast swimming behavior and high

amplitude lateral body undulation, probably indicative of the recruitment of anaerobic muscle. The time maintained at the final speed interval (T , in min) was recorded. U_{\max} (in cm s^{-1}) = $U_{\text{fin}} + 4(T/T_{\text{int}})$, where U_{fin} (in cm s^{-1}) was the speed of the final interval at which steady swimming could be maintained and T_{int} was the time interval between speed increments. This test was not equivalent to a critical swimming speed (U_{crit}) test, as these typically involve exercising the fish until complete exhaustion, indicated by an inability to move from the mesh at the rear of the flume working section (Brett, 1964). Our aim was to determine maximal aerobically supported performance in a similar manner to Claireaux et al. (Claireaux et al., 2006). Speeds approaching U_{crit} involve the recruitment of anaerobic muscle (Burgetz et al., 1998; Lurman et al., 2007), elevating performance above sustainable, aerobic levels. A standard U_{crit} protocol was therefore not compatible with our experimental goals.

Muscle power measurements

The maximum mechanical power output of the largest muscle powering the pectoral fin downstroke (abductor superficialis) was measured *in vitro* using the work loop technique (Josephson, 1985). This technique measures the mechanical power output of cyclically operating muscles under conditions that mimic those experienced by the muscle *in vivo*. Experimental protocols were similar to those previously used to measure pectoral girdle muscle power output in this species (Jones et al., 2007; Kendall et al., 2007). Fish were anesthetized using buffered MS-222 solution at a concentration of 100 mg l^{-1} , and then an L-shaped incision was made in the skin overlying the pectoral girdle muscles, and the skin bluntly dissected from the underlying musculature to expose the abductor superficialis. A loop of silk suture was tied to the distal tendon of a muscle fascicle and the tendon cut distal to the knot. The fascicle was gently freed from the surrounding muscle tissue, the section of the cleithrum around the origin of the fascicle was cut and the intact fascicle removed to a dish of chilled, oxygenated physiological saline at 5°C . The saline contained (in mmol l^{-1}) 109 NaCl, 2.7 KCl, 1.8 CaCl_2 , 0.47 MgCl_2 , 2.5 NaHCO_3 , 5.3 sodium pyruvate and 10.0 Hepes, pH 7.4 at 22°C .

Muscle power measurements were made using a muscle ergometer (300B-LR, Aurora Scientific, Aurora, Ontario, Canada). This controlled muscle length and measured force while the muscle was stimulated electrically (701B, Bi-Phase Current Stimulator, Aurora Scientific). Sinusoidal length change cycles were applied to the fascicle. A sinusoid is a reasonable approximation of the strain patterns experienced by the muscle fascicles *in vivo* (Jones et al., 2007; Kendall et al., 2007). The frequency, amplitude and relative timing and duration of stimulation were controlled using Dynamic Muscle Control software (Version 4.0, Solwood Enterprises Inc., Blacksburg, VA, USA). The force and position data were captured to a PC via a 604A A to D interface, (Aurora Scientific) and a PCI A to D card (PCI-6503, National Instruments, Austin, TX, USA). The net work done per cycle was calculated using Dynamic Muscle Analysis software (Version 3.12, Solwood Enterprises Inc., Blacksburg, VA, USA).

The temperature of the tissue chamber was controlled by circulating water from a Neslab RTE7 thermostatically controlled water bath (Thermo Electric, Chicago, IL, USA) through the water jacket and it was monitored with a thermocouple thermometer (EasyView 10, Exttech, Waltham, MA, USA). Power measurements were made at the same temperatures for which kinematic data were obtained in the flume (14, 18, 22, 26 and 30°C). For a given fascicle the order of progression through the temperatures was randomized. On switching

to a new temperature the fascicle was allowed 5 min rest to ensure that the temperature within the chamber had stabilized. At each temperature an initial set of control work loops was applied to the fascicle. Subsequently, at the fin beat cycle frequency used during maximal labriform swimming at that temperature, muscle strain and relative timing and duration of activation were systematically changed until the maximum power output was measured. Power was then measured at progressively higher cycle frequencies (increased at 0.4 Hz intervals) until a decline in power output was measured. Muscle strain and relative timing and duration of activation were again systematically changed to maximize power output. Optimal strain and stimulus parameters were similar to those previously established for this muscle (Jones et al., 1997). Finally, the control work loops were repeated. Before switching to the next experimental temperature the chamber was brought to the same temperature as was used for the initial set of control work loops at the start of the experiment, and the control loops repeated. This allowed us to control for changes in performance at a given temperature, and across temperatures. The decline between controls was used to correct the power outputs measured during the intervening work loops, assuming a linear change in work output between controls. Data collection was terminated if power output declined by 10% relative to the initial control. After completion of the power measurements, the connective tissue and bone were removed from the fascicle, and the muscle tissue weighed. Power outputs were measured from six muscle fascicles in total, one from each of the fish used for the collection of swimming performance data.

A number of muscles contribute to powering movements of the pectoral fins in labriform swimmers (Drucker and Jensen, 1997; Westneat and Walker, 1997; Lauder et al., 2006; Jones et al., 2007). The abductor superficialis is the largest fin abductor and constitutes 36% of the total muscle mass likely supplying positive power during steady labriform swimming in bluegill (Jones et al., 2007). Its main antagonist, powering the upstroke, is the adductor profundus, constituting another 38% of the power supplying muscle mass. We have not obtained work loop data in relation to changing temperature for this muscle. However, the basic contractile properties, power outputs, and range of operating frequencies are similar in both muscles (Jones et al., 2007). The effects of temperature on the isometric properties of both muscles are also similar (D.J.E., unpublished data). Given these similarities it is reasonable to use the changing mechanical performance of the abductor superficialis in relation to temperature as an indicator of the likely temperature-related changes in the other major power producing muscle.

Statistical analyses

All statistical analyses were carried out using SPSS (Version 14.0, SPSS, Chicago, USA). An individual fish identifier was included in all statistical models as a random factor. General linear models were used to test for differences in U_{trans} and U_{max} between different temperature and oxygen treatments, with temperature and oxygen treatment included in the models as a fixed factor. Similar models, with pectoral and caudal fin beat frequency as the dependent variables, were used to test for differences in these variables between different temperature and oxygen treatments. A general linear model (GLM) was also used to test for differences in abductor superficialis power output in relation to changing temperature and cycle frequency. Temperature and cycle frequency were included in the model as fixed factors. For the muscle power data obtained at a given temperature, power output at higher strain cycle frequencies was compared to that at the *in vivo* cycle frequency using a simple contrasts procedure. This tested whether

there was a significant change in muscle power output at higher cycle frequencies relative to that used *in vivo*. All data are shown as mean \pm s.e.m.

RESULTS

Swimming performance

After the onset of undulatory swimming, caudal fin beat frequency initially increased with swimming speed at a given temperature, but tended to level off as the maximal undulatory swimming speed was approached (Fig. 1). There were significant changes overall in both caudal fin beat frequency during maximal sustained undulatory swimming (GLM, $F=11.52$, $P<0.001$) and maximal sustained undulatory swimming speed (GLM, $F=57.01$, $P<0.001$). At the two lower temperatures, maximal swimming performance and caudal fin beat frequency were reduced relative to performance at T_a (22°C). Caudal fin beat frequency and maximal sustained undulatory swimming speed did not change with increasing temperature relative to their levels at T_a (Fig. 2A,B). This pattern is similar to that previously observed in a number of fish species (Fry and Hart, 1948; Randall and Brauner, 1991; Myrick and Cech, 2000; Ojanguren and Braña, 2000; Lee et al., 2003; Claireaux et al., 2006). Increasing the water oxygen concentration with 100% oxygen at 30°C did not result in detectable changes in maximal swimming speed relative to that measured in air-saturated water at 30°C (Fig. 2A,B). This is similar to the pattern observed in salmonids, where U_{crit} is the same in hyperoxic and normoxic water (Davis et al., 1963; Jones, 1971).

Pectoral fin beat frequency increased approximately linearly with swimming speed at all temperatures (Fig. 3). After the gait transition, for a given temperature, there was little or no further increase in pectoral fin beat frequency. This was similar to the relationship between fin beat frequency and speed previously observed in this species (Gibb et al., 1994; Drucker and Lauder, 1999; Jones et al., 2007). There were significant changes in maximal labriform swimming speed in relation to changing temperature and oxygen

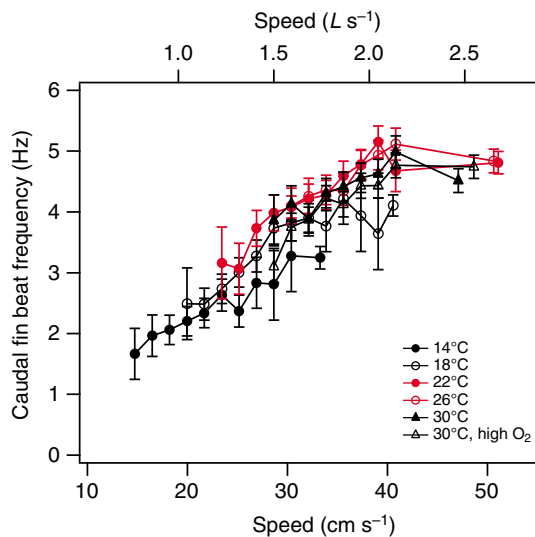


Fig. 1. Relationship between caudal fin beat frequency and swimming speed in bluegill sunfish at a range of water temperatures. All data were collected in air-saturated water except for the high O_2 condition at 30°C where oxygen levels were elevated to 12 mg l^{-1} , using 100% oxygen. Data are shown as mean \pm s.e.m. ($N=6$, air saturated oxygen concentrations; $N=5$, elevated oxygen concentration 30°C).

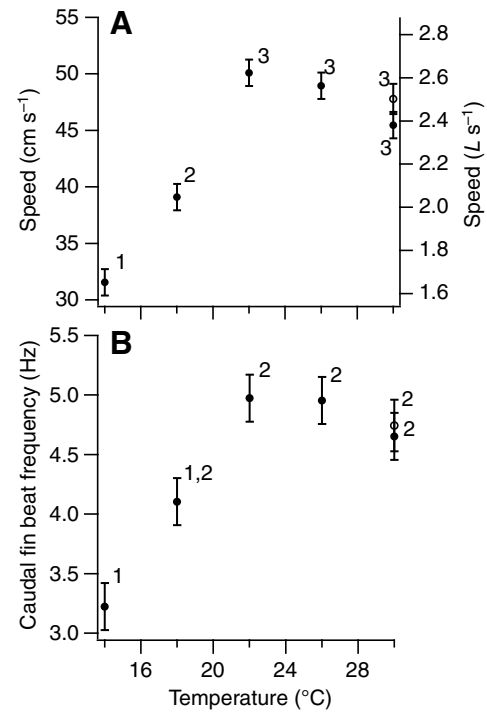


Fig. 2. (A) Relationship between maximal undulatory swimming speed and water temperature. (B) Relationship between caudal fin beat frequency at maximal undulatory swimming speed and water temperature. Filled symbols, air-saturated water; open symbol elevated oxygen concentration at 30°C. Data are shown as mean \pm s.e.m. ($N=6$, air saturated oxygen concentrations; $N=5$, elevated oxygen concentration 30°C). Numbers beside symbols denote homogenous subsets as established by Scheffé *post-hoc* analyses ($P>0.05$).

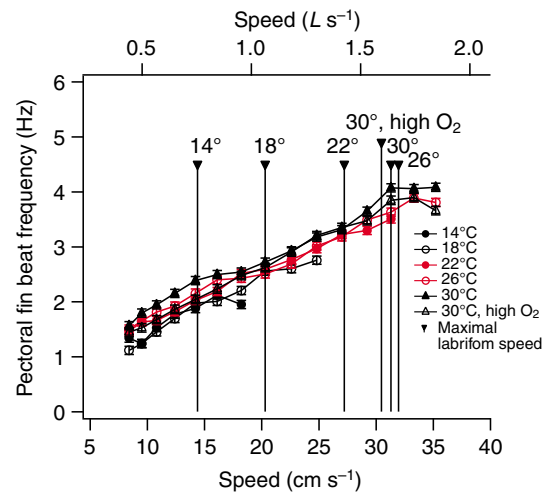


Fig. 3. Relationship between pectoral fin beat frequency and swimming speed in bluegill sunfish at a range of water temperatures. All data were collected in air saturated water except for the high O_2 condition at 30°C, where oxygen levels were elevated to 12 mg l^{-1} using 100% oxygen. Data are shown as mean \pm s.e.m. ($N=6$, air saturated oxygen concentrations; $N=5$, elevated oxygen concentration 30°C).

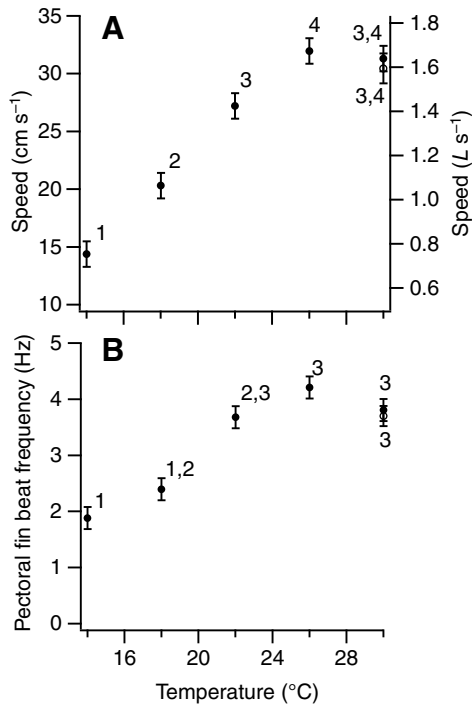


Fig. 4. (A) Relationship between maximal labriform swimming speed and water temperature. (B) Relationship between pectoral fin beat frequency at maximal labriform swimming speed and water temperature. Filled symbols, air saturated water; open symbol elevated oxygen concentration at 30°C. Data are shown as mean \pm s.e.m. ($N=6$, air saturated oxygen concentrations; $N=5$, elevated oxygen concentration 30°C). Numbers beside symbols denote homogenous subsets as established by Scheffé *post-hoc* analyses ($P>0.05$).

treatment (Fig. 2A; GLM, $F=59.52$, $P<0.001$). There were also significant changes in pectoral fin beat frequency at the gait transition speed (Fig. 4B; GLM, $F=21.00$, $P<0.001$). Initially, both gait transition speed and fin beat frequency increased with increasing temperature. Raising the temperature above 22°C did not result in further detectable increases in either variable relative to their values at that temperature (Fig. 4A,B). Increasing the water oxygen concentration with 100% oxygen at 30°C did not result in detectable changes in either gait transition speed or fin beat frequency relative to those measured in air saturated water at 30°C (Fig. 4A,B).

Muscle mechanical performance

Isometric tetanic stress (175 ± 4.8 kN m⁻²), and twitch times at 22°C [twitch rise time (T_r), 40.9 ± 2.2 ms; time from peak force to 90% relaxation (T_{90}), 118.4 ± 3.6 ms] were similar to those previously measured in this muscle at the same temperature (Jones et al., 2007). Peak tetanic stress did not change significantly in relation to changing temperature (GLM, $F=2.7$, $P>0.05$). Both T_r and T_{90} changed significantly with temperature (GLM, $F=50.1$, $P<0.001$, T_r ; GLM, $F=44.0$, $P<0.001$, T_{90}). T_r ranged from 58.0 ± 2.9 to 28.0 ± 2.1 ms, and T_{90} from 190.1 ± 4.6 and 79.1 ± 2.8 ms between 14 and 30°C. The overall Q_{10} for twitch rise times was approximately 1.9 from 14 to 30°C, similar to that observed in other vertebrate muscles across a similar temperature range (e.g. Bennett, 1985; Marsh and Bennett, 1986a; Langfeld et al., 1989). All values for isometric properties are means \pm s.e.m., $N=6$.

Representative fascicle stress, strain and work loop traces from an abductor superficialis fascicle are shown in Fig. 5. Work loop

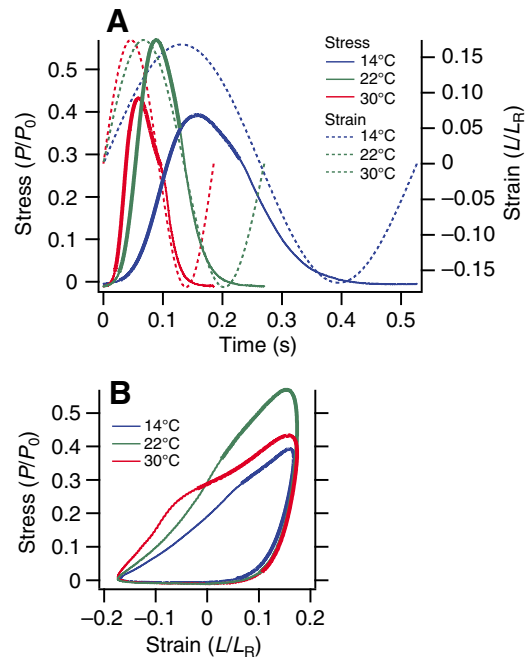


Fig. 5. *In vitro* mechanical performance of a bluegill sunfish abductor superficialis fascicle at a range of temperatures. (A) Applied stimulus and strain and the resulting fascicle stress. Broken lines show fascicle strain, unbroken lines show stress. (B) Work loops showing fascicle stress plotted in relation to strain. In both plots the thickened portions of the stress traces show when the fascicle was being stimulated. The stimulus onsets and durations were those that produced maximal power output. Strains are shown relative to resting length, L_R . Stresses are shown relative to peak isometric tetanic stress, P_0 . Data are representative traces from a single fascicle.

shape changed with temperature (Fig. 5B). As relaxation times decreased with increasing temperature the fascicles could be stimulated for a greater proportion of shortening (Fig. 5B). This resulted in more sustained stress production during shortening, and

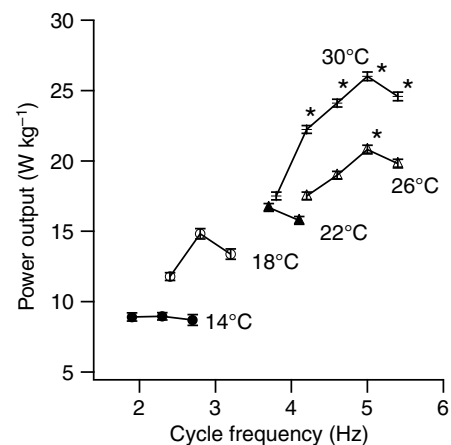


Fig. 6. Relationship between abductor superficialis power output and cycle frequency at a range of temperatures. Data are shown as mean \pm s.e.m. ($N=6$). Values marked with an asterisk are significantly different from the *in vitro* cycle frequency within that temperature treatment ($P<0.05$). Within the data for each temperature, the lowest cycle frequency is equal to the *in vivo* pectoral fin beat frequency.

enhancement of positive work output. Mean net mass specific power outputs of the abductor superficialis fascicles are shown in Fig. 6. Overall there were significant changes in power output in relation to changing temperature (GLM, $F=56.8$, $P=0.001$) and cycle frequency (GLM, $F=17.44$, $P<0.001$). Within the temperature treatments, no change in power output relative to the *in vivo* cycle frequency with increasing cycle frequency were detected at 14, 18 and 22°C ($P>0.05$ for all contrasts). At 26°C power output was significantly higher at a cycle frequency of 5.0 Hz, relative to the *in vivo* frequency of 4.2 Hz ($P=0.047$). At 30°C power output was significantly higher at all higher cycle frequencies relative to that at the *in vivo* cycle frequency of 3.8 Hz ($P<0.001$ for all contrasts).

DISCUSSION

During aerobically powered locomotion the limits to performance are set by a number of factors. In swimming fish, muscle mechanical power is transferred to the water to generate lift and thrust (Rome et al., 1993; Altringham and Ellerby, 1999; Drucker and Lauder, 1999; Coughlin, 2002). The availability of muscle power may therefore limit maximum swimming and gait transition speeds (Korsmeyer et al., 2002; Kendall et al., 2007). The capacity to deliver oxygen and substrates to active muscle, or the muscle to utilize these in the mitochondrial respiratory chain, may also set performance limits (Weibel et al., 1996). This can be due to external factors such as oxygen levels in the surrounding environment, or internal factors such as the rates at which the respiratory surface and cardiovascular system can take up and deliver oxygen (Weibel et al., 1996; Sollid et al., 2005; Farrell, 2002; Farrell, 2007). Determining which factors limit performance in a given locomotor system requires an integrated approach, measuring performance at both the whole organism and tissue levels. By measuring both swimming performance and pectoral girdle muscle power output at a range of temperatures, we have determined how temperature affects both maximal aerobic performance, and a low-speed gait transition in bluegill sunfish. We have also shown that the physiological and mechanical factors limiting the gait transition speed change with temperature.

Most previous studies of the effects of temperature on fish swimming have focused on maximal aerobic performance (Fry and Hart, 1948; Griffiths and Alderdice, 1972; Randall and Brauner, 1991; Myrick and Cech, 2000; Ojanguren and Braña, 2000; Lee et al., 2003; Claireaux et al., 2006). Bluegill exhibited a typical teleost relationship between maximal aerobic swimming speed (U_{\max}) and temperature. At low temperatures, swimming performance was impaired relative to that at the acclimation temperature (Fig. 1, Fig. 2A,B), probably because of a reduction in myotomal muscle contraction velocity and power output (Figs 5 and 6) (Rome, 1990; Rome et al., 1992). Raising the water temperature above the acclimation temperature did not change U_{\max} or the caudal fin beat frequency used at that speed, relative to their acclimation levels (Fig. 1, Fig. 2A,B), despite the potential for enhanced myotomal muscle power output. The dissolved oxygen levels in air-saturated water at 30°C are approximately 70% of those at 14°C (Weiss, 1970). It has been suggested that this may constitute an external limit on oxygen availability at high temperatures, limiting performance (Brett, 1971). Elevated oxygen levels did not increase swimming performance at 30°C (Fig. 1, Fig. 2A,B) suggesting that this was not a factor in the present study. In the absence of external limits of this type, the capacity of the cardiovascular system to maintain systemic oxygen delivery has been identified as a major factor determining

maximal aerobic performance at high temperatures (Farrell, 2002; Farrell, 2007). At high temperatures, cardiac performance may be limited by the oxygen supply to the heart. In many fish the venous blood returning to the heart supplies oxygen to the cardiac muscle in the absence of an extensive coronary circulation. Increased oxygen extraction by skeletal muscle at high temperatures and the resulting reduction in the oxygen content of the venous blood may therefore limit cardiac output (Farrell, 2002). Even if sufficient environmental oxygen is available, the capacity for oxygen uptake at the gills or oxygen and substrate delivery by the cardiovascular system may therefore be insufficient to meet the increased metabolic demands of locomotor muscle at high temperatures (Randall and Daxboeck, 1982; Jain and Farrell, 2003; Farrell, 2002; Farrell, 2007).

Studies of maximal swimming have provided valuable insight into which factors limit aerobic performance in fish with respect to changing temperature. Most locomotor behavior, however, involves sub-maximal energy expenditure. The extent to which temperature changes affect routine locomotor behavior in fish is less well understood. Many fish gait transitions fall into this category of routine behaviors, occurring at relatively low levels of aerobic activity (Brett and Sutherland, 1965; Parsons and Sylvester, 1992; Korsmeyer et al., 2002; Cannas et al., 2006; Kendall et al., 2007). This means that the factors relating to oxygen uptake and delivery that limit U_{\max} are unlikely to apply to U_{trans} . Peripheral factors, such as oxygen and substrate delivery at the muscle level and the relationship between muscle mechanical performance and temperature, rather than systemic maxima for blood flow and respiratory exchange rates, are likely to be significant in determining performance. This led us to hypothesize that, because of the potentially different constraints that applied, the effects of temperature on U_{\max} and U_{trans} would be different.

Contrary to our expectation, the relationship between U_{trans} and temperature was similar to that for U_{\max} (Fig. 3, Fig. 4A,B). At low temperatures this was expected, because of the reduction in pectoral girdle muscle contraction velocity and power output at temperatures lower than the acclimation temperature (Figs 5 and 6). The plateau in U_{trans} at temperatures above the acclimation temperature did not fit with our initial predictions. In bluegill, aerobic activity can be sustained at a level three times greater than that used during maximal labriform swimming (Kendall et al., 2007), so at U_{trans} there is additional capacity to deliver and utilize oxygen, at least at the whole-organism level. Factors other than systemic respiratory and cardiovascular performance must limit U_{trans} at high temperatures.

If the limits to muscle mechanical performance were driving the gait transition, then the power available from the pectoral girdle musculature would be approximately maximal at the fin beat frequency used at U_{trans} . This has previously been observed in bluegill acclimated to, and swimming at 22°C (Kendall et al., 2007). A similar pattern was observed in the present study at 14, 18 and 22°C (Fig. 4B, Fig. 6). At these temperatures, the strain cycle frequency that maximized muscle mechanical power output approximated that used *in vivo* at U_{trans} (Fig. 4B, Fig. 6). By contrast, at temperatures above T_a , there was a mismatch between the *in vivo* cycle frequency, and the optimal cycle frequency for maximizing mechanical power output (Fig. 4B, Fig. 6). At both 26 and 30°C there was considerable scope *in vitro* for increasing operating frequency and power output beyond the *in vivo* maxima (Figs 5 and 6). Something other than the intrinsic mechanical properties of the muscle must therefore be determining U_{trans} and driving the gait transition at higher temperatures.

A similar mismatch between *in vitro* and *in vivo* muscle mechanical performance has been observed in running lizards [*Sceloporus occidentalis* (Marsh and Bennett, 1986b)]. In this species the maximum shortening velocity of the iliofibularis muscle, a hip extensor, increased with increasing temperature (Marsh and Bennett, 1986a; Marsh and Bennett, 1986b). Despite this, both stride frequency and maximal speed plateaued at high temperatures in a similar way to pectoral fin beat frequency and U_{trans} in bluegill (Marsh and Bennett, 1986b). In *Sceloporus* this was attributed to passive mechanical constraints that were not temperature sensitive (Marsh and Bennett, 1986b). Similar factors are also likely to be important in bluegill. Detailed analyses of fin kinematics have revealed the complex twisting and curvature of the fin surface throughout the fin beat cycle (Lauder et al., 2006; Standen and Lauder, 2006; Lauder et al., 2007). Although powered and controlled by muscles, the movement patterns are also caused by the passive mechanical properties of the fin rays, fin membrane and tendons (Lauder et al., 2006; Lauder et al., 2007). The properties of these passive components, which are largely fixed with respect to temperature, may limit fin beat frequency. An additional, external mechanical factor is the interaction of the pectoral fins with the water to generate lift and thrust. In bluegill, as labriform swimming speed increases, the jet flow associated with the pectoral fin down-stroke becomes increasingly laterally oriented (Drucker and Lauder, 2000). This means that as speed, and therefore drag, increase, the capacity to develop thrust as a component of the total momentum transferred to the water decreases, potentially imposing a hydrodynamic limit on labriform swimming that is independent of the available muscle power.

Physiological factors may also limit pectoral girdle muscle performance at high temperatures. Blood flow rates to specific muscles can reach maximum levels at sub-maximal levels of organismal aerobic metabolism (Armstrong et al., 1987; Ellerby et al., 2005). Even though central limits to oxygen uptake and delivery have not been reached at U_{trans} , peripheral limits to blood flow rate, and consequently oxygen and substrate delivery, may prevent the pectoral girdle muscle from reaching its maximum potential power output *in vivo*.

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